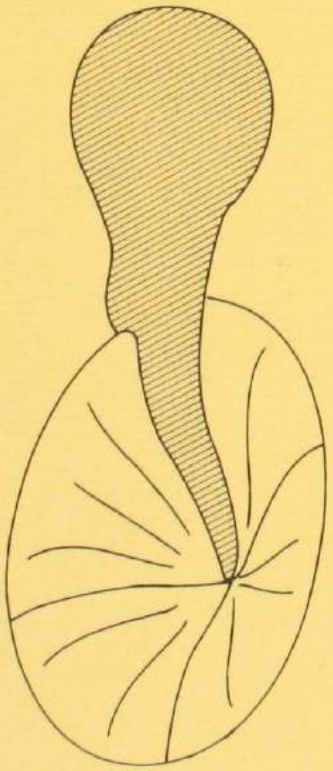
# DEVELOPMENT OF BIODEGRADABLE POLYMERS FOR APPLICATION IN MYRINGOPLASTY



Frits Kohn

DEVELOPMENT OF BIODEGRADABLE
POLYMERS FOR APPLICATION IN
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PROEFSCHRIFT

ter verkrijging van de graad van doctor in de technische wetenschappen aan de Technische Hogeschool Twente, op gezag van de rector magnificus, Prof. ir. W. Draijer, volgens besluit van het College van Dekanen in het openbaar te verdedigen op vrijdag 30 maart te 16.00 uur

door

Frederik Emiel Kohn

geboren op 28 maart 1947 te Hengelo (Ov.)

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1984

Dit proefschrift is goedgekeurd door de promotoren:

PROF. DR. J. FEIJEN
PROF. DR. L. FEENSTRA


Aan mijn moeder
Ter nagedachtenis aan mijn vader

WOORD VOORAF

Het onderzoek beschreven in dit proefschrift bestaat uit twee duidelijk onderscheiden en tegelijkertijd innig verbonden onderdelen. Het polymeerchemische deel kwam vooral tot stand binnen de vakgroep Macromoleculaire Chemie en Materiaalkunde van de afdeling Chemische Technologie van de TH Twente.
De medische component werd begeleid vanuit de afdeling Keel-Neus- en Oorheelkunde van de Vrije Universiteit te Amsterdam.

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CONTENTS

CHAPTER I

INTRODUCTION

After trauma or inflammation an eardrum may become permanently perforated, frequently resulting in some hearing loss and proneness to infection. Until 1950 the treatment of a perforated eardrum consisted of covering the drum permanently with artificial material, -e.g. fish's air bladder, India rubber, egg membrane or, more recently, silicone rubber. Improvement in hearing seldom occurred.
Since then a surgical technique to establish a functional reconstruction of the eardrum was developed. The principle of this technique which is called myringoplasty, is based on covering the drum perforation with a collagenous structure, -i.e. the graft-, which must function as a scaffold for the migrating epithelial tissue from the edges of the perforation. During the healing process which may take several months the scaffolding structure is expected to be absorbed gradually. Almost all grafting materials used until now are of biological origin which implies that their rates of degradation cannot sufficiently be predicted. Myringoplastic operations may fail because of premature desintegration of conventional tissue grafts and also the grafts may cause a remarkable antigenicity or a rather intense inflammatory tissue reaction. Yet another reason why biological tissue grafts may prove to be inadequate is related to the treatment of complicated perforations, -e.g. total or subtotal perforations, atelectatic ears or congenital anomalies. The treatment of such perforations often requires specific grafts which usually cannot be prepared properly from the rather soft biological tissues.
Biomedical research activities over the last twenty years have revealed an increasing interest in the application of synthetic biodegradable polymers in medicine and surgery, having a predictable rate of absorption and loss of strength. This interest originated from an area where natural absorbable collagenous materials were used for over many centuries and concerns the

# CHAPTER I

## INTRODUCTION

After trauma or inflammation an eardrum may become permanently perforated, frequently resulting in some hearing loss and proneness to infection. Until 1950 the treatment of a perforated eardrum consisted of covering the drum permanently with artificial material, -e.g. fish's air bladder, India rubber, egg membrane or, more recently, silicone rubber. Improvement in hearing seldom occurred.

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Biomedical research activities over the last twenty years have revealed an increasing interest in the application of synthetic biodegradable polymers in medicine and surgery, having a predictable rate of absorption and loss of strength. This interest originated from an area where natural absorbable collagenous materials were used for over many centuries and concerns the

repair of traumatic injuries and surgical incisions. Until recently "catgut" derived from the submucosa of the sheep intestine remained unchallenged as the most acceptable form of absorbable suture and ligature material. Although adequate, "catgut" exhibits a number of shortcomings related to the fact that it is a complex natural proteinaceous substance of non-uniform composition. These shortcomings include variable strenght and tissue absorption rate, and local tissue activity.

The number of well-functioning synthetic biodegradable polymers is as yet limited. The poly(α-hydroxy acids), -i.e. poly (glycolic acid), poly(lactic acid) and their co-polymers -, and the poly(α-amino acids) represent two important classes of synthetic biodegradable polymers which have been applied, amongst others, as biodegradable sutures, burn wound covering, sustained release system, and as resorbable prostheses in maxillofacial and orthopedic surgery.

The research activities reported about in this thesis were concentrated on three subjects:

- The chemistry of poly(D,L-lactic acid), the synthetic biodegradable polymer which was extensively studied in experimental myringoplasty (Chapters II, III, IV and V).
- The chemistry of linear, alternating polydepsipeptides, a potentially new class of synthetic biodegradable polymers (Chapter VI).
- The application of a number of biodegradable and also microporous non-degradable synthetic polymers in experimental myringoplasty (Chapters VII and VIII).

The preferred method for the preparation of usable, high molecular weight poly(D,L-lactic acid) is the ring-opening polymerization of the six-membered cyclic diester D,L-lactide (m.p. 126°C) in the melt, initiated with a suitable initiator. The observation that high molecular weight poly(D,L-lactic acid) could not be prepared in a reproducible way by melt polymerizing D,L-lactide with tetraphenyltin initiator, has



led us to a more detailed study of the chemistry of this biodegradable poly(α-hydroxy acid).

In Chapter II the structure of the monomer D,L-lactide having a melting point of 126°C is discussed. X-ray structure elucidation was used to answer the question whether D,L-lactide (m.p. 126°C) is in fact a 1:1 racemate of L-,L-lactide and D-,D-lactide, - both having a melting point of 95°C -, or whether D,L-lactide consists of the meso-lactide molecule, -i.e. each lactide molecule consists of one levorotatory and one dextrorotatory lactate residue.

D,L-lactide can be considered as a modified lactone, that is to say that the ring compound comprises two ester groups instead of usually one. Although in several publications mechanisms have been proposed for the initiation or, - in a broader sense - for the polymerization of lactones, the precise mechanism, - or mechanisms -, are still disputed. In addition to this, there virtually exist no data on the interaction between the initiator and lactone monomer prior to the actual ring-opening. The latter has especially been the subject of the research disclosed in Chapter III. An experimental set-up was chosen which involved the IR-spectroscopical study of the complex formation in solution of a number of initiators or potential initiators, and, - amongst others -, D,L-lactide.

The lack of reproducibility of polymer molecular weight upon the melt polymerization of D,L-lactide with tetraphenyltin initiator was already mentioned. In Chapter IV the results of a study of the relation between melt polymerization conditions and polymer molecular weight and weight distribution is described. Tetraphenyltin initiator concentration was kept constant or was varied, in both "single" and "multiple" (four or eight reactions at the same time) melt polymerizations, while also the effects of impurities were studied. "Multiple"-type polymerizations were also carried out to determine the effect of polymerization time on the degree of conversion of D,L-lactide and on polymer molecular weight. Chapter IV is concluded with a discussion on the possibility of a "living"

mechanism for the melt polymerization of D,L-lactide.

There are strong indications that the degradation behaviour of biodegradable polymers is affected by both molecular weight and molecular weight distribution. Hitherto, however, information on the molecular weight of poly(D,L-lactic acid), - or poly(L-lactic acid) -, is limited. Even less systematic data on the molecular weight distribution as part of the characterization of poly(lactic acid) have been published so far. Therefore a number of poly(D,L-lactic acid) samples prepared in this study were extensively characterized by high-pressure gel permeation chromatography, using a universal calibration curve method. The results are presented in Chapter V which also deals with the effect of <u>in vitro</u> hydrolytic degradation on the molecular weight distribution of two poly(D,L-lactic acid) samples.

As was mentioned before, the poly(α-hydroxy acids) and the poly(α-amino acids) represent two important classes of accepted synthetic biodegradable polymers. Other types of synthetic biodegradable polymers with different degradation characteristics may prove a valuable supplement to these, -e.g. a polymer which combines properties of both α-hydroxy acid homopolymers and of α-amino acid homo-polymers. Therefore in Chapter VI attention is paid to a potentially new class of synthetic biodegradable polymers, the linear, alternating polydepsipeptides, -i.e. polymer chains having ester and amide linkages which succeed each other and which are separated by one carbon atom. Hitherto syntheses of linear, alternating polydepsipeptides, based on succesful multi-step synthetic routes to sequential polypeptides, have only been carried out at a very small scale. It is believed that, in principle, linear, alternating polydepsipeptides can be prepared directly and on a larger scale by the ring-opening polymerization of 2,5-morpholinediones, which are six-membered heterocyclic compounds comprising both an α-hydroxy acid residue and an α-amino acid residue. In Chapter VI the chemistry and preparation of these new heterocyclic monomers are discussed and a few polymerization attempts are described.



The last two chapters are devoted to the application of a number of biodegradable and microporous non-degradable synthetic polymers in experimental myringoplasty.

Chapter VII starts with a description of the anatomy and functioning of the human eardrum and with a historical survey of the treatment of perforated eardrums. Thereafter a justification is given of the idea that synthetic polymers will be a valuable supplement of the existing biological grafting materials. In the second part of Chapter VII the results of the experimental myringoplasty are described. For that purpose artificial eardrums made from several biodegradable synthetic polymers and made from a number of microporous, non-degradable synthetic polymers were implanted into the ears of rats and dogs, and as a reference subcutaneously. Based on these results, at the end of Chapter VII a number of recommendations are given for further research activities in the area of experimental myringoplasty.

Finally in Chapter VIII a part of the experimental myringoplastic studies already mentioned in Chapter VII is presented in greater detail.

CHAPTER II

# THE STRUCTURE OF 3,6-DIMETHYL-1,4-DIOXANE-2,5-DIONE [D-,D-(L-,L-)-LACTIDE]*


## ABSTRACT

$C_6H_8O_4$, $M_r$ = 1.44.1, $P2_1/c$, a = 8.050 (2), b = 9.086 (1), c= 9.713 (2) Å, $\beta$ = 102.86 (3)°, Z = 4, V = 693 Å$^3$, $D_x$ = 1.38 g cm$^{-3}$, $\mu_{Mo\ K}$ = 1.1 cm$^{-1}$.

Data collection was carried out at 293 K. All hydrogen atoms were located. The molecule has approximate $C_2$ symmetry. R = 4.6, $R_w$ = 3.8% for 872 reflexions. The compound D,L-lactide is shown to be the racemate of D-,D- and L-,L-lactide.


## II.1 Introduction

Over the past fifteen years there has been considerable interest in the application of poly(D-,L-lactic acid) in medicine and surgery (Kronenthal, 1975), for example as a grafting material for perforated eardrums (Feenstra, Van der Ven, Kohn and Feijen, 1980). The preferred method of preparation is the ring-opening polymerization of racemic lactide (or dilactide, commonly known as D-,L-lactide (Kulkarni, Pani, Neuman and Leonard, 1966)), a six-membered cyclic diester synthesized from the commercially available racemate of L(+)- and D(-)-lactic acid.

Substantial synthetic evidence exists (Jungfleisch and Godschot, 1906; Deane and Hammond, 1960; Holten, Müller and Rehbinder, 1971) that the D-,L-lactide (m.p. 400 K) routinely used is in fact a racemate of L-,L- and D-,D-lactide (m.p. 368 K). This fact has not been appreciated generally. Some authors (Fouty, 1973; Gregory, Schwope and Wise, 1973; Sinclair, 1977) suggest that D-,L-lactide consists of meso-lactide molecules. In polymers derived from the meso-lactide the stereo sequence cannot contain more than two D- or L-lactate units in succession.


* G.J. van Hummel, S. Harkema, F.E. Kohn and J. Feijen, Acta Cryst., B38, 1679-1681 (1982).




A racemate of D-,D- and L-,L-lactide allows, in principle, the formation of stereo block-type copolymers (Lillie and Schulz, 1975; Schindler and Harper, 1976).

Crude D-,L-lactide was prepared according to Sinclair and Gynn (1972). From this product two compounds could be obtained, one melting at 400 K and one melting at 316 K. Suitable crystals (maximum dimension 0.4 mm) of the compound, melting at 400 K, were obtained by controlled recrystallization from dry ether.

In this paper the first X-ray structure elucidation of racemic lactide is reported

Intensities up to $\theta$ = 25° were collected at 293 K on a Philips PW1100 single-crystal diffractometer, using the $\omega$-2$\theta$ scanning technique (scan width: (2 + tan$\theta$)°), with graphite-monochromatized Mo K$\alpha$ radiation. The scan speed was 0.03° s$^{-1}$. The total background measuring time was scan time x $(I_{backgr}/I_{peak})^{\frac{1}{2}}$, with a minimum of 5 s at each side of the peak.
The number of reflexions measured was 1294, of which 872 were considered significant ($I > \sigma(I)$, where $\sigma(I)$ is the standard deviation from counting statistics).

The stucture was solved by direct methods (Germain, Main and Woolfson, 1971) and refined with a local version of ORFLS (Busing, Martin and Levy, 1962). The function minimized was $\Sigma w(|F_o| - k|F_c|)^2$ with $w = \sigma^{-2}(F)$. $\sigma(F)$ was calculated from $\sigma(I) + 0.01|F_o|$. The atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1974). The parameters refined were the scaling factor, positional parameters for all atoms and anisotropic temperature factors for the non-hydrogen atoms. For H atoms isotropic temperature factors were refined. Extinction corrections were applied. The final unweighted and weighted R factors were 4.6 and 3.8% respectively, for the significant reflexions.

## II.2 Discussion

The asymmetric unit consists of one molecule of lactide. Atomic coordinates are given in Table I (see Fig. 1 for

| | $x$ | $y$ | $z$ | $U_{eq}$* |
|---|---|---|---|---|
| O(1) | 6386 (2) | 9651 (2) | 6789 (2) | 55 (1) |
| O(2) | 6130 (2) | 7170 (2) | 5179 (2) | 58 (1) |
| C(3) | 4863 (3) | 8986 (3) | 6465 (3) | 49 (1) |
| O(4) | 3605 (2) | 9665 (2) | 6522 (2) | 71 (1) |
| C(5) | 7909 (3) | 8742 (3) | 6907 (3) | 56 (1) |
| C(6) | 7692 (3) | 7729 (3) | 5664 (3) | 52 (1) |
| C(7) | 9401 (4) | 9748 (5) | 7070 (5) | 84 (2) |
| C(8) | 4878 (3) | 7394 (3) | 6033 (3) | 55 (1) |
| O(9) | 8831 (2) | 7400 (2) | 5108 (2) | 76 (1) |
| C(10) | 3209 (4) | 6872 (4) | 5169 (5) | 79 (1) |
| H(1) | 796 (3) | 813 (3) | 770 (3) | 66 (8) |
| H(2) | 1049 (4) | 918 (3) | 713 (3) | 74 (8) |
| H(3) | 524 (3) | 684 (3) | 691 (3) | 61 (8) |
| H(4) | 236 (4) | 704 (3) | 566 (3) | 77 (10) |
| H(5) | 328 (4) | 583 (4) | 494 (4) | 93 (11) |
| H(6) | 292 (3) | 738 (4) | 425 (3) | 75 (10) |
| H(7) | 926 (4) | 1035 (4) | 623 (4) | 87 (13) |
| H(8) | 950 (5) | 1032 (4) | 793 (5) | 114 (14) |

* Defined according to Willis & Pryor (1975).

Table 1. Fractional atomic coordinates ($\times 10^4$; H $\times 10^3$) and equivalent isotropic thermal parameter ($Å^3 \times 10$)

| | | | |
|---|---|---|---|
| O(1)–C(3) | 1·340 (3) | C(3)–C(8) | 1·507 (3) |
| O(1)–C(5) | 1·462 (3) | C(5)–C(6) | 1·497 (4) |
| O(2)–C(6) | 1·341 (3) | C(5)–C(7) | 1·490 (5) |
| O(2)–C(8) | 1·455 (3) | C(6)–O(9) | 1·200 (3) |
| C(3)–O(4) | 1·197 (3) | C(8)–C(10) | 1·494 (5) |
| C(3)–O(1)–C(5) | 118·1 (3) | C(6)–C(5)–C(7) | 114·2 (3) |
| C(6)–O(2)–C(8) | 117·5 (3) | O(2)–C(6)–C(5) | 116·6 (3) |
| O(1)–C(3)–O(4) | 119·7 (3) | O(2)–C(6)–O(9) | 119·9 (3) |
| O(1)–C(3)–C(8) | 115·7 (2) | C(5)–C(6)–O(9) | 123·5 (3) |
| O(4)–C(3)–C(8) | 124·5 (3) | O(2)–C(8)–C(3) | 110·0 (2) |
| O(1)–C(5)–C(6) | 109·8 (2) | O(2)–C(8)–C(10) | 106·6 (3) |
| O(1)–C(5)–C(7) | 107·7 (3) | C(3)–C(8)–C(10) | 113·5 (3) |

Table 2. Bond distances (Å) and angles (°)

| | | | |
|---|---|---|---|
| C(5)–O(1)–C(3)–O(4) | 171·3 (2) | C(8)–O(2)–C(6)–O(9) | 169·5 (2) |
| *C(5)–O(1)–C(3)–C(8) | −9·2 (3) | *C(8)–O(2)–C(6)–O(5) | −10·1 (3) |
| *O(1)–C(3)–C(8)–O(2) | −38·2 (3) | *O(2)–C(6)–C(5)–O(1) | −37·1 (3) |
| O(1)–C(3)–C(8)–C(10) | −157·5 (3) | O(2)–C(6)–C(5)–C(7) | −158·2 (3) |
| O(1)–C(3)–C(8)–H(3) | 79 (2) | O(2)–C(6)–C(5)–H(1) | 77 (2) |
| O(4)–C(3)–C(8)–O(2) | 141·3 (2) | O(9)–C(6)–C(5)–O(1) | 143·4 (3) |
| O(4)–C(3)–C(8)–C(10) | 22·0 (4) | O(9)–C(6)–C(5)–C(7) | 22·3 (4) |
| O(4)–C(3)–C(8)–H(3) | −102 (2) | O(9)–C(6)–C(5)–H(1) | −102 (2) |
| *C(3)–C(8)–O(2)–C(6) | 48·4 (3) | *C(6)–C(5)–O(1)–C(3) | 47·2 (3) |
| C(10)–C(8)–O(2)–C(6) | 171·8 (2) | C(7)–C(5)–O(1)–C(3) | 172·2 (3) |
| H(3)–C(8)–O(2)–C(6) | −67 (2) | H(1)–C(5)–O(1)–C(3) | −67 (2) |
| O(1)–C(5)–C(7)–H(2) | −179 (2) | O(2)–C(8)–C(10)–H(4) | −177 (2) |
| O(1)–C(5)–C(7)–H(7) | −62 (2) | O(2)–C(8)–C(10)–H(6) | −56 (2) |
| O(1)–C(5)–C(7)–H(8) | 62 (2) | O(2)–C(8)–C(10)–H(5) | 60 (2) |

* Torsion angles in the cyclic group.

Table 3. Pairwise comparison of torsion angles (°).



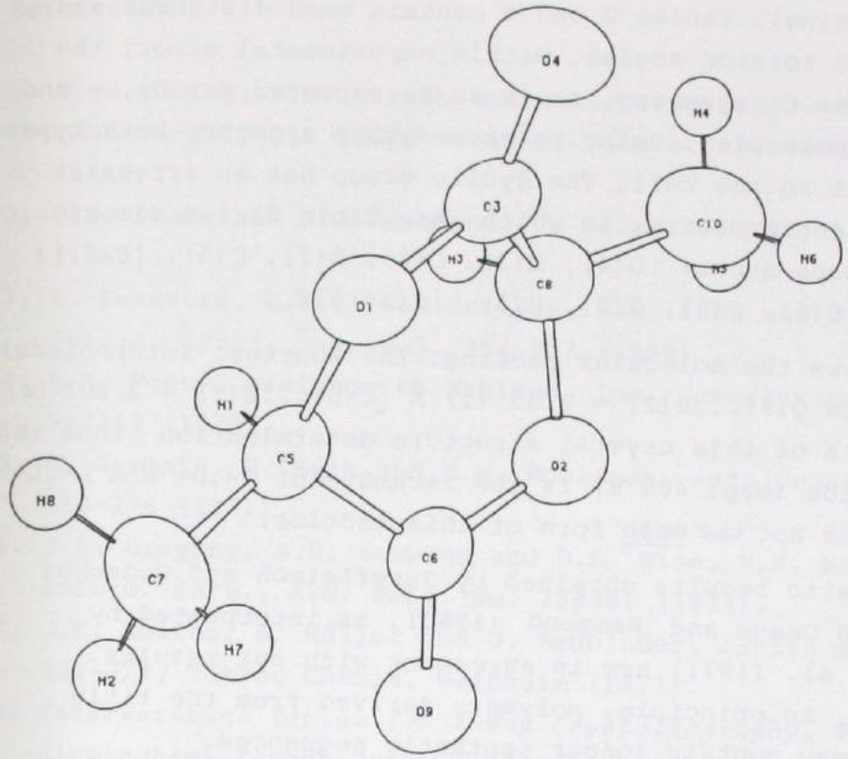



Fig. 1. The D-,D-lactide molecule showing the atomic numbering

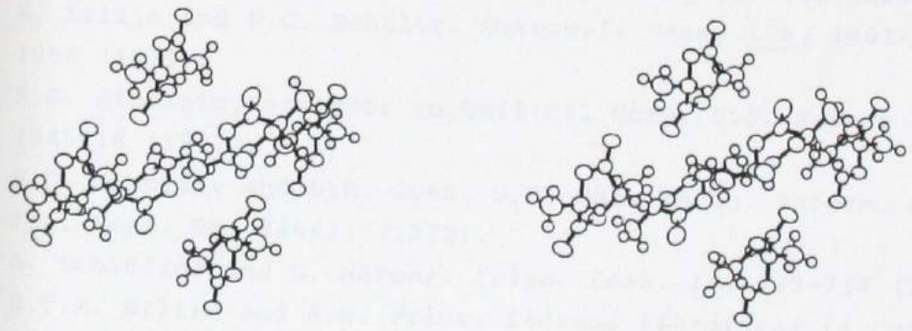


Fig. 2. Stereoscopic view of the molecular packing.

atom numbering); Tables 2 and 3 contain bond distances and angles, and torsion angles. Within experimental error, the molecule has $C_2$ symmetry, as is to be expected for D-,D- and L-,L-type molecules. Owing to space-group symmetry both types are present in the cell. The cyclic group has an irregular skew boat conformation, in which (see Table 3) two almost planar groups occur: {O(4), C(3), C(8), O(1), C(5), |C(7)|} and {O(9), C(6), C(5), O(2), C(8), |C(10)|}.

Fig. 2 shows the molecular packing. The shortest intermolecular distance is O(9)...H(2) = 2.33 (2) Å [C(5)...O(9) = 3.202 (4) Å]. The results of this crystal structure determination show that D-,L-lactide (m.p. 400 K) is the racemate of D-,D- and L-,L-lactide and not the meso form of this lactide.

The synthetic results obtained by Jungfleisch and Godschot (1906) and Deane and Hammond (1960), as interpreted by Holten et al. (1971) are in agreement with our results. Therefore, in principle, polymers derived from the title compound may contain longer isotactic sequences.



References


1. W.R. Busing, K.O. Martin and H.A. Levy, *ORFLS*. Report ORNL-TM-305, Oak Ridge National Laboratory, Tennessee (1962).
2. D.D. Deane and E.G. Hammond, *J. Dairy Sci.* 43, 1421-1429 (1960).
3. L. Feenstra, B.W.C. van der Ven, F.E. Kohn and J. Feijen, *Int. J. Artif. Organs* 3, 354-357 (1980).
4. R.A. Fouty, Assignor to Ethicon, Inc. *Canadian patent* 923245 (1973).
5. G. Germain, P. Main and M.M. Woolfson, *Acta Cryst*, A27, 368-376 (1971).
6. J.B. Gregory, A.D. Schwope and D.L. Wise, U.S. Nat. Techn. Inform. Serv., *A.D. Rept.* No. 759381 (1973).
7. C.H. Holten, A. Müller and D. Rehbinder, *Lactic acid*, pp. 221-224, Verlag Chemie, Weinheim (1971).
8. *International Tables for X-ray Crystallography*. Vol. VI, Birmingham: Kynoch Press (1974).
9. E. Jungfleisch and M. Godschot. *Compt. rendus*, 144, 637-639 (1909).
10. R.L. Kronenthal. *Polymers in Medicine and Surgery*, edited by R.L. Kronenthal, Z. Oser and E. Martin, pp. 119-137, Plenum Press: New York and London (1975).
11. R.K. Kulkarni, K.C. Pani, C. Neuman and F. Leonard. U.S. Nat. Techn. Inform. Serv., *A.D. Rept.* No. 636.716 (1966).
12. E. Lillie and R.C. Schultz. *Makromol. Chem.* 176, 1901-1906 (1975).
13. R.G. Sinclair, assignor to Gulf Oil Corp. *U.S. Patent* 4045418 (1977).
14. R.G. Sinclair and G.M. Gynn, U.S. Nat. Techn. Inform. Serv., *A.D. Rept.* No. 748411 (1972).
15. A. Schindler and D. Harper. *Polym. Lett.* 14, 729-734 (1976).
16. B.T.M. Willis and A.W. Prior. *Thermal Vibrations in Crystallography*, pp. 101-102. Cambridge Unversity Press (1975).

CHAPTER III

# THE MECHANISM OF THE RING-OPENING POLYMERIZATION OF LACTIDE AND GLYCOLIDE*


## ABSTRACT

To obtain more insight in the ring-opening polymerization of lactones, the complex formation of the initiators tetraphenyltin, stannous octoate, tintetrachloride, aluminium bromide and triisobutylaluminium (TIBA), and the monomers L (-) -lactide, D,L-lactide and glycolide was studied by IR-spectroscopy. When equimolar benzene or toluene solutions of initiators and monomers were combined, only complexes of aluminium bromide and D,L-lactide or glycolide, and of TIBA and D,L-lactide or glycolide were observed. The complex formation was studied in detail by varying the initiator and monomer concentrations. From these results and theoretical considerations it is concluded that complexes are formed by the coordination of a carbonyl oxygen of the monomers and the aluminium atom of initiators. The corresponding polymers were formed when TIBA was used as an initiator. When $AlBr_3$ was used only polymers were obtained when traces of water were added. It is concluded that in the case of $AlBr_3$ the actual initiating species is HBr and that the polymerization initiated with TIBA proceeds very likely through a coordinated insertion of the lactone monomer into the aluminium-carbon bond.


## III.1. Introduction

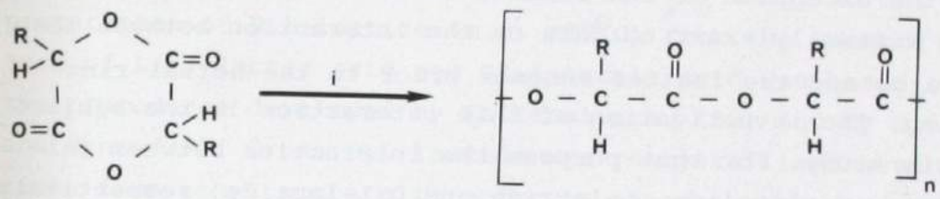
Over the past 15 years there has been considerable interest in the application of poly (glycolic acid) PGA, poly (L- and D,L-


* F.E. Kohn, J.G. van Ommen and J. Feijen,
Eur. Polym. J., 19, 1081-1088 (1983)




lactic acid) PLA and PDLA and of co-polymers of glycolic and L-lactic acid GA/LLA as biodegradable polymers in medicine and surgery (1). Absorbable sutures prepared from PGA (2) and from GA/LLA co-polymers (molar ratio 90/10) (3) are commercially available. In our laboratories we have recently applied PDLA as temporary eardrum grafts in rats (4) and dogs.

Nowadays the preferred method for the preparation of high molecular weight PGA, PLLA, and PDLA or corresponding co-polymers is the ring-opening polymerization of the six-membered cyclic diesters glycolide (m.p. 87°C), L-lactide (m.p. 95°C) and D,L-lactide (m.p. 127°C), respectively, initiated with a suitable initiator, e.g. tintetrachloride (5), stannous octoate or tetraphenyltin (6). The ring-opening polymerization is schematically depicted in Scheme 1.



R = H glycolide

R = $CH_3$ lactide

Scheme 1. Ring-opening polymerization of glycolide and lactide.

The polymerization is preferably performed in the melt at higher temperatures, although lactide has also been polymerized succesfully in solution under mild conditions (7).

Purified D,L-lactide, derived from the racemic mixture of L(+)-lactic acid and D(-)-lactic acid, is the molecular 1:1 compound of both enantiomers L(-)-lactide and D(+)-lactide (8). Lactide and glycolide can be considered as modified lactones and their

polymerization behaviour has been reported to be rather similar to that of δ-valerolactone (9).

In several publications mechanisms have been proposed for the initiation or, in a broader sense, for the polymerization of lactones. These mechanisms can be subdivided into (i) cationic (5,10-15); (ii) anionic (16-20); and (iii) coordination-insertion types (21-27). The latter type is considered by Young e.a. (28) as the intermediate case between the two other modes of initiation. In spite of the many data published on the polymerization of lactones, especially of β-propiolactone and ε-caprolactone (29-31), the question whether a lactone ring is opened by acyl-oxygen bond cleavage or by alkyl-oxygen bond cleavage is still disputed. Another as yet insufficiently answered question is related to the true nature of the initiating species.

With the exception of the results obtained by Kogan e.a. (15) there virtually exist no data on the interaction between the initiator and the lactone monomer prior to the actual ring opening. The investigation of this interaction is the subject of this study. For that purpose the interaction between initiators and glycolide, L-lactide and D,L-lactide, respectively, was studied by IR-spectroscopy. In the study by Kogan e.a. only precipitates which could be isolated after mixing equimolar benzene solutions of a Lewis acid and glycolide were studied by IR-spectroscopy. In this way solid compounds of glycolide and the strong Lewis acids $TiCl_4$ (molar ratio 1:1), $ZrCl_2$ (1:2) and $AlBr_3$ (1:4) were obtained and compared with pure glycolide.

In the present study mainly complex formation in solution (cf. 32) and some isolated precipitates were studied by IR-spectroscopy. Occasionally complex formation in the melt was investigated.



## III.2. Materials and Methods

### III.2.1. Materials

*Initiators and potential initiators.*

Tintetrachloride, tetraphenyltin (purchased from Polysciences) stannous octoate (tin- (II)-salt of 2-ethylhexoic acid; purchased from Polysciences) and triisobutylaluminium (TIBA; purchased from Schuchard, purity 93-95 procent) were used without further purification. Aluminium bromide was sublimed (p=0.1 mm; T=95°C) and stored under nitrogen until use.

*Monomers.*

L(-)-lactide (purchased from Polysciences) was recrystallized twice from ethyl acetate, dried in a vacuum oven at room temperature and stored in a dessicator on $P_2O_5$ (m.p. 95°C). The preparation of D,L-lactide (m.p. 127°C) from the racemic mixture of L(+)-lactic acid and D(-)-lactic acid was based on the procedures developed by Kulkarni e.a. (33) and by Sinclair and Gynn (34). Crude D,L-lactide was recrystallized several times from ethyl acetate in order to separate the compound melting at 127°C from the meso-lactide melting at 43°C (8). Purified D,L-lactide (m.p. 127°C) was dried in a vacuum oven at room temperature and stored in a dessiccator on $P_2O_5$. Glycolide (m.p. 87°C) was prepared according to Lowe (35) and to Sorenson and Campbell (36). It was recrystallized several times from ethyl acetate, dried in a vacuum oven at room temperature and stored in a dessiccator on $P_2O_5$. During the preparation of glycolide a considerable amount of powdered, low molecular weight poly(glycolid acid) mixed with antimony trioxide has to be introduced in small portions into the reaction vessel which is heated at 270°-285°C and maintained at a pressure lower than 15 mm. In the original procedure (35) a supply vessel connected by means of heavy-walled, flexible tubing to a stopcock fitted into the inlet neck of the three-necked reaction flask is recommended. In our experience the use of this equipment led

very soon to accumulation of solidified glycolide distillate and consequent plugging of the stopcock and tubing. Therefore the simple dosage system shown in Figure 1a was developed.

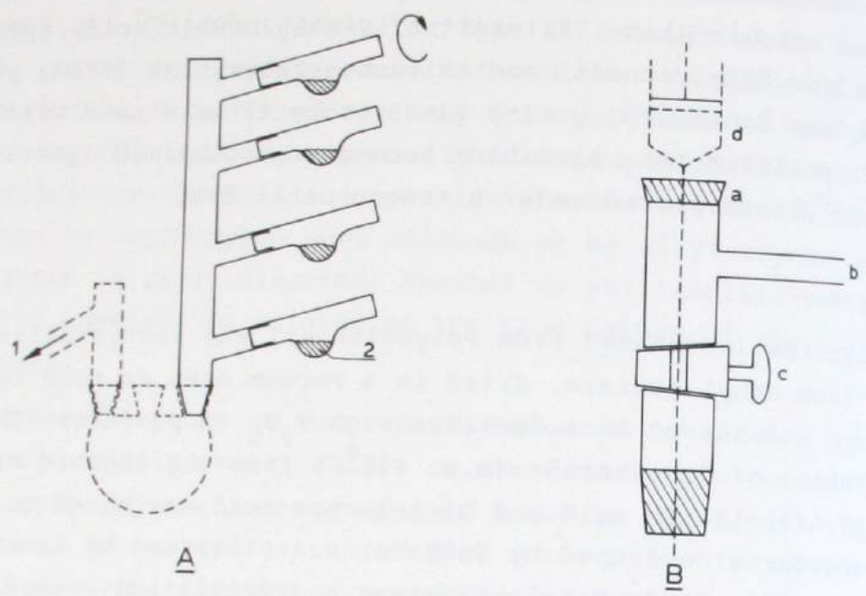



Fig. 1a. Dosage system for the administration of powdered material to an evacuated reaction vessel.

Fig. 1b. Adaptor.

By slowly turning the individual supply vessels one after each other the contents can be administered in a controlled way.

III.2.2. Methods

*Complex formation in solution.*

Toluene or benzene (Merck, pro analysis) was dried on molecular sieves (Union Carbide, 13X) and degassed and saturated with purified nitrogen (32). The $O_2$ and $H_2O$ contents of the purified nitrogen which was used throughout all experiments were less than 1 ppm.



A typical procedure for the preparation and IR-spectroscopical investigation of a complex of D,L-lactide and $AlBr_3$ was as follows. Glassware, syringes and needles were dried overnight at 120°C. A polymerization tube (37) was filled with a small amount of D,L-lactide and connected by means of a special adaptor (Figure 1b) and vacuum tubing to a vacuum system designed for experiments under nitrogen (38). In order to remove traces of water and other contaminating solvents the polymerization tube was placed in an oil bath (maximum temperature 100-120°) and the D,L-lactide was sublimed slowly onto the upper part of the tube at reduced pressure (0,1 mm). The amount of D,L-lactide which is lost during the sublimation process is negligible. Next the tube was purged with nitrogen and toluene was introduced through the septum. After the D,L-lactide had dissolved, the solution was transferred through a septum into a reaction vessel using a syringe which was previously purged with nitrogen. This reaction vessel was previously connected to the vacuum system when still hot, and was purged with nitrogen. A solution of sublimed $AlBr_3$ in toluene was prepared in a dry box and injected through the septum to the reaction vessel which contained the D,L-lactide solution. After 5 minutes a sample of the combined solution was transferred through the septum of the reaction vessel into a syringe which was previously purged with nitrogen. The solution was then quickly injected into an IR-solution cell which was also purged with nitrogen. The IR-spectrum was recorded on a Perkin-Elmer 357 grating IR-spectrophotometer. Toluene was placed in a reference cell to compensate for toluene absorption. When TIBA was used instead of $AlBr_3$, it was added pure or as a solution in toluene. When the complex formation of L(-)-lactide and tetraphenyltin, stannous octoate or $SnCl_4$ was investigated, L(-)-lactide was treated in the same way as D,L-lactide (maximum sublimation temperature 100°C). $SnCl_4$ was added pure or as a solution in toluene. Stannous octoate was added as a solution in toluene. Tetraphenyltin was added as a solution in benzene; in the latter case L(-)-lactide was also dissolved in benzene. For experiments under nitrogen at elevated temperatures including solu-

tion polymerizations, the reaction vessel (see above) was equipped with a condensor which was connected at the top to the vacuum system. When glycolide was used, this monomer was also sublimed (maximum temperature 95°C; p = 0,1 mm). Contrary to the TIBA-glycolide complexes, the $AlBr_3$-glycolide complexes were not soluble in toluene at room temperature. IR-spectra of the complexes in toluene could be recorded at about 90°C.

*Complex formation in the melt.*

Complex formation in the melt was investigated as follows. After the monomer was sublimed onto the upper part of the polymerization tube, an equimolar amount of pure initiator was added. The experiments were carried out in a dry box. Hereafter the tube was sealed under vacuum and kept at elevated temperatures for several hours. In these cases IR-spectra were recorded using KBr discs. The results with Nujol suspensions prepared in a dry box did not differ from those obtained when KBr discs were used.

*Solution polymerization.*

The solution polymerization was based on procedures developed by Dittrich and Schulz (22) and by Kleine and Kleine (39). Typically 1 gram of sublimed monomer was dissolved into 10 ml of dry toluene. After addition of 0.01 g initiator, the reaction mixture was refluxed for 24 hours under nitrogen and the solution was characterized using IR-spectroscopy; the spectra were compared with those of a polymer solution which was previously made. Reaction products (polymer) were isolated by solvent evaporation to dryness and subsequent washing with solvent.

III.3. Results and discussion

In Table 1 the results on the complex formation of monomers and initiators or potential initiators at different temperatures are summarized. Detectable amounts of complexes were only observed with the use of $AlBr_3$ or TIBA. In the experiments



Table 1. Observed complex formation of lactide or glycolide and several (potential) initiators in solution.

| MONOMER | INITIATOR | SOLVENT | C[a] (mol $l^{-1}$) | T (°C) | COMPLEX[b] FORMATION |
|---|---|---|---|---|---|
| L(-)-lactide | tetraphenyltin | benzene | 0.01 | 20 | - |
| L(-)-lactide | tetraphenyltin | benzene | 0.05 | 80 | - |
| L(-)-lactide | stannous octoate | toluene | 0.1 | 20 | - |
| L(-)-lactide | stannous octoate | toluene | 0.1 | 111 | - |
| L(-)-lactide | $SnCl_4$ | toluene | 0.1 | 111 | - |
| D,L-lactide | $SnCl_4$ | toluene | 0.1 | 20 | - |
| D,L-lactide | $AlBr_3$ | toluene | 0.1 | 20 | +, soluble |
| D,L-lactide | TIBA | toluene | 0.1 | 20 | +, soluble |
| Glycolide | $AlBr_3$ | toluene | 0.1 | 80-100 | +, soluble[c] |
| Glycolide | TIBA | toluene | 0.1 | 20 | +, soluble |

a: Equal amounts of monomer and (potential) initiator solutions with initial concentrations as tabulated, were combined.

b: By IR-spectroscopy.

c: Complex precipitated below 80°C.

carried out with L(-)-lactide and D,L-lactide, the absorption band at 935 $cm^{-1}$ which is characteristic of the ring vibration (40) never disappeared. This indicates that ring-opening polymerization did not occur. Using tetraphenyltin, the obsevation of the complex formation was more difficult because of the slight solubility of this initiator in benzene and, in fact, in any other solvent (41,42). Attempts to prepare complexes in the melt between D,L-lactide and tetraphenyltin were also not successful. Contrary to the complex formation found with the aluminium compounds, tin-(II)- and tin-(IV)-complexes were not observed which is in agreement with the fact that alumnium compounds form complexes more easily than tin compounds (43).

*D,L-lactide - $AlBr_3$ complexes*

When equimolar solutions of D,L-lactide and sublimed $AlBr_3$ in toluene were combined, a soluble complex characterized by the shifts of the carbonyl and ether frequencies shown in Table 2, was observed. The positions of the original C=O and C-O absorptions are in accord with those observed for solid L(-)-lactide by Schulz and Schwaab (40). A shift in $\nu$ (C=O) of 100 $cm^{-1}$ towards the long wave lengths is indicative of a weakening of the C=O bond. On the other hand, a shift in $\nu$ (C-O) of 50 $cm^{-1}$ towards the short wave lengths points to an increased strenght of the ether bond. The withdrawel of electrons from ester groups by $AlBr_3$ can be imagined as given in Scheme 2. The partial positive charge on the ether oxygen can be compensated to some extent by the inductive effect of the methyl group present.

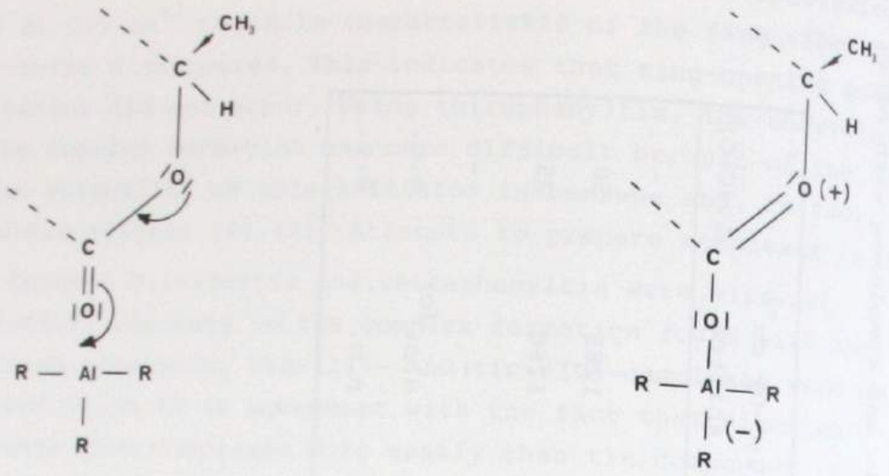
The complex formation between D,L-lactide and sublimed $AlBr_3$ in toluene was studied in more detail by adding successively very small amounts of a highly concentrated $AlBr_3$ solution to a 0.1 M D,L-lactide solution. The results are presented in a qualitative way in Table 3. The addition of the first small portion of $AlBr_3$ resulted in a shift in $\nu$ (C=O) of 100 $cm^{-1}$ towards the long wavelengths and a shift in $\nu$ (C-O) of 50 $cm^{-1}$ towards the short wavelenghts. The surface areas of the shifted



Table 2. Characteristic IR-frequencies of D,L-lactide, glycolide and complexes with $AlBr_3$ and TIBA, dissolved in toluene[a].

| | $\nu$(C=O) $cm^{-1}$ | | $\nu$(C-O) $cm^{-1}$ | |
|---|---|---|---|---|
| | original | shifted | original | shifted | $\Delta\nu$(C-O) |
| D,L-lactide | 1775 | - | 1235 | - | - |
| D,L-lactide-$AlBr_3$ | 1775 | 1675 | 1235 | 1285 | 50 |
| D,L-lactide-TIBA | 1775 | 1690 | 1235 | 1260 | 25 |
| Glycolide | 1780 | - | 1290 (1210) | - | - |
| Glycolide-$AlBr_3$[b] | 1775 | 1700 | 1290 (1210) | n.o.[c] | n.o. |
| Glycolide-TIBA | 1770 | 1710 | 1290 (1210) | n.o. | n.o. |

a: Experimental conditions: see Table 1.
b: IR-spectrum recorded from toluene solution at about 90°C.
c: n.o. not observed.




R = Br aluminum bromide

R = i-Bu TIBA

Scheme 2. Schematic representation of the withdrawel of elctrones from an ester bond of D,L-lactide by $AlR_3$ (R=Br or i-Bu).

absorption bands were as yet very small in comparison with the surface areas fo the original bands. The successive addition of more $AlBr_3$ resulted in almost identical positions of the original and shifted bands, whereas the decrease of the surface areas of the original bands and the increase of that of the shifted bands occurred in a more or less parallel way. The observation that at a molar ratio 1:1 both the original and the shifted bands were present, can be explained by the fact that only half of all ester groups may have formed coordination bonds.

The necessity of using sublimed $AlBr_3$ in these experiments and of working under strictly anhydrous conditions was shown by the observation that a precipitate was formed when solutions of D,L-lactide and non-sublimed $AlBr_3$ in toluene were combined. The precipitate was isolated by evaporation to dryness after it was found that the supernatant solution did not show any



Table 3. IR-spectroscopical investigation of the formation of soluble D,L-lactide-$AlBr_3$ complexes[a] in toluene by the successive addition of small portions of $AlBr_3$ to D,L-lactide.

| ABSORPTION (C=O) | | ABSORPTION (C-O) | | D,L-LACTIDE: $AlBr_3$ (molar ratio) |
|---|---|---|---|---|
| Original ($1775\ cm^{-1}$) | Shifted ($1675\ cm^{-1}$) | Original ($1235\ cm^{-1}$) | Shifted ($1285\ cm^{-1}$) | |
| vs | - | vs | - | 100 : 0 |
| vs | vw | vs | vw | 84 : 16 |
| s | w | s | w | 73 : 27 |
| m | m | m | m | 64 : 34 |
| m | m | w | m | 57 : 43 |
| w | s | vw | vs | 43 : 57 |

vs: very strong; s: strong; m: medium; w: weak; vw: very weak.

a: $C_{complex}$ is approximately 0.1 mol $l^{-1}$.

characteristic absorption band. In the IR-spectrum of the precipitate an absorption band corresponding to the original ν (C=O) of solid D,L-lactide at 1760 $cm^{-1}$ was found together with a new absorption band at 1630 $cm^{-1}$, whereas no other new absorptions were observed. An absorption at 1630 $cm^{-1}$ was also found in the IR-spectrum of non-sublimed $AlBr_3$ and this absorption was not present if sublimed $AlBr_3$ was used. If the latter compound was exposed to the air for only a short time an absorption band at 1630 $cm^{-1}$ appeared. The results of the elemental analysis of the precipitate showed a considerable lower percentage of Br than could be expected on the basis of the amount of Al determined. In conclusion, although D,L-lactide was present in the precipitate, the new absorption at 1630 $cm^{-1}$ must be attributed to partial hydrolysis of $AlBr_3$ (44) instead of to complex formation between D,L-lactide and $AlBr_3$.

*D,L-lactide - TIBA complexes*

When equimolar solutions of D,L-lactide and TIBA were combined, a soluble complex was formed. The IR-spectrum of this complex showed similar, but slightly smaller shifts as observed for the 1:1 D,L-lactide - $AlBr_3$ complex (Table 2). In principle the insertion of D,L-lactide into the Al-C bond can be expected. Under the experimental conditions used, this insertion reaction did not play a major role because the characteristic ring vibration at 935 $cm^{-1}$ was still present. The withdrawel of electrons from an ester group by TIBA may be presented schematically in the same way as in the case of $AlBr_3$ (Scheme 2). The sligthly smaller shifts observed for the complex with TIBA indicate that the Al-atom in $AlBr_3$ is slightly more electron dificient than in TIBA.

The complex formation between D,L-lactide and TIBA was studied in more detail in a similar way as described for D,L-lactide and $AlBr_3$. Apart from the absolute values of the shifts the results were comparable with those of the $AlBr_3$ - D,L-lactide complex formation (Table 3). The addition of the first portion of TIBA resulted in a shift in ν (C=O) of 85 $cm^{-1}$ towards the



long wave lenghts and a shift in ν (C-O) of 25 $cm^{-1}$ towards the short wave lengths. The positions of the original and shifted absorption bands did not change when more TIBA was added, up to a molar ratio of 1:1. Due to the relatively small shift in ν (C-O) of 25 $cm^{-1}$, the shifted band partially overlapped the original band. When the molar ratio TIBA/D,L-lactide was 2, all ester groups present could theoretically form coordination bonds with TIBA. The fact that still weak absorption bands of the original carbonyl and ether groups were present indicated that the equilibrium of the complex formation reaction was not totally to the side of the complex.

*Glycolide - $AlBr_3$ complexes*

Contrary to the complexes of D,L-lactide and sublimed $AlBr_3$, soluble complexes of glycolide and sublimed $AlBr_3$ could not be prepared at room temperature. When an equal amount of a warm solution of sublimed $AlBr_3$ in toluene (c = 0.1 mole/l) was added to a warm solution of glycolide in toluene (c = 0.1 mole/l.), a complex precipitated upon cooling which dissolved again at 80°C. Therefore the IR-spectrum of the complex was recorded from a toluene solution at about 90°C. The characteristic frequency shifts are summarized in Table 2.

The carbonyl group of glycolide absorbs at practically the same wave length as the carbonyl group of D,L-lactide which is in agreement with observations made by Goulden an Millard (45). After complex formation with $AlBr_3$ a shift in ν (C=O) of 75 $cm^{-1}$ towards the long wave lenghts was observed which is slightly less than the shift in ν (C=O) observed for the D,L-lactide-$AlBr_3$ complex.

Whereas the original ether absorption for pure D,L-lactide dissolved in toluene was found at 1235 $cm^{-1}$, the IR-spectrum of pure glycolide in toluene showed an intense absorption at 1290 $cm^{-1}$ and a very weak absorption at 1210 $cm^{-1}$. Kogan e.a. (15) assigned a band at 1215 $cm^{-1}$ to the stretching vibration of the ether group of glycolide, the occurrence of a band at 1290 $cm^{-1}$ was not mentioned. An extensive survey of the liter-

ature with respect to the ether absorption of glycolide only provided us with Raman-spectroscopical data (46). The Raman-spectrum of a glycolide melt showed a medium strong band at 1224 $cm^{-1}$ and a weak band at 1290 $cm^{-1}$. According to Colthup e.a. (47), the ether C-O stretching frequency of esters, which actually involves some interaction with all C-C bonds in the molecule, is near 1200 $cm^{-1}$. Only acetates absorb at 1260-1230 $cm^{-1}$. The ether absorption for the unstrained six-membered D,L-lactide found at 1235 $cm^{-1}$ fits in very well with these data. For glycolide both the absorption at 1210 $cm^{-1}$ (vw) and the absorption at 1290 $cm^{-1}$ (s) can be attributed to the ether group. An explanation for the occurrence of the two bands with different intensities might be a mixing of C-C and C-O stretching vibrations (48).

The IR-spectrum of a solution of the glycolide - $AlBr_3$ complex in toluene at higher temperatures did not show a shift of the original absorption at 1290 $cm^{-1}$. This result is in agreement with the fact that the absorption at 1290 $cm^{-1}$ originates from a coupling of C-O and C-C frequencies and thus responds in a different way to complex formation. The absorption at 1210 $cm^{-1}$ was so weak that significant changes could not be observed.

Kogan e.a. (15) also investigated the formation of complexes between glycolide and several Lewis acids. After mixing equimolar benzene solutions of glycolide and $AlBr_3$ a precipitate was obtained with a molar ratio glycolide: $AlBr_3$ of 1:4. Although Kogan and co-workers had expected a shift of 100 $cm^{-1}$ towards the long wave lengths for the carbonyl group, only a disappearance of the band at 1215 $cm^{-1}$ corresponding to the ether frequency was observed. Consequently Kogan and co-workers concluded that the ether-oxygen was the more active electron donor centre in the glycolide molecule which is not in agreement with our results obtained from IR-studies with solutions.

*Glycolide - TIBA complexes*

Contrary to the complexes between $AlBr_3$ and glycolide, complexes between TIBA and glycolide turned out to be soluble at room



temperature. When equimolar solutions of glycolide and TIBA in toluene were combined, a soluble complex was formed which was characterized by a similar, but slightly smaller shift in $\nu$ (C=O), 60 $cm^{-1}$, than the shift observed for the 1:1 glycolide-$AlBr_3$ complex at higher temperatures (Table 2), TIBA being a slightly weaker Lewis acid than $AlBr_3$. The shift in $\nu$ (C=O) of 60 $cm^{-1}$ is also slightly smaller than the shift in $\nu$ (C=O) of 85 $cm^{-1}$ observed for the soluble complexes of TIBA and D,L-lactide. A similar phenomenon was observed for the $AlBr_3$-glycolide complexes in comparison with their D,L-lactide counterparts. Apparently the glycolide molecule is less capable to donate electrons than the D,L-lactide molecule. It is obvious that in the case with glycolide complexes the induced positive charge on the ether-oxygen cannot be compensated by the electron donating effect of a methyl group as is the case with D,L-lactide (Scheme 2). As a result of this the C=O bond in the glycolide complexes retains more double bond character which is reflected by the smaller shifts in $\nu$ (C=O).

The IR-spectra of the glycolide - TIBA complexes did not show a shift of the absorption at 1290 $cm^{-1}$. Again significant changes of the very weak absorptions at 1210 $cm^{-1}$ could not be observed.

The formation of complexes between glycolide and TIBA was studied in more detail at molar ratio's of TIBA/glycolide >1. If the molar ratio TIBA/glycolide was 2, a weak band corresponding to the original C=O absorption was still present, indicating that complex formation was not complete.

*Solution polymerization studies*

Solution polymerization studies were performed with $SnCl_4$, $AlBr_3$ and TIBA as initiators. $SnCl_4$ has been used succesfully as an initiator for the solution polymerization of lactide (22, 39). Our investigation showed that with $SnCl_4$ moderately high molecular weight poly (D,L-lactic acid) ($\bar{M}_w$ = 2-2.5x$10^4$, $\bar{M}_n$ = 1-1.5x$10^4$) could be obtained.

$AlBr_3$ has not been reported as an initiator for the ring-openin polymerization of lactones. The attempted solution polymerization of D,L-lactide or glycolide in toluene initiated with sublimed $AlBr_3$ failed to produce any polymer. When the polymerization of glycolide was tried with $AlBr_3$ and traces of water, poly(glycolic acid) was formed as inidicated by IR-spectroscopy.

The solution polymerization with TIBA afforded moderately high molecular weight poly (D,L-lactic acid) ($\overline{M}_w$ = 1.5-2x$10^4$, $\overline{M}_n$ = 1-1.5x$10^4$). We also investigated the possibility of preparing poly (D,L-lactic acid) starting from a complex of D,L-lactide and TIBA (molar ratio: 1) dissolved in toluene. The amount of D,L-lactide was increased in a stepwise manner up to a molar ratio 30:1. In between the addition of each new portion of D,L-lactide the reaction mixture was refluxed for several hours and an IR-spectrum was recorded. Upon the successive addition of D,L-lactide the shifted bands corresponding to $\nu$ (C=O) and $\nu$ (C-O) disappeared gradually, whereas the IR-spectra became gradually more alike the IR-spectrum of poly (D,L-lactic acid) dissolved in toluene. IR-spectroscopical analysis of the reaction product formed after the attempted solution polymerization of glycolide initiated with TIBA indicated the formation of poly (glycolic acid).

*Complex formation related to polymerization*

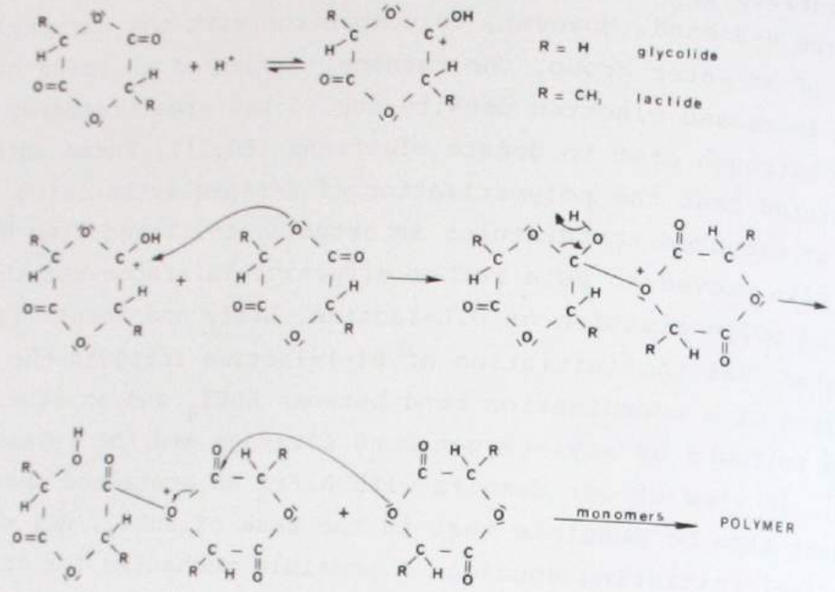
The strong Lewis acid $AlBr_3$ formed complexes with D,L-lactide and glycolide. Polymerization of glycolide using $AlBr_3$ could only be achieved after the addition of traces of water. A similar observation was made by Cherdron e.a. (10) when Lewis acids such as $AlCl_3$ were used as initiator for the polymerization of other lactones. These results strongly suggest that in our case HBr, formed by hydrolysis of $AlBr_3$, was the actual initiating species. In case of $SnCl_4$ no detectable amounts of complexes were observed which seems at first somewhat contradictory to the observation by Amass and Hay (49) that $SnCl_4$ formed a solid 1:2 molar complex with $\varepsilon$-caprolactam which was



characterized by a shift of the carbonyl band and the presence of a free N-H band. However, in comparison with the carbonyl oxygen of an ester group, the carbonyl oxygen of an amide group has an increased electron density due to the great tendency of the nitrogen atom to donate electrons (50,51). These authors also stated that the polymerization of $\varepsilon$-caprolactam using $SnCl_4$ at elevated temperatures is actually initiated with HCl. $SnCl_4$ also proved to be a rather effective initiator for the solution polymerization of D,L-lactide. Lilly and Schulz (5) suggested that the initiation of L(-)-lactide involved the formation of a coordination bond between $SnCl_4$ and an ether oxygen followed by acyl-oxygen bond cleavage and the release of $Cl^-$. In view of our results with $AlBr_3$ as mentioned above, it might also be possible that in the case of $SnCl_4$, HCl was the actual initiating species. A possible mechanism for the initiation and polymerization of lactide and glycolide (in toluene) with strong acids such as HBr or HCl is depicted in Scheme 3. First protonation will take place on one of the two available carbonyl oxygens followed by a proton shift and an $S_n2$-type transesterification reaction. According to Mhala and Mishra (52) the acid catalyzed hydrolysis of D,L-lactide involves as the first step protonation of an ether oxygen which does not seem in agreement with the present weight of physical evidence which shows that esters are initially protonated on the carbonyl and not on the ether oxygen (53).

Using the mechanism depicted in Scheme 3, polymerization with $AlBr_3$ should lead to charge separation which might explain that $AlBr_3$ as such is not a good initiator.

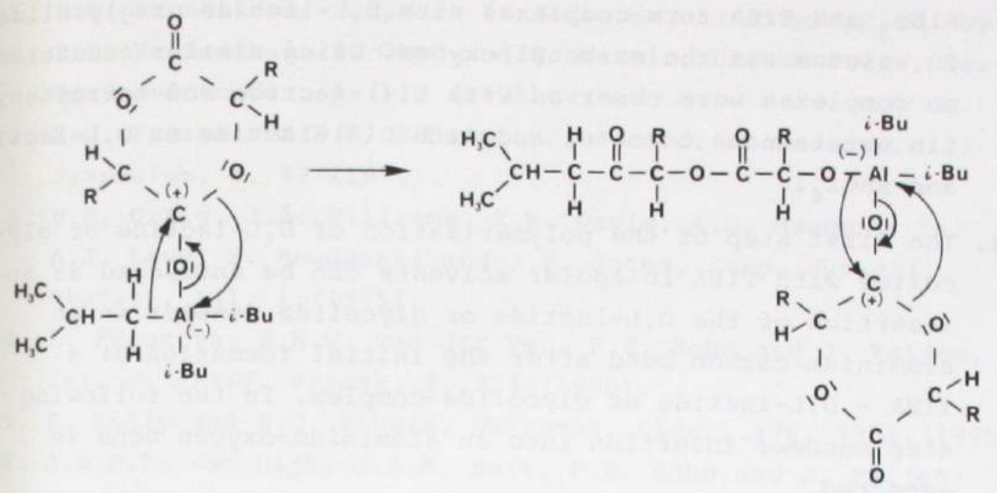
The only compound that could both form complexes with D,L-lactide or glycolide and initiate their polymerization under anhydrous solution conditions was TIBA. According to the often cited (28,31) mechanism proposed by Cherdron e.a. (10), the cationic initiation of a lactone takes place via the attack of a cationic species onto the ether oxygen followed by acyl-oxygen bond cleavage. The propagation step involves the attack of an acylium ion upon the ther oxygen of the next monomer.




Scheme 3. Possible reaction pathway for the ring-opening polymerization of glycolide (R=H) or lactide ($R=CH_3$) initiated with strong protonic acids.

Contrary to this, some authors (11,13,14) proposed as the propagation step the attack of an acylium ion upon the carbonyl oxygen of the next lactone monomer, a satisfactory mechanism for the subsequent ring-opening, however, was not given. Our results show that only the carbonyl group of D,L-lactide or glycolide was involved in the complex formation with TIBA (Scheme 2) which suggests that the classical cationic initiation and propagation mechanism proposed by Cherdron e.a. (10) is not applicable to our system. An acceptable mechanism for the polymerization of D,L-lactide and glycolide with TIBA is the coordination insertion mechanism proposed by Dittrich and Schulz (22) for the polymerization of L(-)-lactide initiated with diethylzinc, involving acyl-oxygen bond breakage (Scheme 4). After the coordination of TIBA with D,L-lactide or glycolide, the insertion of the lactone monomer takes place into an aluminium-carbon bond. The propagation step involves the insertion of monomer into an aluminium-oxygen bond. Other authors (23,24,28) proposed a coordination insertion mechanism






Scheme 4. Coordination insertion mechanism for glycolide (R=H) and lactide ($R=CH_3$) initiated with TIBA.

for lactones which involves a coordination of the monomer to the metal atom of the organometallic compound through the ether oxygen. Our results of the complex formation with TIBA are in disagreement with such a mechanism. Brode and Koleske (31) did not succeed in the synthesis of a low molecular 1:1 molar insertion product of stannous octoate or trimethyltin acetate with caprolactone under a variety of experimental conditions. The fact that also in this study no complex formation was observed with stannous octoate nor with tetraphenyltin which are both very effective initiators in melt polymerizations of lactide or glycolide warrants further research to elucidate the mechanism involved.

## III.4. Conclusions

1. $AlBr_3$ and TIBA form complexes with D,L-lactide or glycolide in toluene via the carbonyl oxygen. Using similar conditions no complexes were observed with L(-)-lactide and tetraphenyl-tin or stannous octoate, and with L(-)-lactide or D,L-lactide and $SnCl_4$.

2. The first step of the polymerization of D,L-lactide or glycolide with TIBA in apolar solvents can be envisaged as an insertion of the D,L-lactide or glycolide monomer in an aluminium-carbon bond after the initial formation of a TIBA - D,L-lactide or glycolide complex. In the following step monomer insertion into an aluminium-oxygen bond is expected.

3. When $AlBr_3$ or $SnCl_4$ are used as the initators for the ring-opening polymerization of D,L-lactide or glycolide in an apolar solvent such as toluene, their hydrolysis products HBr or HCl, respectively, might be the true initiating species.

4. The mechanism of the initiation of D,L-lactide or glycolide with strong protonic acids may be explained as a $S_n2$-type transesterification reaction involving as the first step protonation of a carbonyl oxygen.

Acknowledgement

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References

1. R.L. Kronenthal, *Polymers in Medicine and Surgery*, ed. by R.L. Kronenthal. Z. Oser and E. Martin, Plenum Press, New-York and London, p. 119 (1975).
2. E.J. Frazza and E.E. Schmitt, *J. Biomed. Mater. Res. Symposium*, 1, 43 (1971).
3. P.H. Craig, J.A. Williams. K.W. Davis, A.D. Magoun, A.J. Levy, S. Bogdanski and J.P. Jones, *Surg. Gynecol. Obstet.*, 141, 1 (1975).
4. L. Feenstra, B.W.C. van der Ven, F.E. Kohn and J. Feijen, *Int. J. Artif. Organs*, 3, 354 (1980).
5. E. Lilly and R.C. Schulz, *Makromol. Chem.*, 176, 1901 (1975).
6. J.A.P.P. van Dijk, J.A.M. Smit, F.E. Kohn and J. Feijen, *J. Polym. Sci. Polym. Chem. Ed.*, 21, 197 (1983).
7. R.C. Schulz, *Proc. IUPAC Int. Symp. Makromol. Chem.*, p. 185, Budapest (1969).
8. G.J. van Hummel, S. Harkema, F.E. Kohn and J. Feijen, *Acta Cryst.*, B 38, 1679 (1982).
9. J.L. Brash and D.J. Lyman, *Cyclic monomers, High polymers Vol. XXVI*, ed. by K.C. Frisch, p. 147, Wiley-Interscience, New-York (1972).
10. H. Cherdron, H. Ohse and F. Korte, *Makromol. Chem.* 56, 179 (1962).
11. Y. Yamashita, T. Tsuda, O. Masahiko and Sh. Iwatsuki, *J. Polym. Sci.* Part A-1 4, 2121 (1966).
12. Y. Yamashita, K. Ito, K. Chiba and S. Kosawa, *Polym. J.*, 3, 389 (1972).
13. G.S. Sanina and E.B. Lyudvig, Translated from: *Doklady Akademii Nauk SSSR*, 229, 1400 (1976).
14. G.S. Sanina and E.B. Lyudvig, Translated from: *Doklady Akademii Nauk SSSR*, 230, 153 (1976).
15. V.A. Kogan, N.N. Kharabaev, A.S. Burlov, V.P. Sokolov, O.A. Osipov, V.A. Savin and V.G. Maslov, Translated from: *Zhurnal Obshchei Khimii*, 48, 655 (1978).
16. H. Cherdron, H. Ohse and F. Korte, *Makromol. Chem.*, 56, 187 (1962).

17. Y. Yamashita, T. Tsuda, H. Ishida, A. Uchikawa and Y. Kuriyama, *Makromol. Chem.*, 113, 139 (1968).
18. Y. Yamashita, *Polym. Preprints*, 21, 51 (1980).
19. S. Somkowski and S. Panczek, *Macromolecules* 13, 229 (1980).
20. P. Sigwalt, *Angew. Makromol. Chem.* 94, 161 (1980).
21. J.G. Noltes, F. Verbeek and H.G.J. Overmars, *J. Organomet. Chem.* 24, 257 (1970).
22. W. Dittrich and R.C. Schulz, *Angew. Makromol. Chem.* 15, 109 (1971).
23. T. Ouhadi, Ch. Stevens and Ph. Teyssié, *Makromol. Chem. Suppl.* 1, 191 (1975).
24. A. Hamiton, T. Ouhadi, R. Jerome and Ph. Teyssié, *J. Polym. Sci. Polym. Chem. Ed.* 15, 865 (1977).
25. J.V. Koleske and R.D. Lundberg, *J. Polym. Sci.*, Part A-2 7, 897 (1969).
26. R.A. Fouty, *Can. Patent* 808, 731 (1969).
27. R.G. Sinclair, *US Patent 4,045, 418* (1975).
28. R.H. Young, M. Matzner and L.A. Pilato, *ACS Symposium Ser.* 59, 152 (1977).
29. R.D. Lundberg and E.F. Cox, *Ring-opening polymerization. Vol. 2* ed. by K.C. Frisch and S.L. Reegen, p. 247, Marcel Dekker, New York (1969).
30. R.D. Lundberg, J.V. Koleske and K.B. Wischmann, *J. Polym. Sci., Part A-1*, 7, 2915 (1969).
31. G.L. Brode and J.V. Koleske, *J. Makromol. Sci.-Chem.* A6, 1109 (1972).
32. J.G. van Ommen, H.J. van der Ploeg, P.C.J.M. van Berkeland and P. Mars, *J. Mol. Catal.* 2, 409 (1977).
33. R.K. Kulkarni, K.C. Pani, C. Neuman and F. Leonard, *U.S. Nat. Tech. Inform. Serv.* AD Rept. No. 636, 716 (1969).
34. R.G. Sinclair and G.H. Gynn, *U.S. Nat. Tech. Inform. Serv.* AD Rept. No. 748, 411 (1972).
35. Ch.E. Lowe, *U.S. Patent* 2, 668, 192 (1954).
36. W.R. Sorenson and T.W. Campbell, *Preparative methods of polymer chemistry, 2nd ed.*, p. 363, Interscience Publ., New York (1968).



37. W.R. Sorenson and T.W. Campbell, *Preparative methods of polymer chemistry, 2nd ed.*, p. 12, Interscience Publ., New York (1968).
38. H.J. de Liefde Meyer, M.J. Jansen and G.J.H. van der Kerk, *Studies in organic chemistry of vanadium*, p. 40, Institute for Organic Chemistry, TNO, Utrecht, The Netherlands (1963).
39. J. Kleine and H.H. Kleine, *Makromol. Chem.* 30, 23 (1959).
40. R.C. Schulz and J. Schwaab, *Makromol. Chem.* 87, 90 (1965).
41. W. Strohmeier and K.H. Miltenberg, *Chem. Ber.*, 91, 1357 (1958).
42. M. Vitoria and J. Walkley, *Trans. Faraday Soc.* 65, 57 (1969).
43. G.E. Coates, M.L.H. Green, P. Powell and K. Wade, *Principles of organometallic chemistry*, p. 103, 126 Methuen, London (1968).
44. D.P.N. Satchell and R.S. Satchell, *Chem. Rev.* 69, 251 (1969).
45. J.D.S. Goulden and B.J. Millard, *Org. Mass Spectrom.* 2, 893 (1969).
46. L. Kahovec and K.W.F. Kohlraush, *Z. Phys. Chem.* 193, 188 (1944).
47. N.B. Colthup, L.H. Daly and S.E. Wiberley, *Introduction to infrared and raman spectroscopy*, p. 248, Academic Press, New York and London (1964).
48. L.J. Bellamy, *The infrared spectra of complex molecules*, Chapman and Hall, London p. 196, (1975).
49. A.J. Amass and J.N. Hay, *Makromol. Chem.* 103, 244 (1967).
50. H.C. Brown, J.H. Brewster and H. Shechter, *J. Am. Chem. Soc.*, 76 467 (1954).
51. J. Dale, *Stereochemistry and conformational analysis*, Universitetsforlaget, Oslo p. 82 (1978).
52. M.M. Mhala and J.P. Mishra, *Indian J. Chem.* 8, 243 (1970).
53. J. March, *Advanced organic chemistry, 2nd. ed.*, McGraw-Hill Kagakusha, Tokyo p. 349 (1977).

CHAPTER IV

# THE RING-OPENING POLYMERIZATION OF D,L-LACTIDE IN THE MELT INITIATED WITH TETRAPHENYLTIN


## SYNOPSIS

Melt polymerization conditions for D,L-Lactide initiated with tetraphenyltin were studied with regard to polymer molecular weight and weight distributions. "Single" polymerization, "multiple" polymerization (four or eight reactions at the same time) and time dependent studies are described. "Single" polymerizations using constant initiator concentrations resulted in a broad scattering of non-reproducible molecular weight values. "Multiple" polymerizations at constant initiator concentrations, however, resulted in nearly identical molecular weight profiles.

"Multiple" polymerizations at different initiator concentrations did not show an inverse dependency of initiator concentration on polymer molecular weight. Both the "single" and "multiple" melt polymerizations resulted in rather broad molecular weight distributions. The presence of hydrolysis products of lactide during the melt polymerization most likely has a detrimental effect on molecular weight. After a short induction period the rather slow polymerization of D,L-lactide resulted in a maximal molecular weight followed by a slight decrease in molecular weight to a constant value. It is concluded that the polymerization of D,L-lactide in the melt initiated with tetraphenyltin does not proceed through a "living" mechanism.



F.E. Kohn, J.W.A. van den Berg, G. van de Ridder and J. Feijen, submitted for publication in J. Appl. Polymer Sci.




## IV.1. Introduction

Over the past twenty years there have been studies for the application of poly (D,L-lactic acid) as a biodegradable polyester in medicine and surgery[1], e.g. as sutures[2], burn wound covering[3], sustained release system[4], or as resorbable prostheses in maxillo-facial[5] and orthopedic surgery[6]. In our laboratories we have applied poly (D,L-lactic acid) as temporary eardrum grafts in rats[7] and dogs.

Although poly (D,L-lactic acid) may be prepared by the direct condensation of L(+)- and D(-)-lactic acid[8-10], the preferred method for the preparation of high molecular weight poly (D,L-lactic acid) is the ring-opening polymerization of the six-membered cyclic diester D,L-lactide (m.p. $126^0$C), initiated with a suitable initiator, e.g. tin-(IV)-chloride[11], Zn[12], stannous octoate[5] or tetraphenyltin. The polymerization can be performed in solution under mild conditions[13] or, for the preparation of high molecular weight polymers, preferably in the melt at higher temperatures. Purified D,L-lactide (m.p. $126^0$C), derived from the racemic mixture of L(+)- and D(-)-lactic acid, is the molecular 1:1 compound of both enantiomers L(-)-lactide and D(+)-lactide[14].

It has been reported that for the melt polymerization of lactide the polymer molecular weight can be controlled by varying the amount of initiator[15,16]. This statement, however, is not in agreement with other literature data[3,17-21]. A literature survey on the melt or bulk polymerization of lactide and of other six- and seven-membered ring lactones, i.e. glycolide and ε-caprolactone, revealed that initiators have been employed in a wide concentration range and also that both high and low molecular weights have been obtained with no relationship to initiator concentrations[19,22-36]. Only under solution polymerization conditions does the ring-opening polymerization proceed in a predictable way[37].

The purpose of this study is to find the relation between melt

polymerization conditions for D,L-lactide initiated with tetraphenyltin and polymer molecular weight and weight distribution. In addition to this, the existence of a "living" mechanism for the melt polymerization of D,L-lactide is discussed.

## IV.2. Materials

D,L-lactic acid (a 90% aqueous solution containing the racemic mixture of L(+)-lactic acid and D(-)-lactic acid) and tetraphenyltin were purchased from Polysciences.

D,L-lactide (m.p.126$^0$C) was prepared according to the procedures of Kulkarni et al.[15,38] and of Sinclair and Gynn[29] using zinc oxide as the depolymerization catalyst. Water solvent and part of the condensation water were stripped off for 14-16 hours at 110-140$^0$C pot temperature, starting at 760 mm Hg and stepwise decreasing to about 25 mm Hg. The temperature of the bath was then increased to 180$^0$C and the crude D,L-lactide was distilled at 0,1 mm Hg for about 4 hours. The crude D,L-lactide was recrystallized several times in order to remove the meso-lactide (m.p.43$^0$C)[14].

Approximately 5 ml. of ethyl acetate was used for every 20 grams of crude D,L-lactide[3] and the first recrystallization was carried out in the presence of charcoal. Purified D,L-lactide (yield 15-20%; m.p. 126$^0$C) was dried in a vacuum oven at room temperature in order to prevent sublimation, stored in a desicator over $P_2O_5$ and subsequently over $CaCl_2$. Prior to use a small column (length 5-10 cm; diameter 0,5 cm) containing D,L-lactide was eluted with a small amount of ice-cold dry ether to remove residual impurities.

Poly (D,L-lactic acid). The ring-opening polymerization of D,L-lactide in the melt was also based on the procedures developed by Kulkarni et al.[15,38] and Sinclair and Gynn[29] using tetraphenyltin as the initiator. Pure D,L-lactide (1 gram)



was placed in a clean polymerization tube which was dried for 24 hours. The appropriate amount of initiator dissolved in 0,5 ml or dry benzene was added. The tube was then connected directly ("single" polymerizations) or via an adaptor ("multiple" polymerizations) to a purge valve system with acces to vacuum or dry nitrogen (Fig. 1).

The solvent was evaporated at reduced pressure and the lower part of the tube was placed in an oil bath (temperature range 60-100$^0$C). D,L-lactide was sublimed onto the air cooled portion of the tube (o,1 mm Hg) for removal of residual solvent and water vapor. A negligible amount of D,L-lactide is lost during the sublimation step. During the sublimation step the tube was purged several times with dry nitrogen. After the sublimation the tube was sealed under vacuum and next the tube was immersed in an oil bath which was kept at 180 $\pm$ 1$^0$C for 24 hours or for various time periods, respectively. Apart from the "time variation" experiments, the resulting poly (D,L-lactic acid) (melting from 51-59$^0$C according to DSC) was dissolved in acetone, precipitated in water under vigorous stirring, filtered and dried in vacuo at room temperature. Only very small amounts of monomer are removed by this procedure. For "time variation" experiments four or eight sealed tubes (cf. "multiple" polymerization) were immersed in the oil bath of 180 $\pm$ 1$^0$C at time t=0. At predetermined intervals the tubes were removed and quenched by cooling. After storage at -15$^0$C the contents of the tubes were dissolved in tetrahydrofuran. Aliquots of these solutions were used for gel permeation chromatography (see below). Two peaks were observed in the chromatogram if D,L-lactide monomer was still present. Conversion of D,L-lactide was calculated from the relative peak area's (after correction for the difference in refractive index increments of polymer in THF (dn/dc = 0,050) and of D,L-lactide in THF (dn/dc = 0,046), respectively). $\bar{M}_n$ and $\bar{M}_w$ of the polymers were also calculated. This procedure differs from the one described by Gilding and Reed[19] and by Sanina et al.[33] in which residual

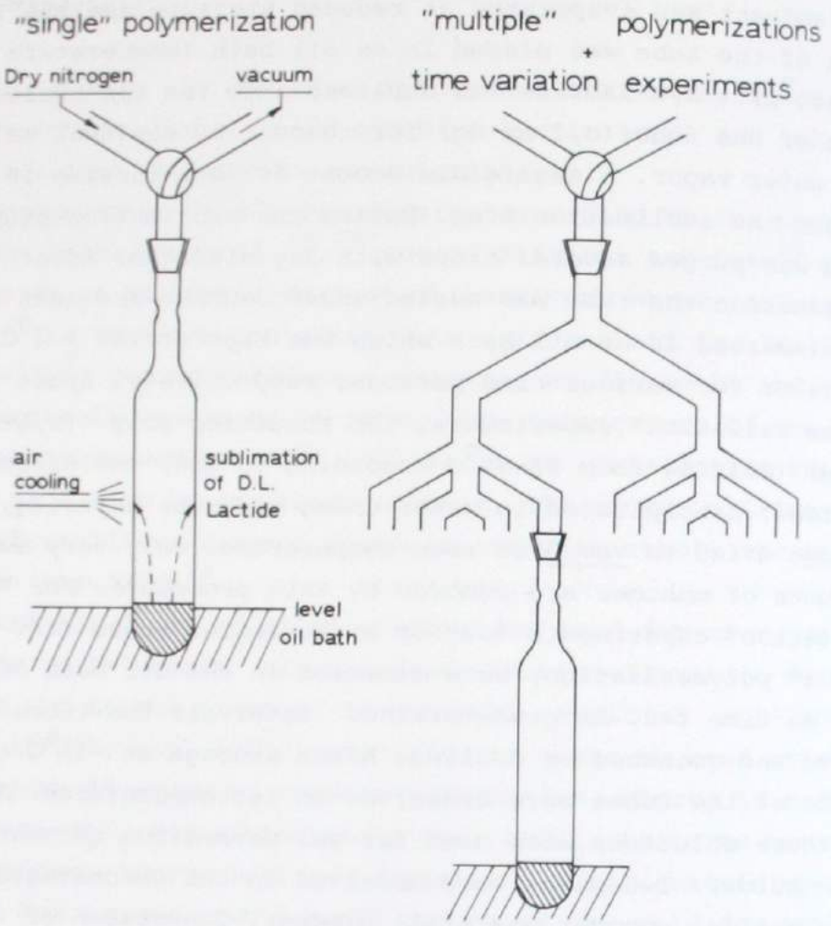


Fig. 1. Experimental set-up for 'single' and 'multiple' melt polymerization.



monomer was removed and the percentage conversion was based on the amount of insoluble polymer obtained.

## IV.3. Methods

### IV.3.1 Gel permeation chromatography

The gel permeation chromatographic measurements were performed with a low-pressure Water's Model GPC-200 apparatus having a 5 ml. syphon. Four analytical columns (4ft. long * 3/8 in.) packed with Styragels of porosity ratings $10^5$, $3x10^4$, $10^3$ and 250 Å had been installed in series. The operational conditions were: flow rate, 1 ml/min.; solvent, THF; temperature, $30^0$C; sample concentration, approximately 0,5%; sample injection time, 60 sec. A differential flow through refractometer was used as a detector. The columns were calibrated by the Q-factor method[40] with poly (D,L-lactic acid) samples of known $\bar{M}_n$- and $\bar{M}_w$-values, previously determined by membrane osmometry and light scattering, respectively. Q, the molecular weight per Angstrom unit was shown to be $33\pm1$ when based on the $\bar{M}_w$-values of the poly (D,L-lactic acid) samples determined by light scattering. Q-values based on osmometrically determined $\bar{M}_n$-values turned out to be higher ($58\pm5$). In another study[41], using a universal calibration curve procedure, we were able to decide that $Q = 33\pm1$ had to be taken as the right value. In the present study columns with a low exclusion limit in the low molecular weight region were chosen to be able to determine monomer and oligomer content in a reliable way. Empirical peak-broadening correction was applied based on polystyrenes for which $\bar{M}_n$, $\bar{M}_w$ and $\bar{M}_z$ were known (membrane osmometry, light scattering and equilibrium distribution in the ultracentrifuge, respectively [42]).

### IV.3.2 Light scattering measurements

Light scattering measurements were performed at 5 concentrations below 0,5% (w/v) in a Fica 50 light scattering photo-

meter, using vertically polarized light of wave length 436 nm. Reliable Zimm-plots were obtained for acetone solutions which had been filtered through Fluoropore 0.2 μ filters (purchased from Millipore Corp). Toluene could not be used as a solvent because of the very low dn/dc-value (-0,02 for poly (D,L-lactic acid) in toluene). Light scattering measurements were performed at 11 angles between $30^0$ and $150^0$ to the incident beam. Refractive index increments were measured on a Brice Phoenix refractometer. The dn/dc-value for poly (D,L-lactic acid) in acetone at $25^0$C was 0.097.

IV.3.3 Membrane osmometry

A Hallikkainen Model 1361 automatic osmometer was used with cellophane membranes (Sartorius membrane filter SM 11539). Measurements at 5 concentrations were carried out in toluene as the solvent.

IV.4 Results

IV.4.1 "Single" melt polymerization

In Table I the results of 20 "single" melt polymerizations of D,L-lactide initiated with tetraphenyltin are presented. Apart from the initiator concentrations, the conditions for these polymerizations were identical. Several polymerizations resulted in very low polymer molecular weights ("failures"). Only one of these values, first result at M/I = 30,000, is included in Table I.
All polymerizations resulted in broad molecular weight distributions. GPC-chromatograms showed that the smallest molecules had molecular weights in the range 100-3,000 (DP=1-20). The largest molecules present ranged from 65,000 - 140,000 (DP= 450-970) for the low molecular weight samples up to 665,000 - 1,450,000 (DP=4,600 - 10,000) for the medium and high molecular weight samples.



TABLE I

Results of "single" melt polymerizations of D,L-lactide

| Monomer/Initiator (mol/mol) | $\bar{M}_w$ | $\bar{M}_n$ | $\bar{M}_w/\bar{M}_n$ |
|---|---|---|---|
| 3,750 | 11,000 | 2,000 | 5,5 |
| 3,750 | 85,000 | 20,000 | 4,2 |
| 7,500 | 13,000 | 2,000 | 6,5 |
| 7,500 | 14,000 | 2,000 | 7,0 |
| 7,500 | 14,000 | 2,000 | 7,0 |
| 7,500 | 118,000 | 31,000 | 3,8 |
| 15,000 | 15,000 | 4,000 | 3,7 |
| 15,000 | 36,000 | 12,000 | 3,0 |
| 15,000 | 36,000 | 14,000 | 2,6 |
| 15,000 | 78,000 | 18,000 | 4,3 |
| 15,000 | 111,000 | 44,000 | 2,5 |
| 15,000 | 117,000 | 30,000 | 3,9 |
| 15,000 | 125,000 | 59,000 | 2,1 |
| 15,000 | 128,000 | 47,000 | 2,7 |
| 15,000 | 166,000 | 39,000 | 4,2 |
| 15,000 | 183,000 | 38,000 | 4,8 |
| 30,000 | 5,000 | 1,000 | 5,0 |
| 30,000 | 20,000 | 11,000 | 1,8 |
| 30,000 | 90,000 | 49,000 | 1,8 |
| 30,000 | 179,000 | 48,000 | 3,7 |

Initiator tetraphenyltin; polymerization time 24 hours; polymerization temperature $180^0$C.

### IV.4.2 "Multiple" melt polymerizations

In the following sets of melt polymerizations the adaptor described in the experimental part (Fig.1) was used and four tubes were prepared for polymerization at the same time. In the first three series (1, 2 and 3) the monomer/initiator ratio was kept constant at 7,500, 15,000 and 30,000 mol/mol, respectively. The results are presented in Table II. The molecular weight distributions of the poly (D,L-lactic acid) samples prepared in this way were rather similar to those observed during the "single" polymerization study. Always a long "tail" towards very low molecular weight species (650-1,500) was observed, whereas at the same time chain molecules having molecular weights of 310,000 - 665,000 were present. In the next three series of "multiple" polymerizations (4, 5 and 6) the initiator concentration was varied in each series. The results are presented in Table III. In series 6 the initiator concentrations were kept very low.



TABLE II

Results of "multiple" melt polymerizations of D,L-lactide at constant initiator concentrations

| $\bar{M}_w$ | $\bar{M}_n$ | $\bar{M}_w/\bar{M}_n$ |
|---|---|---|
| | SERIES 1 | |
| | M/I = 7,500 | |
| 65,000 | 29,000 | 2,2 |
| 67,000 | 20,000 | 3,3 |
| 68,000 | 34,000 | 2,0 |
| 70,000 | 27,000 | 2,6 |
| | SERIES 2 | |
| | M/I = 15,000 | |
| 54,000 | 18,000 | 3,0 |
| 58,000 | 20,000 | 2,9 |
| 58,000 | 22,000 | 2,6 |
| 59,000 | 20,000 | 2,9 |
| | SERIES 3 | |
| | M/I = 30,000 | |
| 88,000 | 31,000 | 2,8 |
| 91,000 | 30,000 | 3,0 |
| 103,000 | 43,000 | 2,4 |
| 127,000 | 43,000 | 3,0 |

Initiator tetraphenyltin; polymerization time 24 hours; polymerization temperature 180$^0$C.

TABLE III

Results of "multiple" melt polymerizations of D,L-lactide at varying initiator concentrations

SERIES 4

| M/I | $\bar{M}_w$ | $\bar{M}_n$ | $\bar{M}_w/\bar{M}_n$ |
|---|---|---|---|
| 7,500 | 27,000 | 5,300 | 5,1 |
| 15,000 | 38,000 | 11,000 | 3,5 |
| 30,000 | 100,000 | 42,000 | 2,4 |
| no initiator | 6,100 | 1,000 | 6,1 |

SERIES 5

| | | | |
|---|---|---|---|
| 7,500 | 39,000 | 19,000 | 2,1 |
| 15,000 | 129,000 | 42,000 | 3,1 |
| 30,000 | 63,000 | 28,000 | 2,2 |

SERIES 6

| | | | |
|---|---|---|---|
| 30,000 | 69,000 | 18,000 | 3,8 |
| 60,000 | 55,000 | 13,000 | 4,2 |
| 90,000 | 53,000 | 19,000 | 2,8 |
| 180,000 | 46,000 | 23,000 | 2,0 |

Initiator tetraphenyltin; polymerization time 24 hours; polymerization temperature $180^0$C.

IV.4.3 Effects of impurities

In one set of "multiple" (four at a time) melt polymerizations, either a small amout of water, recrystallization solvent (ethyl acetate) or of the ethyl acetate mother liquor was added along with D,L-lactide plus initiator. The fourth tube without extra additions served as a reference. After sublimation the tubes were sealed and polymerized in the usual manner.



These results are presented in series 7 of Table IV. In another set of "multiple" polymerizations two tubes had been filled with freshly prepared D,L-lactide whereas the other two tubes contained D,L-lactide which had been stored in a desiccator over $P_2O_5$ and subsequently over $CaCl_2$ for several months. The results are given in series 8 of Table IV.

IV.4.4 Time variation experiments

Several "multiple" polymerizations were carried out to determine the effect of polymerization time on the degree of conversion of D,L-lactide

TABLE IV

Results of "multiple" melt polymerizations of D,L-lactide plus deliberately added impurities (series 7); old and freshly prepared D,L-lactide (series 8)

SERIES 7

I/M = 15,000

| | $\bar{M}_w$ | $\bar{M}_n$ | $\bar{M}_w/\bar{M}_n$ |
|---|---|---|---|
| Initiator only | 30,000 | 9,000 | 3,3 |
| I + $H_2O$ | 28,000 | 4,500 | 6,2 |
| I + ethyl acetate | 51,000 | 9,000 | 5,7 |
| I + ethyl acetate mother liquor | 6,200 | 525 | 11,8 |

SERIES 8

I/M = 15,000

| | $\bar{M}_w$ | $\bar{M}_n$ | $\bar{M}_w/\bar{M}_n$ |
|---|---|---|---|
| Old D,L-lactide | 11,500 | 3,700 | 3,1 |
| Old D,L-lactide | 15,500 | 3,400 | 4,6 |
| Fresh D,L-lactide | 116,000 | 43,000 | 2,7 |
| Fresh D,L-lactide | 129,000 | 40,000 | 3,2 |

Initiator tetraphenyltin; polymerization time 24 hours; polymerization temperatur 180$^0$C.

and on polymer molecular weight. A representative "reaction profile" is shown in Fig. 2 leading to $\bar{M}_w$ = 104,000 and $\bar{M}_n$ = 43,000 after 24 hours of polymerization, using a monomer/initiator ratio of 30,000 mol/mol. After a short induction period (see below) there appear to be three stages of polymerization. During the first 8-9 hours a comparatively rapid rise of $\bar{M}_w$ and $\bar{M}_n$ was observed. In the same period conversion of D,L-lactide mounted up to 80-90%, this conversion being rather slow during the first three hours. Second, after 9 hours the increase of $\bar{M}_w$ and $\bar{M}_n$ levelled off, reaching a maximum after about 15 hours. At this stage conversion of D,L-lactide was approximately 95%. In the next hours a slow decrease of $\bar{M}_w$ and $\bar{M}_n$ was observed, levelling off to constant values after 20-22 hours, as was also established by a few "multiple" polymerizations of long duration. Total conversion of D,L-lactide remained almost constant (95-96%) at this time.
During the first 6.5 - 9 hours of polymerization, polymer molecular chains continued to grow as is indicated by Table V, showing molecular weight values of the largest chain molecules present as a function of polymerization time.



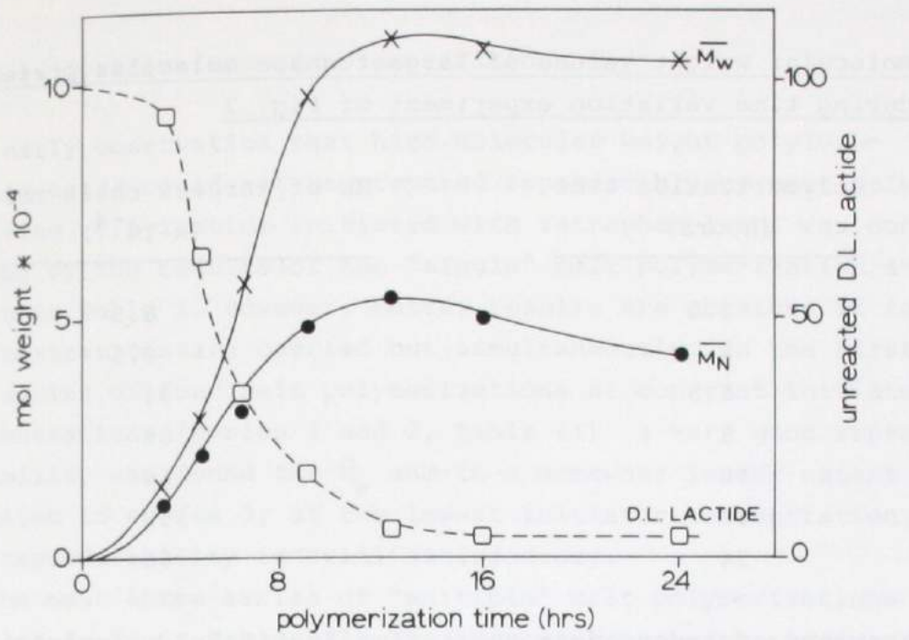


Fig. 2. 'Reaction profile' for a 24 hour melt polymerization of D,L-lactide initiated with tetraphenyltin (T = 180$^o$C; M/I = 30,000).

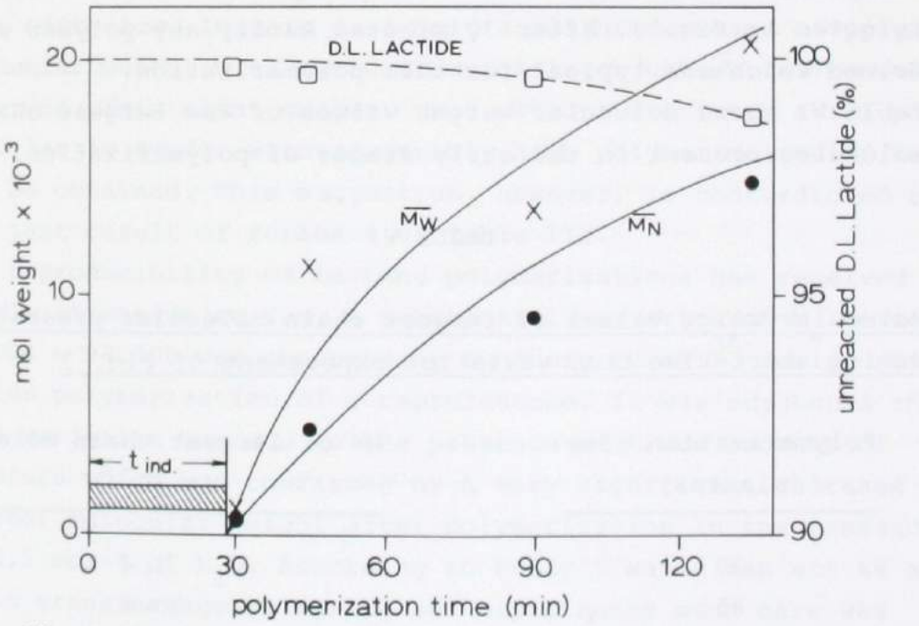


Fig. 3. Short-time 'reaction profile' for a melt polymerization of D,L-lactide initiated with tetraphenyltin (T = 180$^o$C; M/I = 15,000).

TABLE V

Molecular weight values of largest chain molecules present during time variation experiment of Fig. 2

| Polymerization time (hours) | MW of largest chain molecules ($\times 10^{-4}$) |
|---|---|
| 3.5 | 6.5 |
| 5 | 9.5 |
| 6.5 | 31 |
| 9 | 66 |
| 12 | 66 |
| 16 | 150 |
| 24 | 66 |

A number of independent short-time "multiple" polymerizations were carried out to study more closely the induction period preceeding the actual start of the polymerization.
A representative short-time "reaction profile" (M/I = 15,000) is given in Fig. 3. After 30 minutes hardly any polymer was formed which was typical for this polymerization.
Table VI shows molecular weight values of the largest chain molecules present in the early stages of polymerization.

TABLE VI

Molecular weigt values of largest chain molecules present during short-time time variation experiment of Fig. 3

| Polymerization time (minutes) | MW of largest chain molecules ($\times 10^{-3}$) |
|---|---|
| 30 | 1.4 |
| 45 | 30.4 |
| 90 | 66 |
| 135 | 66 |



IV.5. Discussion

IV.5.1. Optimization of polymerization

Our early observation that high molecular weight poly(D,L-lactic acid) could not be prepared reproducibly by melt polymerizing D,L-lactide initiated with tetraphenyltin, was confirmed by the results of the "single" melt polymerization study shown in Table I. However, better results are obtained if four polymerizations are carried out simultaneously. In the first two series of four melt polymerizations at constant initiator concentrations (series 1 and 2, Table II) a very good reproducibility was found for $\bar{M}_w$ and to a somewhat lesser extent for $\bar{M}_n$. Also in series 3, at the lowest initiator concentration, the reproducibility is still satisfactory.
In the next three series of "multiple" melt polymerizations (series 4, 5, 6, Table III) the initiator concentration was varied in each series, but no inverse relationship between initiator concentration and polymer molecular weight was obtained. In series 6 the four different initiator concentrations were kept quite low. In this case only a slight decrease of polymer molecular weight with decreasing initiator concentration was observed. This might suggest that without initiator still poly (D,L-lactic acid) with reasonably high polymer molecular weight can be obtained. This suggestion, however, is contradicted by the last result of series 4 of Table III.
The reproducibility of lactone polymerizations has received little attention so far. A substantial variation of $\bar{M}_w$ between 57,000 - 98,000 was observed by Lundberg et al.[35] during solution polymerization of ε-caprolactone. It was suggested that this variation was due to the presence of trace amounts of moisture which was confirmed by a very significant decrease of polymer molecular weight after polymerization in the presence of 1,5 mol-% of $H_2O$. According to Fouty[23] water can act as a chain transfer agent. During our experiments much care was taken to exclude impurities. Elution of the small column of D,L-lactide with ice-cold, dry ether resulted according to

spectroscopic analysis in the removal of small amounts of low molecular weight poly (D,L-lactic acid) and, most probably, of lactoyllactic acid (c.f. ref. 43) (see below). A number of experiments was carried out to study more closely the possible effects of impurities, including:
(i) the establishment of the optimum depolymerization temperature; (ii) the search for better recrystallization solvents; (iii) the effect of impurities on the polymerization of D,L-lactide in the melt; and (iv) the "aging" effect of D,L-lactide.
In this investigation the maximum depolymerization temperature was $180^0$C (at 0,1 mm) because it was found that crude D,L-lactide formed at higher temperatures as recommended by Kulkarni et al.[15, 38], and Sinclair and Gynn[29] could not effectively be purified by recrystallization. From a number of organic solvents, chloroform and a large excess of ether (c.f. ref. 14) turned out to be effective recrystallization solvents, in addition to ethyl acetate. However, polymers prepared from D,L-lactide recrystallized from ether or from chloroform did not have higher polymer molecular weights.
The poly (D,L-lactic acid) prepared from the D,L-lactide to which the ethyl acetate mother liquor had been added (series 7, Table IV) had a significantly lower polymer molecular weight as compared to the others. This indicates that possible contaminants to the D,L-lactide such as moisture or the recrystallization solvent are effectively removed during the sublimation step. It is believed that the ethyl acetate mother liquor formed during the recrystallization of D,L-lactide (c.f. ref. 14) may contain D,L-lactide, meso-lactide (m.p.$43^0$C), low molecular weight poly (D,L-lactic acid) and lactoyllactic acid (c.f. ref. 43). The latter compound may be formed by hydrolysis of D,L-lactide and especially of meso-lactide which is much more sensitive to moisture than racemic D,L-lactide[44]. We have found that meso-lactide can be polymerized under identical conditions as D,L-lactide, and we suggest that the detrimental effect on the polymerization of D,L-lactide in the presence of ethyl acetate mother liquor was actually caused by the presence of lactoyllactic acid (c.f. ref. 23).



It is concluded from the results presented in series 8 of Table IV that D,L-lactide stored in a desiccator over $P_2O_5$ and subsequently over $CaCl_2$ is subject to an "aging" effect, -i.e. formation of lactoyllactic acid from remnants of the moisture sensitive meso-lactide present in the D, L-lactide.

### IV.5.2. Comments on the reaction mechanism

The two melt polymerization "reaction profiles" shown in Fig's 2 and 3 are representative for a number of other observed "reaction profiles". Our time variation results may be compared with data on the melt polymerization of glycolide initiated with stannous octoate and lauryl alcohol as a catalyst activator at $220^0$C[19] or with antimony trifluoride at 160 - $170^0$C[33]; and with data on the bulk polymerization of ε-caprolactone[45].
It then appears that under our experimental conditions the conversion of D,L-lactide was a comparatively slow process. Gilding and Reed[19] observed that 80% conversion of glycolide had taken place within the first 30 minutes and an additional 3.5 hours yielded a further 16%, 96% being the limit of polymerization. Polymer chains of molecular weights as large as $2x10^6$ were present within 30% of conversion. No induction period was reported although Sanina et al.[33] mentioned a relatively slow initiation process with antimony trifluoride. From a low conversion co-polymerization study[19] it was concluded that glycolide was clearly more reactive than lactide.
Young et al.[45] monitored the bulk polymerization of ε-caprolactone initiated with a coordination-insertion type initiator at $204^0$C, by following the viscosity as a function of polymerization time. Although the polymerization appears to proceed much faster, the shape of the ε-caprolactone "reaction profile" is comparable to the one drawn in Fig. 2, also showing a maximum in polymer molecular weight; however, an induction period is not mentioned.
A "living" mechanism for the ring-opening polymerization of lactones has frequently been discussed[19, 33, 35, 37, 45, 46]. Thus a "living" mechanism was postulated by Sanina et al.[33] for the

melt polymerization of glycolide, whereas Gilding and Reed[19] and Young et al.[45] disagree with such a mechanism for the melt polymerization of glycolide and ε-caprolactone, respectively. Ouhadi et al.[37] got strong indications that under solution polymerization conditions the polymerization of ε-caprolactone indeed proceeded through a "living" mechanism. If the conditions for such a mechanism are met[46] (c.f. ref.47), the number average molecular weight can be calculated theoretically from:

$\bar{M}_n$ = Degree of Polymerization x Molecular Weight $_{D,L\text{-lactide}}$

$$= \frac{[\text{D,L-lactide}]}{[\text{tetraphenyltin}]} \times 144.$$

In addition to this, the resulting poly (D,L-lactic acid) will have a Poisson molecular weight distribution which would be indistinguishable for high molecular weight polymers from a monodisperse system with $\bar{M}_w/\bar{M}_n \approx 1 + 1/DP$. Our results show clearly that theoretical $\bar{M}_n$'s based on a "living" mechanism are very much higher than the $\bar{M}_n$'s actually measured. Thus $\bar{M}_n$'s varying from 4,000 - 59,000 were measured for the "single" melt polymerizations of D,L-lactide carried out at M/I = 15,000 (Table I). On the basis of a "living" mechanism $\bar{M}_n = 2 \times 10^6$ was expected. Molecular weight distributions of the various poly (D,L-lactic acid) samples prepared for this study were considerably broader than could have been expected on the basis of a "living" mechanism, with the exception of the $\bar{M}_w/\bar{M}_n$-values of polymers formed at the early stages of melt polymerization (see "reaction profiles" of Fig's 2 and 3). In the latter case polymer chains were still growing (Tables V and VI). Both facts fit in with a "living" character for the early stages of melt polymerization of D,L-lactide (see, however, below). A "living" mechanism also implies that each tetraphenyltin molecule initiates one polymer chain. The number of initiator molecules present at the start of the polymerization can easily be calculated. The number of polymer chains after the polymerization is completed is equal to:

$$\frac{\text{Weight of poly (D,L-lactic acid) sample}}{\bar{M}_n} \times \text{Avogadro's number.}$$



From these calculations it was found that in the average the poly (D,L-lactic acid) samples having low average molecular weights, contained 400 - 800 times more polymer chains than tetraphenyltin initiator molecules; those having medium average molecular weights contained 100 - 200 times more polymer chains; and those having high average molecular weights contained 25 - 80 times more polymer chains. Also in the early stages of melt polymerization (see Fig's 2 and 3) the number of polymer chains was already a multiplicity of the number of tetraphenyltin initiator molecules originally present which does not fit in with a "living" mechanism. These results might indicate that chain transfer phenomena, ester interchange reactions and perhaps the formation of cyclic compounds in a depolymerization reaction have taken place. It should be realized that in our investigation the polymerization temperature was more or less equal to the depolymerization temperature for the preparation of D,L-lactide from low molecular weight poly (D,L-lactic acid). A few polymerizations of D,L-lactide in the melt were carried out at a considerably lower reaction temperature, 130$^0$C, in the presence of stannous octoate as the initiator, leading to significantly higher $\bar{M}_w$-values but not to very high $\bar{M}_n$-values (e.g. $\bar{M}_w$ = 326,000; $\bar{M}_n$ = 35,000; M/I = 7,000). Also in these cases the number of polymer chains was found a multiplicity of the number of initiator molecules orginally present (30 times more in the above example). On the other hand, it can not be excluded that the tetraphenyltin molecule does not act as a real initiator, but that one tetraphenyltin molecule is able to catalyze the formation of a number of chains. Our study of the initiation mechanism of lactide[48] did not give an answer as to the true initiating nature of tetraphenyltin.

## IV.6. Conclusions

1. Both the results of typical "single" and "multiple" melt polymerizations of D,L-lactide indicate that polymer molecular weights can not be predicted.

2. If "multiple" polymerizations are carried out at constant initiator concentration, a good reproducibility of polymer molecular weight is obtained. For "multiple" polymerizations carried out at different initiator concentrations an inverse dependency of initiator concentration on polymer molecular weight may not be expected.

3. The lack of reproducibility of polymer molecular weights is felt to be related to the presence of contaminants, - especially hydrolysis products of D,L-lactide and meso-lactide.

4. The melt polymerization of D,L-lactide at $180^0$C initiated with tetraphenyltin is a slow process, reaching the maximum polymer molecular weight after about 15 hours, followed by a slight molecular weight decrease to a constant value. A short induction period is noted in the reaction.

5. A "living" mechanism for the ring-opening polymerization of lactones has frequently been discussed. This study (molecular weight distribution, conversion, induction period) contradicts predictions that can be derived from such a "living" mechanism.

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## References

1. R.L. Kronenthal, in "Polymers in Medicine and Surgery", R.L. Kronenthal, Z. Oser, and E. Martin, Eds., *Plenum*, New York, 1975, pp. 119-137.

2. J.W. Hodge, Jr., *U.S. Nat. Tech. Inform. Serv.*, AD Report No. 742, 719 (1971).

3. J.B. Gregory, A.D. Schwope, and D.L. Wise, *U.S. Nat. Techn. Inform. Serv.* AD Report No. 759, 381 (1973).

4. L.C. Anderson, D.L. Wise, and J.F. Howes, *Contraception*, 13, 375 (1976).

5. R.G. Sinclair and G.H. Gynn, *U.S. Nat. Tech. Inform. Serv.*, AD Report No. 748, 410 (1972).

6. R.K. Kulkarni, E.G. Moore, A.F. Hegyeli, and F. Leonard, *J. Biomed. Mater. Res.*, 5, 169 (1971).

7. L. Feenstra, B.W.C. van der Ven, F.E. Kohn, and J. Feijen, *Int. J. Artif. Organs*, 3, 354 (1980).

8. G.T. Peckham and E.M. Filachioni, in *'Kirk-Othmer Encyclop. Chem. Technol.'* 2nd. ed., 12, 170 (1967).

9. E.M. Filachioni and C.H. Fisher, *Ind. Eng. Chem.* 36, 223 (1944).

10. N.A. Higgins, assignor to Du Pont de Nemours, *U.S. Patent* 2, 676, 945 (1954).

11. E. Lillie and R.C. Schulz, *Makromol. Chem.*, 176, 1901 (1975).

12. M. Vert, F. Chabot, J. Leray, and P. Christel, *Makromol. Chem.*, Suppl. 5, 30 (1981).

13. R.C. Schulz, in *'Proceedings, IUPAC International Symposium Macromolecular Chemistry'*, Budapest, 1969, pp. 185-213.

14. G.J. van Hummel, S. Harkema, F.E. Kohn, and J. Feijen, *Acta Crystallogr.*, B38, 1679 (1982).

15. R.K. Kulkarni, K.C. Pani, C. Neuman, and F. Leonard, *U.S. Nat. Tech. Inform. Serv.*, AD Report No. 636, 716 (1966).

16. Assignors to Dynamit Nobel Aktiengesellschaft, *Br. Patent* 932, 382 (1963).

17. Th.M. Jackanicz, H.A. Nash, D.L. Wise, and J.B. Gregory, *Contraception*, 8, 227 (1973).

18. A.K. Schneider, assignor to Ethicon, *U.S. Patent* 3, 797, 499 (1974).

19. D.K. Gilding and A.M. Reed, *Polymer* 20, 1459 (1979).

20. E.S. Nuwayser, D.L. Williams, P.M. Meier, T.C. Wu, S.F. Merchant, and J.H. Kerrigan, in "Proceedings, Drug Delivery Systems", H.L. Gabelnick, Ed., DHEW Publ. No. (NIH) 77-1238 *U.S. Department of Health, Education and Welfare*, Washington, DC, 1976, pp 193-252.

21. A. Schindler and D. Harper, *J. Polym. Sci., Polym. Chem. Ed.*, 17, 2593 (1977).

22. J. Kleine and H.H. Kleine, *Makromol. Chem.*, 30, 23 (1959).

23. R.A. Fouty, assignor to Ethicon Inc., *Can. Patent* 808, 731 (1969).

24. R.A. Fouty, assignor to Ethicon Inc., *Can. Patent* 923,245



(1973).

25. C.G. Pitt, A.R. Jeffcoat, R.A. Zweidinger, and A. Schindler, *J. Biomed. Mater. Res.*, 13, 497 (1979).

26. D. Wasserman and Ch. C. Versfelt, assignors to Ethicon Inc., *U.S. Patent* 3, 839, 297 (1974).

27. R.G. Sinclair, assignor to Gulf Oil Co., *U.S. Patent* 4, 045, 418 (1977).

28. B.Eling, S. Gogolewski and A.J. Pennings, *Polymer*, 23, 1587 (1982).

29. R.G. Sinclair, and G.H. Gynn, *U.S. Nat. Tech. Inform. Serv.*, AD Report, No. 748, 411 (1972).

30. Ch.E. Lowe, assignor to Dupont de Nemours *U.S. Patent* 2, 668, 162 (1954).

31. K. Chujo, H. Kobayashi, J. Suzuki, S. Tokuhara, and M. Tanabe, *Makromol. Chem.*, 100, 262 (1967).

32. E.E. Schmitt, and R.A. Polistina, assignors to American Cyanamid, *U.S. Patent* 3, 463, 158 (1969).

33. G.S. Sanina, M.V. Fomina, A.K. Khomyakov, V.S. Livshits, V.A. Savin, and E.B. Lyudvig, *Vysokomol.soyed. A17*, 2726 (1975) (Translated in English).

34. G.L. Brode and J.V. Koleske, *J. Macromol. Sci., Chem., A6*, 1109 (1972).

35. R.D. Lundberg, J.V. Koleske, and K.B. Wischman, *J. Polym. Sci.: Part A-1:* 7, 2915-2930 (1969).

36. E.F. Cox, F. Hostettler, and R.R. Kiser, *Macromol. Syntheses*, Vol. 3, ed. by N.G.G. Gaylard, Wiley, New York, 1968, pp. 11-113.

37. T. Ouhadi, Ch. Stevens, and Ph. Teyssié, *Makromol. Chem. Suppl.* 1, 191 (1975).

38. R.K. Kulkarni, K.C. Pani, C. Neuman, and F. Leonard, *Arch. Surg.*, 93, 839 (1966).

39. W.R. Sorenson and T.W. Campbell, Preparative methods of polymer chemistry, 2nd. ed., *Inter. Science Publ.* New York, 1968, pp. 12-15.

40. Model 200 Gel Permeation Chromatograph Operation Manual, IM61992, Waters Associates, Inc., *Milford* (Mass. 01757), 1974, pp. C4-C8.

41. J.A.P.P. van Dijk, J.A.M. Smit, F.E. Kohn and J. Feijen, *J. Polym. Sci., Polym. Chem.* Ed., 21, 197 (1983).

42. J.W.A. van den Berg, G. van de Ridder, and C.A. Smolders, *Eur. Polym. J.*, 17, 930 (1981).

43. C.A. Holten, A. Müller, and D. Rehbinder, Lactic acid-properties and chemistry of lactic acid and derivatives, *Verlag Chemie*, Weinheim, 1971, pp. 192-221.

44. D.D. Deane and E.G. Hammond, *J. Diary Science*, 43, 1421 (1960).

45. R.H. Young, M. Matzner, and L.A. Pilato, *ACS Symposium Series*, 59, 152 (1977).

46. R.D. Lundberg and E.F. Cox, in "Ring-opening polymerization", K.C. Frisch and S.L. Reegen, Eds., *Marcel Dekker*, New York and London, 1969, pp. 247-302.



47. P.J. Flory, *'Principles of polymer chemistry'*, Cornell Univ. Press, Ithaca, New York, 1953, pp. 336-339.

48. F.E. Kohn, J.G. van Ommen, and J. Feijen, *Eur. Polym. J.*, 19, 1081-1088 (1983).

CHAPTER V

# CHARACTERIZATION OF POLY(D,L-LACTIC ACID) BY GEL PERMEATION CHROMATOGRAPHY*


## ABSTRACT

A number of samples of poly(D,L-lactic acid) (PLA) with weight-average molecular weights $\bar{M}_w$ in the range 15,000-350,000 were prepared by a ring-opening polymerization. The molecular weight distributions (MWDs) of these samples were determined by gel permeation chromatography (GPC). The method involves a universal calibration of the columns on the basis of polystyrene standards and a rapid iteration algorithm leading to the establishment of the Mark-Houwink relationship. In addition, osmometry and viscometry data are presented. The effect of hydrolytic degradation on the MWD of two PLA samples was studied by GPC.


## V.1. Introduction

Over the past 15 years there has been an increasing interest in the application of PLA as a biodegradable polymer in medicine and surgery (1), e.g., as biodegradable sutures (2), burn wound covering (3), sustained release system (4), or as resorbable prostheses in maxillofacial (5), orthopedic (6) and recently myringoplastic surgery (7).

The preferred method for the preparation of high-molecular-weight PLA is the ring-opening polymerization of the six-membered cyclic diester D,L-lactide (mp 126°C) initiated with a suitable catalyst, e.g., tin (IV) chloride (8), stannous octoate (5), or tetraphenyltin. The polymerization can be performed


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in solution under mild conditions (9), or, preferably, in the melt at higher temperatures. Purified D,L-lactide, derived from the racemic mixture of L(+) lactic acid and D(-) lactic acid, is the molecular 1:1 compound of both enantiomers L(-) lactide and D(+) lactide (10). There are strong indications that the degradation behavior of biodegradable polymers is affected by both molecular weight and MWD (11,12).

Hitherto, however, information on the molecular weight characterization of poly(lactic acids) is limited. Nuwayser et al. (13) determined the $\bar{M}_w$ of four poly(lactic acids), both poly(L-lactic acid) and poly(D,L-lactic acid), by GPC and found a linear correlation when $\bar{M}_w$ (range 20,000-300,000) was plotted against the reduced specific viscosity (0.1% w/v in dioxane). Dittrich and Schulz (14) presented the following values of the Mark-Houwink (MH) parameters for poly(L-lactic acid) in chloroform at 25°C: a = 0.82, K = $1.29 \times 10^{-3}$ ml $g^{-1}$, which were also applied to copolymers of L(-) lactide and D,L-lactide (15). Experimental details, however, were not given. Recently, Schindler and Harper (16) have established a MH equation by measuring the intrinsic viscosity of partially hydrolyzed PLA samples whose number-average molecular weights $\bar{M}_n$ had been derived from carboxyl end-group determinations (a = 0,77, K = $2.21 \times 10^{-2}$ ml $g^{-1}$, chloroform, 30°C). In this MH equation

$$[\eta] = K\Gamma(a + 2)\ \bar{M}_n^a$$

the gamma function of a + 2 was introduced assuming that randomly degraded polymers will possess a most probable MWD in which $\bar{M}_w/\bar{M}_n = 2$. We could not justify this assumption experimentally because homogeneous degradation of PLA samples prepared with both tetraphenyltin and stannous octoate resulted in a MWD in which $\bar{M}_w/\bar{M}_n$ turns out to be lower than 2.

Very few systematic data on the MWD as part of the characterization of PLA have been published so far. Gilding and Reed (17,18) have recently paid attention to this subject. Their work, however, has mainly been concerned with the characterization

of poly(glycolic acid) and copolymers of glycolide and lactide.

This article deals with the quantitative evaluation of the MWD of a number of nonhydrolyzed and partially hydrolyzed PLA samples using GPC, viscometry, and membrane and vapor-pressure osmometry. Besides, it provides a rapid method of determining the MH relationship in THF by combining off-line viscosity data with elution data.

## V.2. Procedure

Among a variety of methods of calibrating CPC columns the universal calibration has become a well-established one. According to this principle the product of the molecular weight M and the limiting viscosity number (LVN) is a universal function of the elution volume v for various polymers:

$$M[\eta] = \mu(v) \qquad (1)$$

Equation (1) is used to determine $\mu(v)$ with narrow-MWD standard of polystyrene (polymer 1). A MH equation for PLA (polymer 2) can then be established even when the samples are polydisperse. This requires the application of an iteration process. If the MH equations for both polymers hold in the molecular weight range studied, eq. (1) may be written as

$$\ln M_2 = \frac{1}{1 + a_2} \ln \frac{K_1}{K_2} + \frac{1 + a_1}{1 + a_2} \ln M_1 \qquad (2)$$

where K and a, respectively, stand for the MH coefficient and exponent. The constants $a_1$ and $K_1$ as well as $\ln M_1$ as a function of v are easily obtained by classical viscometry and primary calibration using the polystyrene standards. In order to evaluate $a_2$ and $K_2$, we proceed as follows. From eq. (1) it can be derived that

$$\ln[\eta]_2 = \frac{a_2}{1 + a_2} \ln\mu + \frac{1}{1 + a_2} \ln K_2 \qquad (3)$$



This equation enables us to estimate the values of $a_2$ and $K_2$ as a first approximation. For the different PLA samples the LVNs can be measured separately. As the corresponding elution volumes, the values at the peak of the chromatograms are taken. The right-hand side of eq. (2) can now be calculated and $\ln M_2$ is obtained as a function of v. Using the latter relationship and the obtained value of $a_2$, the viscosity-average molecular weight $\bar{M}_v$ is calculated according to

$$\bar{M}_v = \left(\int f(v) M_2^{\,a_2}\,(v)\,dv\right)^{1/a_2} \qquad (4)$$

in which f(v) represents the normalized chromatogram. To improve the values of $a_2$ and $K_2$ already found the measured LVNs are correlated with the above obtained values of $\bar{M}_v$, according to a MH relationship, producing new values of $a_2$ and $K_2$. Again these values are inserted in eq. (2) and a second calculation loop is started, resulting in new values of $a_2$ and $K_2$. An iteration performed in this way may be stopped when constant values of $a_2$ and $K_2$ are obtained. With final values of $a_2$ and $K_2$ the correct relationship between $\ln M_2$ and v is established, allowing the calculation of the molecular weight averages.

## V.3. Experimental

### V.3.1. Syntheses

The preparation of the poly(D,L-lactic acid) samples via the ring-opening polymerization in the melt was based on the procedures developed by Kulkarni et al.(19,20) and Sinclair and Gynn (5). With the exception of samples HJH-16 and -23, tetraphenyltin was used as the initiator at concentrations in the range $7 \times 10^{-5}$-$1 \times 10^{-4}$ mol/mol D,L-lactide. For the preparation of HJH-16 and -23 stannous octoate was used at concentrations in the range $(1\text{-}2)\times10^{-4}$ mol/mol D,L-lactide. Careful purification and drying of the D,L-lactide (mp 126°C) is necessary to obtain high-molecular-weight poly(D,L-lactic acid). D,L-lactide was recrystallized several times from ethyl acetate,

dried in a vacuum oven at room temperature, and stored in a dessicator. Prior to use the D,L-lactide was effectively purified by passing ice-cold dry ether through a small column of the compound. D,L-lactide (1-3 g) was placed in a polymerization tube (21) which was dried at 130°C for 24 h and the appropriate amount of initiator dissolved in a small amount of dry benzene (tetraphenyltin) or dry toluene (stannous octoate) was added. The solvent was evaporated at reduced pressure and the lower part of the tube was placed in an oil bath. The D,L-lactide was sublimed onto the upper part of the tube at reduced pressure, which is considered as an essential step to remove residual water and other contaminating solvents. After the sublimation was completed the tube was sealed under vacuum. Tubes containing tetraphenyltin as the initiator were placed in an oven at 178 ± 1°C for 17-24 h, those containing stannous octoate in an oven at 130 ± 1°C for 48 h (HJH-16) or 160 h (HJH-23). The resulting polymers were dissolved in acetone, precipitated in water, separated by filtration, and finally dried in vacuo at room temperature. By this procedure the polymers are not fractionated. Only minor amounts of D,L-lactide are removed. In spite of the rather similar conditions applied during the melt polymerization of PLA initiated with tetraphenyltin the obtained samples differ greatly in MWD, which is not uncommon for this type of bulk polymerizations (21). A limited number of samples were chosen for this characterization study from a large set covering the molecular weight range of 10,000-150,000.

Portions of 100 mg of a poly(D,L-lactic acid) samples were dissolved in 5 ml acetone. To each solution 0.5 ml deionized water was added, yielding clear acetone-water solutions. Thereafter the solutions were kept at 60-70°C for different periods of time. The homogeneously degraded polymers were isolated by solvent evaporation to dryness and in situ dissolved in tetrahydrofuran (THF) for characterization by GPC.



V.3.2. Methods

*Gel Permeation Chromatography*

Measurements were performed on a Waters model 150C high-pressure GPC equipped with four μ-Styragel columns with exclusion limits $10^6$, $10^5$, $10^4$, and $10^3$ Å, respectively. Operating conditions were solvent THF; solute PLA and polystyrenes (standards of Pressure Chemical Company); injected volume 400 μl; sample concentration 0.1 wt%; temperature of columns 31.15°C; flow rate 1 ml/min. The polystyrenes were injected as mixtures of three or four narrow fractions.

The degraded PLA samples were measured on a Waters model 200 GPC in which four columns (4 ft x 3/8 in.) packed with Styragel with exclusion limits $10^5$, 3 x $10^4$, $10^3$, and 250 Å had been installed. The operational conditions were solvent THF; temperature of columns 30°C; injection volume 1 ml; flow rate 1 ml/min; solute concentration 0.5 wt%.

*Viscometry*

Measurements were performed in a semimicroviscometer of the Cannon-Ubbelohde type at 31.15°C with THF as the solvent. For the unfractionated PLA samples and polystyrene standards the LVNs were determined by the usual extrapolation to zero concentration (concentration range 0-1.5 wt%).

*Membrane Osmometry*

Osmotic pressures were measured as a function of PLA concentration in an automatic osmometer (Hallikainen, model 361) in which a cellulose membrane (Schleicher and Schull, type RC 51) was mounted. The measurements were carried out in toluene at 35.8°C.

*Vapor-Pressure Osmometry*

For the PLA samples STPZ-1 and STPZ-3a the number-average molecular weights were obtained by using a Perkin-Elmer vapor-pressure osmometer working at 52.3°C with toluene as the solvent.

Calibrations were performed with tristearin (TRIS, M = 891), sucrose octaacetate (SOA, M = 679), and polystyrenes (PSs) 10,300 and 15,000.

V.4. Results and discussion.

Using the polystyrene standards we carried out a primary calibration with $M_1$ and $[\eta]_1$ as the separation parameters. The corresponding calibration plots are shown in Figures 1 and 2. Both curves may be approximated by a polynomial of the third degree (solid lines). Moreover, numerical results are collected in Table I, showing reasonable agreement of the polynomial approximations. The MH constants obtained by the correlation

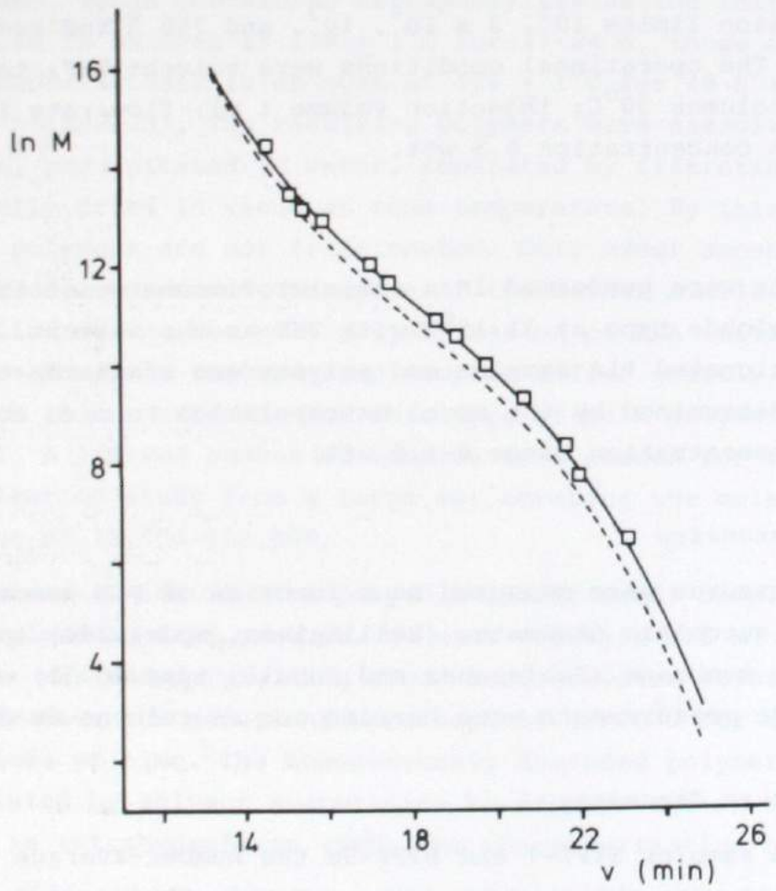



Fig. 1. Calibration curves of polystyrene (solid line) and PLA (dotted line)



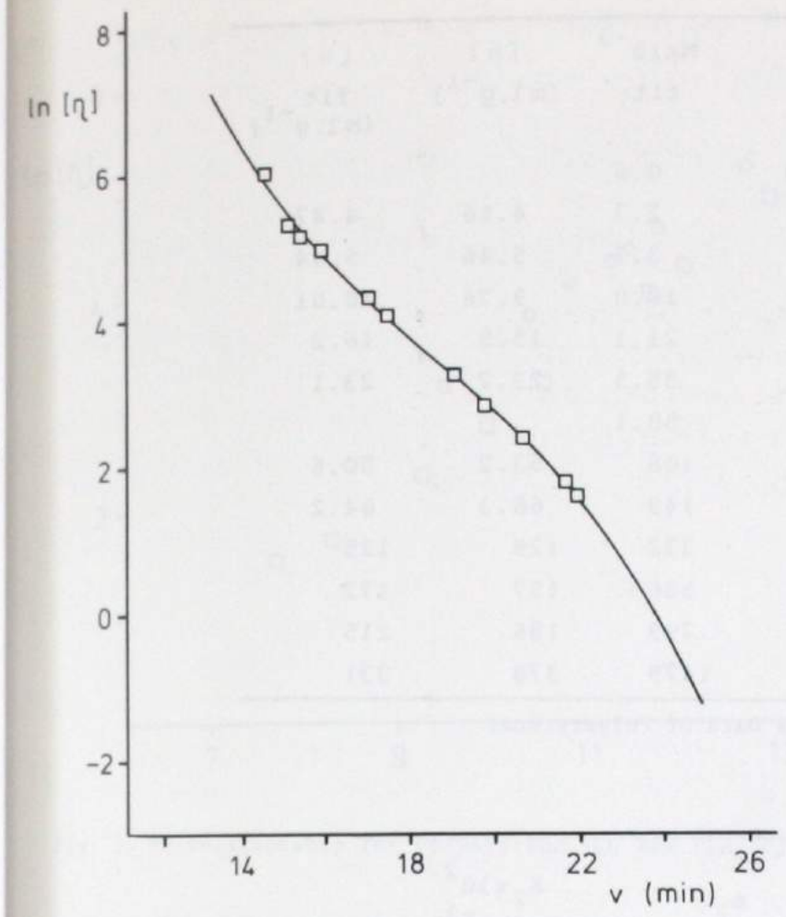



Fig. 2. Viscometric calibration data for polystyrene.

of $[\eta]_1$ and $M_1$ turn out to be a = 0.717 and K = 1.25 x $10^{-2}$ ml $g^{-1}$ for polystyrene in THF at 31.15°C. The range of validity of the MH equation (4,000 < M < 1.8 x $10^6$) is shown in Figure 3. These data agree with earlier data (22-25). According to eq. (1), combination of the $M_1$ and $[\eta]_1$ calibration functions results in the universal calibration function $\mu(v)$. It must be expected that $\ln\mu$ is adequately described by a polynomial of the third degree in v. The values of the $\ln\mu$ function at the peak elution volumes of the PLA chromatograms are of interest because according to eq. (3) they can be correlated to the experimentally determined values of $\ln[\eta]_2$. From a linear

| $v_p$ (min) | $Mx10^{-3}$ | $Mx10^{-3}$ fit | $[\eta]$ ($ml.g^{-1}$) | $[\eta]$ fit ($ml.g^{-1}$) |
|---|---|---|---|---|
| 23.12 | 0.6 | 0.6 | | |
| 21.94 | 2.2 | 2.7 | 4.56 | 4.47 |
| 21.65 | 4.0 | 3.7 | 5.46 | 5.44 |
| 20.65 | 10.3 | 10.0 | 9.76 | 10.01 |
| 19.75 | 20.4 | 21.1 | 15.5 | 16.2 |
| 19.05 | 37.0 | 35.5 | 23.2 | 23.1 |
| 18.56 | 51.0 | 50.1 | | |
| 17.47 | 110 | 108 | 53.2 | 50.6 |
| 17.02 | 160 | 149 | 68.3 | 64.2 |
| 15.89 | 390 | 372 | 129 | 125 |
| 15.40 | 498 | 586 | 157 | 172 |
| 15.09 | 670 | 799 | 186 | 215 |
| 14.53 | 1800 | 1479 | 378 | 331 |

Table I. Calibration Data of Polystyrenes

| iteration | $a_2$ | $K_2x10^2$ ($ml.g^{-1}$) |
|---|---|---|
| 0 | 0.707 | 2.52 |
| 1 | 0.665 | 4.05 |
| 2 | 0.649 | 4.88 |
| 3 | 0.643 | 5.25 |
| 4 | 0.641 | 5.40 |
| 5 | 0.640 | 5.46 |
| 6 | 0.639 | 5.49 |
| 7 | 0.639 | 5.50 |
| 8 | 0.639 | 5.50 |
| 9 | 0.639 | 5.50 |
| 10 | 0.639 | 5.50 |

Table II. Iterative Approach to MH parameters of PLA



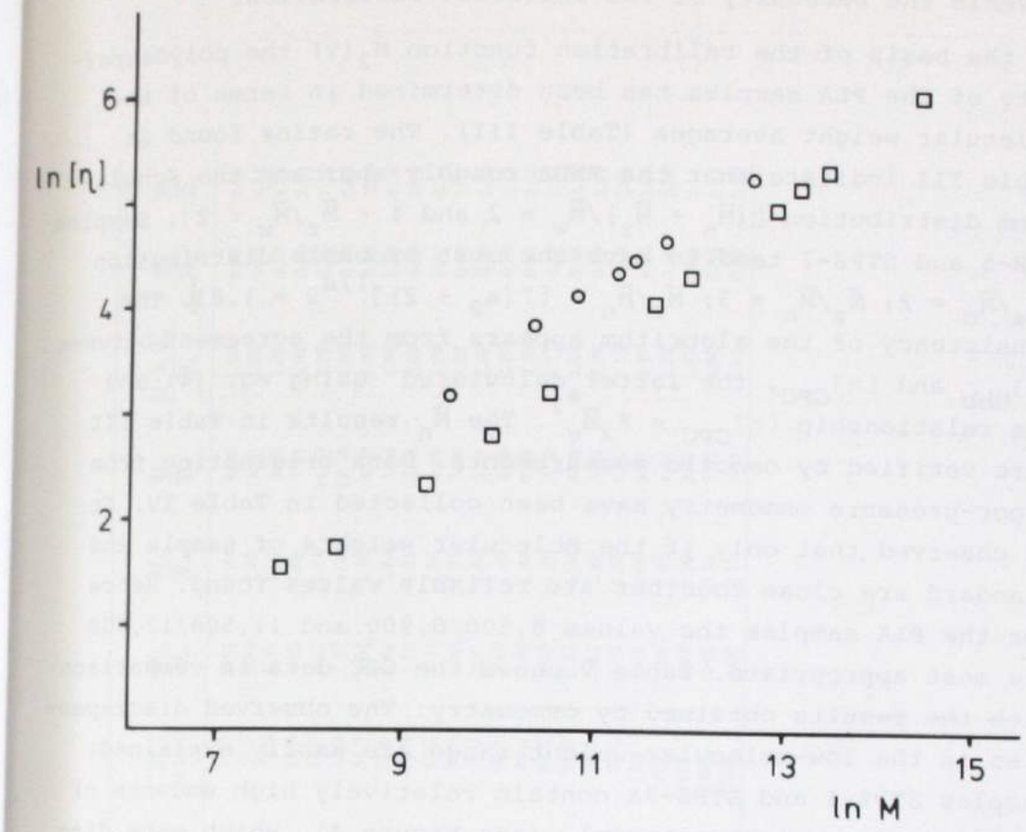



Fig. 3. MH relationship for polystyrene (□) and PLA (o).

regression of seven pairs of points it is found that $a_2$ = 0.707 and $K_2 = 2.52 \times 10^{-2}$ ml $g^{-1}$. With these values the iterative calculation described in the former section is started. Table II shows the values of the MH constants found in the subsequent iterations. The rapid convergence of the iterative calculation leads to constant values of $a_2$ and $K_2$. For further operation they have been fixed at $a_2 = 0.639$ and $K_2 = 5.49 \times 10^{-2}$ ml $g^{-1}$. Using these values and the measured LVNs a MH plot has been constructed in Figure 3.

Once the constants $a_1$, $a_2$, $K_1$ and $K_2$ are known, eq. (2) establishes the relationship between $M_2$ and $M_1$ or $M_2$ and v. As a result the two calibration curves shown in Figure 1 are almost parallel because the factor $(1 + a_1)/(1 + a_2)$ deviates not more than 5% from unity. The fact that the two curves do not coincide

reveals the necessity of the universal calibration.

On the basis of the calibration function $M_2(v)$ the polydispersity of the PLA samples has been determined in terms of the molecular weight averages (Table III). The ratios found in Table III indicate that the MWDs roughly approach the Schulz-Zimm distribution [$(\bar{M}_n + \bar{M}_z)/\bar{M}_w = 2$ and $1 < \bar{M}_z/\bar{M}_w < 2$]. Samples HJH-5 and STPZ-7 tend to have the most probable distribution ($\bar{M}_w/\bar{M}_n = 2$; $\bar{M}_z/\bar{M}_n = 3$; $\bar{M}_v/\bar{M}_n = [\Gamma(a_2 + 2)]^{1/a_2} = 1.83$). The consistency of the algorithm appears from the agreement between $[\eta]_{Ubb}$ and $[\eta]_{GPC}$, the latter calculated using eq. (4) and the relationship $[\eta]_{GPC} = K_2\bar{M}_v^{a_2}$. The $\bar{M}_n$ results in Table III were verified by osmotic measurements. Data originating from vapor-pressure osmometry have been collected in Table IV. It is observed that only if the molecular weights of sample and standard are close together are reliable values found. Hence for the PLA samples the values 8,500/8,900 and 11,500/12,000 are most appropriate. Table V shows the GPC data in comparison with the results obtained by osmometry. The observed discrepancies in the low-molecular-weight range are easily explained. Samples STPZ-1 and STPZ-3a contain relatively high amounts of low-molecular-weight material (see Figure 4), which were disregarded in the evaluation of the chromatograms. Consequently, $\bar{M}_n$ values obtained from vapor-pressure osmometry turn out to be smaller than the corresponding GPC values. Compared to the latter, membrane osmometry yields too high values of $\bar{M}_n$ if solute permeation through the membrane occurs. This effect, reflected by a decline of the experimental osmotic pressure in the course of time, was indeed observed, being more pronounced for the samples possessing the lower $\bar{M}_n$ values. For the high-molecular-weight samples the agreement between GPC and osmotic data is fairly good (see Table V). Although a very slight solute permeation was observed the MWDs did not indicate large amounts of low-molecular-weight substances (Fig. 5).

In the above discussion the effect of zonal dispersion was completely disregarded. As Table VI shows, only small discrepancies were found between the real and the observed values of the



| Sample | $[\eta]_{Ubb}$ (mL/g$^{-1}$) | $[\eta]_{GPC}$ (mL/g$^{-1}$) | $\bar{M}_w \times 10^{-3}$ | $\bar{M}_n \times 10^{-3}$ | $\bar{M}_z \times 10^{-3}$ | $\bar{M}_v \times 10^{-3}$ | $\frac{\bar{M}_w}{\bar{M}_n}$ | $\frac{\bar{M}_z}{\bar{M}_w}$ | $\frac{\bar{M}_z}{\bar{M}_n}$ | $\frac{\bar{M}_z + \bar{M}_n}{\bar{M}_w}$ | $\frac{\bar{M}_v}{\bar{M}_w}$ | $\frac{\bar{M}_v}{\bar{M}_n}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HJH-16 | 22.6 | 23.0 | 14.3 | 3.5 | 23.4 | 12.7 | 4.11 | 1.64 | 6.73 | 1.88 | 0.89 | 3.66 |
| | | 24.0 | 15.2 | 3.0 | 24.8 | 13.5 | 5.10 | 1.63 | 8.33 | 1.83 | 0.89 | 4.54 |
| HJH-19 | | 23.3 | 14.6 | 2.7 | 24.4 | 12.9 | 5.36 | 1.67 | 8.95 | 1.86 | 0.88 | 4.73 |
| | | 23.7 | 15.1 | 3.5 | 25.4 | 13.3 | 4.25 | 1.68 | 7.15 | 1.92 | 0.88 | 3.74 |
| | | 24.5 | 16.0 | 2.0 | 27.9 | 14.0 | 8.12 | 1.74 | 14.15 | 1.87 | 0.88 | 7.10 |
| STPZ-1a | 43.5 | 23.7 | 15.1 | 2.0 | 25.5 | 13.2 | 7.57 | 1.68 | 12.76 | 1.82 | 0.88 | 6.63 |
| | | 41.0 | 34.5 | 13.1 | 53.5 | 31.3 | 2.64 | 1.55 | 4.09 | 1.93 | 0.91 | 2.39 |
| STPZ-3a | 57.2 | 41.2 | 34.7 | 14.0 | 53.7 | 31.6 | 2.48 | 1.55 | 3.84 | 1.95 | 0.91 | 2.26 |
| | | 54.8 | 55.0 | 17.5 | 93.9 | 49.3 | 3.15 | 1.71 | 5.38 | 2.02 | 0.89 | 2.82 |
| HJH-5 | 70.9 | 54.6 | 54.5 | 17.9 | 90.6 | 49.0 | 3.04 | 1.66 | 5.06 | 1.99 | 0.90 | 2.74 |
| | | 71.1 | 82.0 | 41.9 | 140 | 74.0 | 1.96 | 1.71 | 3.34 | 2.22 | 0.90 | 1.77 |
| STPZ-4 | 79.8 | 71.7 | 84.1 | 40.6 | 160 | 75.0 | 2.07 | 1.90 | 3.94 | 2.39 | 0.89 | 1.85 |
| | | 80.0 | 100 | 41.8 | 188 | 89.1 | 2.40 | 1.87 | 4.49 | 2.29 | 0.89 | 2.13 |
| STPZ-7d | | 80.1 | 100 | 43.5 | 185 | 89.3 | 2.30 | 1.84 | 4.24 | 2.28 | 0.89 | 2.05 |
| | | 82.1 | 104 | 50.1 | 185 | 92.6 | 2.07 | 1.79 | 3.70 | 2.27 | 0.89 | 1.85 |
| STPZ-5b | | 81.4 | 104 | 42.8 | 199 | 91.5 | 2.42 | 1.92 | 4.64 | 2.33 | 0.88 | 2.14 |
| | | 85.3 | 111 | 41.6 | 210 | 98.5 | 2.67 | 1.88 | 5.04 | 2.26 | 0.89 | 2.37 |
| HJH-7 | 95.8 | 84.5 | 110 | 36.2 | 204 | 97.1 | 3.03 | 1.86 | 5.65 | 2.19 | 0.88 | 2.68 |
| | | 98.5 | 141 | 59.4 | 289 | 123 | 2.38 | 2.05 | 4.87 | 2.47 | 0.87 | 2.08 |
| FEK-22 | | 97.8 | 138 | 59.4 | 264 | 122 | 2.33 | 1.91 | 4.44 | 2.34 | 0.88 | 2.05 |
| HJH-23 | 173 | 102.7 | 150 | 61.5 | 293 | 132 | 2.44 | 1.96 | 4.77 | 2.37 | 0.88 | 2.14 |
| | | 174.8 | 352 | 143 | 796 | 302 | 2.46 | 2.26 | 5.55 | 2.66 | 0.86 | 2.11 |
| | | 174.9 | 352 | 138 | 758 | 309 | 2.56 | 2.15 | 5.51 | 2.54 | 0.86 | 2.20 |

Table III. Characteristic data of PLA samples

| sample | references | | | |
|---|---|---|---|---|
| | SOA | TRIS | PS10,300 | PS15,000 |
| SOA (M=679) | | 730 | 890 | 830 |
| TRIS (M=891) | 830 | | 1090 | 1130 |
| PS 10,300 | 7800 | 8400 | | 9400 |
| PS 15,000 | 11,000 | 11,800 | 14,400 | |
| PLA STPZ-1 | 6500 | 7000 | 8500 | 8900 |
| PLA STPZ-3a | 8700 | 9400 | 11,500 | 12,000 |

Table IV. Molecular weights from vapor-pressure osmometry

| Sample | $\bar{M}n \times 10^{-3}$ GPC | $\bar{M}n \times 10^{-3}$ vapour pressure osmometry | $\bar{M}n \times 10^{-3}$ membrane osmometry |
|---|---|---|---|
| STPZ-1 | 13.5 | 8.5 | 28.2 |
| STPZ-3a | 17.7 | 11.5 | 35.1 |
| HJH-5 | 41.3 | | 45.2 |
| STPZ-7d | 46.4 | | 58.4 |
| HJH-7 | 59.4 | | 66.0 |
| FEK-22 | 61.5 | | 62.0 |

Table V. Comparison of molecular weights of PLA by vapor-pressure osmometry, membrane osmometry, and GPC.

| $M \times 10^{-3}$ | $\bar{M}w/\bar{M}n$ observed | $\bar{M}w/\bar{M}n$ real |
|---|---|---|
| 4.0 | 1.14 | <1.10 |
| 37.0 | 1.05 | <1.06 |
| 390.0 | 1.12 | <1.10 |

Table VI. Influence of axial dispersion on calculated polydispersity ratio of polystyrenes



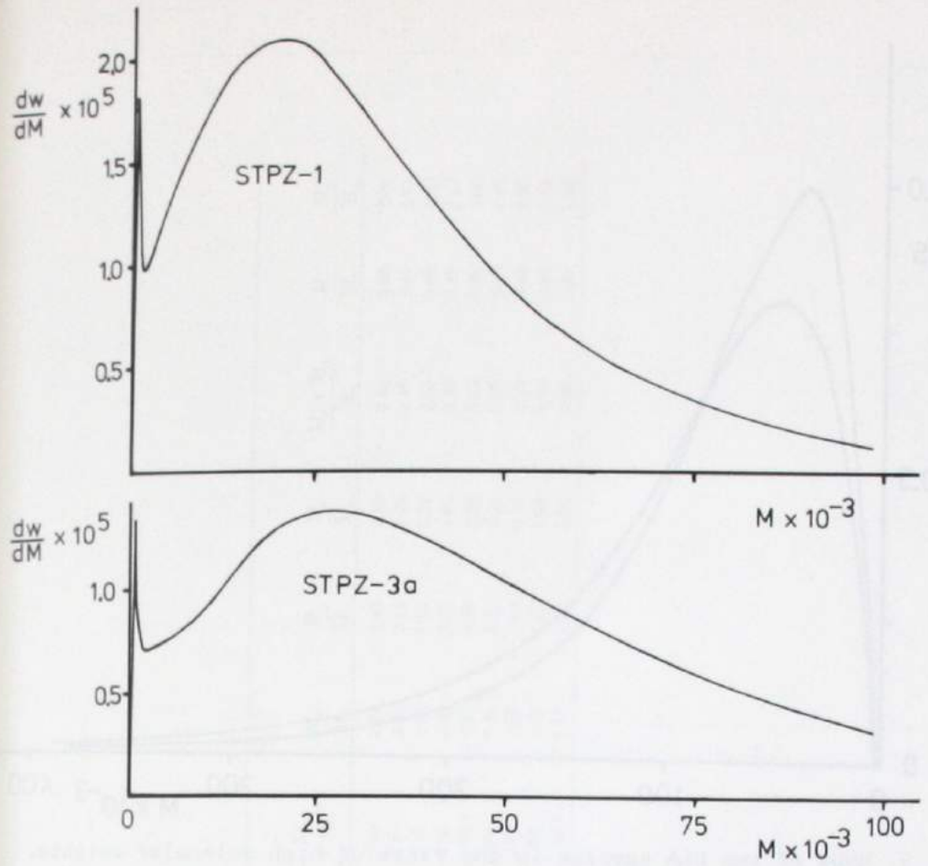



Fig. 4. MWDs of two typical PLA samples showing the presence of material with low molecular weight.

polydispersity of three polystyrene standards. The effect of hydrolysis of PLA and the MWD was studied using the samples HJH-7 and HJH-23. The molecular weight averages of the partially hydrolyzed samples are summarized in Table VII. The chromatograms were measured in this case with a GPC operation at low pressure. According to a method described earlier (26), the obtained normalized chromatograms were transformed into integral distribution curves which were combined with the known MWDs. Comparison of the corresponding values in Tables III and VII shows that no serious errors are introduced by the transformation. For the interpretation of the results in Table VII it is interesting to consider the assumption of Schindler and Harper (16) that the hydrolysis of PLA must proceed as a random chain

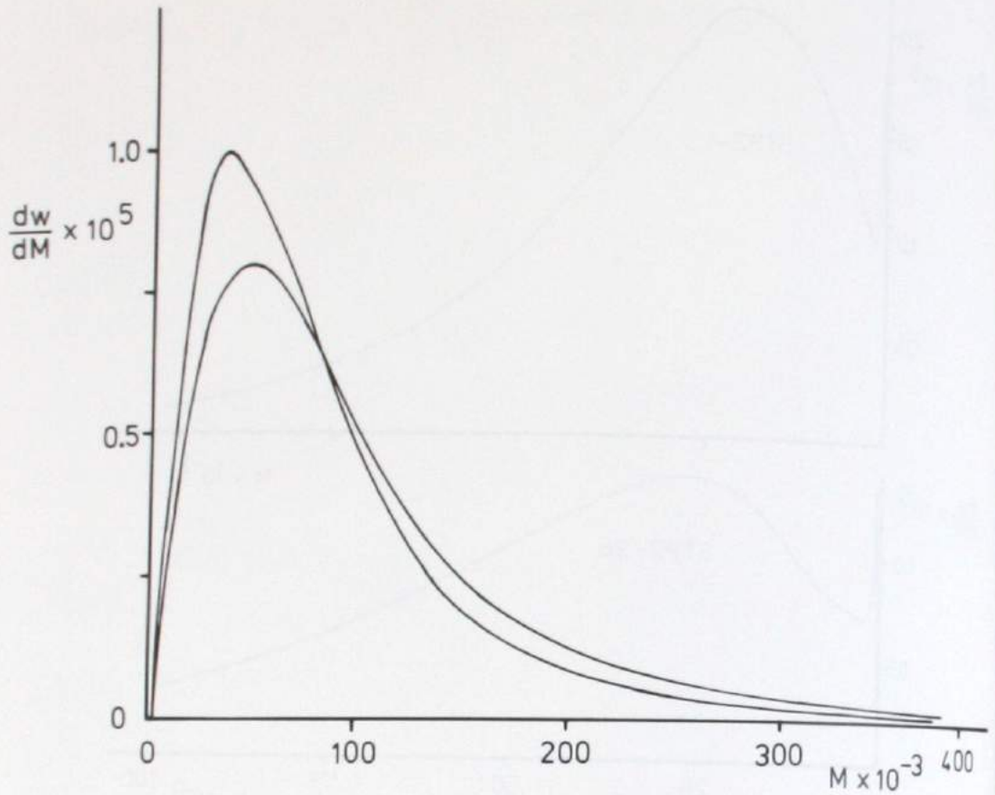



Fig. 5. MWDs of two PLA samples in the range of high molecular weights.

scission process and necessarely must lead to the most probable MWD. This assumption, which indeed is very plausible a priori, is not confirmed by our experiments. Instead it is striking that after hydrolysis MWDs are obtained with $\overline{M}_w/\overline{M}_n < 2$ and in the case of sample HJH-23 rather near unity. This evalution to a narrow MWD, which emerges clearly from Figure 6, warrants further investigation.

We would like to thank Waters Associates (Etten-Leur, The Netherlands) for placing the GPC apparatus at our disposal, Mr. H. Heuvink and Mr. S. Tuinhout for their contributions to the synthetic part of this investigation, Dr. J.W.A. van den Berg and Mr. G. van de Ridder for helpful discussions, and Mrs. T. van Gils for typing the manuscript.



| Sample | Hydrolysis time (h) | $\overline{M}_w$ × $10^{-3}$ | $\overline{M}_n$ × $10^{-3}$ | $\overline{M}_z$ × $10^{-3}$ | $\overline{M}_w/\overline{M}_n$ | $\overline{M}_z/\overline{M}_w$ | $\overline{M}_z/\overline{M}_n$ | $\overline{M}_z + \overline{M}_n/\overline{M}_w$ |
|---|---|---|---|---|---|---|---|---|
| HJH-7 | 0 | 136 | 59.8 | 275 | 2.28 | 2.02 | 4.59 | 2.46 |
| | 5 | 143 | 48.5 | 301 | 2.95 | 2.11 | 6.22 | 2.45 |
| | 17 | 133 | 50.2 | 262 | 2.65 | 1.97 | 5.22 | 2.35 |
| | 41 | 125 | 50.8 | 252 | 2.46 | 2.02 | 4.97 | 2.42 |
| | 170 | 97.6 | 42.7 | 165 | 2.28 | 1.69 | 3.86 | 2.13 |
| HJH-23 | 0 | 338 | 143.6 | 763 | 2.34 | 2.27 | 5.32 | 2.70 |
| | 120 | 200 | 96.9 | 360 | 2.06 | 1.80 | 3.71 | 2.28 |
| | 250 | 94.1 | 72.7 | 119 | 1.29 | 1.27 | 1.64 | 2.04 |
| | 330 | 49.5 | 39.8 | 51.9 | 1.15 | 1.13 | 1.31 | 2.00 |

Table VII. Degradation data of two PLA samples

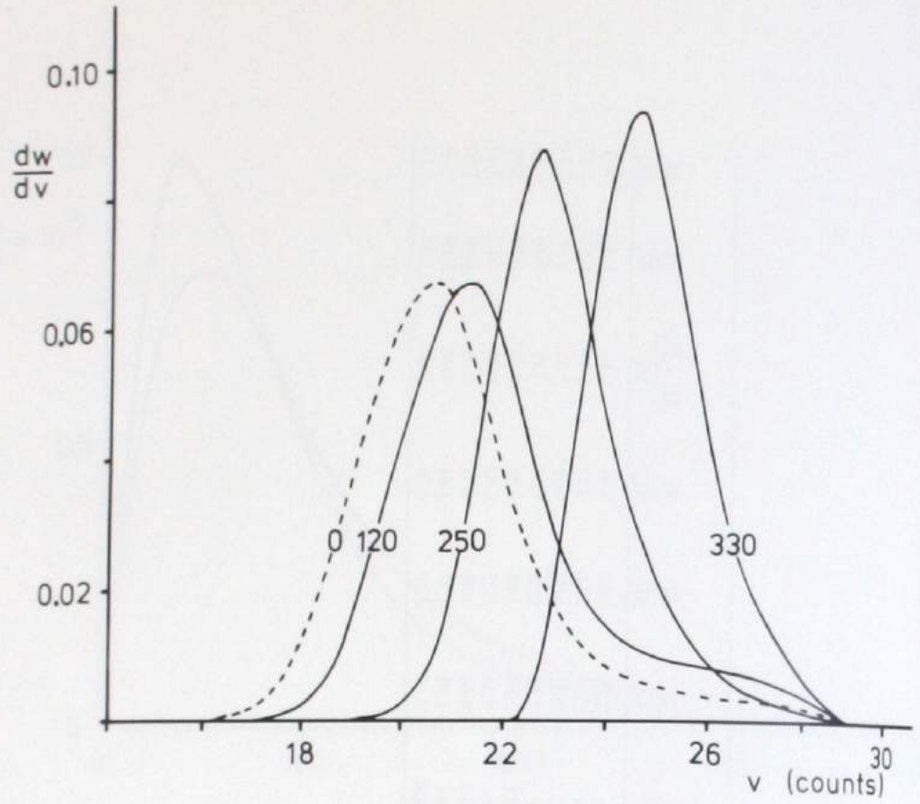



Fig. 6. Degradation behavior of PLA; normalized chromatograms are given as a function of time (0, 120, 210, 330 h).



References


1. R.L. Kronenthal, in: *Polymers in Medicine and surgery*, R.L. Kronenthal, Z. Oser and E. Martin, Eds., Plenum Press, New York, 1975, pp. 119-137.
2. J.W. Hodge, Jr., U.S. Nat. Techn. Inform. Serv. AD Report No. 742719 (1971).
3. J.B. Gregory, A.D. Schwope, D.L. Wise, U.S. Nat. Techn. Inform. Serv., AD Report No. 759381 (1971).
4. L.C. Anderson, D.L. Wise, and J.F. Howes, *Contraception*, 13, 375 (1976).
5. R.G. Sinclair and G.M. Gynn, U.S. Nat. Techn. Inform. Serv. AD Report No. 748411 (1972).
6. R.K. Kulkarni, E.G. Moore, A.F. Hegyeli, and F. Leonard, *J. Biomed. Mater. Res.*, 5, 169 (1971).
7. L. Feenstra, B.W.C. van der Ven, F.E. Kohn, and J. Feijen, *Int. J. Artif. Organs*, 3, 354 (1980).
8. E. Lillie and R.C. Schulz, *Makromol. Chem.*, 176, 1901 (1975).
9. R.C. Schulz, in *Proceedings, IUPAC International Symposium Macromolecular Chemistry, Budapest*, 1969, pp. 185-212.
10. G.J. van Hummel, S. Harkema, F.E. Kohn, and J. Feijen, *Acta Crystallogr.*, B38, 1679, (1982).
11. W.R. Vezin and A.T. Florence, *J. Biomed. Mater. Res.*, 14, 93 (1980).
12. C.G. Pitt, D. Christensen, R. Jeffcoat, G.L. Kimmel, A. Schindler, M.E. Wall, and R.A. Zweidinger, in *Proceedings, Drug Delivery Systems*, H.L. Gabelnick, Ed., DHEW Publ. No. (NIH)77-1238, U.S. Department of Health, Education and Welfare, Washington, DC, 1976, pp. 141-192.
13. E.S. Nuwayser, D.L. Williams, P.M. Meier, T.C. Wu, S.F. Merchant, and J.H. Kerrigan, in *Proceedings, Drug Delivery Systems*, H.L. Gabelnick, Ed., DHEW Publ. No. (NIH)77-1238, U.S. Department of Health, Education and Welfare, Washington, DC, 1976, p. 193.
14. W. Dittrich and R.C. Schulz, *Makromol. Chem.*, 15, 109 (1971).
15. E.W. Fisher, H.J. Sterzel, and G. Wegner, *Kolloid Z. Z. Polym.*, 251, 980 (1973).

16. A. Schindler and D. Harper, *J. Polym. Sci. Polym. Chem. Ed.*, 17, 2593 (1979).
17. D.K. Gilding and A.M. Reed, *Polymer*, 20, 1459 (1979).
18. D.K. Gilding, A.M. Reed, and I.N. Askill, *Polymer*, 22, 505, (1981).
19. R.K. Kulkarni, K.C. Pani, C. Neuman, and F. Leonard, U.S. Nat. Techn. Inform. Serv. AD Report No. 636716 (1966).
20. R.K. Kulkarni, K.C. Pani, C. Neuman, and F. Leonard, *Arch. Surg.*, 93, 839 (1966).
21. R.D. Lundberg, J.V. Koleske, and K.B. Wischmann, *J. Polym. Sci., A-1*, 7, 2915 (1969).
22. H. Benoit, Z. Grubisic, P. Rempp, D. Decker, and J.G. Zilliox, *J. Chim. Phys. Tome*, 63, 1507 (1966).
23. G.V. Schulz and H. Baumann, *Makromol. Chem.*, 114, 122 (1968).
24. Th. Provder and E.M. Rosen, *Sep. Sci.*, 5, 437 (1970).
25. D.J. Goedhard and A. Opschoor, *J. Polym. Sci., A-2*, 8, 1227 (1980).
26. J.A.P.P. van Dijk, W.C.M. Henkens, and J.A.M. Smit, *J. Polym. Sci. Polym. Phys. Ed.*, 14, 1485 (1976).



CHAPTER VI

# 2,5-MORPHOLINEDIONE$^{++}$, A POTENTIALLY NEW HETEROCYCLIC MONOMER FOR A NEW CLASS OF BIODEGRADABLE POLYMERS, - SYNTHESIS, CHARACTERIZATION AND ATTEMPTED POLYMERIZATION

## VI.1 Introduction

### VI.1.1 General introduction

Over the last two decades much progress was made in the development of synthetic absorbable polymeric materials showing a more predictable degradation behaviour than the natural proteinaceous polymeric materials which were mainly used as absorbable sutures, e.g. catgut.
Two important classes of synthetic absorbable polymeric materials can be distinguished: the poly(α-amino acids) and the poly(α-hydroxy acids).

Poly(α-amino acids) which are conveniently synthesized by a ring-opening polymerization of α-amino acid N-carboxyanhydrides (Fig. 1) were developed on the hypothesis that synthetic natural-like materials composed of chemical units which are found in the human body will show a superior biocompatibility (1).
An extensive study on methyl and benzyl esterified L-aspartate-L-leucine copolymers was performed by Sederel and co-workers (2-4). Bio-degradation is controlled to a great extent by the molar

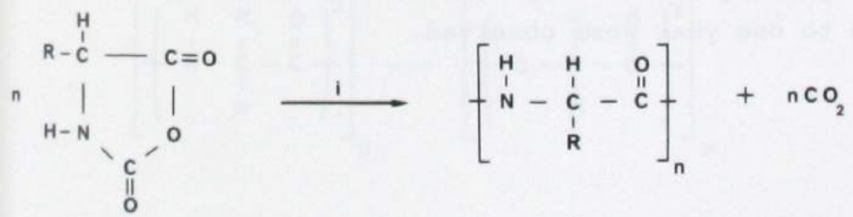


Figure 1. Formation and general formula of poly(α-amino acids).

++IUPAC-name: 1,4-morhpoline-2,5-dione

ratio of free β-carboxyl aspartate residues in the copolymers. Similar results were obtained by Anderson et al. (5) in relation to L-glutamate-L-leucine copolymers. Via an implantation study in rats, Feenstra et al. (6, cf. chapter VIII of this thesis) observed that even a completely benzyl esterified L-aspartate-L-leucine copolymer will be absorbed eventually, most likely after partial hydrolysis in situ. The precise degradation mechanism, -i.e. the mechanism of cleavage of covalent linkages of the polymer backbone (7)-, of synthetic poly(α-amino acids) is hitherto unknown. Proteolytic enzymes seem to promote the degradation of, at least, the more hydrophilic copolymers (1).

The simplest poly(α-hydroxy acids) are poly(glycolic acid) and poly(lactic acid). The chemistry, characterization and biological properties of poly(D,L-lactic acid) have been the subject of a great deal of this thesis. The preferred route for the synthesis of high molecular weight polymers is the ring-opening polymerization of glycolide or lactide in the melt initiated with a suitable initiator (Fig.2) (8,9).

Although there are some indications that certain enzymes may increase the rate of hydrolysis (10), it is generally believed that the degradation of poly(α-hydroxy acids) takes place via non-enzymatic ester hydrolysis of the polymer backbone. Periods up to one year were reported for complete absorption of α-hydroxy acid homo-polymers. Several applications, however, require specific degradation times. Miller et al. (11) synthesized lactic and glycolic acid copolymers of various compositions. Depending on the composition, degradation times of one month up to one year were observed.



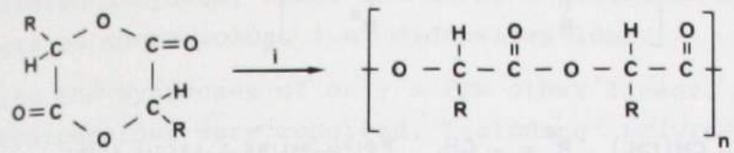


Figure 2: Formation and general formula of poly(glycolic acid) (R=H) and poly(lactic acid) ($R=CH_3$)

An interesting approach to new synthetic biodegradable polymers was reported by Goodman and co-workers (12) who synthesized random block copolymers containing poly(α-amino acid) and poly(α-hydroxy acid) segments (Fig. 3).

The synthesis of these so called polydepsipeptides was achieved by the ring-opening copolymerization of an α-amino acid N-carboxyanhydride and an α-hydroxy acid anhydrosulphite (13). These types of copolymers were said to display excellent physical, chemical and biological properties and further to possess unique hydrolysis characteristics. However, substantial evidence to prove this was not forwarded (14).

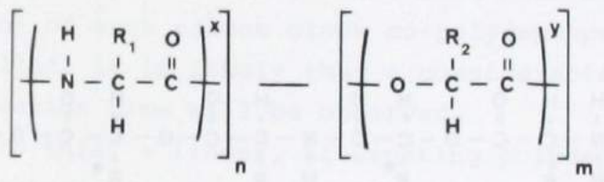
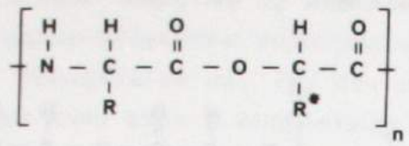


Figure 3: General formula of random block copolymer of α-amino and α-hydroxy acid



| | | |
|---|---|---|
| R = $-CH(CH_3)$ | $R^* = -CH_3$ | POLY(L-VALINE-L-LACTIC ACID) |
| R = $-CH_3$ | $R^* = -CH_3$ | POLY(L-ALANINE-L-LACTIC ACID) |
| R = $-H$ | $R^* = -H$ | POLY(L-GLYCINE-L-GLYCOLIC ACID) |

Figure 4: General formulas of linear alternating polydepsipeptides synthesized by Goodman et al. (15-17)

Soon after the disclosure of these block copolymers Goodman et al. described the synthesis of some linear, alternating, high molecular weight ($\bar{M}_w$ = 20,000 - 100,000) polydepsipeptides (15-17) (Fig. 4).
These polymers were synthesized at a very small scale only to study the conformational properties (17,18). The best results, -$\bar{M}_w \approx$ 100,000-, were obtained by the thermal polymerization of the trifluoro acetate salt of the pentachlorophenyl ester of a tetradepsipeptide on an inert, celite matrix (16) (Fig. 5).

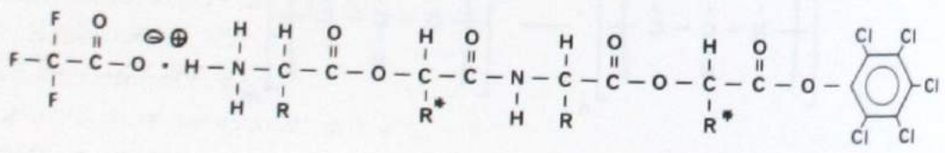


Figure 5: General formula of a trifluoro acetate salt of a pentachlorophenyl ester of a tetradepsipeptide



The synthesis of this tetradepsipeptide derivative was based on general coupling procedures (19,20) for the formation of peptide and depside linkages, using N-protected α-amino acids, C-protected α-hydroxy acids and N- or C-protected reaction products of these acids, i.e. didepsipeptides.

Hitherto the syntheses of only a few other linear, alternating polydepsipeptides were reported, including poly(L-leucine-L-2-hydroxy-4-methyl pentanoic acid) ($\bar{M}_n$ = 24,000) (21) and poly (glycine-glycolic acid), the molecular weight of which was probably quite low (22,23). The starting material for the first polymer was a preformed tetradepsipeptide, the carbonyl group of which was activated through an acid chloride. For the second polymer a didepsipeptide was used. In this case the carboxyl group was activated with p-nitrophenol.
Contrary to activation of the carboxyl group of a preformed depsipeptide, Kunz and Lorenz (24) have recently described the oligomerization of a L-leucine-L-lactic acid-L-leucine-L-lactic acid tetradepsipeptide which was protected and activated by 4-methylthiophenoxycarbonyl as the N-terminal group.

It is plausible that the biodegradation properties of linear, alternating polydepsipeptides having regular sequences of α-amino acid and α-hydroxy acid residues, will be different to a certain extent from those of the random block co-polydepsipeptides mentioned previously.
We believe that the degradation rates of the latter polymers will be determined by both the degradation rates of the comprising α-hydroxy acid and α-amino acid homo-blocks. As the composition of such random block co-polydepsipeptides cannot be controlled, it is likely that a considerable variation of the degradation time will be observed.
Contrary to this, a linear, alternating polydepsipeptide has a well characterized composition. Therefore the rate of degradation of this polymer is expected to be more predictable. The last stage of biodegradation of a polymer involves complete removal of polymer residues from the tissue (7). This may be

the result of simple solubilization of low molecular weight species into the intracellular fluids or small fragments may be removed from the implant site by phagocytosis and eventually carried to the lymphatic system. If block co-polydepsipeptides are degraded, the low molecular weight species in the last stage of degradation are, -mainly-, α-hydroxy acid or α-amino acid oligomers. In the case of linear, alternating polydepsipeptides they contain both α-hydroxy acid and α-amino acid residues. This may imply different solubilization or phagocytosis phenomena.

A substantial amount of a linear, alternating polydepsipeptide is needed for a successful evaluation of its biodegradation behaviour. The synthetic routes applied hitherto and based on successful multi-step synthetic routes to sequential polypeptides, are inadequate for the preparation of sufficient amounts of polymer.



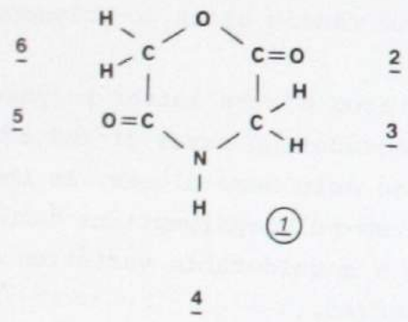



Figure 6: Formula of 2,5-morpholinedione (1)

In this chapter we will report on the synthesis and characterization of 2,5-morpholinedione (1, Fig. 6) and some of its



derivatives. In principle, linear, alternating polydepsipeptides can be prepared directly by a ring-opening polymerization of this new heterocyclic monomer. Attempts to polymerize the monomers will be reported. For reasons that will be outlined in section VI.1.2 the polymerization studies were restricted to either non- or partially substituted 2,5-morpholinedione derivatives.

VI.1.2 Ring-opening polymerization of 2,5-morpholinedione

From our experience with the ring-opening polymerization of

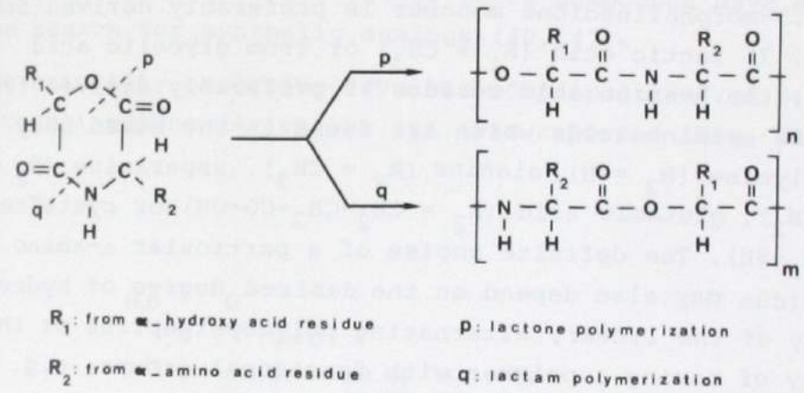



Figure 7: Formation of linear, alternating polydepsipeptides by the ring-opening polymerization of 2,5-morpholinedione or its derivatives

D,L-lactide (Fig. 2) as outlined in other parts of this thesis, it was rationalized that, in principle, linear, alternating polydepsipeptides can be synthesized by a ring-opening polymerization of 2,5-morpholinedione (1) or its derivatives (Fig. 7).

2,5-Morpholinedione can be considered both as a 6-membered lactam as well as a 6-membered lactone.
For a long time 6-membered lactams were considered as non-polymerizable (25, 26). Newer developments in lactam polymerization, however, made the anionic ring-opening polymerization of the

simplest 6-membered lactam, 2-piperidinone, feasible (27, 28). A few ring-opening polymerizations of the 6-membered cyclic dipeptide (dilactam) 2,5-piperazinedione (16) in the presence of water were reported (29-31). In the presence of aqueous ammonia oligomers were obtained (32). Attempted ring-opening polymerizations of substituted 2,5-piperazinedione failed to give any polymer. As is extensively outlined in this thesis, 6-membered dilactones such as D,L-lactide can easily be polymerized under various conditions. The same applies to the 6-membered monolactone δ-valerolactone.

From a biodegradation point of view of the linear, alternating polydepsipeptides to be synthesized, the α-hydroxy acid residue of the 2,5-morpholinedione monomer is preferably derived from (see Fig. 7) lactic acid ($R_1 = CH_3$) or from glycolic acid ($R_1 = H$); the α-amino acid residue is preferably derived from one of the α-amino acids which are found in the human body (33), - e.g. glycine ($R_2 = H$), alanine ($R_2 = CH_3$), asparagine ($R_2 = CH_2\text{-}CO\text{-}NH_2$), glutamic acid ($R_2 = CH_2\text{-}CH_2\text{-}CO\text{-}OH$) or cysteine ($R_2 = CH_2\text{-}SH$). The definite choise of a particular α-amino acid residue may also depend on the desired degree of hydrophilicity of the linear, alternating polydepsipeptide or the necessity of having a polymer with functional groups, e.g. for coupling drugs (34).

2,5-Morpholinedione monomers with substituted α-amino acid or α-hydroxy acid residues, however, may easily cause problems as to their polymerizability. Hall and co-workers (25,35) made the general observation that the tendency of both lactams and 6-membered lactones to polymerize, always diminishes if alkyl or aryl substituents are present in the ring.
For that reason only the polymerizability of unsubstituted or partially substituted 2,5-morpholinedione was investigated in this study.

### VI.1.3 Chemistry of 2,5-morpholinedione (1)

Although at first sight a rather simple heterocyclic compound,



2,5-morpholinedione and its derivatives have only been mentioned occasionally in the literature, whereas their synthesis has not been studied systematically. These compounds can be considered as the simplest representatives of the so called (36) cyclodepsipeptides, which are, -often large-, ring systems involving regularly or irregularly alternating ester and amide linkages (37, 38). Since their first discovery in 1947 (36), cyclodepsipeptides have attracted increasing attention because of the biological activity exhibited by various naturally occurring members of this group (39-46). This includes antibiotic activity and the ability to transport selectively alkali metal cations in biological systems. The unusual biological activities of these compounds have stimulated the search for synthetic analogs (40, 47).

One naturally occurring "cyclomonodepsipeptide", i.e. a 2,5-morpholinedione derivative (2), was reported (Fig. 8). The

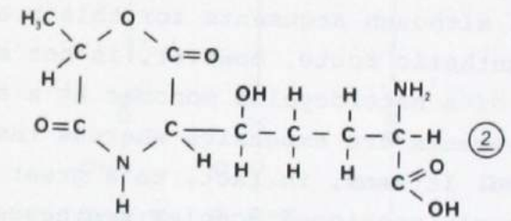


Figure 8: Chemical formula of toxin of Pseudomonas tabaci (2)

phytopathogenic toxin of Pseudomonas tabaci isolated by Woolley et al. (48) has been shown to be most probably a 2,5-morpholinedione derived from lactic acid and from α,ε-diamino-β-hydroxypimelic acid.

The synthesis and characterization of unsubstituted 2,5-morpholinedione has not been published previously. From an extensive

survey of the literature with respect to the chemistry of derivatives of 2,5-morpholinedione (see also 49-57), it was concluded that the possible synthetic routes to unsubstituted 2,5-morpholinedione (1) could roughly be divided into the following (Fig. 9):

Route a:

Lactonization (36, 58-65) of N-hydroxyacetylglycine (3). This route, however, is hardly useful because pure 'α-hydroxyacetyl-α-amino acids can only be prepared with great difficulty, e.g. by hydrolysis of 2,5-morpholinedione which was prepared in a different way (60).

Route b:

Amide bond formation (15,16,21,22,40,66) by cyclization of an activated ester, acid chloride or azide of 'α-aminoacetyl-α-hydroxyacetic acid (4). According to Thamm (67) cyclization of a linear didepsipeptide through the formation of an amide bond (route b) is preferred to the formation of an ester bond (routes a, c and d), although arguments for this preference are not given. Such a synthetic route, however, is not suitable for the preparation of a heterocyclic monomer at a somewhat larger scale. The reagents are expensive whereas the synthesis is rather complicated. It runs, in fact, to a great extent parallel to the previously mentioned complex syntheses of linear, alternating polydepsipeptides described by Goodman et al. (15-18), Ridge et al. (21) and Stewart (22), who obtained often 2,5-morpholinediones as side-products.

Route c:

Sublimation (68-70) of the sodium (5) or alkaloid salt of N-chloroacetylglycine. Kaźmierczak and Kupryszewski (70) reported a yield of 57% of 6-methyl-2,5-morpholinedione (13) after the sublimation of N-D,L-α-bromopropionylglycine (10) on a 25-grams scale. N-'α-halogenacyl-α-amino acids such as N-chloro-



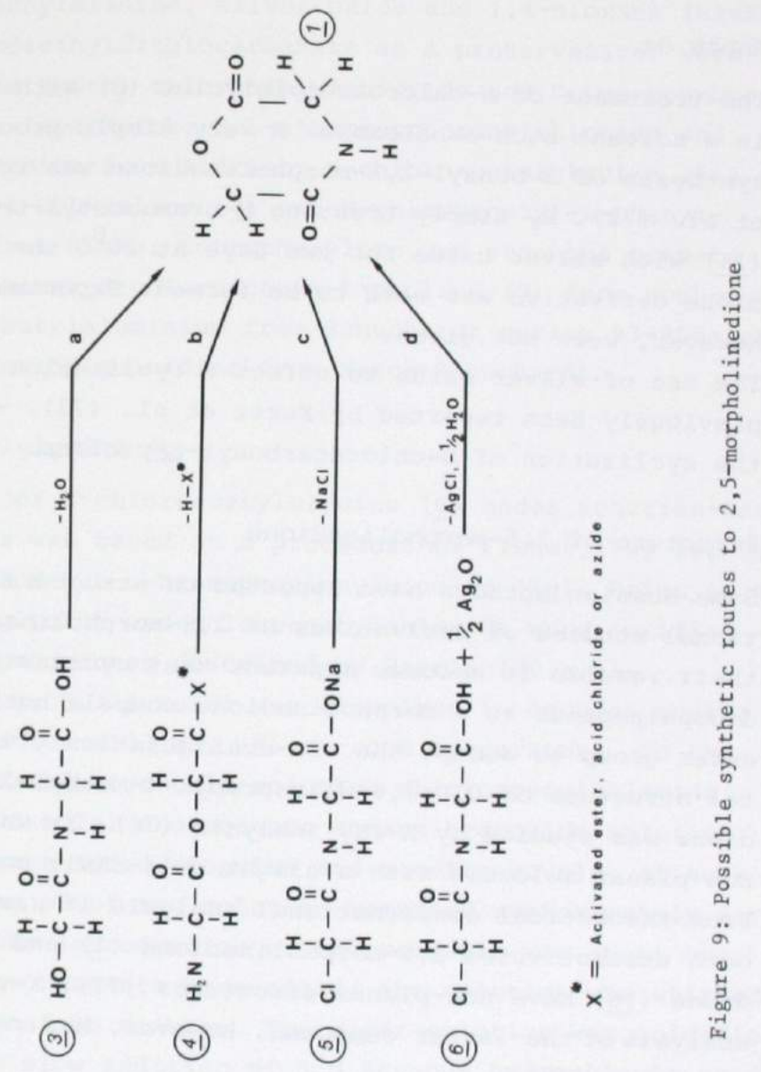



Figure 9: Possible synthetic routes to 2,5-morpholinedione

acetylglycine (6) are technically simply prepared by acylation of an α-amino acid with an acyl halide under Schotten-Baumann conditions (71). Solid salts of N-'α-halogenacyl-α-amino acids are easily prepared by dissolving the acids in aqueous sodium hydroxide and evaporating to dryness in vacuo (69).

Route d:

The treatment of N-chloroacetylglycine (6) with silver oxide in a solvent such as dioxane. A very simple procedure for the synthesis of 3-benzyl-2,5-morpholinedione was reported by Rumsh et al. (72). By simply treating N-bromoacetyl-L-phenylalanine (11) with silver oxide for two days at 20°C the 2,5-morpholinedione derivative was said to be formed. Experimental details, however, were not given.
The use of silver oxide to effect a cyclization reaction had previously been reported by Kurtz et al. (73), -i.e. to effect the cyclization of N-chlorocarbonyl-L-proline.

Structure of 2,5-morpholinedione

Some Russian authors have reported on structural and conformational studies of derivatives of 2,5-morpholinedione. From their results it becomes apparent that cyclization of a linear didepsipeptide to a morpholinedione compels both the amide and ester group to accept the cis-configuration (74-76). The crystal structure of D,D-3,6-diisopropyl-4-methyl-2,5-morpholinedione was studied by X-ray analysis (76). It was found to be a non-planar molecule with a slight half-chair conformation.
From theoretical conformational analysis it was concluded that both unsubstituted 2,5-morpholinedione (1) and 2,5-piperazinedione (16) have non-planar structures (77). X-ray diffraction analysis of the latter compound, however, has revealed a planar structure (78).

VI.2 Experimental

All melting points are uncorrected. IR-spectra were recorded in tablets with KBr on a Perkin-Elmer 357 grating IR-spectrophotometer. $^1$H-NMR spectra were recorded by Miss J.L.M. Vrielink and Miss J.M. Visser on a $^1$H-NMR apparatus Bruker WP 80. Mass spectra were recorded by Mr. T.W. Stevens on a mass spectrometer Varian MAT 311A. Elemental analyses were carried out at TNO, Utrecht.

Glycine, L-phenylalanine, silver oxide and 1,4-dioxane (having 0.001% sodiumdiethyldithiocarbamate as a preservative) were purchased from Baker; chloroacetylchloride and D,L-α-bromopropionylbromide from Merck; D,L-α-bromopropionylchloride and bromoacetylbromide from Aldrich; N-chloroacetylglycine (6), N-chloroacetyl-D,L-valine (7) and N-chloroacetyl-L-phenylalanine (8) from Sigma; tetraphenyltin and stannous octoate from Polysciences; N-chloroacetyl-L-proline (9) from Koch-Light; triisobutylaluminium from Schuchardt, purity 93-95%; and N,N,N',N'-tetramethylguanidine from Merck-Schuchardt.

N-chloroacetylglycine (6)

The synthesis of N-chloroacetylglycine (6) under Schotten-Bauman conditions was based on a procedure of Fisher (79) for the preparation of N-D,L-α-bromopropionylglycine (10). Prior to the preparation, glycine and chloroacetylchloride were purified according to procedures described by Perrin (80,a,b).
Glycine (10.0 g, 0.133 mole) was dissolved in aqueous sodium hydroxide solution (133 ml, 1 N) at room temperature. To the vigorously stirred, ice-cold solution chloroacetylchloride (12.5 ml, 0.175 mole) and aqueous sodium hydroxide solution (200 ml, 1 N) were added dropwise and simultaneously, at such a rate that the pH of the solution remained approximately 9. The time for the introduction of the reagents was about one hour. After the reagents were added, the solution was stirred for an additional 30 minutes. The clear solution was acidified to pH 3 by the slow addition of 5 N aqueous hydrochloric acid solution. During the addition the solution was continuously stirred and cooled with an ice-water mixture. No N-chloroacetylglycine precipitated upon acidification. The solution was

evaporated to dryness and the residue was extracted 5 times with warm ether (100 ml). The combined ether layers were concentrated to 50 ml, whereupon a part of crude N-chloroacetylglycine precipitated. After filtration, more crude crystals were isolated by the addition of a large excess of petroleum ether (b.p. 40-60$^0$C). Recrystallization from a large volume of ether gave pure N-chloroacetylglycine (6). The yield was 14.1 g (70%); m.p. 104-106$^0$C; lit. 100$^0$C (81).

Various substituted 'α-bromoacyl-α-amino acids were prepared in good yields by Cook and Cox (69) by shaking the acid chloride with two equivalents of α-amino acid suspended in dry and ethanol-free chloroform. In our hands this procedure failed to produce any N-chloroacetylglycine.

N-D,L-α-bromopropionylglycine (10)

The procedure for the preparation of N-D,L-α-bromopropionylglycine (10) from glycine (10.0 g, 0.133 mole) and D,L-α-bromopropionylbromide (27,4 g, 0.16 mole) under Schotten-Bauman conditions was similar to the procedure for the preparation of N-chloroacetylglycine (6). The crude product (19.2 g, 0.09 mole) was recrystallized from a large volume of chloroform (250 ml). The yield was 14.7 g (53%); m.p. 94-97$^0$C; lit. 100-103$^0$C (68).

N-bromoacetyl-L-phenylalanine (11)

N-bromoacetyl-L-phenylalanine (11) was prepared from L-phenylalanine (16,5 g, 0.10 mole) and bromoacetylbromide (22.2 g, 0.11 mole) according to Steiger's (82) detailed procedure for the benzoylation of α-amino acids. Contrary to this procedure a slight excess (10%) of the acid bromide was used and the reaction mixture was acidified to pH 2 whereupon the N-bromoacetyl-L-phenylalanine (11) crystals precipitated. The yield was 8.3 g (29%). The crystals melted sharp at 134$^0$C (no literature data were found). Attempted recrystallization from several solvents failed. The structure was confirmed by IR-spectroscopy and mass spectrometry. IR-spectrum: N-H stretch 3360 cm$^{-1}$; amide I 1635 cm$^{-1}$; amide II trans 1545 cm$^{-1}$; amide V trans



700 cm$^{-1}$; C=O carboxylic acid 1720 cm$^{-1}$; C-Br 690 cm$^{-1}$; monosubstituted benzene: around 3000 cm$^{-1}$; the spectrum was very similar to that recorded from N-chloroacetyl-L-phenylalanine (8), purchased from Sigma. The mass spectrum showed significant peaks at m/e = 285 (parent peak), 240.5, 240, 205, 148 and 91, and was very similar to that recorded from N-chloroacetyl-L-phenylalanine except for bromine instead of chlorine.

Active copper (12)

Active copper (12) was prepared according to a slightly modified procedure described by Ingram (83). The starting material was a mixture of copper-(I)-oxide and copper-(II)-oxide, in wire form, with lengths of about 5 mm (Merck, no. 2767). First the slightly alkaline copper oxide was washed with an aqueous solution of acetic acid (10% w/v), washed with distilled water and dried in an oven. Then the dried copper oxide was packed in a clean piece of combustion tubing made of quarz for its reduction with hydrogen. Before this was done the copper oxide war purged with nitrogen to remove oxygen. The actual reduction was carried out by heating the copper oxide at 220-230$^0$C by means of an electric furnace, using a moderately slow stream of hydrogen. The reduced copper was allowed to cool in a slow stream of hydrogen and then stored under nitrogen. Prior to use the active copper was pulverized under a blanketing atmosphere of dry nitrogen resulting in a mixture of fine and coarse powder.

Sodium salts of 'α-halogenacyl-α-amino acids (cf. 5) (69)

Typically 2-5 grams of the 'α-halogenacyl-α-amino acid were dissolved in an equivalent of a 1N aqueous sodium hydroxide solution (the solution may not become basic in order to prevent substitution of the halogen atom by a hydroxyl group). The water was evaporated in vacuo yielding the very hygroscopic sodium salt (cf. 5) as a compact mass. This was crushed to a fine powder, in situ, under nitrogen. The fine powder was dried for several days in a vacuum oven at 1 mm and stored in a dessiccator over $P_2O_5$.

In order to check whether the preparation of the sodium salt in an aqueous environment would be disadvantageous as was suggested by Chadwick and Pascu (68), the sodium salts were also prepared, with considerable difficulty, as follows:

-a- In an ethanolic sodium hydroxide solution, followed by precipitation with ether.

-b- In an ethanolic sodium hydroxide solution and isolation by evaporation of the ethanol to dryness.

IR-spectra of the sodium salts prepared in different ways did not show significant differences.

VI.2.1 Synthesis of 2,5-morpholinedione (1)

a. Dry distillation of the sodium salt (5) of N-chloroacetylglycine

Dry distillation of the sodium salt (5) of N-chloroacetylglycine occurred in a conventional apparatus for distillation under reduced pressure (0.05 mm) prepared from standard semi-micro glassware. The stillhead, very short condensor and neck of the receiver were warmed during distillation. Experimental results are given in Table 1. Experiments 1 and 2 show that dry distillation was not a suitable procedure for the preparation of 2,5-morpholinedione (1) from the sodium salt (5) of N-chloroacetylglycine.

b. Sublimation of the sodium salt (5) of N-chloroacetylglycine

Most sublimations were carried out in a conventional sublimator (diameter exterior wall 5 cm; diameter "cold finger" 3 cm). Depending on the loading of the sublimator, the distance between the "cold finger" and the bottom of the sublimator was 1.5 or 2.5 cm. The temperature of the "cold finger" was kept at $-20^0$C. The sublimator was connected via two cold traps ($-196^0$C) to a mercury diffusion pump which was used in series with a conventional oil pump. A typical sublimation procedure was as follows. The sublimator was quickly loaded with the hygroscopic sodium salt (5) of N-chloroacetylglycine or with the



sodium salt and copper powder (12). After the sublimator was purged 5 times with dry nitrogen, it was connected to the oil pump and evacuated for about 15 hours. Thereafter the mercury diffusion pump was used and the actual sublimation was started. The temperature of the oil bath was raised at a rate of 30-$40^0$C/15 minutes to $170^0$C if copper was added. The temperature of the oil bath was kept at $170^0$C for one or two hours until the collection of the reaction product on the surface of the "cold finger" had almost stopped. Thereafter the temperature was raised at a rate of about $10^0$C/30 minutes to maximal $220^0$C. From $140^0$C onwards a gradual discoloration and melting of the sodium salt was always observed. At $170^0$C the sodium salt was completely molten and light-brown. If no copper was added the temperature had to be raised to about $200^0$C before the first amount of reaction product collected on the surface of the "cold finger". In a few occasions a larger sublimator was used to investigate whether an increased contact surface between the salt and the heating medium would improve the yield (diameter exterior wall 10 cm; diameter "cold finger" 9 cm).
In Table 1 the results of the sublimation experiments with the sodium salt (5) of N-chloroacetylglycine are summarized.

Recrystallization

Crude 2,5-morpholinedione (1) obtained after the sublimation experiments was recrystallized from dry methanol (less than 0.05% of water) under a blanketing atmosphere of dry nitrogen. All the glassware used was dried over night at $130^0$C. Typically 0.5 g of crude 2,5-morpholinedione was placed in a 25 ml flask fitted with a condensor. The flask was warmed by a water bath with a thermostatic control. While the bath temperature was kept just under the boiling temperature of methanol ($65^0$C), methanol was added gradually until most of the crude 2,5-morpholinedione (slightly yellow-green coloured) had dissolved. The period of addition may not be too long, otherwise undesired side reactions will occur. The last remnants of crude 2,5-morpholinedione were dissolved by refluxing the solution for a

very short time. After filtration the solution was set aside, first at room temperature and later at about $-4^0$C.
The results of the recrystallization of several portions of crude 2,5-morpholinedione are summarized in Table 2.
Attempts to avoid the recrystallization procedure by re-subliming the crude 2,5-morpholinedione were not successful. The resublimed compound still contained a substantial amount of yellow-green contaminants.

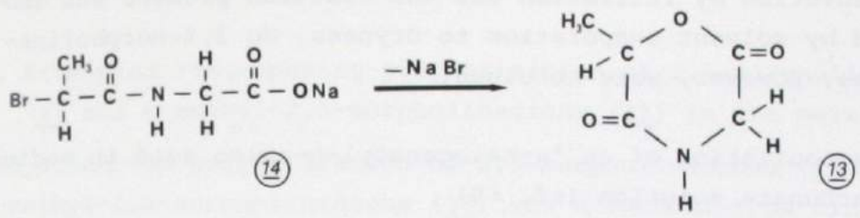
Characterization

Unsubstituted 2,5-morpholinedione (1) has a fairly high melting point: 191-193$^0$C. This melting point is considerably higher than that of the dilactone glycolide (87$^0$C) (see chapter III of this thesis), but lower than that of the dilactam 2,5-piperazinedione (16) (320$^0$C). Sublimation of 2,5-morpholinedione (1) at 760 mm Hg starts from 145$^0$C onwards.
Elemental analysis:
Found : C = 41.81 H = 4.56 N = 12.13 O = 41.27
Required: C = 41.74 H = 4.38 N = 12.17 O = 41.70
Spectroscopic data:
Table 3 shows the characteristic infrared absorption frequencies of 2,5-morpholinedione (1) in the condensed phase. The $^1$H-NMR spectral data for 2,5-morpholinedione are compiled in Table 4. The mass spectrum of 2,5-morpholinedione showed significant fragment peaks at m/e = 115 (parent ion), 87, 71, 59, 42, 31, and 30.

VI.2.2 Synthesis of 6-methyl-2,5-morpholinedione (13)

Dry distillation of the sodium salt (14) of N-D,L-α-bromopropionylglycine

Dry distillation of the sodium salt (14) of N-D,L-α-bromopropionylglycine (Fig. 10) occurred in a conventional apparatus for distillation under reduced pressure (0.05 mm) prepared from standard semi-micro glassware. The stillhead, very short condensor and neck of the receiver were warmed during distillation. In a typical experiment 6.1 g sodium salt (0.062 mole) was subjected to dry distillation at 160-180$^0$C for about one hour. The crude, yellow-coloured 6-methyl-2,5-morpholinedione (13) was dissolved in ethyl acetate (30 ml). The solution was stirred for one hour in the presence of charcoal. After filtration of the charcoal, white 6-methyl-2,5-morpholinedione was recovered



(14) (13)

Figure 10: Schematical representation of the formation of 6-methyl-2,5-morpholinedione (13) from the sodium salt (14) of N-D, L-α-bromopropionylglycine

by solvent evaporation of ethyl acetate to dryness. The crude yield was 1.85 g (55%). The laborious recrystallization from a large excess of ether, however, resulted in a considerable loss. Yield: 0.67 g (20%); m.p. 96$^0$C; lit. 98$^0$C (68). IR- and NMR-spectroscopical data of 6-methyl-2,5-morpholinedione (13) are given in Tables 3 and 4, respectively.
The crude yield of 6-methyl-2,5-morpholinedione was not improved if the reaction was carried out in a sublimator.

VI.2.3 Other attempted synthetic routes to 2,5-morpholinedione and its derivatives

a. Treatment of 'α-halogenacyl-α-amino acids with silver oxide in dioxane (cf. 72)

The 'α-halogenacyl-α-amino acids used in these experiments included N-chloroacetylglycine (6), N-chloroacetyl-D,L-valine

(7), N-chloroacetyl-L-phenylalanine (8), N-D, L-α-bromopropionylglycine (10) and also the compound used by Rumsh e.a. (72), N-bromoacetyl-L-phenylalanine (11).
Typically the 'α-halogenacyl-α-amino acid (1 g) was dissolved in dioxane (50-100 ml). After the addition of $Ag_2O$ (excess of 50 mole-% (73)), the suspension of $Ag_2O$ in the dioxane solution was stirred for at least two days in the dark. After the reaction had been stopped, solid products were separated from the solution by filtration and the reaction product was isolated by solvent evaporation to dryness. No 2,5-morpholinediones, however, were obtained.

b. Lactonization of an 'α-halogenacyl-α-amino acid in sodium carbonate solution (cf. 69)

The lactonization in sodium carbonate solution was attempted for the following 'α-halogenacyl-α-amino acids: N-chloroacetylglycine (6), N-chloroacetyl-D,L-valine (7), N-chloroacetyl-L-phenylalanine (8), N-chloroacetyl-L-proline (9) and N-D,L-α-bromopropionylglycine (10). The 'α-halogenacyl-α-amino acid (2 g) was dissolved in 2 equivalents of an aqueous sodium carbonate solution (1 N) and shaked for about 10 minutes. Next the aqueous solution was extracted several times with ether (100 ml). The combined ether layers were dried over $MgSO_4$. Reaction products were isolated by solvent evaporation to dryness. The previous lactonization route which was described by Cook and Cox (69) for a number of N-alkylated 'α-bromoacyl-α-amino acids, did not afford 2,5-morpholinediones in case of the 'α-halogenacyl-α-amino acids used in these experiments.

c. Attempted ring-closure of N-chloroacetyl-L-phenylalanine (8) in the presence of N,N,N',N'-tetramethylguanidine (cf. 84)

The reaction was performed in dry glassware under a blanketing atmosphere of dry nitrogen. A solution of N-chloroacetyl-L-phenylalanine (8) (1 g, 4 mmole) in dry N,N-dimethylformamide (5 ml) and a solution of N,N,N',N'-tetramethylguanidine (0.6 ml, 4.8 mmole) in dry N,N-dimethylformamide (5 ml) were prepared. At regular intervals of 30 minutes 1 ml of both solutions were added to the reaction vessel which originally contained 20 ml of stirred dry N,N-dimethylformamide. After both solutions had been added, the reaction mixture was stirred for an additional 3 hours. No 2,5-morpholinedione formation could be detected on T.L.C. (silica gel, THF or chloroform, UV- or ninhydrin visualization)



VI.2.4 Polymerization

a. Attempted ring-opening polymerization of 2,5-morpholinedione (1) and 6-methyl-2,5-morpholinedione (13) in the melt

The attempted polymerization of 2,5-morpholinedione (1) and 6-methyl-2,5-morpholinedione (13) via a lactone type ring-opening polymerization in the melt was based on the procedures developed by Kulkarni e.a. (8, 85) and Sinclair and Gynn (86, 87) for the melt polymerization of the dilactones lactide or glycolide (see also other chapters of this thesis).
2,5-Morpholinedione or 6-methyl-2,5-morpholinedione (200-500 mg) was placed in a small polymerization tube (88) which had been dried at 130$^0$C for 24 hours, and the corresponding amount of initiator dissolved in a small quantity (0.1-0.2 ml) of dry benzene (tetraphenyltin) or dry toluene (stannous octoate) was added. The solvent was evaporated at a reduced pressure and the lower part of the polymerization tube was placed in an oil bath. While the polymerization tube was evacuated (0.05 mm), the temperature of the oil bath was raised gradually until the 2,5-morpholinedione (maximum temperature 160$^0$C) or its 6-methyl derivative (maximum temperature 125$^0$C) had sublimed onto the upper part of the polymerization tube which was cooled by air. During the sublimation step the tube was purged several times with dry nitrogen. By this procedure residual water and other contaminating solvents are removed (see also chapter IV of this thesis). Finally the polymerization tube was sealed under reduced pressure and placed in an oven with thermostatic control or in an oil bath for a certain period of time.

Thereafter the tube was cooled to room temperature and the content of the tube was dissolved in tetrahydrofuran (if possible). Aliquots of this solution were measured by gel permeation chromatography (cf. chapters IV and V of this thesis). The results of the attempted melt polymerization of 2,5-morpholinedione (1) and 6-methyl-2,5-morpholinedione (13) via a lactone type polymerization (Fig. 7, route a) are summarized in Table 5.

b. Attempted ring-opening polymerization of 6-methyl-2,5-morpholinedione (13) via a solution polymerization

The procedure for the attempted solution polymerizations of 6-methyl-2,5-morpholinedione (13), initiated with triisobutylaluminium was based on procedures described by Kleine and Kleine (89) and by Dittrich and Schulz (90) for the solution polymerization of lactide (see also chapter III of this thesis). The reaction was performed under a blanketing atmosphere of dry nitrogen. Typically 6-methyl-2,5-morpholinedione (0.5 g, $4x10^{-3}$ mole) was dissolved in dry toluene (5 ml, $C_{monomer}$ = 0.8 mole/l). After the addition of triisobutylaluminium ($5x10^{-3}$ g, M:I = 150 mole/mole), the reaction mixture was refluxed for 24 hours at $111^{0}$C. Reaction products were isolated by solvent evaporation to dryness and characterized by melting point, IR-spectroscopy and gel permeation chromatography. No polymer was formed and most of the monomer was recovered.

## VI.3 Results and discussion

### VI.3.1 Starting compounds for 2,5-morpholinedione formation

If we wish to prepare 2,5-morpholinedione or its derivatives via a ring-closure reaction of an 'α-halogenacyl-α-amino acid (route c or d of Fig. 9), a number of 'α-halogenacyl-α-amino acids is already commercially available. These compounds, however, are rather expensive. They can be prepared without much difficulties by acylation of an α-amino acid with an acyl halide, -preferably in a slight excess-, under Schotten-Bauman conditions (71). In the last step of this synthesis the 'α-halogenacyl-α-amino acid crystals are precipitated by acidification of the reaction mixture. Although it is recommended by a number of authors to acidify to pH 3-4 (cf. 79,82), better results are obtained if the reaction mixture is acidified to pH 2.



### VI.3.2 Synthesis of 2,5-morpholinedione and its derivatives

a. Synthesis of 2,5-morpholinedione (1) by dry distillation and sublimation of the sodium salt (5) of N-chloroacetylglycine

From the dry distillation and sublimation results summarized in Table 1 it was concluded that dry distillation of the sodium salt (5) of N-chloroacetylglycine (route c of Fig. 9) was not a suitable procedure for the preparation of 2,5-morpholinedione (1). Clearly better results were obtained when the sodium salt was heated under reduced pressure in a sublimator (Table 1, experiments 3 and 4). The amount of 2,5-morpholinedione which collected on the surface of the cold finger, however, was low in comparison with the theoretical amount.

Active copper (12)

Sporzyński e.a. (91), when attempting to prepare glycolide (cf. chapter III of this thesis) by dry distillation of the sodium salt of chloroacetic acid under reduced pressure at high temperatures, observed excessive charring of the sodium salt, whereas the yield of glycolide was very low. According to these authors this charring was most probably due to the very low thermal conductivity of sodium chloroacetate. When this sodium salt was mixed with copper turnings resulting in a considerable increase of the thermal conductivity of the mass to be heated, the yield of glycolide was always more than 70% of the theoretical amount. The quality of the copper turnings was not specified.
As the low yield of 2,5-morpholinedione (1) in the first experiments, in addition to the observed formation of a large

TABLE 1: Synthesis of 2,5-morpholinedione (1) via dry distillation or sublimation of the sodium salt (5) of N-chloroacetylglycine

| NO. | PROCEDURE | SODIUM SALT (mg) | ACTIVATED COPPER (mg) | SALT : Cu (g/g) | REACTION TEMP. ($^0$C) | PRESSURE(a) (mm) | REACTION(b) (hours) | CRUDE YIELD (mg) | CRUDE YIELD(c) (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | dry dist (d) | 2000 | - | - | 200-220 | 0.1 | 5 | (e) | - |
| 2 | dry dist | 3000 | - | - | 180-220 | 0.003 | 5 | (e) | - |
| 3 | subl | 2440 | - | - | 200-220 | 0.002 | 4 | 154 | 9.5 |
| 4 | subl | 490 | - | - | 200-220 | 0.003 | 4 | 90 | 28 |
| 5 | subl | 900 | 6000 | 1:6$^5$ | 170-210 | 0.004 | 4 | 176 | 30 |
| 6 | subl | 650 | 5500 | 1:8$^5$ | 170-210 | 0.003 | 4 | 227 | 53 |
| 7 | subl | 2100 | 22000 | 1:10 | 170-210 | 0.001 | 4 | 522 | 37 |
| 8 | subl | 1100 | 12000 | 1:11 | 170-210 | 0.003 | 4 | 299 | 41 |
| 9 | subl | 1200 | 14000 | 1:12 | 160-200 | 0.003 | 5 | 302 | 38 |
| 10 | subl | 2000 | 24000 | 1:12 | 170-210 | 0.003 | 4 | 472 | 37 |
| 11 | subl (f) | 1400 | 11000 | 1:8 | 170-210 | 0.002 | 4$^5$ | 496 | 53 |
| 12 | subl (f) | 3200 | 12200 | 1:4 | 170-210 | 0.003 | 4$^5$ | (g) | - |
| 13 | subl | 1100 | 14000 | 1:12 | 170 | 0.004 | 6 | 272 | 37 |
| 14 | subl | 1100 | 11000 | 1:10 | 170 | 0.003 | 5 | 161 | 22 |
| 15 | subl (h) | 3000 | - | - | 190-220 | 0.002 | 4 | 520(i) | 26 |
| 16 | subl (h) | 8000 | 53000 | 1:6$^5$ | 160-190 | 0.0005 | 4$^5$ | 767 | 14.5 |
| | | continued: | | | 170-210 | 0.0005 | 5 | 521(i) | 10 |

a: Maximum pressure; b: Corresponding to the reported reaction temperatures, not including warming-up time; c: Percentage of theoretical yield; d: This reaction was performed twice, giving identical results; e: Small amount of crude 2,5-morpholinedione in stillhead; f: Sodium salt was treated with ice-cold, dry acetone prior to use;g: A waxy product with an acid smell was obtained in stead of 2,5-morpholinedione; h: Sublimator with large contact surface; i: 2,5-Morpholinedione was contaminated with a considerable amount of a waxy, acid smelling product.



amount of tar-like products, could also have been the result of a low heat conductivity of the sodium salt (5) of N-chloroacetylglycine, it was decided to investigate the thermal decomposition of this salt in the presence of active copper. From the results of the sublimation experiments 3-14 of Table 1 it can be concluded that the addition of the active copper resulted in a decrease of the reaction temperature range and a considerable increase of the yield of 2,5-morpholinedione. Presumably the lower reaction temperature range had diminished the tendency for side reactions, i.e. charring, resulting in a higher yield of the desired reaction product. On the other hand, a decrease of the reaction temperature range also indicates a catalytic effect of the added active copper upon the ring-closure reaction. The data of the sodium salt - active copper ratios might suggest that the best results are obtained if this ratio is approximately 1:8. The number of experiments, however, is too small to substantiate this suggestion. The experiments do suggest, however, that a certain minimum amount of copper (12) has to be added.

Attempts to improve the yield

Dale e.a. (92) who prepared di- and higher cyclic esters of glycolide by the sublimation of sodium chloroacetate powder, purified the sodium salt by treating it with acetone. Prior to the experiments 11 and 12 of Table 1 a small column of the sodium salt (5) of N-chloroacetylglycine was treated with dry, ice-cold acetone and dried. This procedure did not improve the yield. In the sublimation experiments the temperature of the salt-copper mixture was always raised rather quickly to 170$^0$C. Thereafter in most experiments the temperature was raised very slowly, in a stepwise manner, to 210$^0$C. This latter temperature rise was attended with an increasing tendency for charring. In the experiments 13 and 14 of Table 1 the reaction temperature was kept at 170$^0$C in order to oppress this tendency and hopefully increase the yield of 2,5-morpholinedione (1). Although the charring in these experiments was indeed less excessive than in the others, the yield was not increased. This

might imply that the ring-closure reaction at 170$^0$C occurred so slowly that the concurrent charring was still predominant. The sublimator used in most of the experiments could only be loaded with relatively small portions of the sodium salt - copper mixture. In two experiments larger portions of the sodium salt of N-chloroacetylglycine were sublimed. For this purpose a special sublimator was used which afforded a greater contact surface between the salt and the heating medium. In spite of the increased contact surface the yield of 2,5-morpholinedione was rather low when the pure sodium salt was sublimed (experiment 15 of Table 1). The sublimation of the largest amount of the sodium salt (5), in the presence of copper (12) (experiment 16 of Table 1), resulted in a yield which was only 14.5% of the theoretical amount. After the first reaction product was removed from the "cold finger", the sublimation reaction was continued for another 5 hours at a slightly higher temperature range. The 2,5-morpholinedione (1), however, which collected at the surface of the "cold finger" was strongly contaminated with a waxy, acid smelling product.

Extraction

According to Chadwick and Pascu (68), only a small amount of 6-methyl-2,5-morpholinedione (13) distilled upon the dry distillation (150$^0$C; 1.5 mm) of the sodium salt (14) of N-D,L-α-bromopropionylglycine, whereas the remainder of the reaction products sintered down to a hard solid lump. By the extraction of this finely ground substance using solvents such as alcohol, acetone or chloroform, about 40% of the theoretical amount of 6-methyl-2,5-morpholinedione was isolated. The attempted extractions of the finely ground substances which stayed behind after the sublimation reactions of the sodium salt of N-chloroacetylglycine, mostly in the presence of active copper, never yielded any 2,5-morpholinedione. This might have been due to the very low solubility of 2,5-morpholinedione in almost all common organic solvents or it might not have been present at all in the remaining tar-like sublimation products.



Concluding remark

The results of all our experiments indicate that the synthetic route to unsubstituted 2,5-morpholinedione (1) via the sublimation of the sodium salt (5) of N-chloroacetylglycine is only useful if small amounts of the sodium salt are sublimed in combination with a considerable amount of active copper (12).

b. Synthesis of 6-methyl-2,5-morpholinedione (13) by dry distillation of the sodium salt (14) of N-D,L-α-bromopropionylglycine

In comparison with the synthesis of non-substituted 2,5-morpholinedione (1) via dry distillation of the sodium salt (5) of N-chloroacetylglycine, the synthesis of 6-methyl-2,5-morpholinedione (13) via dry distillation of the sodium salt (14) of N-D,L-α-bromopropionylglycine (Fig.10) was clearly more successful. As was mentioned before, Chadwick and Pascu (68) reported that upon dry distillation of the sodium salt of N-D,L-α-bromopropionylglycine, a substantial amount of 6-methyl-2,5-morpholinedione was obtained by the extraction of the finely ground residue. In our hand solvent extraction of the residue using various organic solvents yielded no products. Contrary to the observation done by the same authors, in our hands no 6-methyl-2,5-morpholinedione (13) was formed spontaneously upon storage of the sodium salt (14) of N-D,L-α-bromopropionylglycine at room temperature.

c. Other attempted synthetic routes to 2,5-morpholinedione and its derivatives

Treatment of 'α-halogenacyl-α-amino acids with silver oxide in dioxane

In spite of the results claimed by Rumsh e.a. (72), in our hands the treatment of various 'α-halogenacyl-α-amino acids (including N-bromoacetyl-L-phenylalanine (11) which was used by Rumsh himself) with $Ag_2O$ in dioxane (route d of Fig. 9) did

not produce any 2,5-morpholinedione (1) or its derivatives. Instead, the solid silver salts were obtained.

Lactonization in sodium carbonate solution

The attempted lactonization (route a of Fig. 9) in aqeous sodium carbonate solution described by Cook and Cox (69) for a number of N-alkylated α-bromoacyl-α-amino acids did not afford 2,5-morpholinediones if N-chloroacetylglycine (6), N-chloroacetyl-D,L-valine (7), N-D,L-α-bromopropionylglycine (10), N-chloroacetyl-L-phenylalanine (8) or N-chloroacetyl-L-proline (9) were used. It should be mentioned in this respect that Abderhalden and Haase (93) reported that the rate of removal of the bromine ion in the N-bromoacetyl-L-proline ion in diluted alkali solution was much higher than in a great number of other N-bromoacetyl-α-amino acid ions under identical conditions. In spite of our own results, in our opinion this might have been the result of a fast internal bimolecular ring-forming reaction between the carboxylate group and the carbon-bromine group for N-bromoacetyl-L-proline (cf. 68).

Attempted ring-closure in the presence of N,N,N',N'-tetramethylguanidine (cf. 84).

A few ring-closure reactions of N-chloroacetyl-L-phenylalanine (8) were attempted in the strong dipolar solvent N,N-dimethylformamide. In this case, however, the use of alkali-metal salts gives rise to suspensions which are, generally, less suitable for this type of reactions. Therefore the strong base N,N,N',N'-tetramethylguanidine was used in DMF, the generated salts being generally more soluble than the corresponding alkali-metal salts in strong dipolar organic solvents. No 2,5-morpholinedione formation, however, could be detected on thin layer chromatographic examination.

d. Recrystallization of 2,5-morpholinedione (1)

Substituted 2,5-morpholinediones have been recrystallized from organic solvents such as chloroform, ether or light petroleum-ether (69, 70). Unsubstituted 2,5-morpholinedione (1) appeared



to be very soluble only in water and could not be recrystallized from these solvents. After many other organic solvents had been tried, only methanol turned out to be a moderately effective recrystallization solvent if it was used in a large excess. The great differences in the recrystallization results summarized in Table 2 can not easily be explained.

TABLE 2: Recrystallization of 2,5-morpholinedione (1) from methanol[a]

| NO. | QUANTITY (mg) | VOLUME OF METHANOL (ml) | YIELD (mg) | PERCENTAGE OF THEORETICAL AMOUNT | MELTING POINT ($^0$C) |
|---|---|---|---|---|---|
| 1 | 496 | 17 | 247 | 50 | 191-193 |
| 2 | 782[b] | 22 | 38 | 5 | 189-192 |
| 3 | 557 | 16 | 262 | 47 | 191-192 |
| 4 | 440[c] | 10 | failure | - | - |
| 5 | 450[c] | 10 | failure | - | - |
| 6 | 710 | 18 | failure[d] | - | - |

a: Contained less than 0.05% water; b: No water-bath with thermostatic control was used, erlenmeyer containing crude 2,5-morpholinedione and dry methanol was heated directly on a hot plate; c: Crude 2,5-morpholinedione was contaminated with a waxy, acid smelling product; d: Upon cooling of the filtrate a small quantity of crystals was formed which dissolved again.

With the exception of recrystallization no. 2, all recrystallizations were performed under exactly the same conditions. The crude 2,5-morpholinedione (1) samples used in the recrystallizations 4 and 5 were strongly contaminated. On the other hand, recrystallization no. 6 involving crude 2,5-morpholinedione which was not strongly contaminated, was also a failure. If no crystals were formed upon cooling of the solutions of crude 2,5-

morpholinedione in methanol (experiments 4-6), it was always attempted, however in vain, to recover the crude product by evaporation of the methanol at a low temperature and pressure. Unfortunately, the residues which remained behind after solvent evaporation, could never be characterized properly. Likewise, when the mother liquors obtained in the recrystallizations 1-3 were carefully evaporated, in stead of the crude 2,5-morpholinedione a product of an unknown composition was recovered. The instability of 2,5-morpholinedione (1) observed during the attempted recrystallizations may be explained as follows. Although the methanol used was very dry, acid- or base catalyzed hydrolysis of the lactone part of 2,5-morpholinedione can not be ruled out, followed by an esterification of the formed N-hydroxyacetylglycine in excess methanol if traces of acid were present. Apart from hydrolysis, acid- of base catalyzed transesterification (methanolysis) of the lactone part of 2,5-morpholinedione in excess methanol at higher temperatures might also have occurred during the attempted recrystallizations (94). On the other hand, the $^1$H-NMR spectrum taken from a 9 days old solution of 2,5-morpholinedione (1) in $D_2O$ containing 0.1% of $H_2O$, did not differ from that taken from a freshly prepared solution.



TABLE 3: Characteristic infrared absorption frequencies of 2,5-morpholinedione (1), 6-methyl-2,5-morpholinedione (13) and related compounds

| COMPOUND[a] | BAND POSITION $cm^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | N-H stretching vibration | AMIDE I | AMIDE II trans | AMIDE V trans | C=O carboxylic acid | C=O lactone |
| N-chloroacetylglycine (6) | 3320 | 1645 | 1550 | 690 | 1705 | - |
| 2,5-morpholinedione (1) | 3205 | 1690 | - | - | - | 1755 |
| N-D,L-α-bromopropionylglycine (10) | 3320 | 1625 | 1550 | 690 | 1755 | - |
| 6-methyl-2,5-morpholinedione (13) | 3210 (3270)[b] | 1695 (1700)[b] | - (-) | - (-) | - (-) | 1765 (1760)[b] |
| acyclic precursor esters (17)[c] | (d) | 1650-1660 | 1540-1550 | 690-700 | 1740[e] 1750 | - |
| substituted 2,5-morpholinediones (15)[c] | (d) | (d) | - | - | - | (d) |
| 3,6-dimethyl-2,5-morpholinedione (19)[f] | 3215 3405 | 1702 | - | - | - | 1760 |
| 3-isopropyl-6-methyl-2,5-morpholinedione (20)[f] | 3215 3405 | 1692 | - | - | - | 1758 |
| acyclic dipeptide precursors (18) | ~3300 (trans) | ~1650[g] (trans) | 1540-1570 | 690-720 | (h) | (h) |
| 2,5-piperazinediones (16) | 3180-3190 (cis) | 1670-1690 (cis) | - | - | (h) | (h) |

a: In the condensed phase unless stated otherwise; b: Recorded by Kaźmierczak and Kupryszewski (70) by the Nujol suspension thin film technique; c: Recorded by Tul'chinskii e.a. (75) with KBr

or paraffin oil in the 2000-600 $cm^{-1}$ region; d: Relevant band position not reported; e: Carboxylic acid ester; f: Recorded by Nissen e.a. (16) in chloroform (0.1%); g: Or lower; h: Not present.

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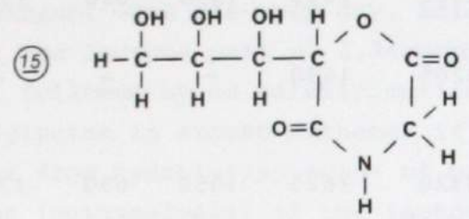


Fig. 11. Example of a derivative of 2,5-morpholinedione (15) studied by Tul'chinskii et al. (75).

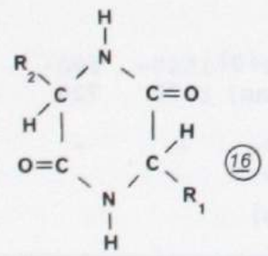


Fig. 12. General formula of 2,5-piperazinediones (16).



e. Characterization of 2,5-morpholinedione (1) and 6-methyl-morpholinedione (13)

Infrared spectroscopic data (condensed phase)

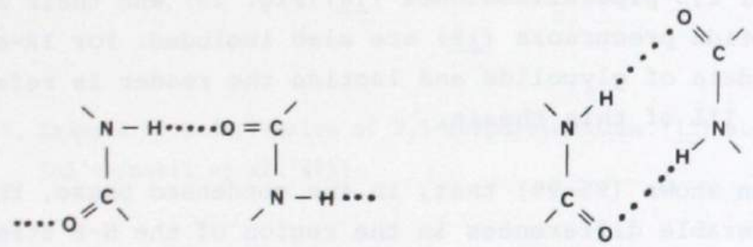
In table 3 characteristic infrared absorption frequencies of 2,5-morpholinedione (1) and of the starting compound N-chloroacetylglycine (6) are presented, together with data on 6-methyl-2,5-morpholinedione (13) and its starting compound N-D, L-α-bromopropionylglycine (10). Tul'chinskii e.a. (75) have reported on an IR-study of several substituted 2,5-morpholinediones (15), in the region 2000-600 $cm^{-1}$. An example is given in Fig. 11. These spectra were compared with those of their acyclic precursors (17). The results are also presented in Table 3, together with data reported by Nissen e.a. (16) on IR-spectra of some substituted 2,5-morpholinediones (19,20) dissolved in chloroform. For comparison, characteristic infrared absorption frequencies of 2,5-piperazinediones (16)(Fig. 12) and their acyclic dipeptide precursors (18) are also included. For IR-spectroscopic data of glycolide and lactide the reader is referred to chapter III of this thesis.

It has been shown (95-99) that, in the condensed phase, there are considerable differences in the region of the N-H stretching frequencies for the amide bonds of 2,5-piperazinedione (16) and its derivatives, in comparison with their acyclic dipeptide precursors (18):

N-H $stretch_{cis}$ = 3180-3195 $cm^{-1}$, N-H $stretch_{trans}$ = ~3300 $cm^{-1}$; in that of the amide II vibrations (N-H plane deformation vibrations): amide $II_{cis}$ = 1440-1455 $cm^{-1}$, amide $II_{trans}$ = 1540-1570 $cm^{-1}$; and in that of the amide V vibrations (N-H non-planar vibrations): amide $V_{cis}$ = 760-850 $cm^{-1}$, amide $V_{trans}$ = 690-720 $cm^{-1}$.

The CONH-groups in 2,5-piperazinedione (16) are in the cis-

configuration, whereas in most acyclic amides the trans-configuration is predominant. Only in sterically hindered amide compounds such as N-tert.-butylphenylacetamide, the cis-configuration is present to the extent of approximately 70 percent. The shift of the N-H stretching vibration, in the condensed phase, has been associated with the N-H stretching mode in hydrogen bonding. It has been suggested that the 3300 $cm^{-1}$ absorption is due to amide bonds which are hydrogen bonded in the trans form (Fig. 13a), whereas the 3180-3195 $cm^{-1}$ absorption arises from the cis-, cyclic dimer, arrangement (Fig. 13b). These data provide an easy tool to distinguish between 2,5-piperazinediones (16) and their acyclic dipeptide precursors (18).



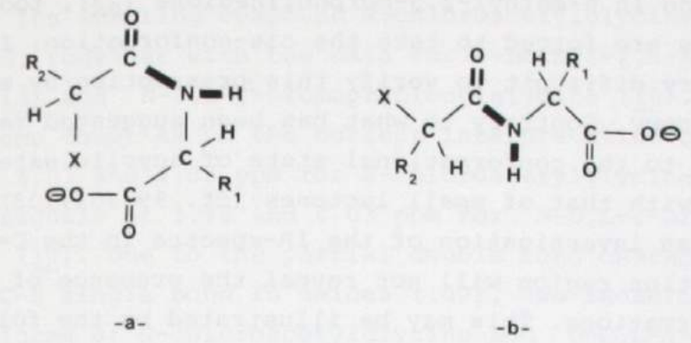
-a- -b-

Figure 13: Hydrogen bonding in (a) trans- and (b) cis-amide bonds

The data presented in Table 3 indicate that for the vibration of the CONH-groups their exists a close similarity between the 2,5-morpholinediones (1,13) and their acyclic precursors (6,10) on the one side, and the 2,5-piperazinediones (16) and their acyclic precursors (18) on the other side. This most probably implies that, in order to form a 2,5-morpholinedione, the 'α-halogenacyl-α-amino acid has to adopt a folded conformation (Fig. 14a) in which the amide bond is in the cis-conformation,



rather than the more stable, extended form (Fig. 14b) in which the amide bond is in the favoured trans-conformation (cf. 97).



-a- -b-

Figure 14: Folded (a) and extended (b) form of 'α-halogenacyl-α-amino acids

When an amide both exists in the cis- and trans-conformation, the various amide vibrations show twin bands in the IR-spectrum. In principle it is possible to estimate the relative proportions of the cis- and trans-rotational isomers by comparison of the relative intensities of the twin bands. However, as the various cis-amide bands in the spectra of N-chloroacetylglycine (6) and N-D,L-α-bromopropionylglycine (10), respectively, were in part overlapped by other bands, we were not able to estimate the percentages cis- and trans-conformation of the amide groups of these 'α-halogenacyl-α-amino acids.

The N-H stretching vibrations had shifted from 3320 $cm^{-1}$ for the two 'α-halogenacyl-α-amino acids (6,10) to approximately 3205 $cm^{-1}$ for the 2,5-morpholinediones (1,13). This indicates that, in the condensed phase, in 2,5-morpholinedione, too, the amide groups are hydrogen bonded in a cis-, cyclic dimer, arrangement (compare Fig. 13a).

Till so far the conformational state of the ester (lactone) group (C=O stretching vibration at 1755 $cm^{-1}$) of 2,5-morpholinedione (1) and 6-methyl-2,5-morpholinedione (13) has not been discussed. In acyclic compounds the ester group has a strong

trans-preference (100). Zhuklistova e.a. (76) have shown by X-ray structural analysis that in D,D-3,6-diisopropyl-2,5-morpholinedione both the amide and ester group are in the cis-conformation. It is therefore very likely that in 2,5-morpholinedione (1) and in 6-methyl-2,5-morpholinedione (13), too, the ester groups are forced to take the cis-conformation. It is, however, very difficult to verify this presumption by means of IR-spectroscopy. Contrary to what has been suggested regularly in relation to the conformational state of acyclic esters in comparison with that of small lactones (cf. 99,101,102), we believe that an investigation of the IR-spectra in the C=O stretching vibration region will not reveal the presence of cis- or trans-conformations. This may be illustrated by the following example. An X-ray structure elucidation of the 6-membered dilactone D,L-lactide (103), the results of which are presented in chapter II of this thesis, showed that both ester groups had been forced to take the cis-configuration. After ring-opening polymerization of D,L-lactide, the ester groups in the resulting acyclic poly(D,L-lactic acid) take the favoured trans-conformation (cf. 104). A comparison of the IR-spectra of D,L-lactide and poly(D,L-lactic acid), in the condensed phase, shows that the C=O stretching vibrations appear almost in the same position, at 1755 $cm^{-1}$ and at 1750 $cm^{-1}$, respectively. Similar observations were done by Ōki and Nakanishi (105) in relation to small and large lactone rings. In small lactone rings the ester groups have the cis-conformation, whereas in large lactones the trans-form is the preferred conformation. This difference, however, could not be shown by differences of the C=O stretching vibrations. In principle the cis- or trans-conformational state of the ester group in a 2,5-morpholinedione could be determined by an investigation in the region 1300-1000 $cm^{-1}$ of the $C_{c=o}$-$O_{ether}$ skeletal deformation vibrations which are known to be sensitive to conformational changes (100,105). However, this C-O band could not be assigned properly in the IR-spectrum of 2,5-morpholinedione (1) or 6-methyl-2,5-morpholinedione (13) because it appeared in a region of



the spectrum where several other strong bands appeared.

$^1$H-NMR spectral data

In Table 4 the $^1$H-NMR spectral data for 2,5-morpholinedione (1) and its starting compound N-chloroacetylglycine (6) are compiled, together with the data for 6-methyl-2,5-morpholinedione (13) and N-D,L-α-bromopropionylglycine (10). There may exist some doubt as to the correct interpretation of the signals at 4.03 and 4.08 ppm for N-chloroacetylglycine (6), and of the signals at 3.96 and 4.03 ppm for N-D,L-α-bromopropionylglycine (10). Due to the partial double bond character of the formal C-N single bond in amides (100), two isomeric (cis- and trans-)forms of N-chloroacetylglycine and N-D,L-α-bromopropionylglycine can theoretically be distinguished (Fig. 15). The slightly different magnetic environment for the two protons "b" might be reflected by two different signals, -i.e. at 4.03 and 4.08 ppm, and at 3.96 and 4.03 ppm, respectively. Nevertheless the signals were interpreted as a doublet (4.05 ppm, J = 5.8 Hz and 4.00 ppm, J = 5.6 Hz, respectively), resulting from a coupling between the two protons "b" and proton "c". Arguments

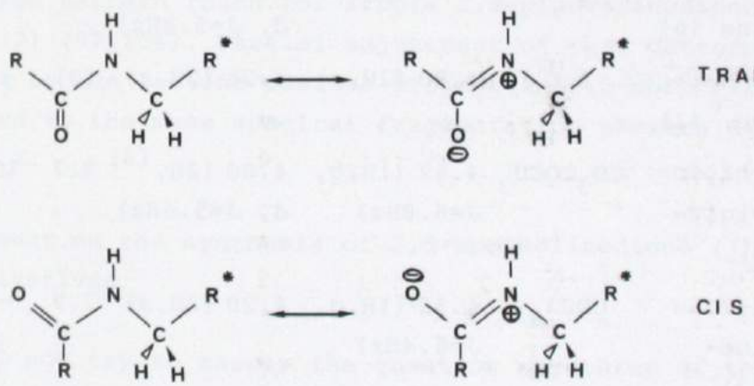
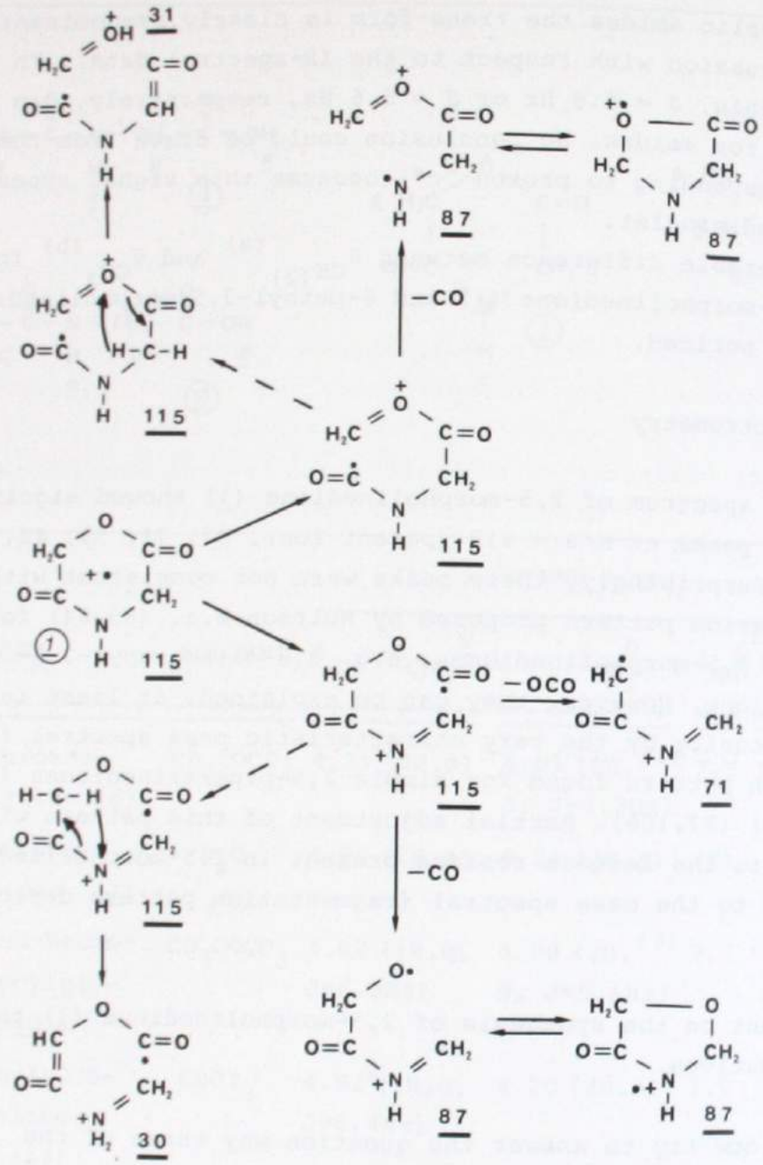



Figure 15: (Cis- and trans-) isomeric forms of N-chloroacetylglycine and N-D,L-α-bromopropionylglycine




Scheme 1: Mass spectral fragmentation pattern for 2,5-morpholinedione (1)



distillation.

Trans - cis energy barrier

According to Thamm (67), cyclization of a linear didepsipeptide through the formation of an amide bond is preferred to the formation of an ester bond. Reasons for this preference, however, were not given. It has been shown (see characterization study above) that in most acyclic amides the trans-conformation of the CONH-group is (by far) predominant. The barrier to rotation for the C-N bond is about 90 kJ/mole (100). The conformational situation in acyclic esters very much resembles that for the N-monosubstituted amides, with a strong trans-preference. The energy barrier, however, is only 42 kJ/mole (100), that is, half the barrier of related amides.
In 2,5-morpholinediones both the amide and the ester bond are in the cis-conformation. If we assume that, in order to form 2,5-morpholinediones via a cyclization reaction, the activation energy for the formation of an amide bond does not differ very much from that for the formation of an ester bond. Then the formation of the 2,5-morpholinediones is largely determined by the easiness of the trans-cis isomerization of the "central unit" of the precursor, -i.e. the amide bond in case of an 'α-halogenacyl-α-amino acid or the ester bond in case of an 'α-aminoacetyl-α-hydroxyacetic acid. The high trans-cis energy barrier of the amide "central unit" may explain that we were not able to lactonize several 'α-chloro- and bromoacyl-α-amino acids,- neither in sodium carbonate solution or a strong dipolar solvent, nor by the treatment with $Ag_2O$. However, it was rather surprising that we did not succeed in the lactonization of N-chloroacetyl-L-proline in aqueous sodium carbonate solution, because this compound is already forced to adopt the folded cis-amide conformation by the presence of the pyrrolidine ring. The possibility that the 2,5-morpholinedione ring had actually been formed but was immediately opened by a consecutive hydrolysis reaction can not be excluded.

N-alkylation

It has been pointed out (107-110) that, due to the similarity between the $CH_3$ - group and $CH_2$ - group, the strong trans preference for the amide group disappears in linear dipeptides derived from N-methylglycine, -i.e. the folded cis-amide conformation required for cyclic dipeptide formation is obtained much more easily in N-$CH_3$ amides than in N-H amides. Moreover, hydrogen bonding is excluded. Therefore there are good reasons to assume that, upon N-alkylation, the trans-preference of the amide group in 'α-halogenacyl-α-amino acids also disappears. This, perhaps, explains the easy lactonization of several N-alkylated 'α-bromoacyl-α-amino acids in aquous sodium carbonate solution reported by Cook and Cox (69), although these investigators particularly emphasize the effects of alkyl groups attached to the carbon atom of the α-amino acid part of the molecule and to the carbon atom carrying the bromine atom.

High sublimation temperature

High temperatures had to be applied during the sublimation and dry distillation of the sodium salts of N-chloroacetylglycine or N-D,L-α-bromopropionylglycine. As values for the activation energy of the transition state preceding the formation of a 2,5-morpholinedione from an 'α-halogenacyl-α-amino acid were not estimated, we do not know for sure whether such high reaction temperatures were inevitable in order to afford the trans-cis isomerization of the amide bond. The low yield of desired reaction product may be explained in part by the rather excessive charring in the course of the sublimation or dry distillation reaction. Moreover, several concurrent side reactions might have taken place, e.g. the formation of oxazolines, α-lactams, 2,6-morpholinediones, or other oxygen and nitrogen containing heterocycles.

It was shown that the addition of active copper to the sodium salt of N-chloroacetylglycine, initially intended to increase the thermal conductivity of the salt, resulted in a significantly lower reaction temperature range. Therefore a catalytic



effect upon the ring-closure reaction, in addition to an increased thermal conductivity, is suggested. No attempts were made to clarify the mechanism of this catalytic effect experimentally. We do not preclude the possibility that a complex between the active copper and the nitrogen of the amide group was formed which promoted the folded cis-amide conformation of N-chloroacetylglycine by diminishing the involvement of the nitrogen's pair of unshared electrons in a C-N "double bond".

### VI.3.3 Attempted polymerization of 2,5-morpholinedione (1) and 6-methyl-2,5-morpholinedione (13)

a. Melt polymerization

The results of the attempted polymerization of 2,5-morpholinedione (1) and 6-methyl-2,5-morpholinedione (13) in the melt via a lactone type polymerization (Fig. 7, route a) are summarized in Table 5.

All three attempts to polymerize 2,5-morpholinedione (1) using various concentrations of tetraphenyltin, an effective initiator for the ring-opening polymerization of lactones (cf. chapter IV of this thesis), were failures. Rather soon after the attempted polymerization was started, a considerable discoloration of the reaction product was always observed. Eventually tar-like products were obtained. The third polymerization was discontinued after three hours. By that time, however, an excessive charring had already occurred. The high reaction temperature was inevitable because of the high melting point of 2,5-morpholinedione (1), 191-193$^0$C.

6-Methyl-2,5-morpholinedione (13) melts at a considerably lower temperature, 96$^0$C. Therefore the polymerization of 6-methyl-2,5-morpholinedione could be tried under much milder conditions. In the fourth polymerization attempt of Table 5 tetraphenyltin was used as the initiator. An oligomer, $M_n$ = 300, was obtained in combination with a large amount of unreacted monomer. If tetraphenyltin is used as an initiator for the polymerization of lactones, e.g. D, L-lactide (m.p. 126$^0$C), in the melt, the reaction temperature is usually 170$^0$C or somewhat higher.

TABLE 5: Attempted melt polymerization of 2,5-morpholinedione (1) and 6-methyl-2,5-morpholinedione (13) via a lactone type polymerization (Fig. 7, route a)

| MONOMER | QUANTITY (mg) | INITIATOR | I : M mole/mole | REACTION TEMP. ($^0$C) | REACTION TIME (hours) | RESULT |
|---|---|---|---|---|---|---|
| 2,5-morpholinedione | 300 | tetraphenyltin | 1:3750 | 195 | 15 | failure (a) |
| 2,5-morpholinedione | 150 | tetraphenyltin | 1:5000 | 200 | 15 | failure (a) |
| 2,5-morpholinedione | 200 | tetraphenyltin | 1:5000 | 195 | 3 | failure (a) |
| 6-methyl-2,5-morpholinedione | 250 | tetraphenyltin | 1:15000 | 130 | 24 | oligomer (b) $\bar{M}_n$=300 |
| 6-methyl-2,5-morpholinedione | 250 | tetraphenyltin | 1:15000 | 170 | 24 | failure (a) |
| 6-methyl-2,5-morpholinedione | 250 | stannous octoate | 1:15000 | 130 | 68 | (c) |
| 6-methyl-2,5-morpholinedione | 250 | stannous octoate | 1:15000 | 150 | 49 | (c) |
| 6-methyl-2,5-morpholinedione | 150 | (d) | - | 150 | 73 | oligomer (b) $\bar{M}_n$=300 |

a: Formation of tar-like products

b: In combination with a large amount of unreacted monomer

c: Reaction product was insoluble in tetrahydrofuran and could therefore not be characterized by gel permeation chromatography; trans-amide band at 1540 $cm^{-1}$ in IR-spectrum; slight discoloration of reaction product obtained at 150$^0$C

d: No initiator added



The fifth attempted melt polymerization of 6-methyl-2,5-morpholinedione, however, failed under such temperature conditions because of excessive charring.
Another effective initiator for the ring-opening polymerization of lactones in the melt which is usually used at lower reaction temperatures is stannous octoate (cf. Ch. IV and V of this thesis). Rather long polymerization times are required to obtain high molecular weight poly(lactones). Stannous octoate was used in the polymerization experiments number six and seven. No charring occurred, albeit that at 150$^0$C a slight discoloration was observed. Glassy, "polymeric-like" products were obtained, which, unfortunately, could not be characterized by gel permeation chromatography because they could not be dissolved in tetrahydrofuran. A distinct band at 1540 $cm^{-1}$ corresponding to the trans-amide II vibration was observed in the IR-spectra of the reaction products indicating the formation of a linear compound. For comparison in the last polymerization experiment (no. 8) no stannous octoate was added. An oligomer in combination with a large amount of unreacted monomer was obtained.

b. Solution polymerization

No polymer was formed upon solution polymerization of 6-methyl-2,5-morpholinedione (13) initiated with triisobutylaluminium. This initiator was chosen because it had proven to be an effective initiator for the solution polymerization of D,L-lactide under the conditions reported here for the solution polymerization of 6-methyl-2,5-morpholinedione (see also chapter III of this thesis).

c. Comment on the polymerization studies

As only small amounts of 2,5-morpholinedione (1) and of 6-methyl-2,5-morpholinedione (13) could be synthesized, the polymerizability of these potential new heterocyclic monomers, utilizing some effective initiators for lactones, could not be investigated systematically and therefore the results of this

polymerization study are not yet conclusive. Moreover, no attempts could be made to polymerize the 2,5-morpholinediones by techniques commonly used for lactam polymerizations. Nevertheless, the results obtained when stannous octoate was used as the initiator, are intriguing. This initiator might prove to be a suitable initiator for the ring-opening polymerization of 2,5-morpholinediones.

VI.4 In conclusion

1. Because of their specific molecular composition it is believed that linear, alternating polydepsipeptides will be a valuable supplement to the existing synthetic biodegradable polymers.

2. The synthetic routes applied hitherto which are based on multi-step synthetic routes to sequential polypeptides are considered to be only suitable for the preparation of small quantities of polymer.

3. For the preparation of larger amounts of linear, alternating polydepsipeptides, a new synthetic route is suggested which involves the lactone or lactam type ring-opening polymerization of 2,5-morpholinedione or its derivatives.

4. No systematic study of the synthesis of 2,5-morpholinedione and its derivatives has been reported in the literature.

5. In the present study it was found that 2,5-morpholinediones could only be prepared with considerable difficulties via a cyclization reaction of an $'\alpha$-halogenacyl-$\alpha$-amino acid.

6. Small quantities of unsubstituted 2,5-morpholinedione could be prepared via the sublimation of the sodium salt of N-chloroacetylglycine in the presence of active copper. It is presumed that copper increases the thermal conductivity of the mass to be heated and therefore diminishes charring of



the sodium salt. In addition to this, the active copper may have a catalytic effect upon the ring-closure reaction.

7. 6-Methyl-2,5-morpholinedione could be prepared somewhat easier via dry distillation of the sodium salt of N-D,L-$\alpha$-bromopropionylglycine.

8. Unsubstituted 2,5-morpholinedione appears to be very soluble in water. Dry methanol used in a large excess was found to be an acceptable recrystallization solvent.

9. The molecular structure of 2,5-morpholinedione, 6-methyl-2,5-morpholinedione and their precursors could be verified by IR-, $^1$H-NMR- and mass spectroscopy. Based on these results and theoretical considerations it is expected that the cyclization of an $'\alpha$-halogenacyl-$\alpha$-amino acid is impeded by the high trans-cis energy barrier for the (non-alkylated) amide bond.

10. As only small quantities of 2,5-morpholinedione and 6-methyl-2,5-morpholinedione could be synthesized, the polymerizability could not yet be investigated systematically and was restricted to a number of lactone type polymerization attempts.

11. The attempted melt polymerization of unsubstituted 2,5-morpholinedione initiated with tetraphenyltin only resulted in excessive charring probably due to the high reaction temperature which was necessary because of the high melting point of 2,5-morpholinedione (191-193$^0$C).

12. As 6-methyl-2,5-morpholinedione melts at a much lower temperature, -96$^0$C-, melt polymerizations could be carried out under milder conditions. If stannous octoate was used as the initiator, glassy, "polymeric-like" products were obtained which, unfortunately, could not be characterized properly by g.p.c.. IR-spectroscopic analysis indicated the formation of

a linear product. If no initiator was added, an oligomer in combination with a large amount of unreacted monomer was obtained.

## Postcript

After completion of the experimental work which led to this chapter, recently the following Chemical Abstract (98: 143823 e) was revealed: Hwang, S.S., Hong, S.I., and Choi, N.S.: Synthesis and characterization of morpholine-2,5-dione, Hanguk Sumyu Konghakhoe Chi, 18 (4), 200-206 (1981) (Eng.). "The title compound (I) was prepared by acylating glycine by $BrCH_2COBr$ and cyclizing the resulting $BrCH_2CONHCH_2CO_2H$ in 1N KOH at room temperature. (I) was characterized by its IR and NMR spectrum. (I) is a cyclic depsipeptide and can be used as a monomer for polydepsipeptides". As we were not able to get access to the full paper before completion of this chapter, no more experimental details were available. It should be mentioned, however, that our own attempts to prepare 2,5-morpholinediones according to the above mentioned procedure were not successful.

## Acknowledgement

We would like to thank Mr. T. Otten for his contribution to the synthesis and attempted polymerization of 6-methyl-2,5-morpholinedione.



## REFERENCES

1 J. Feijen, W.L. Sederel, T. Beugeling, and A. Bantjes, Synthetic polypeptides as biomaterials, *Proc. ESAO*, Vol. I, 37-41 (1974).

2 W.L. Sederel, The potential of copoly (α-amino acids) based on L-aspartic acid and its esters as biomaterials, *Doctors Thesis*, Twente University of Technology, Enschede, The Netherlands, 1977.

3 W.L. Sederel, A. Bantjes, and J. Feijen, A route to anionic hydrophilic films of copolymers of L-leucine, L-aspartic acid and L-aspartic acid esters, *Polymer*, 16, 735-738 (1975).

4 K.W. Marck, Ch.R.H. Wildevuur, W.L. Sederel, A. Bantjes, and J. Feijen, Biodegradability and tissue reaction of random copolymers of L-leucine, L-aspartic acid and L-aspartic acid esters, *J. Biomed. Mater. Res.*, 11, 405-422 (1977).

5 J.M. Anderson, D.F. Gibbons, R.L. Martin, A. Hiltner, and R. Woods, The potential for poly (α-amino acids) as biomaterials, *J. Biomed. Mater. Res.* Symposium, No. 5 (Part 1), 197-207 (1974).

6 L. Feenstra, B.W.C. van der Ven, F.E. Kohn, and J. Feijen, Experimental myringoplasty, *Int. J. Artif. Organs*, 3, 354-357 (1980).

7 R.L. Kronenthal, Biodegradable polymers in medicine and surgery. In: Polymers in medicine and surgery, editors R.L. Kronenthal, A. Oser and E. Martin, *Plenum Press*, New York and London, 1975, pp. 119-137.

8 R.K. Kulkarni, K.C. Pani, C. Neuman, and F. Leonard, Polylactic acid for surgical implants, *U.S. Nat. Techn. Inf. Serv.*, AD-Report 636,716, Apr. 1966.

9 E.J. Frazza, and E.E. Schmitt,
A new absorbable suture,
*J. Biomed. Mater. Res. Symposium*, 1, 43-58 (1971).

10 D.F. Williams, and E. Mort,
Enzyme-accelerated hydrolysis of polyglycolic acid,
*J. Bioengineering*, 1, 231-238 (1977).

11 R.A. Miller, J.M. Brady, and D.E. Cutright,
Degradation rates of oral obsorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios,
*J. Biomed. Mater. Res.*, 11, 711-719 (1977).

12 M. Goodman, Ch. Gilon, G.S. Kirshenbaum, and Y. Knobler,
Polydepsipeptides. I. Synthesis and characterization of copolymers of α-amino and α-hydroxy acids,
*Israel J. Chem.*, 10, 867-879 (1972).

13 G.P. Blackbourn, and B.J. Tighe,
Studies in ring-opening polymerization. I. 5,5-Dietyl-1,3,2-dioxathiolan-4-one-2-oxide,
*J. Polym. Sci.*: Part A-1, 8, 3591-3605 (1970).

14 M. Goodman, and G.S. Kirshenbaum, assignors to Sutures, Inc.,
Hydrolyzable polymers of amino acids and hydroxy acids,
*U.S. Patent* 3,773,737 (1971).

15 M. Goodman, Ch. Gilon, M. Palumbo, and R.T. Ingwall,
Polydepsipeptides II: Synthesis and preliminary conformational studies of an alternating α-amino and α-hydroxy acid polymer, *Isr. J. Chem.*, 12, 67-77 (1974).

16 D. Nissen, Ch. Gilon, and M. Goodman,
Polydepsipeptides 4. Synthesis of the alternating polydepsipeptides poly(ala-lac) and poly(val-lac),
*Makromol. Chem.*, Suppl. 1, 23-53 (1975).



17 R.T. Ingwall, and M. Goodman,
Polydepsipeptides. III. Theoretical conformational analysis of randomly coiled and ordered depsipeptide chains,
*Makromolecules*, 7, 598-605 (1974).

18 M. Goodman,
Matrix-controlled synthesis and conformational studies of polydepsipeptides,
*J. Polym. Sci.*: Polym. Symposium, 62, 173-188 (1978).

19 B.F. Gisin, R.B. Merrifield, and D.C. Tosteson,
Solid-phase synthesis of the cyclododecadepsipeptide Valinomycin, *J. Am. Chem. Soc.*, 91, 2691-2595 (1969).

20 G. Losse, and H. Klengel,
Synthese des Depsipeptides Valinomycin nach der Festphasenmethode, *Tetrahedron*, 27, 1423-1434 (1971).

21 B. Ridge, H.N. Rydon, and C.R. Snell,
Polypeptides. Part XXI. Synthesis of some sequential macromolecular polypeptolides of L-leucine and L-2-hydroxy-4-methylpentanoic acid, *J. Chem. Soc.*, Perkin I, 1972, 2041-2046.

22 F.H.C. Stewart,
Synthesis of polydepsipeptides with regularly repeating unit sequences, *Aust. J. Chem.*, 22, 1291-1298 (1968).

23 F.H.C. Stewart,
Depsipeptide synthesis by accelerated active ester coupling,
*Aust. J. Chem.*, 21, 1639-1649 (1968).

24 H. Kunz, and K. Lorenz,
Synthese alternierender Depsipeptide mit der 4-Methylthiophenoxycarbonyl Gruppe als Schutz- und Aktivierungsprincip für die Aminofunktion,
*Angew. Chem.*, 92, 953-954 (1980).

25 H.K. Hall, Structural effects on the polymerization of lactams, *J. Am. Chem. Soc.*, 80, 6404-6409 (1958).

26 K.C. Frish, and S.L. Reegen, Introduction. In: 'Kinetics and mechanism of polymerization. Vol. 2 Ring-opening polymerization', edited by K.C. Frish and S.L. Reegen, *Marcel Dekker*, New York, 1969, pp. 1-11.

27 H. Sekiguchi, and B. Coutin, Polymerizability and related problems in the anionic polymerization of lactams, *J. Polym. Sci.: Polym. Chem. Ed.*, 11, 1601-1614 (1973).

28 B. Coutin, and H. Sekiguchi, Dissociation of the alkaline and quarternary ammonium salts of 2-piperidone in the polymerization of 2-piperidone, *J. Polym. Sci.: Polym. Chem. Ed.*, 15, 2539-2541 (1977).

29 A.B. Meggy, Glycine peptides, Part I. The polymerization of piperazine-2,5-dione at 180°C, *J. Chem. Soc.*, 1953, 851-855.

30 A.B. Meggy, Glycine peptides, Part II, The heat and entropy of formation of the peptide bond in polyglycine, *J. Chem. Soc.*, 1956, 1444-1454.

31 K. Haga, K. Uno and Y. Iwakura, Polymerization of piperazine-2,5-dione, *Kobunshi Kagaku*, 29, 634-637 (1972) (through Chem. Abstracts 78, 16552 c).

32 Y. Kozai, N. Hayashi, and Y. Ikeda, Ring-opening polymerization of piperazine-2,5-dione in the presence of aqueous ammonia, *Kobunshi Ronbunshu*, 32, 49-54 (1975) (through Chem. Abstracts 82, 171482 f).

33 G.E. Schulz, and R.H. Schirmer, 'Princinples of protein structure', *Springer Verlag*, New York, 1979, Ch. 1.

34 S.W. Kim, R.V. Petersen, and J. Feijen, Polymeric Drug delivery system, in: 'Drug design, Vol. x', ed. by E.J. Ariëns, *Academic Press*, New York, 1980, pp. 193-250.

35 H.K. Hall, and A.K. Schneider, Polymerization of cyclic esters, urethanes, ureas and imides, *J. Am. Chem. Soc.*, 80, 6409-6412 (1958).



36 M.M. Schemjakin,
Die Chemie der Depsipeptide,
*Angew. Chem.*, 72, 342-345 (1960).

37 C.H. Hassall, and W.A. Thomas,
Conformations of cyclic and cylindrical peptides,
*Chem. Brit.* 7, 145-153 (1971).

38 U. Schmitt, J. Haüsler, E. Öhler, and H. Poisel,
Dehydroamino acids, α-hydroxy-α-amino acids and α-mercapto-α-hydroxy acids,
*Fortschr. Chem. Naturstoffe*, 37, 251-327 (1979).

39 W.A. Taber, and L.C. Vining,
Amidomycin, a new antibiotic from streptomyces species. Production, isolation, assay and biological properties,
*Can. J. Microbiology*, 3, 953-965 (1957).

40 M.M. Shemyakin, Yu.A. Ovchinnikov, V.T. Ivanov, A.V. Evstratov, A. Mikhaleva, and I.D. Ryabova,
Synthesis and antimicrobial activity of analogs of Enniatin antibiotics, *J. Gen. Chem. USSR*, 42, 2316-2328 (1972).

41 M.M. Shemyakin,
The structure of depsipeptides and their biological function. In: "Some recent developments in the chemistry of natural products", ed. by S. Rangaswani and S. Rao, Prentice-Hall, *New Delhi*, 1972, pp. 265-286.

42 M.M. Shemyakin, Yu.A. Ovchinnikov, V.T. Ivanov, V.K. Antonov, E.I. Vinogradova, A.M. Shkrob, G.G. Malenkov, A.V. Evstratov, I.A. Laine, E.I. Melnik, and I.D. Ryabova,
Cyclodepsipeptides as chemical tools for studying ionic transport through membranes,
*J. Membrane Biol.*, 1, 402-430 (1969).

43 Yu.A. Ovchinnikov, and V.T. Ivanov,
Recent developments in the structure-functional studies of peptide ionophores,
*FEBS-Symposium series (Biochem. Membr. Transp.)*, 42, 123-146 (1977).

44 Yu.A. Ovchinnikov,
The chemistry of membrane active peptides and proteins. "Frontiers in physiological biology: Proc. Int. Symposium", ed. by B. Pullman, *Academic Press*, New York, 1978, pp. 81-113.

45 D.G. Davis, and B.F. Gisin,
Structural, kinetic and mechanistic aspects of cation complexation by cyclo-(L-Pro-L-Val-D-Ala-D-Val)$_3$,
*J.Am. Chem. Soc.*, 101, 3755-3762 (1979).

46 D.W. Russell,
Cyclodepsipeptides, *Quart. Rev.*, 20, 559-576 (1966).

47 M. Rohthe, and W. Kreiss,
Cyclodepsipeptide-Synthese durch Cyclooligomerisierung,
*Angew. Chem.*, 89, 117-118 (1970).

48 D.W. Woolley, G. Schaffner, and A.C. Braun,
Studies on the structure of phytopatogenic toxin of Pseudomonas tabaci, *J.Biol.Chem.*, 215, 485-493 (1955).

49 Cited in "Rodd's chemistry of carbon compounds", Vol. IV, Heterocyclic compounds Part H, ed. by S. Coffey, Amsterdam, 1978, p. 454.

50 E. Fisher, and G. Reif,
Derivate des Prolins, *Ann.*, 363, 118-135 (1908).

51 E. Fisher, and W. Gluud,
Synthese von Polypeptiden XXXI: Derivate des Leucins, Alanins und N-Phenylglycins, *Ann.*, 369, 247-275 (1909).



52 S.G.P. Plant, and J.E. Facer, The synthesis and reaction of 1-anilino-cyclopentane-1-carboxylic acid, *J. Chem. Soc.*, 127, 1210-1213 (1926).

53 S.M. Oakeshott, and S.G.P. Plant, Some reactions of 1-p-toluidino-cyclopentane-1-carboxylic acid. A new carbazole synthesis, *J. Chem. Soc.*, 128, 1210-1213 (1926).

54 A. Ya. Khorlin, I.M. Privalova, L.Ya. Zakstelskaya, E.V. Molibog, and N.A. Evstignuva, Synthetic inhibitors of vibriocholerar neuraminidase and neuraminidases of some influenza virus strains, *FEBS Letters*, 8, 17-19 (1970).

55 Th. Wieland, B. Heinke, and K.H. Shin, Eine Umlagerungsreaktion von Pyruvyl-aminosäurethiophenylestern, *Chem. Ber.*, 93, 3027-3035 (1960).

56 Th. Wieland, and J. Stärk,
Thioarylwanderung bei Oxocarbonsäurethioestern,
*Chem. Ber.*, 96, 2410-2419 (1963).

57 R. Pfleger, and G. Markert,
Die Einfluss des Lösungsmittels und des an der Kohlenstoff-Doppelbindung stehenden Halogen auf die Halogenierung der Methylgruppen der α-Acetamino-Zimtsäure und der α-p-Toluylamino-Zimtsäure-Azlactone, *Ber.*, 90, 1482-1488 (1957).

58 H. Brockmann, and H. Geeren,
Valinomycin II; Antibiotika aus Actinomyceten XXXVII: die Konstitution des Valinomycin, *Ann.*, 603, 216-232 (1957).

59 A.M. Cook, S.F. Cox, and T.H. Farmer,
Antibiotics produced by fungi, and a new phenomenon in optical resolution, *Nature*, 162, 61 (1948).

60 A.H. Cook, S.F. Cox, and T.H. Farmer,
Production of antibiotics by fungi. Part IV. Lateritiin-I, Lateritiin-II, Avenacein, Sambucinin, and Fructigenin,
*J. Chem. Soc.*, 1949, 1022-1028.

61 Pl.A. Plattner, and U. Nager,
Werkstoffe und Antibiotika. Über die Konstitution von Enniatin B, *Helv. Chim. Acta*, 31, 665-671 (1948).

62 Pl.A. Plattner, and U. Nager,
Werkstoffe und Antibiotika. Über die Konstitution von Enniatin A, *Helv. Chim. Acta*, 31, 2192-2203 (1948).

63 N.S. Wulfson, V.A. Puchkov, V.N. Bochkarev, B.V. Rozinov, A.M. Zyakoon, M.M. Shemyakin, Yu.A, Ovchinnikov, V.T. Ivanov, A.A. Kiryushkin, E.I. Vinogradova, M.Yu. Feigina, and N.A. Aldanova, Mass spectrometric study on cyclodepsipeptides, *Tetrahedron Letters*, 1964, 951-960.

64 V.A. Puchkov, N.S. Wulfson, B.V. Rozinov, Yu.V. Desinov, M.M. Shemyakin, Yu.A, Ovchinnikov, and V.T. Ivanov,
The thermal effect in mass spectrometry of organic compounds, *Tetrahedron Letters*, 1965, 543-553.

65 L.C. Vining, and W.A. Taber,
Amidomycin, a new antibiotic from a streptomyces species, chemical structure, *Can. J. Chem.*, 35, 1109-1116 (1957).

66 A. Yasutake, K. Miyazaki, H. Aoyagi, T. Kato, and N. Izumiyua,
Hydrolysis of cyclic depsipeptides by tripsin or chymotripsin, *FEBS Letters*, 100, 241-243 (1979).

67 P. Thamm,
Herstellung heteromerer und O-(S-)Peptidbindungen. In: "Synthese von Peptiden II. Houben Weyl, Methoden der Organischen Chemie", 4th edition, ed. by Eugen Müller, *Thieme Verlag, Stutgart*, 1974, pp. 369-396.



68 A.F. Chadwick, and E. Pascu,
The resolution and rates of hydrolysis of d,l-α-bromopropionic acid and its glycine derivatives,
*J. Am. Chem. Soc.*, 65, 392-403 (1943).

69 A.H. Cook, and S.F. Cox,
2:5-Diketomorpholines, their synthesis and stability,
*J. Chem. Soc.*, 1949, 2347-2351.

70 R. Kaźmierczak, and G. Kupryszewski,
On intramolecular interaction in peptide-like systems. Part I. Rearrangement of the N-(α-hydroxypropionyl)-3-methyl-2,5-diketomorpholine to cyclic tridepsipeptide,
*Roczniki Chemii Ann. Soc. Chim. Polonorum*, 41, 103-108 (1967).

71 A.L.J. Beckwith,
Acylation of amines and amides. In: "*The chemistry of amides*", ed. by J. Zabicky, Interscience Publ., New York, 1970, pp. 77-86.

72 L.D. Rumsh, L.I. Volkova, and V.K. Antonov,
Hydrolysis of L-3-benzyl-2,5-diketomorpholine by chymotripsin, *FEBS Letters*, 9, 64-66 (1970).

73 J. Kurtz, G.D. Fasman, A. Berger, and E. Katchalski,
Poly-hydroxy-L-proline, *J. Am. Chem. Soc.*, 80, 393-397 (1958).

74 Yu.A. Ovchinnikov, V.T. Ivanov, G.Yu. Peck, and M.M. Shemyakin, Approaches to the conformational study of depsipeptides, *Acta Chemica Academiae Scientiarum Hungarica*, 44, 211-218 (1965).

75 V.M. Tul'chinskii, G.A. Kogan, L.D. Kvaratskheliya, I.M. Privalova, and A.Ya. Khorlin,
Study of N-aldonoylamino acids by IR spectroscopy,
*Khimiya Prirodnykh Sodinenii*, 3, 381-384 (1974).

76 N.E. Zhuklistova, V.I. Smirnova, G.N. Tishchenko, and V.I. Andrianov, Structural study of cyclic dipeptides and didepsipeptides.II. Crystal and molecular structure of the cyclodepsipeptide diketomorpholine (-D-MeVal-D-HyIv-), *Sov. Phys. Crustallogr.*, 22, 41-44 (1977).

77 E.M. Popov, V.Z. Pletnev, S.L. Portnova, V.T. Ivanov, P.V. Kostetskii, and Yu.A. Ovchinnikov, Conformations of 3,6-disubstituted 2,5-morpholinediones and 2,5-piperazinediones, *J. Gen. Chem. USSR*, 41, 420-427 (1971).

78 I.L. Karle, Crystal structure and conformation of the cyclic dipeptide cyclo-L-prolyl-L-leucyl, *J. Am. Chem. Soc.*, 94, 81-84 (1972).

79 E. Fisher, α-Brompropionyl-glycin, *Ann.*, 340, 128-129 (1905)

80 D.D. Perrin, W.L.F. Amarego, and D.R. Perrin, "Purification of laboratory chemicals", *Pergamon Press*, Oxford, 1966, (a) p. 66; (b) p. 349.

81 E. Ronwin, Direct acylation of α-amino acids and of α-hydroxy acid derivatives, *J. Org. Chem.*, 18, 127-132 (1953).

82 R.E. Steiger, Benzoylation of amino acids, *J. Org. Chem.*, 9, 396-400 (1944).

83 G. Ingram, The microdetermination of nitrogen in organic compounds by the rapid combustion process, *Mikrochim. Acta*, 1953(2), 131-147.



84 W.A.R. van Heeswijk, M.J.D. Eenink, and J. Feijen, An improved method for the preparation of γ-esters of glutamic acid and β-esters of aspartic acid, *Synthesis*, 1982(9), 743-747.

85 R.K. Kulkarni, K.C. Pani, C. Neuman, and F. Leonard, Polylactic acid for surgical implants, *Archives in Surgery*, 93, 839-843 (1966).

86 R.G. Sinclair, and G.M. Gynn, Preparation and evaluation of glycolic and lactic acid-based polymers for implant devices used in management of maxillofacial trauma. Part I. *U.S. Nat. Tech. Inform. Serv.*, AD Rept. No. 748,410 (1972).

87 R.G. Sinclair, and G.M. Gynn, Preparation and evaluation of glycolic and lactic acid-based polymers for implant devices used in management of maxillofacial trauma. Part II. *U.S. Nat. Tech. Inform. Serv.*, AD Report. No. 748,411 (1972).

88 W.R. Sorenson, and T.W. Campbell, "Preparative methods of polymer chemistry", 2nd ed., *Interscience Publ.*, New York, 1968, pp. 8-10.

89 J. Kleine, and H. Kleine, Über hochmolekulare, insbesondere optisch aktive Polyester der Milchsäre, ein Beitrag zur Stereochemie makromolekularer Verbindungen, *Makromol. Chem.*, 30, 23-38 (1959).

90 W. Dittrich, and R.C. Schulz, Kinetik und Mechanismus der ringöffnenden Polymerisation von L(-)-Lactid, *Angew. Makromol. Chem.*, 15, 109-126 (1971).

91 A. Sporzyński, W. Kocay, and H.V.A. Briscoe, A new method of preparing glycollide,

*Recueil*, 68, 613-618 (1949).

92 J. Dale, O. Sevaldsen, and K. Titlestad,
Cyclic oligo-esters of glycolic acid,
*Acta Chem. Scand.*, B32, 306-307 (1978).

93 E. Abderhalden, and E. Haase,
Fortgesetzte Studien über die Abspaltbarkeit von Halogen aus Halogenacylaminosäuren und -polypeptiden durch verdüntes Alkali, *Fermentforschung*, 12, 313-328 (1931).

94 J. March,
"*Advanced organic chemistry*", 2nd ed.,
McGraw-Hill Kogakusha Ltd., Tolyo, 1977, pp. 349-353; 363-367.

95 Y. Koyamata, and T. Shimanouchi,
Conformation of model compounds of proteins.I. Acetylglycine N-methylamide, *Biopolymers*, 6, 1037-1076 (1968).

96 S. Karplus, and S. Lifson,
Consistent force field calculations on 2,5-diketopiperazine and its 3,6-dimethyl derivatives,
*Biopolymers*, 10, 1973-1982 (1971).

97 P.G. Sammes,
Naturally occurring 2,5-dioxopiperazines and related compounds. In: "Fortschritte der Chemie organischer Naturstoffe", Vol. 32, ed. by W. Herz, H. Grisebach and G.W. Kirby,
*Springer Verlag*, Wien-New York, 1975, pp. 51-118.

98 K. Bláha, J. Smolíková, and A. Vítek,
Amino acids and peptides. LXIV. Infrared spectra of substituted 2,5-piperazinediones and the detection of cis-peptide bonds in diastomeric cyclohexapeptides,
*Coll. Czech. Chem. Comm.*, 31, 4296-4311 (1966).



99 L.J. Bellamy,
"*The infrared spectra of complex molecules*", Methuen & Co.,
London, 1958, Chapter 12.

100 J. Dale,
"*Stereochemistry and conformational analysis*",
Universitetsforlaget, Oslo, 1978, pp. 83-85.

101 H.K. Hall, and R. Zbinden,
Infrared spectra and strain in cyclic carbonyl compounds,
*J. Am. Chem. Soc.*, 80, 6428-6432 (1958).

102 J.L. Brash, and D.J. Lyman,
Lactones.
In: "*Cyclic monomers*" (High polymers series Vol. XXVI), ed. by K.C. Frish, John Wiley, New York, 1972, pp. 147-178.

103 G.J. Hummel, S. Harkema, F.E. Kohn, and J. Feijen,
Structure of 3,6-dimethyl-1,4-dioxane-2,5-dione (D-,D-(L-, L-)Lactide),
*Acta Cryst.*, B38, 1679-1681 (1982).

104 P. De Santis, and A.J. Kovacs,
Molecular conformation of poly(S-lactic acid),
*Biopolymer*, 6, 299-306 (1968).

105 M. Ōki, and H. Nakanishi,
Conformations of the ester group,
*Bull. Chem. Soc. Japan*, 43, 2558-2566 (1970).

106 H.J. Svec, and G.A. Junk,
The mass spectra of dipeptides,
*J. Am. Chem. Soc.*, 86, 2278-2282 (1964).

107 J. Dale, and K. Titlestad,
Cyclic oligopeptides of sarcosine (N-methylglycine),

*Chem. Comm.*, 1969, 656-659.

108 J. Dale, and K. Titlestad,
A common conformation for five cyclic tetrapeptides,
*Chem. Comm.*, 1970, 1403-1404.

109 K. Titlestad,
Cleavage of linear tetrapeptides into cyclic dipeptides,
*Chem. Comm.*, 1971, 1527-1529.

110 M.Y. Ali, J. Dale, and K. Titlestad,
Bi- and tricyclic products from tetra- and penta-peptides of α-methylalanine,
*Acta Chem. Scand.*, 27, 1509-1518 (1973).



CHAPTER VII

# NEW PERSPECTIVES IN MYRINGOPLASTY*

ABSTRACT

Until 1950 the treatment of a perforated eardrum consisted of covering the drum permanently with artificial material. Since then a surgical technique to establish a functional reconstruction of the eardrum was developed (myringoplasty). A survey of the biological grafting materials used in this technique is given.

Biodegradable and non-degradable synthetic materials may prove to be a valuable supplement to the existing biological grafting materials. Artificial eardrums made from several biodegradable poly(α-hydroxy acids) and poly(α-amino acids) and made from a number of microporous poly(tetrafluoroethylene) membranes and from a microporous bisphenol-A poly(carbonate) membrane were implanted into the ears of rats and dogs and as a reference subcutaneously. The implants were histologically examined for periods up to one year. From the biodegradable polymers studied poly(β-benzyl-L-aspartate-co-L-leucine) 50/50 evoked the least tissue reaction and the newly formed eardrums were the best in terms of thickness and overall integrity. The formation of a reinforced eardrum may be accomplished by the support of an inert, very thin, highly porous poly(tetrafluoroethylene membrane) preferably implanted as composite graft with a biodegradable polymer.

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## VII.1. Introduction

After trauma or inflammation an eardrum may become permanently perforated, frequently resulting in some hearing loss and proneness to infection. Attempts to close a perforated drum date back to 1640 when Marcus Banzer tried a piece of pig's bladder as a prosthetic covering for a drum defect. Since then two major approaches have been developed which can roughly be divided into: (a) prosthetic covering (1640-1950) - the perforated drum is covered permanently with artificial material; (b) myringoplasty (1950-present) - a surgical technique to establish a functional reconstruction of the tympanic membrane.

In this paper we will discuss briefly the anatomy and functioning of the normal eardrum, followed by a survey of methods and materials for treatment of perforated eardrums. Finally we will deal specifically with the use of biodegradable and microporous non-degradable polymeric materials. These synthetic materials might provide a promising supplement to the various biological materials used in myringoplasty hitherto.

## VII.2. Anatomy and functioning of the human eardrum

The human tympanic membrane is a conical, almost circular membrane (Fig. 1). It maintains its conical shape because of a cobweb-like structure of radial, circular and parabolic collagen fibres. The handle of the malleus, the first bone of the ossicular chain in the middle ear, is firmly attached to the drum. The collagen fibres are incorporated in soft tissue. Therefore the material which constitutes the human drum is strongly anisotropic with an average elasticity comparable to slightly vulcanized rubber (Young's moduli range from 2-4 x $10^8$ dynes/$cm^2$) (Kirikae, 24). The function of the drum and the ossicles is to transfer soundwaves, i.e. vibrations of the surrounding air particles, into vibrations of the perilymph liquid in the inner ear. Drum and ossicles together can be considered as a mechanical impedance matching transformer (Dallos, 7). The way the transformer action of the middle ear



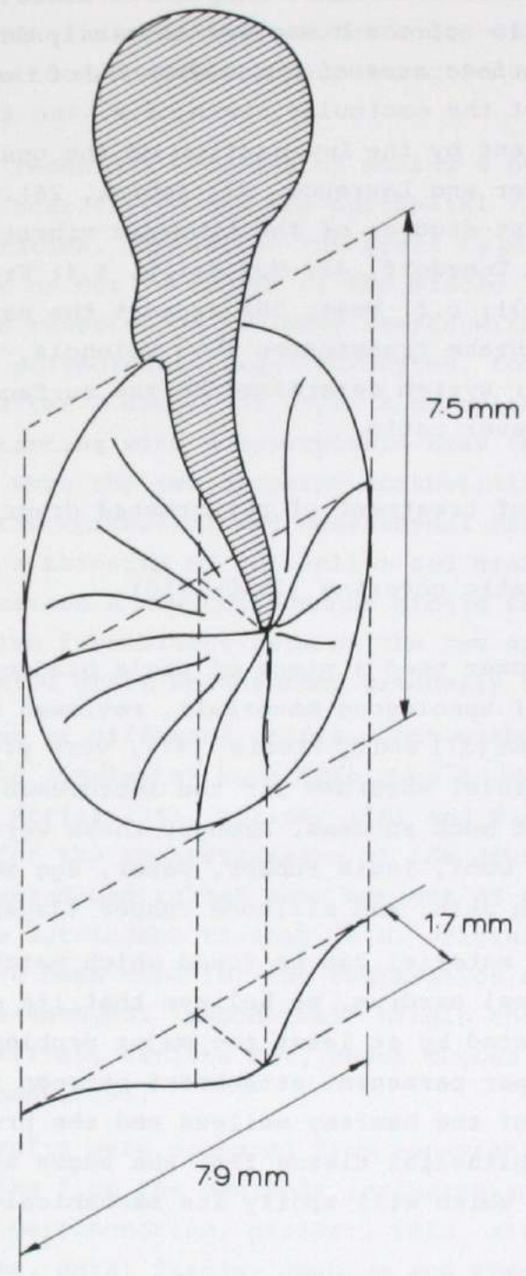


Fig. 1. Schematic presentation of a human tympanic membrane.

takes place is still under discussion. It is postulated that the eardrum moves as an essentially stiff plate and that the transformer ratio of the human ear is mainly determined by the ratio of the surface area of the eardrum and of the footplate of the last bone of the ossicular chain, i.e. the stapes, and to a lesser extent by the lever action of the ossicular chain (Békésy, 5; Wever and Lawrence, 53; Kobrak, 26). On the other hand, more recent studies of the tympanic vibrations (Tonndorf and Khanna, 48; Tonndorf, 49; Von Bally, 3,4; Fritze, 15; Løkberg et al, 31; c.f. Vest, 50) support the early concept of a curved membrane transformer (Von Helmholz, 19) in addition to a transformer system determined by the surface area ratio and ossicular lever ratio.

### VII.3. History of treatment of performated drums

#### VII.3.1. Prosthetic covering (1640-1950).

Since Marcus Banzer used a piece of pig's bladder in 1640 a great variety of xenologous materials, reviewed by Schrimpf (42), by Örtegren (37) and by Storrs (47), were proposed as permanent artificial eardrums for the improvement in hearing, however, without much success. Amongst these were fish's air bladder, cotton wool, India rubber, paper, egg membrane, silver, cellophane, fish skin, and silicone rubber (Jayapathy, 22).

Assuming that a material can be found which matches the properties of the normal eardrum, we believe that its application will be complicated by at least two major problems: the realization of a proper permanent attachment between the prosthesis and the handle of the healthy malleus and the prevention of the growth of new epithelial tissue from the edges across the eardrum prosthesis which will modify its mechanical-acoustical properties.

#### VII.3.2. Myringoplasty (1950-present)

The principle of myringoplasty is based on covering a drum perforation with a more or less permanent collagenous structure, i.e. the graft, which must function as a scaffold for the migrating epithelial tissue from the edges of the perforation. The objective is a functional reconstruction of the tympanic membrane.



The surgical technique consists of making a new wound by removing the scar tissue and the epithelial rim from the edges of the perforation. Thereafter the graft is placed underneath (underlay) or on top (overlay) of the middle layer of the drum. Then the wound reaction of the body starts with infiltration of the graft by polymorphonuclear leukocytes, followed by capillary ingrowth after a couple of days. Also epithelial cells cross the graft, starting with a hyperplasia near the rim of the perforation. When the two opposing epithelial edges meet, the activity of the epithelial and mesenchymal tissue declines resulting in a thinning of epithelium and graft. After closure of the perforation a new collagenous middle layer is formed by the invading fibroblasts between the two epithelial layers. The transplanted graft is absorbed gradually during this process.

A large number of different grafts, both viable (transplants) and non-viable (implants) have been used in myringoplasty. The pioneers Von Moritz (35), Zöllner (58) and Wullstein (57) used skin grafts for the reconstruction of the drum membrane. Skin grafts were abandoned rather soon because of the high failure rate. Various autologous tissues, i.e. originating from the own body, have been used for the preparation of grafts. These tissues of mesenchymal (mesodermal) origin enclose vein, perichondrium, periost, earlobe fat, cheek mucosa and above all fascia (Heermann, 18).

Allologous grafts were prepared from non-viable human tissues not originating from the own body including amnion, cornea, pericardium, perichondrium, periost, vein, arteria umbilicalis, cardiac valves, dura, fascia, omentum and tympanic membrane (Örtegren, 37; Storrs, 47; Feenstra and Feenstra, 9; Feenstra et al, 10). The xenologous materials used for the preparation of grafts are conveniently divided into those originating from

an animal source (heterologous) and non-biological materials (exogenous). Cardiac valve, mesenterium (sheep mesentry), drum membrane, serosa and also reconstituted collagen are included in the first group.

Schrimpf (42) has re-evaluated the applicability of some exogenous materials like paper, cotton, cellophane and gold, however, with little success. The use of a few potentially resorbable exogenous materials was reported. Watson and Maguda (51) used methyl-α-cyanoacrylate adhesive for securing a fascial graft to the drum during the healing period. Johnson (23) performed six myringoplasties in cats using poly(vinyl alcohol) grafts. Gelfoam® (gelatine sponge) discs were used by Goodhill (16) in cases where the presence of an active infection would contra-indicate the use of a conventional tissue graft.

In the current otologic literature autologous fascia, or, occasionally, a preserved allologous material of collagenous tissue origin is preferred. The reason for this preference is not entirely obvious. Of course, a grafting material must not cause a severe inflammation and must not be rejected by the host tissue. It must stay in close contact with the drum during the healing process. Above all, however, it must possess the necessary scaffolding ability: surgical closure of a perforated eardrum fails if the epithelial migration is slower than the desintegration of the scaffolding graft. It is somewhat doubtful, however, that autologous fascia offers the best scaffold. The actual scaffolding ability of the various mesodermal tissue grafts is in fact determined by the properties of the collagen matrix they possess. This fact has led to the study of grafts prepared from reconstituted collagen. Thus, Salén and Simbach (41) and Patterson (38) prepared heterologous collagen grafts from the tail of rat and from the deep flexor tendon of cattle, respectively. Attempts to close perforated eardrums of cats using these grafts gave variable results. Abbenhaus and Hemenway (1) reported good results when using bovine collagen sheets as a tympanic membrane graft in dogs.



Feenstra et al. (11) when performing experimental myringoplasties in rats, obtained good results with heterologous collagen of human origin*, whereas homologous collagen prepared from rat tail tendon caused serious anomalies.

## VII.4. Synthetic grafts

It is estimated that about 15% of the myringoplastic operations fail because of premature desintegration of a conventional tissue graft, i.e. autologous fascia. The development of a synthetic graft material which could reduce this failure rate seems attractive. Moreover, the commercial availability of such a graft reduces operation time.

The treatment of complicated perforations (e.g. total or subtotal perforations, atelectatic ears or congenital anomalies) often requires specific grafts which usually can not be prepared properly from the rather soft autologous or allologous tissue. Pre-fabricated, tailor-made synthetic grafts might contribute to a more successful treatment of these types of perforations.

Our search for new synthetic grafting materials was based on two approaches: (a) the development of synthetic, non-collagenous grafting materials which possess sufficient inherent mechanical strength and which show a controlled rate of degradation; (b) the application of non-degradable microporous synthetic materials which could function as a permanent reinforcement of a healed drum by replacing the absent collagen middle layer.

A commonly used material like collagen was not further investigated because the medical aspects of the application of collagen as a biomaterial are still very poorly understood (Stenzel et al., 46) and it is as yet impossible to control sufficiently the rate of degradation of modified collagen. Besides it may cause a remarkable antigenicity or a rather

* Received with thanks from Braun, Melsungen, Germany.

intense inflammatory reaction most probably depending on preparation and sterilization methods (Patterson, 38).

Johnson (23) performed six myringoplasties in cats using grafts prepared from poly(vinyl alcohol). The results, after a maximum follow-up of 11 months, were disappointing: none of the poly(vinyl alcohol) grafts showed overgrowth of new epithelial tissue and they were finally recovered free in the middle ear. There were no indications of biodegradation of the poly(vinyl alcohol) grafts (Kronenthal, 28). Poly(methyl-α-cyanoacrylate), a biodegradable polymer, was used by Watson and Maguda (51) to secure a fascial graft to the drum. Interestingly, when using the considerably less toxic isobutyl-α-cyanoacrylate adhesive (Hastings, 17) in the middle ear of rats, we observed an acute very severe inflammatory reaction. The attempts by Goodhill (16) to induce 'spontaneous' neotympanic membranes utilizing porous Gelfoam® discs as a scaffold can be compared to a certain extent with our approach. The resorption rate of Gelfoam®, however, is very high and can not be controlled.

### VII.4.1. Biodegradable grafts

A synthetic biodegradable eardrum graft must meet a number of specific requirements. It must be thin and it must also have sufficient mechanical strenght and consistency to be able to function as a cover of the perforation. At the same time it must be relatively soft and pliable to minimize tissue irritation. The grafting material has to allow the overgrowth of new epithelial tissue which should adhere to it. The graft must retain its integrity during the healing process of the perforated drum. Ideally, the synthetic biodegradable graft remains unchanged during this process and it degrades in a short period after it has fullfilled its task. In actual practice partial degradation of the graft will inevitably occur during the healing process. However, sufficient inherent mechanical strength has to be retained for several months. Eventually the graft must degrade completely and the degradation products have to be removed from the implant site. These degradation products should not cause any adverse effects to the environmental tissue, nor cause adverse systemic effects (Williams and Roaf, 55), and they must not retard or impede the healing process of the perforated drum. The tissue environment of the eardrum is relatively poor in blood as a result of which the removal of degradation products might be hampered and accumulation of degradation products might occur. Degradation products of resorbable polymers which are relatively harmless at low concentrations, may be harmful to the environmental tissue at high concentrations. A typical example of such a degradation product is formaldehyde which is formed from alkyl-α-cyanoacrylate tissue adhesives (Hastings, 17).



Biomedical research activities over the last twenty years have revealed an increasing interest in the application of synthetic biodegradable polymers in medicine and surgery (Kronenthal, 28). This interest originated from the often impredictable degradation behaviour of resorbable sutures prepared from natural proteinaceous polymeric materials (catgut). The number of well-functioning synthetic biodegradable polymers, however, is as yet limited. The synthetic poly(α-hydroxy acids) and poly(α-amino acids) represent two important classes of accepted biodegradable polymers.

In the period 1955-1970 poly(glycolic acid), poly(lactic acid) and their co-polymers became well-known by the extensive research of, amongst others, Frazza (14; c.f. Mennie, 33) and Kulkarni et al. (29, 30). These polymers are degraded by hydrolysis (Kronenthal, 28) releasing glycolic acid and lactic acid as harmless degradation products. The rates of degradation have been established up to twelve months after implantation in soft tissue of bone (Miller et al., 34). Although glycolic- and lactic acid as such are harmless degradation products, the possibility that local pH-changes are induced cannot be ruled out. Such pH-changes may influence the growth of new epithelial tissue when eardrum grafts prepared from these poly (α-hydroxy acids) are implanted.

Much effort was put into the development of synthetic biodegradable poly(α-amino acids) (Feijen et al., 13; Sederel et al., 43; Sederel, 44; Marck et al., 32; Anderson et al., 2). A well characterized set of co-poly(α-amino acids) having various degradation times was synthetized and evaluated. For the development of synthetic biodegradable eardrum grafts we have concentrated on the types of polymers mentioned above.

VII.4.2. Development of synthetic biodegradable eardrum grafts

In the initial stage of our studies one poly(α-hydroxy acid), i.e. poly(L-lactic acid), and several co-poly(α-amino acids), see table I, were selected as potential candidates for synthetic biodegradable eardrum grafts. A co-polymer of L-lactic acid and glycolic acid is clinically applied as a synthetic resorbable suture material (Craig et al., 6). From implantation experiments in rats (Marck et al., 32) it was concluded that the hydrophobic co-poly(α-amino acids) degraded very slowly when implanted subcutaneously (table I).

| Compound* | Hydrophilicity | Degration rate** |
|---|---|---|
| Poly[Asp(OBz)$^{50}$Leu$^{50}$] | hydrophobic | slow |
| Poly[Asp(OBz)$^{50}$Glu(OBz)$^{50}$] | hydrophobic | slow |
| Poly[Asp(OBz)$^{10}$Asp(OH)$^{25}$Leu$^{65}$] | intermediate | intermediate |
| Poly[Asp(OBz)$^{15}$Asp(OH)$^{50}$Leu$^{35}$] | hydrophilic | fast |
| Poly[Asp(OBz)$^{15}$Asp(ONa)$^{50}$Leu$^{35}$] | hydrophilic | fast |

* Asp: L-aspartic acid; Leu: L-leucine; Glu: L-glutamic acid; OBz: benzyl ester; OMe: methyl ester; OH: acid; ONa: sodium salt; 50,50 etc.: molar ratio; molecular weights: 50,000-100,000

** After subcutaneous implantation in rats. Slow: hardly any degradation was observed after three months of implantation. Intermediate: a substantial amount of degradation was observed over the same period. Fast: implants could not be detected after a few days of implantation.

Table I. Relation between structure and degradation behaviour of co-poly (α-amino acids).



These implants remained more or less unchanged during an implantation period of three months. Somewhat hydrophilic implants had degraded to a certain extent after this period whereas the hydrophilic implants had disappeared within a few days. Small discs prepared from poly(L-lactic acid) and from the co-poly (α-amino acids) were tested in an experimental study which involved a small number of rats, maximum follow-up was four months. From this study we learned that the hitherto unknown reaction of the tissue of the middle ear evoked by these polymer implants did not differ very much from the reaction of subcutaneous tissue, i.e. a mild or very mild tissue reaction was evoked by the poly(L-lactic acid) and the hydrophobic co-poly(α-amino acid) grafts and a more pronounced adverse tissue reaction was evoked by the other grafts. Also the rates of degradation of the co-poly(α-amino acid) discs implanted subcutaneously as a reference did not differ very much from those implanted in the environment of the eardrum which is rather poor in blood. This means that the hydrophilic and partially hydrophilic co-poly (α-amino acids) are not suitable for the application as synthetic absorbable eardrum grafts: their degradation times are too short.

In addition, more information about the design requirements of the eardrum graft was obtained, which resulted in the replacement of the rather stiff eardrum grafts fabricated from commercially available poly(L-lactic acid) (Polysciences, 40) by more pliable ones made from poly(D,L-lactic acid) (Feenstra et al., 12; Van Hummel et al., 21; Van Dijk et al., 9; Kohn et al, 27).

The second stage involved an extensive, long-lasting experimental study in rats - maximum follow-up twelve months - a detailed description of which is reported elsewhere (Feenstra et al., 12). Eardrum grafts were prepared from poly(D,L-lactic acid), from poly[Asp(OBz)$^{50}$Leu$^{50}$] and also from poly(glycolic acid) which has found clinical application as Dexon® synthetic absorbable suture material (Frazza, 14; Mennie, 33). Each of the three resorbable polymers was implanted in 35 rats, both

as eardrum graft and subcutaneously. In this animal study the quality of newly formed eardrums in terms of thickness and overall integrity was compared. Significant differences in the quality of newly formed eardrums in relation to the type of implanted polymers were observed. Moreover, the reaction evoked by the subcutaneous tissue was similar to that evoked by tissue of the middle ear. Therefore our results do not confirm the opinion of other investigators that the reaction of the middle ear tissue in response to foreign materials is considerably milder than that of other kinds of tissue.

Grafting the perforated eardrum with the 50/50 random co-polymer of β-benzyl-L-aspartate and L-leucine resulted in newly formed eardrums (Fig. 2b) which were comparable to a normal drum (Fig. 2a). The quality of the newly formed eardrums induced by poly(D,L-lactic acid) grafts was also good. With poly(glycolic acid) grafts a violent cellular reaction in addition to a rapid desintegration of the grafts into sharp needles was observed. (Fig. 2c). Films had been prepared with great difficulty from the highly crystalline (Sinclair and Gynn, 45) poly(glycolic acid) and the desintegration could well have been the result of a poor quality of the films from which the grafts were cut.

The third stage of testing preceeding a possible clinical trial was performed with dogs having eardrums similar in size to that of the human eardrum. Grafts (diameter 7,4 mm) prepared from poly(D,L-lactic acid), poly(glycolic acid) and poly[Asp(OBz)$^{50}$ Leu$^{50}$] films were tested in a long-lasting experimental study. With each of the three different polymers nine myringoplasties were performed whereas subcutaneous implants served as a reference. Postoperatively the quality of the newly formed eardrums was examined after 1, 4, 8, 12, 18, 26, 32, 40 and 52 weeks, both visually and histologically. The results of this study confirmed to a large extent the results of the study in rats although the poly(D,L-lactic acid) grafts did less well in dogs than was anticipated. A marked adverse tissue reaction in the middle ear as well as in the subcutis evoked by poly



(D,L-lactic acid) was regularly observed.

Although the initial quality of the poly(glycolic acid) film from which the grafts were prepared had seemingly improved, soon after implantation sharp needles were observed which caused a severe tissue reaction. The behaviour of the poly [Asp(OBz)$^{50}$Leu$^{50}$] grafts in dogs resembled that in rats. No adverse tissue reaction or only a very mild one was observed and after complete degradation of the grafts the newly formed tympanic membranes were comparable to a normal tympanic membrane.

### VII.4.3. Non-degradable, microporous grafts

Non-degradable microporous grafts which are meant for a permanent reinforcement of a healed eardrum have to fullfil to a certain extent the same requirements as mentioned before with respect to their synthetic biodegradable counterparts. There are, however, important differences. Instead of being biodegradable, they must remain completely 'inert' for an indifferent period of time. Neither their chemical structure, nor their inherent mechanical properties may change after implantation. During the healing process they must become incorporated into the newly formed eardrum, preferably by the ingrowth of tissue or by the covering of tissue on both sides. Eventually they must function as a permanent reinforcement of the healed drum and improve its acoustical functioning. The ingrowth of tissue, including collagen fibres, into a microporous material is governed by at least two parameters: the pore diameter and the degree of porosity. Too small a pore diameter will prevent the tissue from growing into the pores whereas a low degree of porosity, i.e. the absence of a large amount of intercommunicating pores, will obstruct the nourishment of ingrowing tissue.

It is difficult to estimate the ideal pore diameter and degree of porosity (Wesolowski et al., 52; Klawitter and Hulbert, 25; Winter, 56; Howe et al., 20; White et al., 54; Pollock et al., 39; Nelson et al., 36). Nowadays a considerable number of micro-

porous membranes prepared from different polymeric materials is commercially available, e.g. filtration membranes or non-woven fabrics. Considering only the pore diameter and the degree of porosity, several of these commercial membranes appear to be potential candidates for the application as permanent eardrum grafts. Most of these membranes, however, have been prepared from polymeric materials which are either not sufficiently 'inert' themselves, e.g. cellulose triacetate, or which contain additives which are toxic to the human body, e.g. non-medical grade poly(vinyl chloride). Therefore a careful investigation of commercially available microporous membranes resulted only in the selection of bisphenol-A poly(carbonate) (Nuclepore®).

Since long poly(tetrafluoroethylene) has been recognized as an almost perfectly 'inert' biomaterial which has, for instance, resulted in its clinical application in vascular prostheses. We have tested some experimental microporous PTFE membranes* as reinforcing eardrum grafts, including a membrane which was used successfully by Winter (56) as a trancutaneous implant in pigs.

VII.4.4. Development of non-degradable, microporous grafts

In our first study small discs cut from microporous bisphenol-A poly(carbonate) (Nuclepore®) membranes (diameter 2½ mm, thickness 8 microns, porosity 10%, pore size 10 microns, pores run parallel; see Fig. 3a); from a microporous PTFE membrane (diameter 2½ mm, thickness 13 microns, porosity 95%, pore size 10-15 microns, pores are intercomminucating, see Fig. 3b); and from some thicker (25-500 microns) microporous PTFE membranes with considerably smaller pore sizes (maximum 2 microns; porosity 75-85%) were tested in rats, with a maximum follow-up of four months.

From this study we obtained the following information. Both the bisphenol-A poly(carbonate) and the thinnest PTFE membrane

*Kindly donated by W.L. Gore & Co, München, Germany.



evoked no adverse or only a very mild tissue reaction. Tissue had clearly grown into and through the pores of the latter membrane whereas the small sized pores of the other PTFE membranes were found free of tissue. At the same time the sides of these membranes were not completely covered with tussue. Moreover, the thickest PTFE membranes gave sometimes rise to moderate inflammatory reactions. The small pore sized PTFE membranes were not used for further studies.

The PTFE membrane successfully applied, however, had a serious drawback. During surgery the membranes creased and curled up very easily. Therefore it was decided to use this membrane in combination with biodegradable poly(D,L-lactic acid) as a 'double-layer' graft in the second stage of this study (Feenstra et al., 12).

Discs were cut from the bisphenol-A poly(carbonate) membrane and from the PTFE membrane placed on a poly(D,L-lactic acid) film. Each of these materials were implanted in 35 rats as eardrum grafts and subcutaneously as a reference. From this long-lasting study we got the following information. The microporous PTFE discs, implanted in combination with biodegradable poly(D,L-lactic acid) film as a 'double-layer' graft evoked hardly any adverse tissue reaction, neither did the poly(D,L-lactid acid) film. The PTFE discs became a real part of the newly formed eardrum or of the subcutis after degradation of the poly(D,L-lactic acid) layer. Connective tissue covered both sides of the membrane and had grown into and through the pores (Figs. 4a,b). This observation is in full agreement with the results obtained by Winter (56) who used a similar membrane as a transcutaneous implant. The tissue reaction evoked by the rather stiff microporous bisphenol-A poly(carbonate) discs was also very mild. They had become surrounded with a very thin capsule of fibrous tissue. However, contrary to what was observed in case of the PTFE membranes, hardly any tissue had invaded the pores (Fig. 4c). It turned out that a pore size of 10 microns was too small to allow the ingrowth of tissue. At first sight the small difference between the pore sizes of the bisphenol-A

poly(carbonate) membrane and of the PTFE membrane can hardly explain why tissue had grown abundantly into the pores of the latter membrane. It it, however, plausible that the combined effects of the pore patterns, the degree of porosity and the material resilience caused the observed difference between the ingrowth of tissue into the bisphenol-A poly (carbonate) membrane and into the PTFE membrane.

The third stage of testing served to explore the justification of clinical trials of grafts prepared from microporous PTFE. For that purpose nine myringoplasties were performed in dogs and the functioning of the PTFE grafts (diameter 7,4 mm) was examined as mentioned before with respect to the third stage of the development of biodegradable grafts.

In stage 1 pure microporous PTFE grafts were implanted with little success as far as the design of the graft was concerned. In stage 2 they were implanted in combination with poly(D,L-lactic acid) film as 'double-layer' grafts. Although functioning satisfactorily in rats, these double-layers which are connected rather loosely to each other, were not considered to be optimally designed. It was then attempted to impregnate the microporous PTFE membrane with poly(D,L-lactic acid) by a casting technique. A scanning electron micrograph of the resulting microporous PTFE-poly(D,L-lactic acid) composite graft (thickness approximately 18 microns) is shown in Fig. 5a. For comparison a scanning electron micrograph having the same magnification of microporous PTFE is shown in Fig. 5b.

After degradation of the poly(D,L-lactic acid), the microporous PTFE grafts were nicely incorporated into the newly formed eardrums by the ingrowth of tissue which was also observed with the rat experiments of stage 2. However, the degradation of the poly(D,L-lactic acid) showed a more pronounced adverse tissue reaction than was observed in the middle ear of rats. This observation was in agreement with the observed tissue reaction evoked by pure poly(D,L-lactic acid) implanted in the middle ear of dogs mentioned above.



## VII.5. Conclusion

The functioning of several potential synthetic biodegradable eardrum grafts and non-degradable, microporous eardrum grafts was examined both visually and histologically in three consecutive experimental studies with animals. After the first and second stage experimental study in rats, poly(D,L-lactic acid), poly (glycolic acid) and poly[Asp(OBz)$^{50}$Leu$^{50}$] were selected as potential synthetic biodegradable grafting materials, whereas a microporous PTFE membrane showed potential for the reconstruction of a missing fibrous middle layer of an eardrum.

After the third stage experimental study with dogs only the synthetic biodegradable graft prepared from poly[Asp(OBz)$^{50}$ Leu$^{50}$] and the porous PTFE membrane were finally selected for possible clinical trials. The PTFE membrane was implanted as a microporous PTFE - poly(D,L-lactic acid) composite graft fabricated by the impregnation with poly(D,L-lactic acid). This biodegradable polymer, however, is no longer considered as a suitable grafting material because of the marked adverse tissue reaction it evoked in dogs. Therefore, if clinical trials will be performed with microporous PTFE, it is proposed to use this membrane as a composite with poly[Asp(OBz)$^{50}$Leu$^{50}$].

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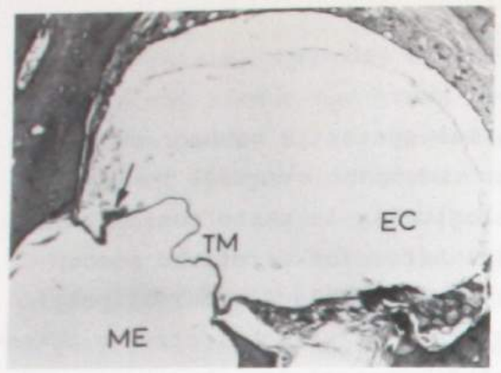



Fig. 2a. A normal ear of a rat; at the right the ear canal (EC), at the left the middle ear (ME), running in between the drum (TM) with part of the handle of the malleus (hammer), see arrow.

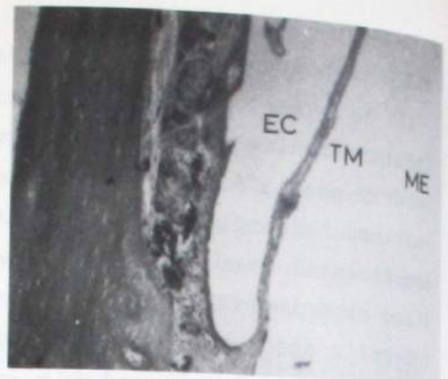



Fig. 2b. A tympanic membrane a year after implantation of a graft of a random copolymer of L-leucine and γ-benzylaspartate (molar ratio 50/50).

Fig. 2c. A poly(glycolic acid film one week after subcutaeous implantation Desintegration into a needle-like structure can be clearly observed (arrow).

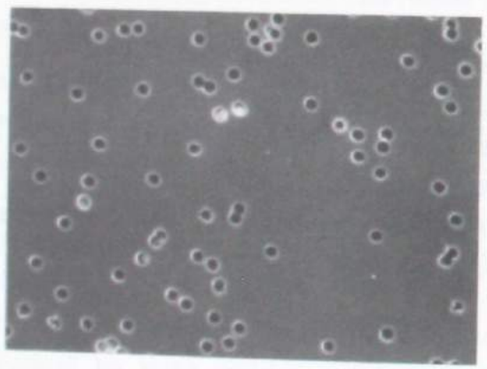


Fig. 3a. Scanning electron micrograph of a microporous bisphenol - A poly-(carbonate) membrane (Nuclepore$^{R}$). Magnification 300x.

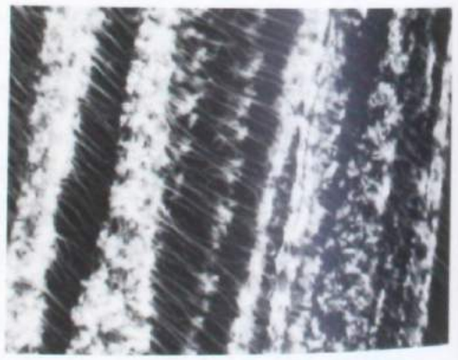


Fig. 3b. SEM picture of a microporous PTFE membrane. Magnification 400x.



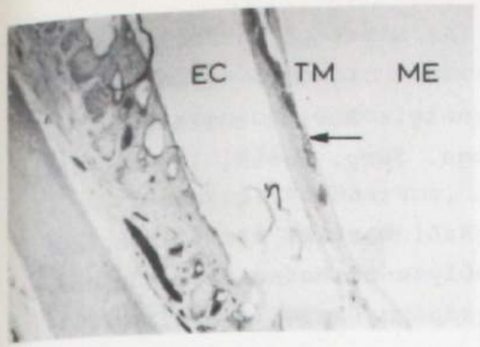



Fig. 4a. PTFE tympanic membrane (TM) one year after implantation. EC (ear canal), ME (middle ear), arrow crinckled PTFE membrane.

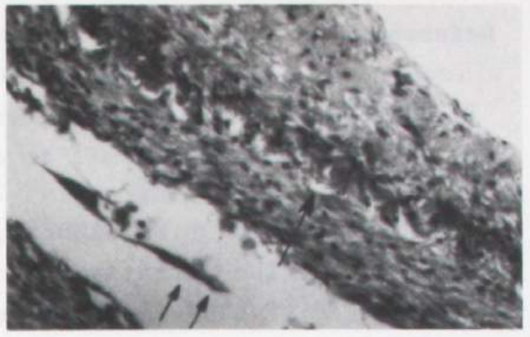


Fig. 4b. PTFE-PLA-composite membrane, 26 weeks after implantation into the subcutis of a rat; arrow PTFE, double arrow space occupied by the PLA. Hardly any reaction can be seen.

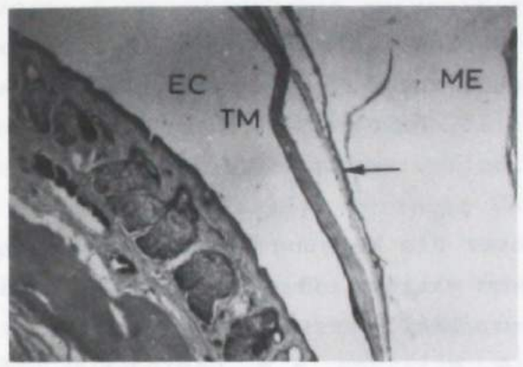



Fig. 4c. Polycarbonate tympanic membrane (TM) one year after implantation; arrow indicates polycarbonate; tympanic membrane slightly thicker than normal (c.f. Fig. 2a).

Fig. 5a. Scanning electron micrograph of PTFE-poly(D,L-lactic acid) composite (2000x).

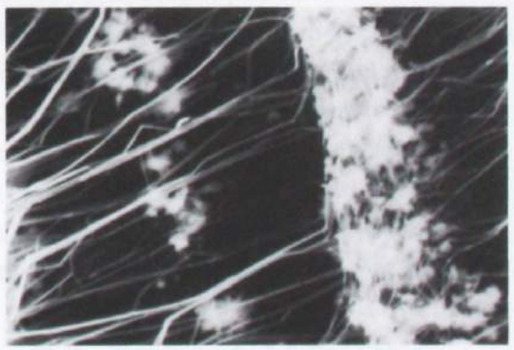


Fig. 5b. Scanning electron micrograph of microporous PTFE (2000x).

References


1. J.I. Abbenhaus, and W.G. Hemenway,: Bovine collagen as a tympanic membrane graft in dogs. *Surg. Forum*, 18, 490-491, 1967.
2. J.M. Anderson, D.F. Gibbons, R.L. Martin, A. Hiltner, and R. Woods: The potential for poly(α-amino acids) as biomaterials. *J. Biomed. Mater. Res. Symposium*, 5 (Part 1), 197-207, 1974.
3. G. von Bally: Holography in otology, In: *Springer Series in Optical Sciences*, Vol. 18, Holography in medicine and biology, editor G. von Bally, Springer Verlag, Berlin, 1979, pp. 183-197.
4. G. von Bally: Otological investigations in living man using holographic interferometry, In: *Springer Series in Optical Sciences*, Vol. 18, Holography in medicine and biology, editor G. von Bally, Springer Verlag, Berlin, 1979, pp. 198-205.
5. G. Békèsy,: Ueber die Messung der Schwingungsamplitude der Gehörknöchelchen mittels einer kapazitiven Sonde. *Akust. Ztschr.*, 6, 1-6, 1941.
6. P.H. Craig, J.A. Williams, K.W. Davis, A.D. Magoun, A.J. Levy, S. Bogdanski and J.P. Jones: A biologic comparison of polyglactin 910 and polyglycolic acid synthetic absorbable sutures, *Surg. Gynec & Obstet.*, 141, 1-10, 1975.
7. P. Dallos: The auditory periphery-biophysics and physiology, *Academic Press*, New York and London, 1973, Chapter 3.
8. J.A.P.P. van Dijk, J.A.M. Smit, F.E. Kohn, and J. Feijen: Characterization of poly(D,L-lactic acid) by gel permeation chromatography, *J. Polym. Sci.*, Polym. Chem. Ed., 21, 197-208, 1983.
9. L. Feenstra and K. Feenstra,: Studies on tympanic transplants, I. Tympanic homografts, *J. Oto-Rhino-Laryngol.*, 37, 149-160, 1975.





10. L. Feenstra, K. Feenstra, B. Haagsma and H.B. Kuipers: Studies on tympanic membrane transplants. II. Cartilage, bone and other homografts, *J. Oto-Rhino-Laryngol.*, 37, 161-168, 1975.
11. L. Feenstra, R. Baarsma, J. Feijen and H.J. Houthoff: Experimental myringoplasty, *J. Bioeng.*, 2, 251-257, 1978.
12. L. Feenstra, B.W.C. van der Ven, F.E. Kohn and J. Feijen: Experimental myringoplasty, *Int. J. Artif. Organs*, 3, 354-357, 1980.
13. J. Feijen, W.L. Sederel, T. Beugeling and A. Bantjes: Synthetic polypeptides as biomaterials, *Proc. ESAO*, Vol. I, 37-41, 1974.
14. E.J. Frazza: A new absorbable suture. *J. Biomed. Mater. Res.*, Symposium, 1, 43-58, 1971.
15. W. Fritze: On holographic-interferometric investigations of the membrana tympani (living man). In: *Springer Series in Optical Series*, Vol. 18, Holography in medicine and biology, editor G. von Bally. Springer Verlag, Berlin, 1979, pp. 206-211.
16. V. Goodhill: Deliberate 'spontaneous' tympanoplasty: roles of annular induction and basement membranes. *Ann. Otol.*, 75, 866-880, 1966.
17. G.W. Hastings: Adhesives and tissues. In: *Biocompatibility of implant materials*, editor D. Williams, Sector Publ. Ltd. London, 1976, pp. 144-151.
18. J. Heermann: Erfahrungen met freitransplantiertem Fazienbindergewebe des Musculus temporalis bei Tympanoplastik und Verkleinerung der Radikalhöhle. *Ztschr. Laryng. Rhin. Otol.*, 41, 141, 1962.
19. H. Helmholz, von: Die Mechanik des Gehöhrknöchelchen und Trommelfells. *Pflügers Archiv*, 1, 1-60 (1868).
20. D.F. Howe, C.W. Svare and R.W. Tock,: Some effects of pore diameter on single pore bony ingression patterns in Teflon, *J. Biomed. Mater. Res.*, 8, 399-406, 1974.
21. G.J. van Hummel, S. Harkema, F.E. Kohn and J. Feijen: Structure of 3,6-dimethyl-1,4-dioxane-2,5-dione (D-,D-(L-,L-) lactide), *Acta Cryst.*, B38, 1679-1681 (1982).

22. B. Jayapathy: Silastic patch prosthesis in the management of central perforations of the eardrum. *The Bulletin of the Dow Corning Center for Aid to Medical Research*, Vol. 2, number 3, July 1960.
23. F. Johnson: Polyvinyl in tympanic membrane perforations. *Arch. Otolaryng.*, 86, 152-155, 1967.
24. I. Kirikae: The Structure and function of the middle ear. *The University of Tokyo Press*, Tokyo, 1960
25. J.J. Klawitter and S.F. Hulbert: Application of porous ceramics for the attachment of load bearing internal orthopedic applications, *J. Biomed. Mater. Res.*, Symposium, 2 (Part 1), 161-229, 1971.
26. H.G. Kobrak: The middle ear. *The University of Chicago Press*, Chicago, 1959, Chapter 3.
27. F.E. Kohn, J.G. van Ommen and J. Feijen: The mechanism of the ring-opening polymerization of lactide and glycolide. *Eur. Polym. J.*, 19, 1081-1088, 1983.
28. R.L. Kronenthal: Biodegradable polymers in medicine and surgery. In: *Polymers in medicine and surgery*, editors R.L. Kronenthal, Z. Oser and E. Martin, Plenum Press, New York and London, 1975, pp. 119-137.
29. R.K. Kulkarni, K.C. Pani, C. Neuman, and F. Leonard: Polylactic acid for surgical implants, *Arch. Surg.*, 93, 839-843, 1966.
30. R.K. Kulkarni, K.C. Pani, C. Neuman and F. Leonard: Polylactic acid for surgical implants., *U.S. Nat. Tech. Inf. Serv.*, AD-report 636716, Apr. 1966.
31. O.J. Løkberg, K. Høgmoen and T. Gundersen: Use of ESPI to measure the vibration of the human eardrum in vivo and other biological movements. In: *Springer series in Optical Sciences*, Vol. 18. Holography in medicine and biology, editor G. von Bally. Springer Verlag, Berlin, 1979, pp. 212-217.
32. K.W. Marck, Ch.R.H. Wildevuur, W.L. Sederel, A. Bantjes and J. Feijen: Biodegradability and tissue reaction of random copolymers of L-leucine, L-aspartic acid and L-aspartic acid esters, *J. Biomed. Mater. Res.*, 11, 405-422, 1977.



33. A.T. Mennie, (Editor): Polyglycolic acid sutures. *Report of the Proceedings of the Symposium Royal College of Surgeons* (held on June 30th, 1970). Davies and Geck, London, 1972.
34. R.A. Miller, J.M. Brady and D.E. Cutright, Degradation rates of oral resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios, *J. Biomed. Mater. Res.*, 11, 711-719, 1977.
35. W. Moritz, Hörverbessernde Operationen bei chronisch-entzündlichen Prozessen beider Mittelohren. *Ztschr. Laryng. Rhin. Otol.*, 29, 578-583, 1950.
36. R.J. Nelson, R.A. White, R.S. Lawrence, M.D. Walkinshaw, F.L. Fiaschetti, E.W. White and F.M. Hirose: Development of a microporous tracheal prosthesis, *Trans. Am. Soc. Artif. Int. Organs*, Vol. XXV, 8-13, 1979.
37. U. Örtegren: Myringoplasty (four years' experience of temporal fascia grafts), *Acta Otolaryng.* (Stockholm), Suppl. 193, 1964.
38. M.E. Patterson: Experimental tympanic membrane closure with collagen film, *Arch. Otolaryng.*, 86, 486-489, 1967.
39. E. Pollock, E.J. Andrews, D. Lentz and K. Sheikh: Tissue ingrowth and porosity of Biomer, *Trans. Am. Soc. Artif. Intern Organs*, Vol. XXVII, 405-409, 1981.
40. Polysciences, Inc., Warrington, USA (catalog 1982-83, nr. 6529).
41. B. Salén and I. Simbach, Exogenous collagen in the closure of tympanic membrane perforations, *J. Laryngol. Otol.*, 79, 159-165, 1965.
42. W.J. Schrimpf: Repair of tympanic membrane perforations with human amniotic membrane, *Ann. Otol.*, 63, 102-115, 1954.
43. W.L. Sederel, A. Bantjes and J. Feijen, A route to anionic hydrophilic films of copolymers of L-leucine, L-aspartic acid and L-aspartic acid esters, *Polymer*, 16, 735-738, 1975.

44. W.L. Sederel: The potential of copoly (α-amino acids) based on L-aspartic acid and its esters as biomaterials, *Doctora Thesis*, Twente University of Technology, Enschede, The Netherlands, 1977.
45. R.G. Sinclair and G.M. Gynn: Preparation and evaluation of glycolic and lactic acid-based polymers for implant devices used in management of maxillofacial trauma, Part I., *U.S. Nat. Tech. Inf. Serv.*, AD-report 748410, February 1972.
46. K.H. Stenzel, T. Miyata and A.L. Rubin: Collagen: a biological plastic. In: *Polymers in medicine and surgery*, editors R.L. Kronenthal, Z. Oser and E. Martin, Plenum Press, New York and London, 1975, pp. 109-118.
47. L. Storrs: Myringoplasty, *Laryngoscope*, 76, 185-195 (1966).
48. J. Tonndorf and S.M. Khanna: The role of the tympanic membrane in middle ear transmission, *Ann. Otol.*, (St. Louis), 79, 743-753, 1970.
49. J. Tonndorf: New aspects of middle ear transmission, *Acta Oto-Rhino-Laryng.*, Belg., 28, 425-441, 1974.
50. Ch.M. Vest: *Holographic interferometry*, John Wiley and Sons, New York, 1979.
51. D.R. Watson and T.A. Maguda: An experimental study for closure of tympanic perforations with fascia and an adhesive, *South. Med. J.*, 58, 844-847, 1965.
52. S.A. Wesolowski, Ch.C. Fries, K.E. Karlson, M. de Bakey and Ph.N. Sawyer: Porosity: primary determinant of ultimate fate of synthetic vascular grafts, *Surg.* (St. Louis), 50, 91-96, 1961.
53. E.G. Wever and M. Lawrence: Physiological acoustics, *Princeton University Press*, Princeton, New York, 1954.
54. R.A. White, F.M. Hirose, R.W. Sproat, R.S. Lawrence and R.J. Nelson: Histopathologic observations after short-term implantation of two porous elastomers in dogs, *Biomaterials*, 2, 171-176, 1981.
55. D.F. Williams and R. Roaf: *Implants in surgery*, W.B. Saunders Co., London, 1973, pp. 203-297.



56. G.D. Winter, Transcutaneous implants: reactions of the skin implant interface, *J. Biomed. Mater. Res.*, Symposium, 5, 99-113, 1974.
57. H. Wullstein: Funtionelle Operationen im Mittelohr mit Hilfe des freien Spaltlappen-Transplantates, *Arch. Ohren-Nasen- un Kehlkophf*, 161, 422, 1952.
58. F. Zöllner, Die Radikal-Operation mit besonderem Bezug auf die Hörfunktion, *Ztschr.Laryng. Rhin. Otol.*, 30, 104, 1951.

CHAPTER VIII

# EXPERIMENTAL MYRINGOPLASTY*


## ABSTRACT

Artificial eardrums made from biodegradable poly(D,L-lactic acid), poly(glycolic acid) and poly(β-benzyl-L-aspartate-co-L-leucine) 50/50, and made from the microporous poly(tetrafluoroethylene) and bisphenol-A poly(carbonate) membranes were implanted into the ear and as a reference subcutaneously in rats.

The implants were histologically examined for periods up to one year. From the biodegradable polymers studied the poly(β-benzyl-L-aspartate-co-L-leucine) 50/50 evoked the least tissue reaction and the newly formed tympanic membranes are the best in terms of thickness and overall integrity. The microporous poly(tetrafluoroethylene) membrane can be considered as a valuable support for the formation of a reinforced tympanic membrane.


* L. Feenstra, B.W.C. van der Ven, F.E. Kohn and J. Feijen, Int. J. Artif. Organs, 3, 354-357 (1980).



## VIII.1 Introduction

A great number of autologous, homologous and heterologous grafts are currently used for the repair of a defect in tympanic membranes. These grafts act as a scaffold for the migrating epithelium from the border of the perforation. Usually the grafts disappear partially or completely after fulfilling their task. The resulting membrane may be satisfying, but sometimes it is too stiff or on the other hand too flabby. Moreover in the case of atelectatic ears or congenital anomalies one would like to do better than with the commonly used fascia or sometimes used homograft drum. Especially the late results obtained with the latter in our hands were very disappointing.

In this experimental study in rats we have tested a series of biodegradable synthetic polymers, poly(D,L-lactic acid) PLA, poly(glycolic acid) PGA and poly(β-benzyl-L-aspartate-co-L-leucine) 50/50 PBzAL as a temporary scaffold for the migrating epithelium.

Non-degradable microporous membranes made from poly(tetrafluoroethylene) PTFE and from bisphenol-A poly(carbonate) BAPC were also tested as a permanent reinforcement for a newly formed tympanic membrane.

The materials implanted in the ear were also implanted subcutaneously as a reference.

## VIII.2 Methods and Materials

For our experiments we used rats(Wistar, CPB-TNO-Rijswijk, 200 gm) free from otitis media. Under hypnorm® (0,1 ml/100 gm, i.m.) anaesthesia the right ear drum together with malleus and annulus was removed transmeatally. Subsequently a myringoplasty was performed using a circular artificial drum with a diameter of 2½ mm. The left ear served as a control.

Thereafter four artificial drums of the same material were implanted subcutaneously on the back. The wound was sutured with Dexon® 5.0.

Post-operatively and during the whole experiment strict aseptic precautions were taken to prevent mycoplasma infections. Also tetracycline was added (1 mg/100 gm/24 h) to the drinking water during the whole experiment. Each of the 5 different materials specified in Table I were implanted in 35 rats

After 1, 3, 5, 7, 12, 26, 52 weeks five rats per material were sacrificed with ether.

Subcutis of the back and both the bullae were removed and fixed in Bouin. The osseous tissue was decalcified in $HNO_3$ 6% during 24 hours, in $Na_2SO_4$ 5% during 24 hours and embedded in paraffin. Sections 7 µm thick were prepared in such a manner that the external auditory meatus was cut lenghtwise in cranio-caudal direction. The sections were stained with Azan.

POLY(D,L-LACTIC ACID) - The preparation of PLA via the ring-opening polymerization in the melt was based on the procedures developed by Kulkarni et al. (1966, a, b) and Sinclair and Gynn (1972).

Tetraphenyltin was used as the initiator ($7.10^{-5}$ mole/mole D,L-lactide). Careful purification and drying of the D,L-lactide proved to be necessary to obtain high molecular weight PLA, m.p. = 53°C. D,L-lactide was recrystallized several times from ethyl acetate and finally dried in a vacuum desiccator. Subsequently the D,L-lactide was treated with ice-cold dry ether prior to use. The D,L-lactide was placed in a polymerization tube (Sorenson and Campbell 1968) which was dried at 130°C for 24 hrs and the corresponding amount of initiator dissolved in a small amount of dry benzene was added. The benzene was removed under vacuum and the tube was placed in an oil bath. The D,L-lactide was sublimated onto the upper part of the tube at reduced pressure. After the sublimation was completed the tube was sealed under vacuum and placed in an oven at 178°C for about 15 hours. The resulting polymer was dissolved in acetone, precipitated in water and dried in vacuo at room temperature.

Thin films of high molecular weight PLA were cast from a 3.5% (w/v) solution in chloroform onto a glass plate using a doctor



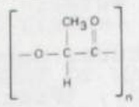
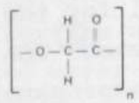
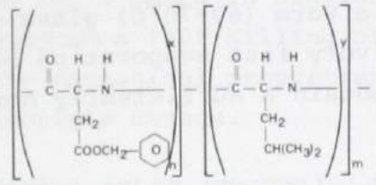
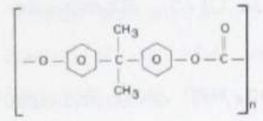
| MATERIALS | FORMULA | MOLECULAR WEIGHT | BIODEGRAD-ABLE | POROSITY (%) PORE DIAM. (MICRONS) | FILM THICKNESS (MICRONS) |
|---|---|---|---|---|---|
| Poly(D,L-lactic acid) |  | $M_w$ = 155.000 | + | non porous | 15 ± 2 |
| Poly(glycolic acid) |  | $M_w$ range 20 — 145.000 | + | non porous | 21 ± 3 |
| Poly(β-benzyl-L-aspartate-co-L-leucine) |  | ηspec/c = 0.8 dlg⁻¹ (c = 0.2 in $CHCl_3$) | + | non porous | 14 ± 3 |
| Polytetrafluoro-ethylene |  | | — | 95 10-15 | 13 ± 2 |
| Bisphenol-A poly(carbonate) |  | | — | 10 10 | 8 ± 1 |

Table 1. Some properties of the implanted materials.

knife (Sorenson and Campbell 1968).

The solvent was evaporated slowly in an atmosphere of saturated chloroform vapour. The dried film was removed from the glass plate after a short immersion in deionized water.

POLY(GLYCOLIC ACID) - Dexon® absorbable sutures purchased from Davis & Geck, Cyanamid of Great Britain Ltd., (Frazza 1971, Mennie 1972) were used as starting material for the PGA films. A warm (50-60°C), 2,5% (w/v) solution of Dexon® PGA in hexafluoroacetone sesquihydrate or hexafluoroisopropanol (Schadt et al. 1974) was spread onto a warm (60-70°C) glass plate. This procedure results in a very fast evaporation of the solvent which is necessary to obtain a sufficiently homogenous PGA film.

POLY(β-BENZYL-L-ASPARTATE-CO-L-LEUCINE) 50/50 - PBzAL, a 50/50 random copolymer of β-benzyl-L-aspartate and L-leucine was synthesized by co-polymerizing their N-carboxyanhydrides in a ratio of 50:50 mole % (Sederel et al. 1975, Stenneke 1976, Sederel 1977).

Films of PBzAL were cast from a 2% (w/v) chloroform solution in a similar way as the PLA films. Some properties of the above mentioned biodegradable polymers are summarized in Table I.

MICROPOROUS POLY(TETRAFLUOROETHYLENE) - A microporous Gore-Tex® PTFE membrane (Winter 1974) as specified in Table I was kindly donated by W.L. Gore & Co., Germany. As circular pieces of this thin membrane could hardly be manipulated, the material was implanted in combination with the above mentioned PLA as a support. For that purpose the Gore-Tex® membrane was spread over the PLA film and next circular pieces were cut from the double layer with a dinking punch. By this procedure the Gore-Tex® was folded slightly around the edges of the underlying PLA film yielding a stable 'double graft'. The great porosity and the pore sizes of the Gore-Tex® favour ingrowth of tissue.

MICROPOROUS BISPHENOL-A POLY(CARBONATE) - Microporous Nuclepore® BAPC membranes as specified in Table I were purchased from Nuclepore Corporation, California, U.S.A.. The pores in these Nuclepore® membranes are near perfectly round cylinders normal within ± 29°) to the surface with evenly random dispersion over the surface. The rather low porosity of Nuclepore® compared with that of Gore-Tex® is less favourable for ingrowth of tissue.

PRETREATMENT OF MATERIALS - The Nuclepore® membranes were washed with ethyl alcohol, the other materials with distilled water. After the artificial drums had been well moistened in a saturated water-vapour atmosphere for 24 hours, they were sterilized for 3 hours in ethylene oxide at 33°C. This procedure garantees a 100% killing of micro-organisms while neither the mechanical properties nor the chemical structure of the materials change.

VIII.3 Results

No otitis media was observed and no rat died untimely. No substantial differences were noted between the groups after one week. Some extravasation, an epithelial tongue progressing from the margins of the wound and cellular proliferation in the middle layer of the few remnants of the drum were seen. Repair activity was most important.

Biodegradable synthetic grafts

POLY(D,L-LACTIC ACID)

SUBCUTIS: After one week in the midst of a great cellular activity macrophages are noted joining into giant cells. The material is gradually encapsulated. The resulting capsular membrane grows thicker. Macrophages appear along the margins and in the middle of the material. After 26 weeks only some traces of material are left, after 52 weeks no foreign material is noted.

MIDDLE EAR: The same cellular activities are seen as in the subcutis. The resulting eardrum is rather thick in the beginning consisting of fibrous tissue, but gets thinner after 26 and 52 weeks and is eventually slightly thicker than a

normal drum.

POLY(GLYCOLIC ACID)

SUBCUTIS: In the beginning a very violent cellular acivity is observed. The PGA graft desintegrates rapidly into sharp needles. After 3 weeks no material could be detected at the site of implantation.

MIDDLE EAR: The cellular reaction is somewhat less than in the subcutis. After 3 weeks some material is still noted. After 5 weeks a slightly thickened tympanic membrane without foreign material is seen. The membranes remain, even after 52 weeks slightly thicker than a normal drum.

POLY(β-BENZYL-L-ASPARTATE-CO-L-LEUCINE) 50/50

SUBCUTIS: Due to an error the animals meant for sacrificing after 3 weeks were killed after 52 weeks. After 5 weeks a more or less stationary picture is observed consisting of some cellular activity with only a few giant cells. Very slowly a rather firm capsular membrane is formed. The material is degraded very slowly. After 26 weeks some foreign material is clearly seen, after 52 weeks none.

MIDDLE EAR: The same happens in the drum as subcutaneously but with slightly more giant cell formation. After 52 weeks only some traces of the foreign material are left.

The resulting membrane has a near normal appearance.

Non-degradable microporous membranes

MICROPOROUS POLY(TETRAFLUOROETHYLENE)

SUBCUTIS: Always both the tissue reaction on the PLA and PTFE had to be taken into account. In the beginning the tissue reaction caused by the PLA is predominant with lots of giant cells. Sometimes, however, both the materials showed a rumpled appearance in the histological slide. This led to difficulties in interpretation if the reaction was directed against the one or the other. In some places however it was obvious that the PTFE did not give rise to much cellular reaction. The material



is inert with hardly any giant cell formation.

Tissue has grown into the pores of the PTFE membrane.

MIDDLE EAR: Both types of reaction were also observed in the drum. With one drum a small polyp had formed with extrusion of the PLA. No PTFE was found in that case. After one year a medium thick membrane is present with only inert PTFE and no PLA. The PTFE has become part of the eardrum by ingrowth of tissue.

MICROPOROUS BISPHENOL-A POLY(CARBONATE)

SUBCUTIS: After one week already a thin syncytial layer is formed around the implant. Gradually this layer changes into a very thin fibrous tissue capsule with only sporadically a giant cell and no other cellular activity. It remains unaltered from the fifth week on. Hardly any tissue has grown into the pores of the BAPC membrane.

MIDDLE EAR: An identical picture is seen. Incidentally some connective tissue seems to be present in the pores of the material. Occasionally some phagocytic activity is noted. The BAPC membrane was not incorporated in the eardrum by ingrowth of tissue.

## VIII.4 Discussion

BIODEGRADABLE SYNTHETIC GRAFTS

The PLA synthetic graft causes a moderately intense cellular activity with gradual encapsulation and absorption of the implant. Although in the beginning the tympanic membrane is quite thick at the end it compares favourably with a normal tympanic membrane. PLA might be considered as an acceptable grafting material.

The PGA graft gives rise to a rather intense cellular reaction presumably as a result of its rapid desintegration into sharp needles. The resulting tympanic membranes are slightly thickened. On the basis of this we do not consider PGA as a good grafting material for eardrum reconstruction.

The PBzAL graft evokes only little tissue reaction and sporadic giant cell formation. After half a year a significant amount, after one year only traces of the implant are left. The resulting tympanic membranes are the best in terms of thickness and overall integrity.

NON-DEGRADABLE MICROPOROUS MEMBRANES

Microporous PTFE membranes were implanted in combination with PLA film because the pure material could not be handled as such. After degradation of the PLA hardly any tissue reaction was present near the PTFE membrane. It therefore becomes part of the newly formed eardrum. In the future this membrane might be used to reconstruct a missing fibrous layer of an eardrum.

The microporous BAPC membranes were stiff and became surrounded with a very thin capsule of tissue. Hardly any tissue had invaded the pores. Although they do not have the mechanical qualities necessary for an eardrum they might be used as a frame to strengthen an atrophic part or to secure an ossicular chain.

## VIII.5 Conclusion

In the past we have studied tympanic membrane grafting in rats using homologous eardrum (Feenstra and Feenstra 1975), and also using autologous fascia, homologous and heterologous collagen, heterologous amnion and proplast (Feenstra e.a. 1978). The methods used in our former experiments were identical except for a few minor details. Only the results obtained with heterologous collagen seem comparable to those obtained with the synthetic PBzAL grafts.

In conclusion from this series the synthetic grafts made from PBzAL and the microporous PTFE membranes are the most promising for tympanic membranes grafting. These grafts are currently evaluated in dogs.



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References


1. Feenstra, L., and Feenstra, K.: Studies on tympanic membrane transplants. *ORL 37*, 149-60 (1975).
2. Feenstra, L., Baarsma, R., Feijen, J., Houthoff, H.J. Experimental myringoplasty. *J. Bioengineering* 2, 251-58 (1978).
3. Frazza, E.J. A new absorbable suture. *J. Biomed. Mater. Res.*, Symposium, 1, 43-58 (1971).
4. Kulkarni, R.K., Pani, K.C., Neuman, C., and Leonard, F. Polylactic acid for surgical implants. *Archives in Surgery*, 93, 839-43 (1966).
5. Kulkarni, R.K., Pani, K.C., Neuman, C. and Leonard, F. Polylactic acid for surgical implants. *U.S. AD-report* 636716, Apr. 1966 .
6. Mennie, A.T.,(ed). Polyglycolic acid sutures. *Report of the Proceedings of the Symposium Royal College of Surgeons* (held on June 30th, 1970). Davis and Geck, London (1972).
7. Schadt, F.L., Schleyer, P.v.R., and Bentley, T.W. Hexafluoroisopropanol - a solvent of high ionizing power and low nucleophilicity. *Tetrahedron Letters*, 27, 2335-8 (1974).
8. Sederel, W.L., Bantjes, A., and Feijen, J. A route to anionic hydrophilic films of copolymers of L-leucine, L-aspartic acid and L-aspartic acid esters. *Polymer*, 16, 735-8 (1975).
9. Sederel, W.L. The potential of copoly(α-amino acids) based on L-aspartic acid and its esters as biomaterials. *Doctorsthesis*, Twente University of Technology, Enschede, The Netherlands (1977).
10. Sinclair, R.G., and Gynn, G.M. Preparation and evaluation of glycolic and lactic acid-based polymers for implant devices used in management of maxillofacial trauma. *Part. II U.S. AD-report*, 748411, July 1972.
11. Sorenson, W.L., and Campbell, T.W. Preparative methods of polymer chemistry, *2nd ed. Interscience Publ. New York* (1968).





12. Stenneke, B. *Ingenieurs-Thesis*, Twente University of Technology, Enschede, The Netherlands (1976).
13. Winter, G.D. Transcutaneous implants: reactions of the skin implant interface. *J. Biomed. Mater. Res.*, Symposium, 5, 99-113 (1974).

SUMMARY

After trauma or inflammation an eardrum may become permanently perforated, frequently resulting in some hearing loss and proneness to infection. Until 1950 the treatment of a perforated eardrum consisted of covering the drum permanently with artificial material.
Improvement in hearing seldom occurred. Since then a surgical technique to establish a functional reconstruction of the eardrum was developed. The principle of this technique which is called myringoplasty, is based on covering the drum perforation with a collagenous structure - i.e. the graft - which must function as a scaffold for the migrating epithelial tissue from the edges of the perforation. During the healing process which may take several months the scaffolding structure is expected to be absorbed gradually. There are a number of reasons why grafting materials of biological origin may prove to be inadequate - e.g. the myringoplastic operation may fail because of premature desintegration of the graft.

Biomedical research activities over the last twenty years have resulted in an - as yet - limited number of well-functioning synthetic biodegradable polymers, having a predictable rate of absorption and loss of strength. Amongst these are the poly(α-hydroxy acids) and the poly(α-amino acids).

The research activities reported about in this thesis were concentrated on three subjects:

- The chemistry and characterization of poly(D,L-lactic acid), the synthetic biodegradable poly(α-hydroxy acid) which was extensively studied in experimental myringoplasty (Chapters II, III, IV and V).
- The chemistry of linear, alternating polydepsipeptides, a potentially new class of synthetic biodegradable polymers (Chapter VI).
- The application of a number of biodegradable and also microporous non-degradable synthetic polymers in experimental myringoplasty.



The preferred method for the preparation of high molecular weight poly(D,L-lactic acid) is the ring-opening polymerization of the six-membered cyclic diester D,L-lactide (m.p. 126°C) in the melt, initiated with a suitable initiator, - e.g. tetraphenyltin.

In Chapter II the structure of the monomer D,L-lactide (m.p. 126°C) was discussed. From X-ray structure elucidation results it was concluded that D,L-lactide (m.p. 126°C) is in fact a 1:1 racemate of L-,L-lactide (m.p. 95°C) and D-,D-lactide (m.p. 95°C).

To obtain more insight in the mechanism of the ring-opening polymerization of D,L-lactide or - in a broader sense - of lactones, in Chapter III complex formation of the (potential) initiators tetraphenyltin, stannous octoate, tin tetrachloride, aluminium bromide and triisobutylaluminium (TIBA), and the monomers L(-)-lactide, D,L-lactide and glycolide was studied by IR-spectroscopy. When equimolar benzene or toluene solutions of initiators and monomers were combined, only complexes of aluminium bromide and D,L-lactide or glycolide, and of TIBA and D,L-lactide or glycolide were observed. It is concluded that complexes are formed by coordination of a carbonyl oxygen of the monomer and the aluminium atom of the initiator - whereas on the basis of existing theories a coordination with the ether oxygen of the monomer had been more likely. From concomitant solution polymerization studies it is concluded that in the case of $AlBr_3$ the actual initiating species is HBr and that the polymerization initiated with TIBA proceeds very probably through a coordinated insertion of the lactone monomer into the aluminium-carbon bond.

In Chapter IV melt polymerization conditions for D,L-lactide initiated with tetraphenyltin were studied with regard to polymer molecular weight and weight distribution. 'Single' polymerizations using constant initiator concentrations resulted in a broad scattering of non-reproducible molecular weight values. 'Multiple' polymerizations (four or eight reactions at the same time) at constant initiator concentrations, however,

resulted in nearly identical molecular weight profiles. 'Multiple' polymerizations at different initiator concentrations did not show an inverse dependency of initiator concentrations on polymer molecular weight. Both the 'single' and 'multiple' melt polymerizations resulted in rather broad molecular weight distributions. The presence of hydrolysis products of lactide during the melt polymerization most likely has a detrimental effect on molecular weight. After a short induction period the rather slow polymerization of D,L-lactide resulted in maximal molecular weight followed by a slight decrease in molecular weight to a constant value. It is concluded that the polymerization of D,L-lactide in the melt initiated with tetraphenyltin does not proceed through a 'living' mechanism.

In Chapter V the molecular weight distribution of a number of poly(D,L-lactic acid) samples having $\bar{M}_w$'s in the range of 15,000-350,000 were studied in greater detail by gel permeation chromatography. The method involved a universal calibration of the columns on the basis of polystyrene standards and a rapid iteration algorithm leading to the establishment of the Mark-Houwink relationship. In addition, osmometry and viscometry data were presented. The effect of hydrolytic degradation on the molecular weight distribution of two poly(D,L-lactic acid) samples was studied by gel permeation chromatography.

In Chapter VI it is argued that linear, alternating polydepsipeptides will be a valuable supplement to the existing synthetic biodegradable polymers. For their preparation a new synthetic route is suggested which involves the lactone or lactam type ring-opening polymerization of 2,5-morpholinediones. It was found that these rather unknown heterocyclic compounds could only be prepared with considerable difficulties via a cyclization reaction of an 'α-halogenacyl-α-amino acid - e.g. via a sublimation of the sodium salt of N-chloroacetylglycine or N-D,L-α-bromopropionylglycine. It is demonstrated that the cyclization of an 'α-halogenacyl-α-amino acid is impeded by the high trans-cis energy barrier for the (non-alkylated) amide bond. A small number of lactone type polymerization attempts were performed. The attempted melt polymerization of unsubstituted



2,5-morpholinedione at about 190°C, initiated with tetraphenyltin, only resulted in excessive charring. There were some indications that the attempted melt polymerization of 6-methyl-2,5-morpholinedione at 130 or 150°C, initiated with stannous octoate, had led to polymeric products.

In Chapter VII it is shown that biodegradable and microporous non-degradable synthetic polymers may prove to be a valuable supplement to the existing biological grafting materials used in myringoplastic operations. Artificial eardrums made from several biodegradable poly(α-hydroxy acids) and poly(α-amino acids) and made from a number of microporous poly(tetrafluoroethylene) membranes and from a microporous bisphenol-A poly(carbonate) membrane were implanted into the ears of rats and dogs and as a reference subcutaneously. The implants were histologically examined for periods up to one year. From the biodegradable polymers studied, poly(β-benzyl-L-aspartate-co-L-leucine) 50/50 evoked the least tissue reaction and the newly formed eardrums were the best in terms of thickness and overall integrity. The formation of a reinforced eardrum may be accomplished by the support of an inert, very thin, highly porous poly(tetrafluoroethylene) membrane preferably implanted as a composite graft with a biodegradable polymer.

Finally in Chapter VIII a part of the experimental myringoplastic studies already mentioned in Chapter VII is presented in greater detail - e.g. the fabrication methods for the synthetic eardrum grafts.

## SAMENVATTING *

Als gevolg van letsel of als gevolg van een ontsteking kan een trommelvlies blijvend een perforatie oplopen, hetgeen dikwijls resulteert in een verslechtering van het gehoor en een verhoogde vatbaarheid voor infekties. Tot 1950 kwam de behandeling van een geperforeerd trommelvlies neer op een blijvend bedekken van het vlies met lichaamsvreemd materiaal, bijvoorbeeld met de luchtblaas van vissen, India rubber, ei-membraan en ook siliconenrubber is wel toegepast. Deze behandeling leidde zelden tot gehoorverbetering.
Sinds die tijd kwam een chirurgische techniek in zwang die tot doel heeft een funktionele rekonstruktie van het trommelvlies te bewerkstelligen. Het principe van deze techniek, genaamd myringoplastiek, is gebaseerd op het bedekken van de trommelvliesperforatie met een dun collageen-bindweefselachtig vlies, de zogenaamde 'plastieklap'. De plastieklap moet funktioneren als een ondersteunende struktuur voor de aangroei van nieuw epitheelweefsel vanaf de -geaktiveerde- wondranden van de perforatie. Gedurende het helingsproces, dat een aantal maanden in beslag kan nemen, behoort de ondersteunende struktuur geleidelijk aan geabsorbeerd te worden.

Bijna alle materialen die tot nu toe als plastieklap werden gebruikt zijn van biologische oorsprong -bijvoorbeeld fascie van de patiënt zelf- hetgeen in de praktijk betekent dat de snelheid waarmee ze geabsorbeerd worden onvoldoende controleerbaar is. Myringoplastiek-operaties kunnen dan ook falen vanwege een voortijdige desintegratie van plastieklappen van het konventionele weefsel-type en ook kunnen zulke plastieklappen een aanmerkelijke antigeniteit veroorzaken of een vrij intense ontstekingsreaktie van het aanliggende weefsel. Nog een andere reden waarom plastieklappen van biologische oorsprong minder geschikt kunnen blijken te zijn, houdt verband met de behandeling van gekompliceerde trommelvliesdefekten, bijvoorbeeld in geval van totale of subtotale perforaties, atelektatische oren of congenitale afwijkingen. Ten behoeve van de behandeling van dergelijke defekten worden aan de plastieklappen dikwijls zodanig



specifieke mechanische eisen en vormeisen gesteld, dat deze niet op bevredigende wijze geprepareerd kunnen worden uit de tamelijk zachte weefsels van biologische oorsprong.

Biomedisch onderzoek gedurende de afgelopen 20 jaar laat zien dat er vanuit de genees- en heelkunde een toenemende belangstelling is voor de toepassing van synthetische, in het lichaam afbreekbare kunststoffen die op een voorspelbare wijze geabsorbeerd worden en daarbij op een voorspelbare wijze hun mechanische sterkte verliezen.
Het aantal goed funktionerende synthetische, biodegradeerbare kunststoffen is vooralsnog beperkt. De poly(α-hydroxyzuren) -met name poly(glycolzuur), poly(melkzuur) en hun co-polymeren- en de poly(α-aminozuren) vertegenwoordigen twee belangrijke klassen van synthetische, biodegradeerbare kunststoffen die, -ten dele alleen nog in de dier-experimentele situatie, maar ten dele ook reeds in de klinische situatie- toegepast zijn, of nog steeds worden: bijvoorbeeld als in het lichaam afbreekbaar hechtmateriaal ter vervanging van het reeds eeuwen gebruikte hechtmateriaal op natuurlijke eiwitbasis, als afdekking van brandwonden, als gecontroleerd doseringssysteem voor medicijnen, en als biodegradeerbare protheses in de orthopedische en plastische aangezichtschirurgie.

Het onderzoek waarover in dit proefschrift gerapporteerd wordt -en in samenhangend verband beschreven in hoofdstuk I- heeft zich gericht op de volgende drie hoofd-onderwerpen:

- De chemie en karakterisering van poly(D,L-melkzuur), een synthetische, in het lichaam afbreekbare kunststof die uitvoerig toegepast en geëvalueerd werd als tijdelijk trommelvliessubstituut in dier-experimenteel onderzoek (hoofdstukken II, III, IV en V);
- De chemie van lineaire, alternerende polydepsipeptiden, een potentieel nieuwe klasse van synthetische, in het lichaam afbreekbare kunststoffen (hoofdstuk VI);
- De toepassing en evaluatie van een aantal in het lichaam afbreekbare, en ook microporeuze, niet-afbreekbare synthetische

kunststoffen als (tijdelijke) trommelvliessubstituten in dier-experimentele studies (experimentele myringoplastiek).

Hoogmolekulair poly(D,L-melkzuur), dat geschikt is om verwerkt te worden tot implantaten, wordt bij voorkeur gesynthetiseerd door middel van de ring-openingpolymerisatie van de heterocyclische 6-ring diester D,L-lactide (smeltpunt 126°C) in de smeltfase, geïnitieerd door een geschikte initiator. De opgedane ervaring dat hoogmolekulair poly(D,L-melkzuur) niet op een reproduceerbare wijze gesynthetiseerd kon worden via de smeltfase polymerisatie van D,L-lactide in de aanwezigheid van tetrafenyltin als de initiator, heeft geleid tot een meer gedetailleerde studie van de chemie van dit biodegradeebare poly(α-hydroxyzuur).

Uit literatuurgegevens was tot op heden niet eenduidig te konkluderen of D,L-lactide (smeltpunt 126°C) bestaat uit het zogenaamde meso-lactide molekuul -dat wil zeggen dat ieder lactide molekuul gevormd wordt door één linksdraaiend en één rechtsdraaiend lactaat residu- dan wel dat D,L-lactide in feite een 1:1 racemaat is van L-,L-lactide en D-,D-lactide, die beide een smeltpunt 95°C hebben. Op basis van röntgendiffraktie-onderzoek, beschreven in hoofdstuk II, kon eenduidig vastgesteld worden dat D,L-lactide (smeltpunt 126°C) inderdaad het bedoelde 1:1 racemaat is, hetgeen onder meer impliceert dat poly(D,L-melkzuur) stereo-isotaktische ketendelen kan bevatten van meer dan twee links- of rechtsdraaiende lactaat residuen.

D,L-lactide kan beschouwd worden als een gemodificeerd lacton, in die zin dat de ringverbinding twee estergroepen bevat en een lacton-ring gewoonlijk één. Hoewel in diverse publikaties mechanismen zijn voorgesteld voor de initiatie of -in ruimere zin- voor de polymerisatie van lactonen, is het precieze mechanisme -of zijn de precieze mechanismen- nog steeds in diskussie. Daarenboven zijn er zo goed als geen gegevens beschikbaar over de interaktie tussen de initiator en het lacton monomeer juist voor de feitelijke ring-opening plaats vindt. Dit laatste is met name het onderwerp geweest van het onderzoek besproken in hoofdstuk III. De gevolgde werkwijze hield in dat



met behulp van IR-spektroskopie de komplexvorming in oplossing onderzocht werd van een aantal initiatoren met D,L-lactide, L-lactide of glycolide. De (potentiële) initiatoren waren tetrafenyltin, het tin-(II)-zout van 2-ethylhexaanzuur, tintetrachloride, aluminiumbromide en triïsobutylaluminium (TIBA). Wanneer equimolaire oplossingen van initiator en monomeer in benzeen of tolueen bijeengevoegd werden, werden alleen complexen waargenomen van aluminiumbromide met D,L-lactide of glycolide en van TIBA met D,L-lactide of glycolide. De complexvorming werd meer gedetailleerd bestudeerd door de initiator en monomeer concentraties te variëren. De resultaten van de complexvormingsstudie in kombinatie met overwegingen van theoretische aard, leidde tot de konklusie dat komplexen gevormd worden door koördinatie van het aluminiumatoom van de initiator met een carbonylzuurstof van het monomeer, terwijl op basis van op dat moment bekende theoriën koördinatie met een etherzuurstof van het monomeer meer in de lijn der verwachting lag. De overeenkomstige polymeren werden gevormd wanneer onder water-vrije omstandigheden TIBA werd toegepast als initiator. Wanneer aluminiumbromide als iniitator werd toegepast, werden alleen polymeren verkregen na toevoeging van spore-hoeveelheden water. Dit leidde tot de konklusie dat in geval van $AlBr_3$ het werkelijk initiërende deeltje HBr is, en dat de polymerisatie geïnitieerd door TIBA met een grote waarschijnlijkheid start via een koördinatie-insertie van het lacton monomeer in de aluminium-koolstofband.

De niet-reproduceerbaarheid van het -gemiddelde- polymeer-molekuulgewicht tijdens de smeltfase polymerisatie van D,L-lactide geïnitieerd door tetrafenyltin werd reeds genoemd. In hoofdstuk IV worden de resultaten beschreven van een studie die tot doel had de relatie te onderzoeken tussen de omstandigheden waaronder de smeltfase polymerisatie wordt uitgevoerd èn het molekuulgewicht en de molekuulgewichtsverdeling van de gevormde polymeren. De studie betrof zogenaamde 'enkelvoudige' polymerisaties, 'meervoudige' polymerisaties (waarbij 4 of 8 polymerisatiereakties gelijktijdig werden voorbereid en uitgevoerd) en 'tijdsafhankelijke' polymerisaties. 'Enkelvoudige' polymerisaties uitgevoerd bij konstante initiatorkoncentraties resulteerden

in een grote spreiding van niet-reproduceerbare molekuulgewichten. "Meervoudige' polymerisaties uitgevoerd bij konstante initiatorkoncentraties resulteerden daarentegen in bijna identieke molekuulgewichtsverdelingen. Wanneer 'meervoudige' polymerisaties bij verschillende initiatorkoncentraties werden uitgevoerd, werd geen inverse relatie gevonden tussen initiatorkoncentratie en polymeer-molecuulgewicht. Zowel de 'enkelvoudige' als de 'meervoudige' smeltfase polymerisaties resulteerden in tamelijk brede molekuulgewichtsverdelingen. Op basis van resultaten van smeltfase polymerisaties van D,L-lactide uitgevoerd in de aanwezigheid van hydrolyse-produkten van lactide werd vastgesteld dat deze laatste de hoogte van het polymeer-molekuulgewicht vermoedelijk sterk nadelig beïnvloeden. Polymerisaties van het 'meervoudige'-type werden ook uitgevoerd om de invloed te bestuderen van de polymerisatietijd op de omzetting van D,L-lactide en op het polymeer-molekuulgewicht. Uit de resultaten van deze 'tijdsafhankelijke' polymerisatiestudies werd gekonkludeerd dat een tamelijk langzame polymerisatie van D,L-lactide, die voorafgegaan wordt door een korte induktieperiode, resulteert in een maximum polymeer-molekuulgewicht dat vervolgens enigermate afneemt tot een konstante waarde. Tenslotte wordt aan het eind van hoofdstuk IV beredeneerd waarom de polymerisatie van D,L-lactide in de smeltfase, geïnitieerd door tetrafenyltin niet volgens een 'levend' mechanisme verloopt.

Er bestaan duidelijke aanwijzingen dat de wijze van degradatie van in het lichaam afbreekbare kunststoffen mede beïnvloed wordt door het polymeer-molekuulgewicht en de molekuulgewichtsverdeling. Tot op heden zijn gegevens over het molekuulgewicht van poly(D,L-melkzuur) -of van poly(L-melkzuur)- maar in beperkte mate voorhanden. Nog minder systematische gegevens zijn tot nu toe gepubliceerd over de molekuulgewichtsverdeling als onderdeel van de karakterisering van poly(melkzuur). Daarom werden meerdere poly(D,L-melkzuur) monsters uit deze studie met gewichtsgemiddelde molekuulgewichten $\overline{M}_w$ in de orde 15.000 - 350.000 uitvoerig gekarakteriseerd. De resultaten zijn weergegeven in hoofdstuk V. Molekuulgewichtsverdelingen van de monsters werden bepaald door middel van hoge-druk-gel-permeatie-chromatografie. De gevolgde methode bestond uit een universele kalibratie van de GPC-kolommen op basis van polystyreen-standaards met zeer smalle molekuulgewichtsverdelingen en een wiskundig iteratieproces met behulp waarvan de parameters van de Mark-Houwink relatie vastgesteld konden worden. Van negen op deze wijze gekarakteriseerde monsters met gewichtsgemiddelde molekuulgewichten 35.000 - 350.000 werden waarden voor $\overline{M}_w/\overline{M}_n$ gevonden tussen 2 en 3. Twee monsters met lagere $\overline{M}_w$'s hadden aanzienlijk bredere verdelingen. Daarnaast werden de relevante osmometrie en viskosimetrie gegevens gepresenteerd. Tenslotte werd het effekt van <u>in vitro</u> hydrolytische degradatie op de molekuulgewichtsverdeling van twee monsters poly(D,L-melkzuur) door middel van GPC bestudeerd. Verrassenderwijs werden toen ook waarden voor $\overline{M}_w/\overline{M}_n < 2$ gevonden en zelfs een waarde zeer dicht bij één.



Zoals reeds vermeld in de aanhef van deze samenvatting, vertegenwoordigen de poly(α-hydroxyzuren) en de poly(α-aminozuren) twee belangrijke klassen van geaccepteerde, in het lichaam afbreekbare kunststoffen. Anderssoortige in het lichaam afbreekbare synthetische kunststoffen met verschillende degradatiekarakteristieken zouden een waardevolle aanvulling hierop kunnen betekenen -bijvoorbeeld een kunststof die in zich eigenschappen kombineert van zowel een α-hydroxyzuur homo-polymeer en van een α-aminozuur homo-polymeer. Daarom wordt in hoofdstuk VI aandacht besteed aan een potentieel nieuwe klasse van in het lichaam afbreekbare synthetische kunststoffen: de lineaire, alternerende polydepsipeptiden, dat wil zeggen polymeerketens waarin ester en amide groepen elkaar afwisselen terwijl ze gescheiden zijn door steeds één koolstofatoom. Tot nu toe konden lineaire, alternerende polydepsipeptiden slechts op zeer kleine schaal gesynthetiseerd worden volgens bepaalde multi-step syntheses, ontwikkeld voor de bereiding van polypeptiden. In hoofdstuk VI wordt beredeneerd dat, in principe, lineaire, alternerende polydepsipeptiden ook in één stap en op grotere schaal gesynthetiseerd kunnen worden via een lacton of lactam type ring-opening polymerisatie van 2,5-morfolinedionen. Deze laatste heterocyclische 6-ring verbindingen, die in de literatuur maar sporadisch beschreven zijn, bevatten zowel een α-hydroxyzuur residu als een α-aminozuur residu. In

de huidige studie werd gevonden dat 2,5-morfolinedionen slechts met aanzienlijke moeite gesynthetiseerd konden worden via een cycliseringsreaktie van een 'α-halogeenacyl-α-aminozuur. Kleine hoeveelheden ongesubstitueerd 2,5-morfolinedion konden worden bereid via sublimatie van het natrium zout van N-chlooracetylglycine in de aanwezigheid van 'aktief' koper; door de aanwezigheid van het koper wordt vermoedelijk de thermische geleidbaarheid van de te verwarmen massa sterk verhoogd waardoor teervorming duidelijk vermindert, terwijl niet uitgesloten wordt dat het koper ook een katalyserend effekt heeft op de ringsluitingsreaktie. 6-Methyl-2,5-morfolidion kon wat eenvoudiger gesynthetiseerd worden via droge destillatie van het natriumzout van N-D,L-α-broompropionylglycine. Ongesubstitueerd 2,5-morfolinedion bleek niet of nauwelijks oplosbaar in 'gewone' organische oplosmiddelen. Het is daarentegen zeer goed oplosbaar in water. Ongesubstitueerde 2,5-morfolinedion kon gezuiverd worden via omkristallisatie uit een ruime overmaat droge methanol. De molekulaire struktuur van 2,5-morfolinedion, 6-methyl-2,5-morfolinedion en hun lineaire uitgangsverbindingen konden geverifieerd worden door middel van IR-, $^{1}$H-NMR —, en massaspektrometrie. Gebaseerd op deze resultaten en theoretische overwegingen werd gekonkludeerd dat de ringsluiting van een 'α-halogeenacyl-α-aminozuur vermoedelijk gehinderd wordt door de hoge trans-cis energie barrière voor de (niet-gealkyleerde) amide band. Aangezien slechts geringe hoeveelheden 2,5-morfolinedion en 6-methyl-2,5-morfolinedion gesynthetiseerd konden worden, kon de polymeriseerbaarheid van deze heterocyclische verbindingen vooralsnog niet systematisch onderzocht worden en werd slechts een beperkt aantal lacton-type ring-opening-polymerisatiepogingen uitgevoerd. De pogingen ongesubstitueerde 2,5-morfolinedion in de smeltfase te polymeriseren met tetrafenyltin als initiator resulteerden slechts in sterke teervorming, vermoedelijk als gevolg van de hoge reaktietemperatuur die noodzakelijk was vanwege het hoge smeltpunt van 2,5-morfolinedion (191-193$^{o}$C). Aangezien 6-methyl-2,5-morfolinedion bij een veel lagere temperatuur smelt -96$^{o}$C- konden smeltfasepolymerisaties onder aanzienlijk mildere omstandigheden worden uit-



gevoerd. Wanneer het tin-(II)-zout van 2-ethylhexaanzuur werd gebruikt als initiator, werden glasachtige, 'kunststofachtige' produkten verkregen, die helaas niet op molekuulgewicht gekarakteriseerd konden worden vanwege onoplosbaarheid in tetrahydrofuraan, het standaardoplosmiddel van de beschikbare lage-druk-gel-permeatie-chromatograaf. IR-spektroskopisch onderzoek wees op de vorming van een lineair produkt. Wanneer geen initiator werd toegevoegd, werd, onder dezelfde polymerisatieomstandigheden, naast een oligomeer een grote hoeveelheid niet-gereageerd monomeer teruggewonnen.

De laatste twee hoofdstukken zijn gewijd aan de toepassing van een aantal in het lichaam afbreekbare en microporeuze, niet-afbreekbare synthetische kunststoffen als (tijdelijke) trommelvliessubstituten in dier-experimentele studies (experimentele myringoplastiek).

Hoofdstuk VII begint met een beschrijving van de anatomie en funktie van het menselijk trommelvlies en met een historisch overzicht van de behandeling van geperforeerde trommelvliezen. Vervolgens wordt een verantwoording afgelegd van de filosofie dat (tijdelijke) trommelvliessubstituten van synthetische kunststoffen een waardevolle aanvulling kunnen betekenen op de momenteel toegepaste plastieklappen van biologische oorsprong (zie ook de aanhef van deze samenvatting). In het tweede deel van hoofdstuk VII worden de resultaten beschreven van de experimentele myringoplastiek-studies. Daartoe werden trommelvliessubstituten gemaakt van diverse, in het lichaam afbreekbare poly (α-hydroxyzuren) en poly(α-aminozuren), èn gemaakt van een aantal microporeuze poly(tetrafluoretheën) membranen en van een microporeus bisfenol-A poly(carbonaat) membraan, geïmplanteerd in oren van ratten en honden, en als een referentie onderhuids. Weefselreakties werden gedurende een periode van totaal een jaar bestudeerd. Van de onderzochte, in het lichaam afbreekbare synthetische kunststoffen bleek poly(β-benzyl-L-aspartaat-co-L-leucine) 50/50 de geringste weefselreaktie te veroorzaken, terwijl het nieuw gevormde trommelvlies het best overeenkwam met een normaal trommelvlies. Een andere doelstelling, de vorming van een 'versterkt' trommelvlies, lijkt in

principe gerealiseerd te kunnen worden door als weefselingroei-ondersteunende struktuur een inert, zeer dun en zeer poreus poly(tetrafluoretheen) membraan te gebruiken dat bij voorkeur geïmplanteerd wordt als een 'composiet-substituut' met een in het lichaam afbreekbare synthetische kunststof. Op basis van de gevonden resultaten worden enkele aanbevelingen gedaan voor verder (klinisch) onderzoek.

Tenslotte wordt in hoofdstuk VIII een deel van het myringoplastiek dier-experimenteel onderzoek, reeds beschreven in hoofdstuk VII, in meer gedetailleerde vorm uitgewerkt, waaronder de fabrikage van de (tijdelijke) synthetische trommelvliessubstituten.

*TITEL: Ontwikkeling van in het lichaam afbreekbare kunststoffen voor toepassing in de myringoplastiek.



LEVENSLOOP

Frederik Emiel Kohn werd op 28 maart 1947 geboren te Hengelo (o) alwaar de openbare kleuterschool, lagere school en het openbaar gymnasium De Bataafse Kamp werden bezocht. Na het behalen van het diploma gymnasium-β ging hij in 1965 chemische technologie -voorafgegaan toen nog door één jaar algemene propadeuse- studeren aan de TH Twente. In 1969 werd het baccaleureaatsdiploma en in 1972 het ingenieursdiploma behaald, beide met als hoofdrichtingen macromoleculaire materiaalkunde en -techniek. Vervolgens werden de militaire verplichtingen vervuld en was hij in 1974 en 1975 werkzaam als research fellow in de Biomedical Engineering Unit van het North Staffordshire hospital in Stoke-on-Trent, Engeland. In de periode medio 1976 - medio 1981 werd het onderzoek verricht dat ten grondslag ligt aan dit proefschrift en was er een tijdelijk dienstverband van 3½ jaar als wetenschappelijk ambtenaar aan de afdeling Keel- Neus- en Oorheelkunde van de VU te Amsterdam, terwijl de overige promotietijd gefinancierd werd door de TH Twente.
Gedurende bijna de gehele onderzoekperiode was Frits Kohn gestationeerd binnen de vakgroep Macromoleculaire Chemie en Materiaallunde, afdeling Chemische Technologie, TH Twente.
Sinds 1 oktober 1981 is hij werkzaam binnen de afdeling produktontwikkeling van Firet B.V. te Veenendaal, producent van non-woven vliezen.

# DEVELOPMENT OF BIODEGRADABLE POLYMERS FOR APPLICATION IN MYRINGOPLASTY

**STELLINGEN**

**Frits kohn**
30 maart 1984

**1.** Voor de productie van nonwoven vliezen wordt tegenwoordig veel gebruik gemaakt van de zogenaamde random-kaard als vliesvormer.
De vezeloriëntatie in de nog ongebonden vliezen gevormd op de random-kaard is duidelijk minder random dan op basis van de naam van deze kaard verwacht mag worden.

**2.** De bewering van Brydson, dat van nylon-4,6 via het smeltspinproces geen goede vezels te produceren zijn, is aan bedenkingen onderhevig.

J.A. Brydson, Plastic materials, 4th ed., Butterworth Scientific, London 1982, p. 460.

**3.** De Stokes-straal is waarschijnlijk geen goede maat voor het door een macromolecuul in oplossing meegesleepte volume aan oplosmiddel, dat aan het macromolecuul 'gebonden' is door de zogenaamde "hydrodynamische interactie".

P.F. Mijnlieff en F.W. Wiegel, Physica, 85a, 207 (1976).

**4.** Day en Wright hebben bij hun onderzoek naar de thermische degradatie van polyepichloorhydrine rubbers de invloed van de hoeveelheid zuuracceptor onderschat.

J. Day en W.W. Wright, Brit. Polym. J., March 1977, 66-71.

**5.** Verschillende onderzoekers hebben getracht bloedcompatibele materialen te vervaardigen door heparine aan het materiaaloppervlak te binden. In de literatuur worden voor zulke zogenaamde geheparineerde oppervlakken geen eensluidende resultaten vermeld m.b.t. de bloedplaatjesadhesie en de contact-activering van het stollingsproces. De bedoelde verschillen kunnen grotendeels worden verklaard door het al dan niet weglekken van heparine van het oppervlak.

R. Larsson, G. Edwall, H. Lagergren, E. Nilsson en P. Olsson, Proc. Eur. Soc. Artif. Organs, VII, 76-79 (1980).

N.A. Platé en L.I. Valuev, Biomaterials, 4, 14-20 (1982).

E.W. Salzman, R.D. Rosenberg, M.H. Smith, J.N. Lindon en L. Favreau, J. Clin. Invest, 65, 64-73 (1980).

M.F.A. Goosen and M.V. Sefton, J. Biomed Mater. Res., 17, 359-373 (1983).

**6.** Het dient vermeden te worden dat de financiering van fundamenteel onderzoek van hoge wetenschappelijke kwaliteit teruggedrongen wordt ten gunste van onderzoek waarvan de maatschappelijke relevantie direct aantoonbaar is.

**7.** Het tegenwoordig zo in zwang geraakte begrip 'innovatie' verhoudt zich tot het ouderwetse begrip 'onderzoek en ontwikkeling' als een sandwich tot een snee brood.

8. Indien de economische groei in de jaren '84-'86 niet sneller toeneemt dan in het regeeraccoord van het kabinet Lubbers wordt verondersteld, dient het financieringstekort minder snel te dalen dan in hetzelfde accoord ten doel wordt gesteld.

9. Dat de regel: "Rechtdoorgaand verkeer op dezelfde weg heeft voorrang" ook geldt ten opzichte van voetgangers en dat een zebrapad er is om bescherming te bieden aan voetgangers, schijnt bij grote groepen van de Nederlandse bevolking niet bekend te zijn.

10. Door de huidige opzet van het promotiereglement van de Technische Hogeschool Twente (vastgesteld door het College van Decanen d.d. 14 september 1982) is het openbare karakter van de promotie onvoldoende gewaarborgd.

11. Aan de promovendus wordt, omdat hij/zij een promotie-onderzoek afrondt, vaak ten onrechte een grote deskundigheid toegeschreven op gebieden die buiten het onderwerp van de promotie liggen.

Vergelijk Saskia van der Stoel, OPZIJ, 11e jrg. nr. 12, december 1983, p. 31.

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