Enzyme Membrane Based Upon Polyamide-6 for Oil Hydrolysis

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SYNOPSIS

This work presents the preparation of the enzyme membrane based upon polyamide-6 (PA-6) destined for the hydrolysis of oils. The transport properties of the base PA-6 membranes, i.e., their porosity and permeability coefficients, as well as the mode of their modification, aimed at the chemical binding of lipase molecules have been estimated. © 1992 John Wiley & Sons, Inc.

INTRODUCTION

In recent years, microporous membranes have gained considerable significance not only for separation purposes but also as the carriers for catalytic active substances. The most advanced is their application for the immobilization of biocatalysts—enzymes or the whole microorganisms in the form of the so-called enzyme membranes and their usage in bioreactors.

The membranes ought to fulfill many conditions both of physical and chemical character. First, their porous structure must be strong enough to withstand an enhanced pressure usually applied in the membrane forced-flow bioreactors. In addition, the membrane material must be chemically and thermally resistant. Moreover, it ought to be also resistant to biodegradation.

The above conditions are well fulfilled by many aromatic and aliphatic polyamides.[1-3]

In our research, we have chosen the widely accessible aliphatic polyamide-6 (PA-6) as the base material for the preparation of enzyme membranes. The important advantage of the polymer is its strong hydrophylicity, which enables the storage of prepared ultrafiltration membranes in a dry state without any changes in their transport properties.

The aim of the work is to present the mode of preparation of the base microfiltration PA-6 membrane and its modification, enabling the immobilization of lipase.

EXPERIMENTAL

To prepare the enzyme membrane with the constant in time catalytic activity, a lipase immobilization was accomplished by a chemical bonding.

A commercial PA-6 produced by Stilon, Gorzow Wielkopolski, and Tarnow (Poland) were used as the membrane polymer. All other chemicals of reagent grade were purchased by POCH (Gliwice, Poland).

The enzyme lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) from *Candida rugose* was the product of Sigma Chemical Co. (St. Louis, MO). Its activity equals 400–900 U/mg protein. Commercial olive oil produced by Izdas dis Ticaret (Istanbul, Turkey) was used in the form of a fine emulsion. Arabic gum used for the stabilization of the emulsion was the product of Fisher Scientific Co. (Fair Lawn, NJ).

The olive oil emulsion was prepared by homogenization of olive oil in a buffer solution of pH 5.0–9.0 with arabic gum in the amount of 10% b/w of a mass of olive oil. The pH value was estimated by adding the proper quantity of HCl solution to the solution of Tris (0.24% b/w with 0.24% b/w of sodium benzoate). The prepared emulsion was stable and could be stored in a refrigerator during 2 weeks.

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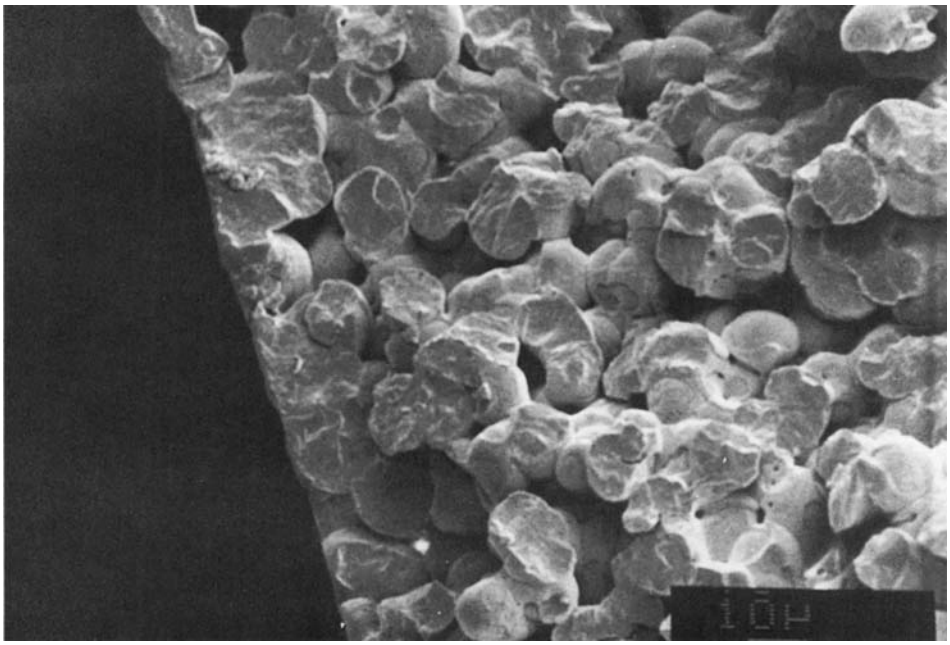

Figure 1 Fracture of the polyamide-6 membrane (PA 15/3) on the SEM photomicrograph.

PREPARATION OF THE BASE PA-6 MEMBRANE

The preparation of membranes has been accomplished by a classical phase inversion process, which involves the conversion of polymer (PA-6) solution into a two-phase system with a polymer phase constituting the microporous membrane structure and a liquid phase forming the membrane pores.[1-3]

Formic acid has been chosen[1,4] as the solvent. The casting solution of PA-6, 15–20% b/w, was prepared in advance and prefiltered. Membranes were formed on glass plates.

The following parameters of the inversion process were estimated:

Initial gelation—5–20 min, temperature 278 K, in air atmosphere.
Main gelation—3–5 h, in water, at controlled temperature (275–315 K).

The structure of prepared membranes was recognized on the base of micrographs of the membrane fractures in an electron scanning microscope. One of the typical micrographs of the membranes is presented in Figure 1.

As can be seen, the structure is asymmetric, with the distinct dense skin layer while a bulk layer exhibits a (well defined) sponge-like structure.

For the quantitative characterization of the membranes, such properties as porosity and hydrodynamic permeability coefficient, as well as pore mean sizes and pore sizes distribution, were estimated. Some results are presented in Table I.

The number of pores per unit membrane surface area changes from $1*10^9$ in PA 15/3 membrane to $1*10^{10}$ in PA 22/1 membrane.

Pore sizes distribution and mean pore radius were estimated according to the modified bubble point

Table I Characteristics of PA-6 Membranes

Membrane Symbol[a]	Temp. of Main Gelation (K)	Permeability (m/s*Pa) 10^9	Mean Pore R_{av} (nm)
PA 22/1	278	1.3	180
PA 22/2	288	28	331
PA 22/3	298	63	430
PA 18/1	278	17	240
PA 18/2	288	60	380
PA 18/3	298	88	710
PA 15/1	278	69	450
PA 15/2	288	84	610
PA 15/3	298	105	950

Initial gelation at 293 K.
[a] The first number marks the concentration of the casting solution; the second points to one of the three levels of temperature of the main gelation.

method.[5] As an example, the results of such an estimation for a membrane PA 22/2 are shown in Figure 2.

According to the presented data, two opposite effects exist: Together with the increase of mean pore radius and the permeability coefficient, the mechanical properties of the membranes are still worse.

It is known that the addition of some components to the casting solution can distinctly change the gel structure of a membrane and consequently also its mechanical properties. That is why in the next step the optimal parameters of membrane formation were estimated. It was done by means of simplex planning method for multifactor experiments, choosing the hydrodynamic permeability coefficients and mechanical strength of the membrane as the quality criterion.[6-8] The optimization procedure was aimed at the estimation of the composition of the casting solution, i.e., the concentration of PA-6, $CaCl_2$ or $MgCl_2$, CH_3COOH, and water.

The following values have been estimated:

13.5% b/w PA-6
52.4% b/w HCOOH
8.3% CH_2COOH
8.3% $CaCl_2$ or $MgCl_2$
17.5% H_2O.

Some properties of the membranes prepared with the optimum parameters are shown in Table II.

MODIFICATION OF THE PA-6 MEMBRANES

All the above experiments were aimed at the preparation of base membranes that after lipase immobilization ought to be suitable for the hydrolysis of oils in buffered emulsion. So, it was reasonable to assume that the membrane ought to have a high hydrodynamic permeability coefficient and high mean pore radius to avoid fouling of its surface by oil droplets. That is why for the next chemical modification the two last membranes $PA_{Ca}40$ and $PA_{Ca}47$ have been chosen.

The structure of one of the mentioned membranes is well visible upon scanning electron microscopy (SEM) micrograph of its fracture (Fig. 3). It is worth pointing out the distinct difference of the structure in comparison to that presented in Figure 1.

The modification of membranes was aimed at introducing to their structure the proper amount of function groups active in the chemical bonding of enzyme molecules. It was necessary as the amount of active end groups in that polymer is insufficient for obtaining membranes with accessible catalytic activity.

The modification of polyamide in the prepared membranes was performed by one of the methods[9,10] commonly used for modification of polyamides. It was performed in three consecutive steps:

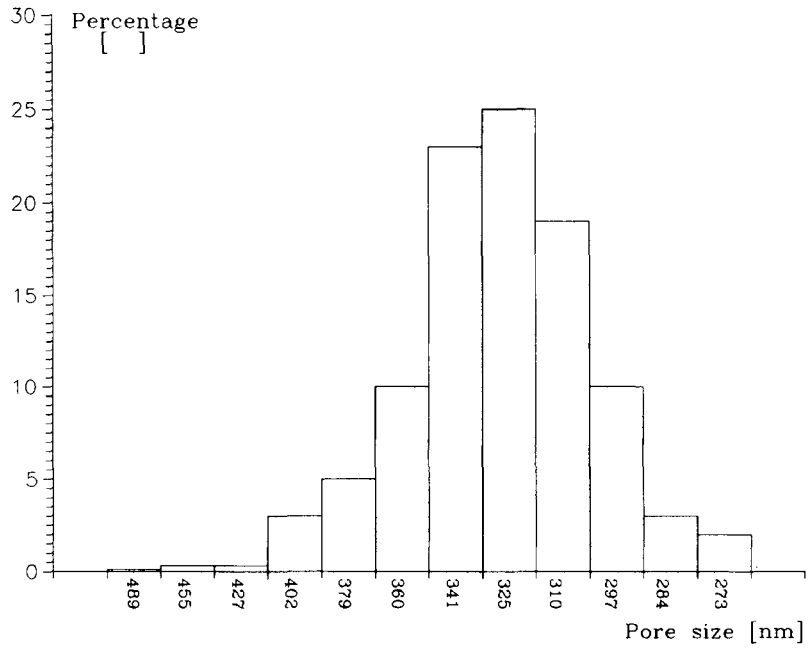

Figure 2 Diagram of pore size distribution of the polyamide-6 membrane (PA 22/2).

Table II Main Parameters of PA-6 Membranes Obtained with Optimal Parametes ($MgCl_2$ and $CaCl_2$)

Membrane[a]	Temp. of Main Gelation (K)	$Lp*10^9$ (m/s*N)	R_{av} (nm)	Density of Pores (m^{-2})	Water Content (%)
$PA_{Mg}29$	302	2.18	331	$1*10^9$	67.9
$PA_{Mg}37$	310	2.76	368	$1*10^9$	67.7
$PA_{Mg}40$	313	3.30	386	$1*10^9$	67.9
$PA_{Mg}47$	320	4.00	397	$1*10^9$	67.9
$PA_{Ca}29$	302	60.70	713	$1*10^8$	72
$PA_{Ca}37$	310	74.50	989	$1*10^8$	76
$PA_{Ca}40$	313	86.70	1090	$1*10^8$	79
$PA_{Ca}47$	320	98.12	1500	$1*10^7$	81

[a] Subscripts in the membrane symbols point to the kind of salt added to casting solution: Mg, $MgCl_2$; Ca, $CaCl_2$.

1. dissolving the part of amorphous PA-6 with a mixture containing 18.6% $CaCl_2$ in 81.4% methanol,[11]
2. controlled acidolise of amide bounds with 3.6*M* HCl, and
3. activation by 1,4-butanediamine.

All the steps of the above process were accomplished directly in the base PA-6 membranes recirculating (two to three times) the active substances through the membranes (forced by nitrogen pressure).

The results of the modification are presented in Table III.

The determination of hydrodynamic permeability coefficients and mean pore sizes of the above membrane point to the fact that the values do not differ remarkably from those estimated before the modification. So, the modification proceeds mainly on the bulk membrane structure (support layer). Consequently, lipase molecules ought to be attached mainly to this bulk membrane structure.

As can be seen from Table III, the modification caused more than 30% increase of the active group

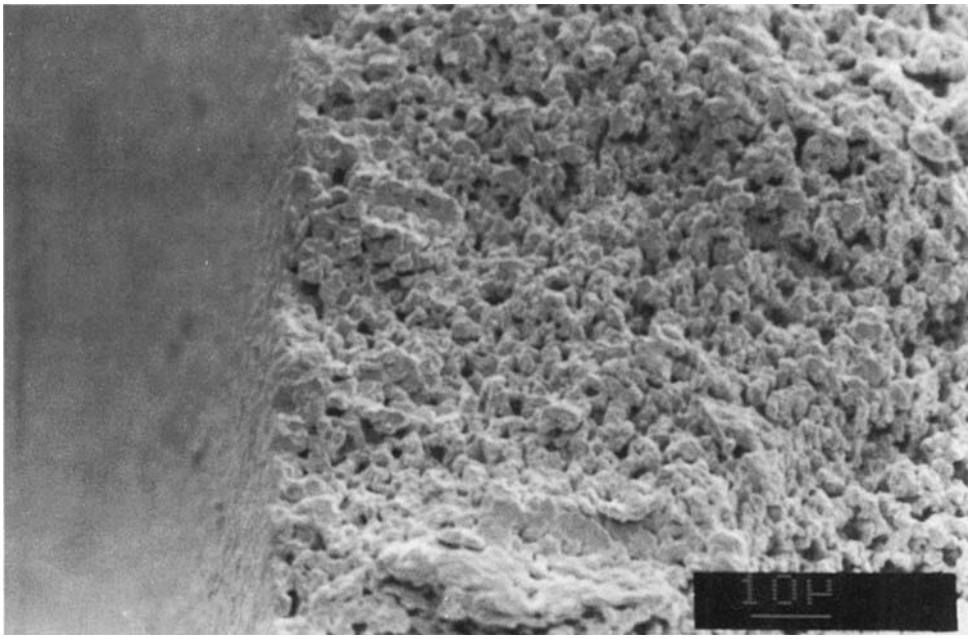

Figure 3 Fracture of the polyamide-6 membrane ($PA_{Ca}29$) on the SEM photomicrograph.

Table III Results of the Chemical Modification of PA-6 Membranes Prepared with $CaCl_2$ in the Casting Solution

Membrane	Molecular Weight Before and After Acidolisis[a]		Content of Amine Groups After Modification[b]
$PA_{Ca}40$	21,000	15,500	73%
$PA_{Ca}47$	21,000	15,500	75%

[a] Molecular weights were estimated by the end-group method.[15]
[b] Content of amine was estimated by the end-group method.

content. Moreover, the concentration of amine groups in comparison to that of carboxylic groups increased from 1 : 1 to 3 : 1, which is important for the further process of chemical bonding of enzymes.

The process of lipase immobilization was performed directly on the modified membranes in two steps:

1. activation in 5% glutaraldehyde of pH 8.5 at 293 K for 3 h and
2. coupling of lipase (0.3–1.2 mg/mL) in the phosphate buffer solution of pH 7.8 at 278 K for 24 h.

The above processes were performed by several recirculations of the reacting solutions.

The strong ability of polyamides to adsorb proteins is well known. The property is often exploited, e.g., in different kinds of chromatography and for the preparation of porous PA beads with adsorbed enzymes for column bioreactors.[12,13] In that case, however, the continuous process of desorption causes the decrease of catalytic activity of the column.

That is why after the coupling of lipase the membranes were carefully washed until no traces of proteins were present in the effluent, which was controlled by light absorption at $\lambda = 280$ nm. Nevertheless, we cannot exclude the fact that part of the lipase can be immobilized in membrane by the specific adsorption.[14] Detailed analysis of this phenomena will be performed in our future works.

The load density of chemically bonded lipase in the membranes has varied from 0.1–0.5 mg/cm^2 of the membrane surface. The above values were determined on the basis of light absorption measurements ($\lambda = 595$ nm) of the complex of lipase with Coomasie brilliant blue.

The quality of elaborated membranes was proved in the process of olive oil (in emulsion) hydrolysis performed in a forced-flow reactor. The scheme of the reactor is shown in Figure 4.

The details of the hydrolysis process are:

1. concentration of oil in the emulsion 18% b/w,
2. pH of the emulsion 7.0, and
3. temperature of the process 300 K.

Concentration of free fatty acids was estimated by titration of the effluent from continuously "working" reactor. The results are presented in Figures 5 and 6. As can be seen from Figure 5, the yield of the reaction is limited by reaction kinetics only at the smallest reaction times, whereas at longer times the transport processes started to be the limiting effects of the whole hydrolysis process. The distinct drop in reactor performance in long test work (Fig. 6) seems to be caused by the mentioned effects.

CONCLUSIONS

1. The elaborated base PA-6 membrane exhibits sufficient transport and mechanical properties.
2. The estimated procedure of chemical modification is an effective method of preparation of the membrane for lipase immobilization.
3. The enzyme membranes enable the performance of an effective hydrolysis process; however, an attempt ought to be made to eliminate the limiting transport processes diminishing the yield of a reactor.

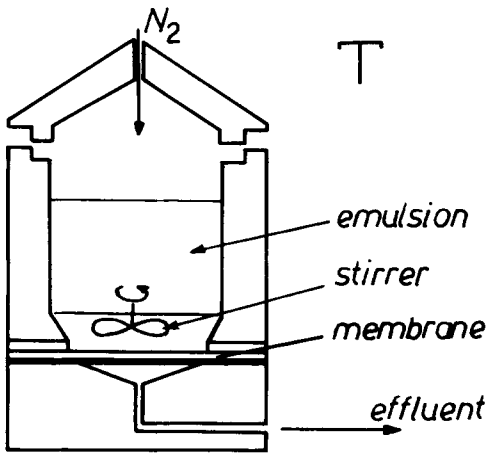

Figure 4 Scheme of the reactor for oil hydrolysis.

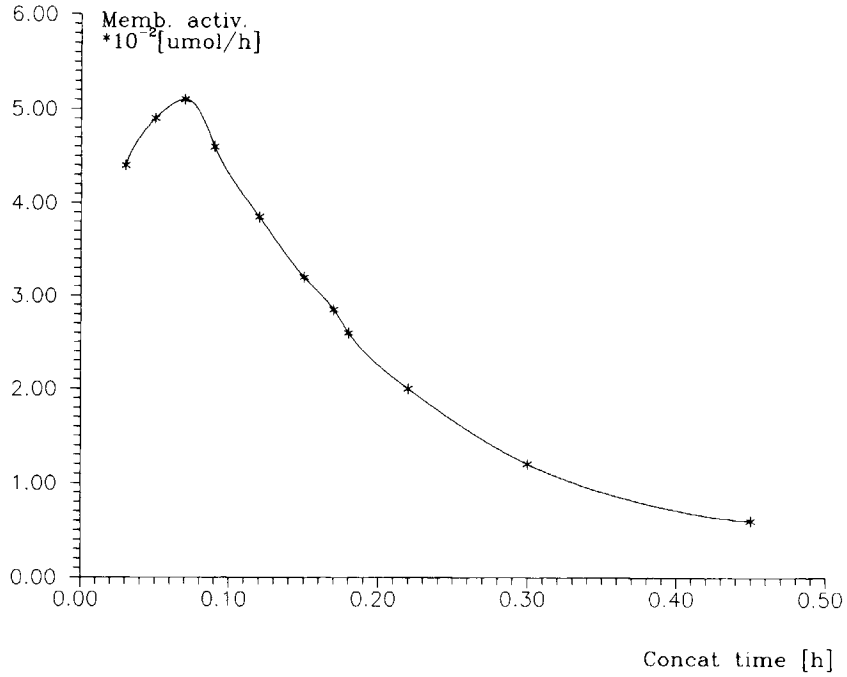

Figure 5 Dependence of the hydrolysis yield upon contact time in the membrane. (Contact time = the quotient of volume porosity of the membrane against the flow rate of effluent.)

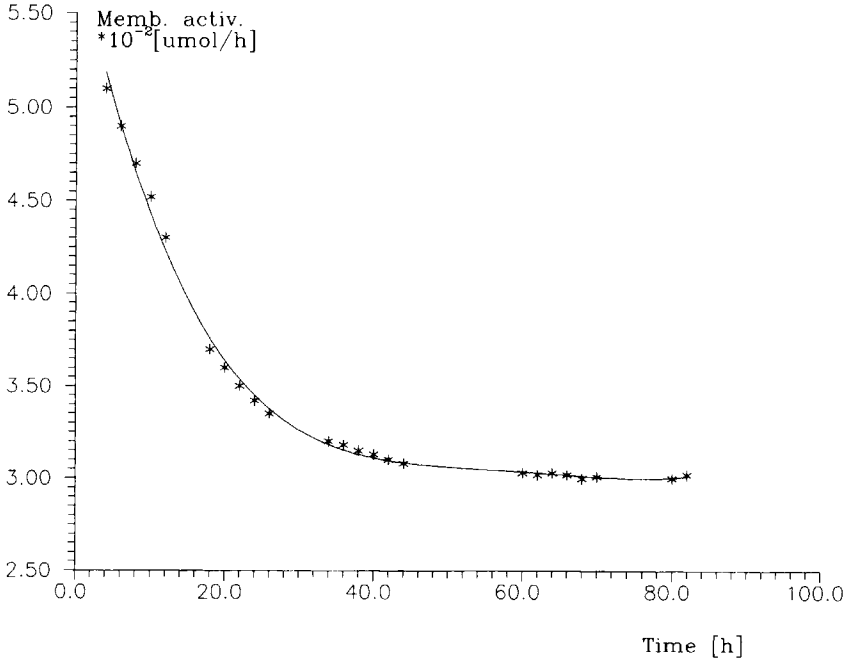

Figure 6 Yield of hydrolysis at the optimum contact time in a long-time continuous experiment.

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