β-Galactosidase Immobilization on Premodified Teflon Membranes Using γ-Radiation Grafting

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ABSTRACT: γ-Radiations have been used to immobilize, by mutual grafting, the β-galactosidase enzyme and monomers of 2-hydroxyethyl methacrylate on Teflon (polytetrafluoroethylene) membrane previously grafted with acrylic acid monomers. This double grafting technique improved the catalytic activity of the membrane. Membrane activity has been studied as a function of some of the most relevant parameters affecting the grafting degree and of the amount of enzyme used for immobilization. Experimental conditions producing the best membrane activity have been characterized. The advantages in using Teflon catalytic membrane in nonisothermal bioreactors, more efficient than the analogous isothermal bioreactors, are also discussed. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 625–636, 1998

Key words: radiation grafting; Teflon; immobilized enzymes; β-galactosidase; 2-hydroxyethyl methacrylate; acrylic acid

INTRODUCTION

Polymer membranes are widely used as enzyme carriers. In many cases, modifications have been introduced on the polymeric matrices to increase their catalytic activity. Graft copolymerization is a well-known method for introducing such modifications.[1] Grafting is conducted chemically[2–4] or by irradiation.[5–18]

When the grafted membranes are biocatalytic, they are generally used in the construction of biosensors. Other practical applications of grafted membranes are the immobilization of drugs for the controlled release, of antigens and antibodies for the immunodiagnostic, and of microorganisms for biomass conversion. All of these applications are performed under isothermal conditions.

When biocatalysts are not immobilized, the grafted membranes have been used in water desalination[8] and in azeotropic mixtures separation by pressure gradients under isothermal conditions[17,18] or by temperature gradients.[19,20]

Our recent research activity has been focused on the employment of biocatalytic membranes in nonisothermal reactors, where the presence of temperature gradients across a membrane system favorably affects the rate of enzyme reaction.[21–26] As a matter of fact, the bioreactor efficiency increases increasing the temperature gradient applied across the catalytic membrane. In all cases, the membrane system used was a biocatalytic membrane coupled with a Teflon [polytetrafluoroethylene (PTFE)] membrane. The role of the hydrophobic Teflon membrane is to induce mass transport (including substrate and products) when it is interposed between two liquid solu-

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tions, of equal or different composition, kept at different temperatures. This nonisothermal matter transport process is known as thermodialysis.[27–31]

The aim of this work is to immobilize an enzyme directly on a suitably grafted Teflon membrane so that the membrane system used in the nonisothermal bioreactor is simplified, making easier the study of the physical causes that affect the enzyme reaction in the presence of temperature gradients. β-Galactosidase has been chosen as an enzyme model, also in view of its application to lactose hydrolysis in milk and whey to render these foods suitable for lactose-intolerant people.

EXPERIMENTAL

Materials

PTFE membranes of the type TF-450, manufactured by the Gelman Instrument Company (Ann Arbor, MI) have been used as solid support to perform the grafting process on. These membranes, constituted by a Teflon film supported by a polypropylene net, have a thickness of 150 μm and are endowed with anastomizing pores of 450 μm in diameter.

The monomers used for the grafting were 2-hydroxyethyl methacrylate (HEMA) and acrylic acid (AA). Ferrous ammonium sulfate (FAS) was used as inhibitor for the formation of AA homopolymers, because the radiation grafting is performed without oxygen using the mutual technique.

The enzyme used was a β-galactosidase (EC 3.2.1.23) from *Aspergillus oryzae.* The enzyme hydrolyzes lactose in glucose and galactose. The β-galactosidase activity was colorimetrically assayed by the GOD-Perid method for glucose determination (Boehringer GmbH, Mannheim, Germany) and expressed as μmol min^{-1}.

All chemical products, including the enzyme, were purchased from Sigma Chemical Company (St. Louis, MO) and used without further purification.

Catalytic Membrane Preparation

Membrane grafting was performed by irradiation with γ-rays. The radiation source was cesium 137 in a γ-cell 1000 Elite by Nordion International, Inc. (Kanata, ON, Canada). The average dose rate in the core of the radiation chamber (central dose rate) was 2.35×10^4 rad h^{-1}. Because direct grafting of HEMA in the presence of enzyme solution has produced membranes endowed with low catalytic activity, we used a "double grafting technique" that allowed us to obtain more active membranes in the presence of the same initial concentration of enzyme.

First Grafting: Modification of PTFE Membrane by AA Grafting

First grafting was performed by irradiating in the γ-cell the PTFE membranes with a solution of AA monomers and FAS, thus obtaining a PTFE-AA membrane. Experimental conditions used are specified according to the following scheme: a = % AA concentration (v/v); b = % FAS concentration (w/v); and $c = t_1$ = irradiation time used during first grafting (h).

Second Grafting: Immobilization of β-Galactosidase by HEMA Grafting

Second grafting was performed by irradiating in a solution of HEMA and enzyme the previously grafted membranes. The experimental conditions used are specified according to the following scheme: d = % HEMA concentration (v/v); e = enzyme concentration (mg mL^{-1}); and $f = t_2$ = irradiation time used during second grafting (h).

Determination of Grafting Degree

As for the percentage of grafting, we adopted the classical definition used for this parameter. The degree of grafting (X, %) was determined by the difference between membrane masses before, G_B, and after, G_A, the grafting, according to the expression

$$X(\%) = \frac{G_A - G_B}{G_B} \times 100 \qquad (1)$$

In the case of AA grafting, G_B is the membrane mass before the first irradiation, and G_A is the mass of the dry membrane after the irradiation. Similarly, in the case of the HEMA grafting G_B is the dry membrane mass before the second irradiation and G_A is the mass of the dry membrane after the second irradiation.

Determination of Activity of the Catalytic Membrane

For activity determination, catalytic membranes were put in 35 mL of a well-stirred 200 mM lac-

tose: $0.1M$ phosphate buffer solution (pH 6.5) and maintained at 40°C. Glucose production was followed in the course of time. Membrane activity is given by the angular coefficient of the linear plot of the glucose production as a function of time.

Determination of Time Stability of the Catalytic Membrane

Time stability of the biocatalytic membranes was assessed by analyzing their activity every day under the same experimental conditions. After 3 days, during which the membranes lost some activity, a stable condition was reached that remained unchanged for more than 2 months. Only these stabilized membranes were used in the comparative experiments reported in the following. When not used, the membranes were stored at 4°C in $0.1M$ phosphate buffer (pH 6.5).

Treatment of Experimental Data

Every experimental point reported in the figures represents the average of three experiments performed under the same conditions. The experimental errors never exceeded 6%. For each of the three experiments, the procedures in the various steps were performed according to the following methodology. Twenty-four Teflon membrane disks, 2.5 cm in diameter, were weighed and put in the solution for first grafting. After irradiation, the membrane disks were repeatedly washed under vigorous stirring in abundant double distilled water to remove the adherent homopolymers. Then, eight disks were taken for determining the AA grafting degree and the remaining were used for the second grafting. At the end of this operation, disks were washed using $0.1M$ phosphate buffer solution (pH 6.5), then separated in two groups of eight membranes each for determining the HEMA grafting degree and catalytic activity, respectively.

RESULTS AND DISCUSSION

Because the activity of the biocatalytic membranes is affected by the irradiation time during first and second grafting, by the concentrations of the enzyme, HEMA, FAS, and AA, we have studied the glucose production in dependence of each of these parameters.

Dependence on Irradiation Time During First Grafting

The first information to optimize the performance and evaluate the cost/benefit of the grafted membranes is to know the dependence of their enzyme activity on the irradiation time. To this aim, we have performed first grafting by immersing membrane disks in an aqueous solution of 15% AA (v/v) and 2.5% FAS (w/v) and putting the container in the γ-cell for the desired times. After washing the disks and following the previously described procedure, a new grafting for 16 h with a solution of 10% HEMA (v/v) in $0.1M$ phosphate buffer at pH 6.5 and 0.5 mg mL^{-1} enzyme concentration is performed. The results obtained, reported in Table I, indicate that the AA grafting degree is independent of irradiation time. Because catalytic activity of the membrane is practically constant, we can observe that the amount of the immobilized enzyme depends on the AA grafting degree that also controls second grafting. For irradiation times smaller than 6 h during first grafting, we have found scarcely reproducible, generally smaller, values of AA grafting.

Dependence on Enzyme Concentration

The dependence of catalytic membrane activity on enzyme concentration has been studied, considering that 6 h of irradiation time during first grafting are sufficient to obtain the maximum and constant value of the AA grafting degree. Results relative to this series of experiments are reported in Table II. The table shows how glucose production increases with the amount of enzyme used in the second grafting. When the membrane activity is reported in the graphical form of Figure 1, we obtain a linear dependence of the activity on the enzyme concentration. This behavior seems to suggest that, under the experimental conditions used, protein–protein interaction that can inactivate some bound enzymes does not occur.

To be sure that protein–protein interaction is absent, we have performed another series of experiments, identical to the ones just reported, but changing only the FAS concentration to 0.1%. Results of this experiment are reported in Table III and in Figure 1, where it is possible to see how the activities of the membranes prepared using 0.1% FAS are surprisingly higher than that ones prepared using 2.5% FAS. To explain this behavior, some considerations must be done. Let us indicate with 1 and 2 the membranes treated with two different FAS concentrations: FAS_1 and

Table I Effect of Irradiation Time During First Grafting

Irradiation Time Used During First Grafting (h)	AA Grafting Degree (%)	Irradiation Time Used During the Second Grafting (h)	HEMA Grafting Degree (%)	Catalytic Membrane Activity $\left(\frac{\mu mol}{min} \times 10^3\right)$
6	20	16	51	7.5
12	22	16	49	8.2
18	21	16	48	7.0

Results reported herein were obtained under the following experimental conditions: a = 15% AA concentration; b = 2.5% FAS concentration; $c = t_1$ = 6, 12, and 18 h; d = 10% HEMA concentration; e = 0.5 mg mL^{-1} enzyme concentration; and $f = t_2$ = 16 h.

FAS_2, respectively. Calling R_{FAS} the ratio FAS_1/FAS_2 and R_G the ratio $(G\ \%)_{FAS1}/(G\ \%)_{FAS2}$ between the AA grafting degrees obtained at the two corresponding FAS concentrations, we can experimentally obtain $R_{FAS} \gtrless R_G$. If $R_{FAS} = R_G$, the length of the AA branches on the two membranes are equal. If $R_{FAS} < R_G$, the AA branches on membrane 1 are longer than the ones on the membrane 2; if $R_{FAS} > R_G$, the contrary occurs. Inspection of the values in Tables II and III shows that, in our case, identifying FAS_1 and FAS_2 with the 0.1 and 2.5% FAS concentration, respectively, R_G is about 1 whereas R_{FAS} is 0.04. This means that the length of the AA branches on the membrane treated with 0.1% FAS concentration is greater than the one on the membranes treated with 2.5% FAS concentration and that the first ones are probably 25 times longer than the second ones. This circumstance suggests that, at 0.1% FAS concentration, protein–protein interaction does not occur, whereas it occurs at 2.5% FAS concentration.

To optimize the performance of the biocatalytic membrane, having ascertained that 0.1% FAS concentration gives better results, we have tested the dependence on the first irradiation time in these last conditions. We have performed a series of experiments at 45 mg mL^{-1} enzyme concentration and at different irradiation times, with all other parameters being the same as those that have led to the results reported in Figure 1. These new results are reported in graphical form in Figure 2, which shows how both AA grafting percentage and membrane activity linearly increase with the duration of the first irradiation time.

Dependence on Irradiation Time During Second Grafting

We proceed now to test the dependence of membrane activity on the second irradiation time, the step during which the membranes, previously grafted with AA, become biocatalytic. To this aim,

Table II Effect of Enzyme Concentration Used for Preparing the Catalytic Membrane at Fixed 2.5% FAS Concentration

Enzyme Concentration (mg mL^{-1})	Irradiation Time Used During First Grafting (h)	AA Grafting Degree (%)	Irradiation Time Used During Second Grafting (h)	HEMA Grafting Degree (%)	Catalytic Membrane Activity $\left(\frac{\mu mol}{min} \times 10^2\right)$
5	6	18.2	16	30.2	8.3
10	6	19.4	16	25.0	16.6
15	6	17.0	16	27.3	23.0
20	6	18.0	16	28.4	31.6
25	6	21.5	16	31.1	37.2
30	6	19.0	16	22.5	44.4
35	6	18.5	16	30.6	53.4
45	6	18.1	16	27.2	68.3

Results reported herein were obtained under the following experimental conditions: a = 15% AA concentration; b = 2.5% FAS concentration; $c = t_1$ = 6 h; d = 10% HEMA concentration; e = 5, 10, 15, 20, 25, 30, 35, and 45 mg mL^{-1} enzyme concentration; and $f = t_2$ = 16 h.

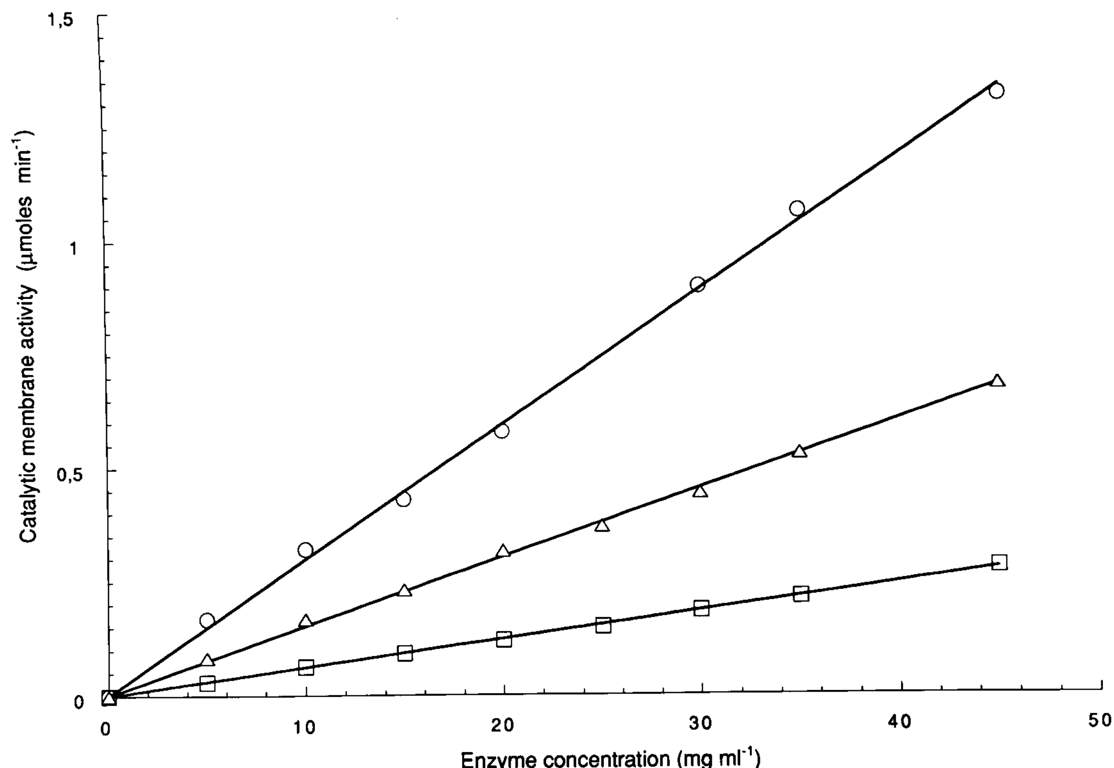


Figure 1 Membrane activity as a function of initial enzyme concentration used for the grafting. (○) 0.1% FAS concentration; (△) 2.5% FAS concentration; (⊡) HEMA directly grafted onto the Teflon membrane. Experimental conditions used are reported in the footnote to Table II, with the exception of the points identified with the symbol □, which were obtained in the absence of AA and FAS.

we have performed a series of experiments under the best experimental conditions found in the previous section and exposing the samples to different irradiation times during second grafting. The results, reported in Table IV and in Figure 3, show that the membrane activity linearly increases

Table III Effect of Enzyme Concentration Used for Preparing the Catalytic Membrane at Fixed 0.1% FAS Concentration

Enzyme Concentration (mg mL^{-1})	Irradiation Time Used During First Grafting (h)	AA Grafting Degree (%)	Irradiation Time Used During Second Grafting (h)	HEMA Grafting Degree (%)	Catalytic Membrane Activity $\left(\frac{\mu mol}{min} \times 10\right)$
5	6	21.3	16	50.1	1.7
10	6	20.5	16	47.2	3.2
15	6	21.7	16	49.0	4.0
20	6	21.0	16	52.1	5.8
25	6	21.0	16	51.3	7.5
30	6	21.2	16	50.4	9.5
35	6	21.2	16	44.3	10.6
45	6	21.7	16	50.2	12.9

Results reported herein were obtained under the following experimental conditions: a = 15% AA concentration; b = 0.1% FAS concentration; $c = t_1$ = 6 h; d = 10% HEMA concentration; e = 5, 10, 15, 20, 25, 30, 35, and 45 mg mL^{-1} enzyme concentration; and $f = t_2$ = 16 h.

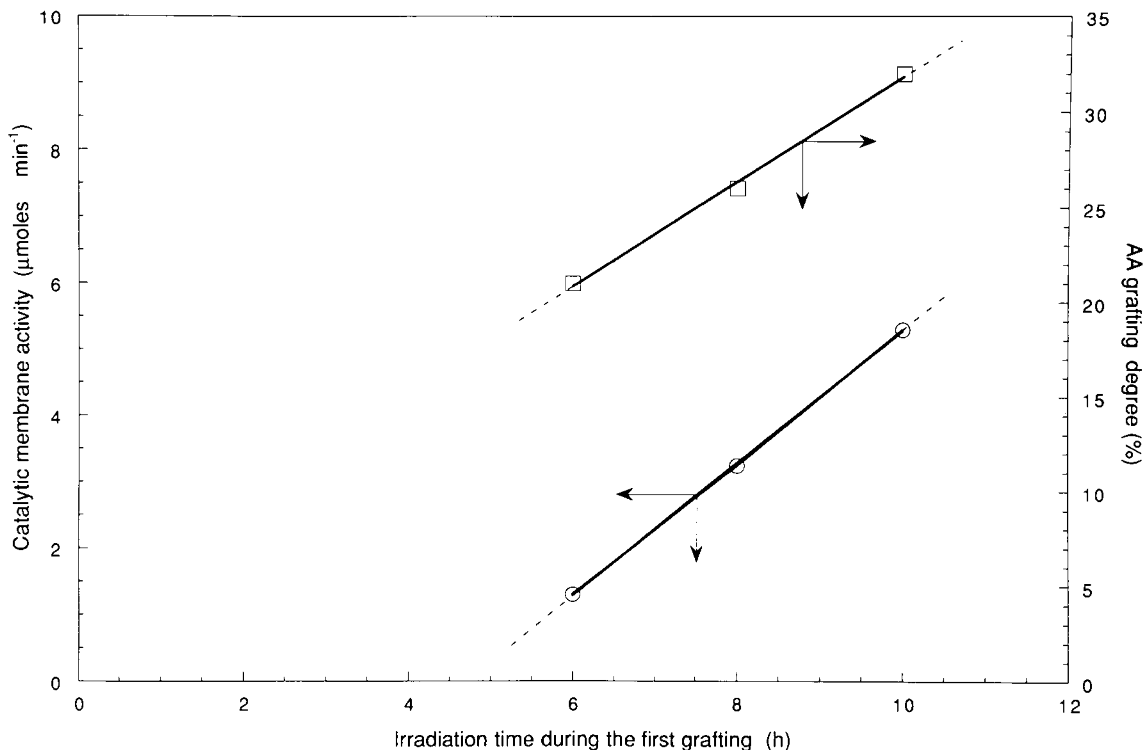


Figure 2 Catalytic membrane activity (○) and AA grafting degree (⊡) as a function of irradiation time used during first grafting. Experimental conditions used are reported in the footnote to Table III.

with the second irradiation time, thus indicating that the amount of the immobilized enzymes depends, at least in the time intervals used, on the time employed for immobilization.

Dependence on FAS Concentration

Investigating the effect of the enzyme concentration on the membrane activity, we concluded that FAS concentration plays an important role. Now, we have to study this role in more detail using different FAS concentrations in the range from 0.1 to 2.5%. Initial enzyme concentration was 45 mg mL^{-1}. The results, reported in Table V and in Figure 4, show that the change in the FAS concentration has no significant effects on both AA and HEMA grafting degrees accordingly to the results obtained by other authors.[7] The second

Table IV Effect of Irradiation Time During Second Grafting

Irradiation Time Used During First Grafting (h)	AA Grafting Degree (%)	Irradiation Time Used During Second Grafting (h)	HEMA Grafting Degree (%)	Catalytic Membrane Activity (μmol min^{-1})
10	31.6	8	41.2	5.1
10	32.5	16	43.0	5.8
10	30.7	24	44.5	6.9
10	32.0	32	49.1	8.1

Results reported herein were obtained under the following experimental conditions: a = 15% AA concentration; b = 0.1% FAS concentration; $c = t_1 = 10$ h; d = 10% HEMA concentration; e = 45 mg mL^{-1} enzyme concentration; and $f = t_2 =$ 8, 16, 24, and 32 h.

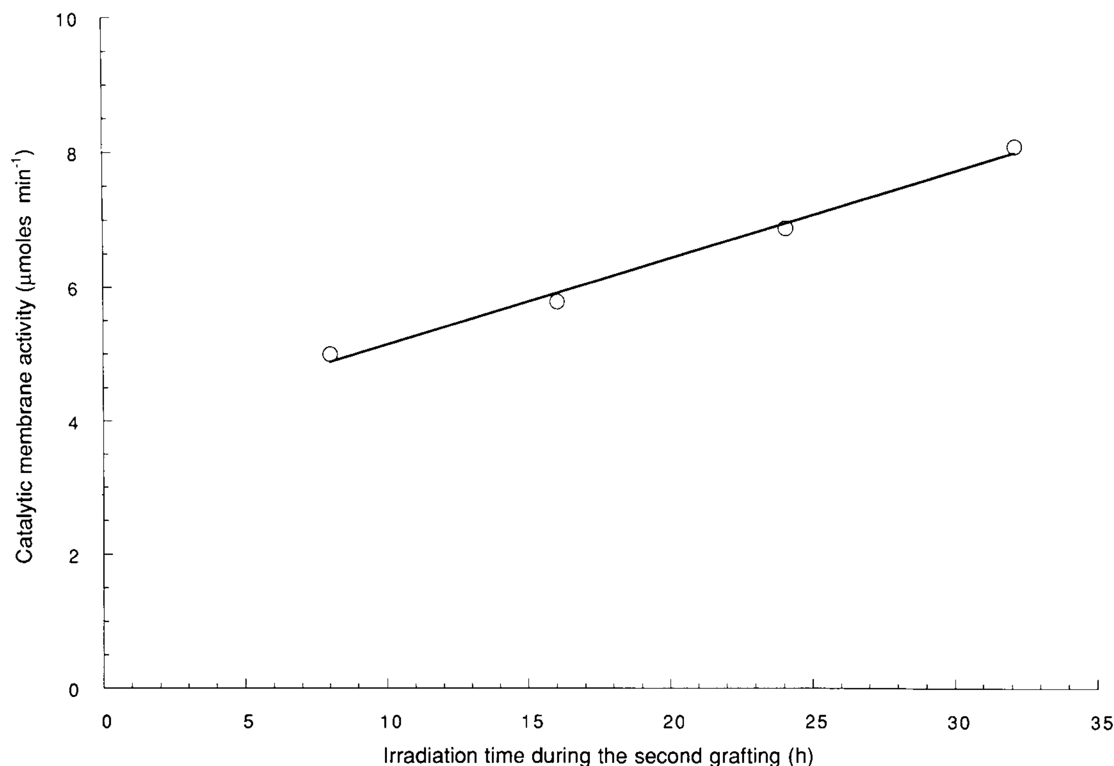
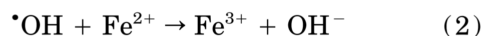


Figure 3 Catalytic membrane activity as a function of irradiation time used during second grafting. Experimental conditions used are reported in the footnote to Table IV.

grafting has, of course, the same behavior, owing to its dependence on first grafting. Much different is the effect on membrane activity that decreases exponentially with the increase of FAS concentration. These results agree with the hypothesis of the occurrence of protein–protein interaction, which becomes relevant at FAS concentrations higher than 0.5%. As is well known, Fe^{2+} ions react with hydroxyl radicals, produced from radiolysis of water, that is responsible for initiation of homopolymerization. According to the reaction



$$^{\bullet}OH + Fe^{2+} \rightarrow Fe^{3+} + OH^{-} \qquad (2)$$

the increase of Fe^{2+} concentration reduces the ho-

Table V Effect of FAS Concentration During First Grafting

FAS Concentration (%)	Irradiation Time Used During First Grafting (h)	AA Grafting Degree (%)	Irradiation Time Used During Second Grafting (h)	HEMA Grafting Degree (%)	Catalytic Membrane Activity (μmol min^{-1})
0.1	10	32.0	16	43	5.20
0.3	10	30.0	16	43	3.00
0.5	10	31.0	16	41	2.13
1.0	10	30.0	16	42	1.33
1.5	10	29.5	16	41	1.20
2.0	10	28.0	16	40	1.07
2.5	10	20.0	16	47	0.98

Results reported herein were obtained under the following experimental conditions: a = 15% AA concentration; b = 0.1, 0.3, 0.5, 1.0, 1.5, 2.0, and 2.5% FAS concentration; $c = t_1$ = 10 h; d = 10% HEMA concentration; e = 45 mg mL^{-1} enzyme concentration; and $f = t_2$ = 16 h.

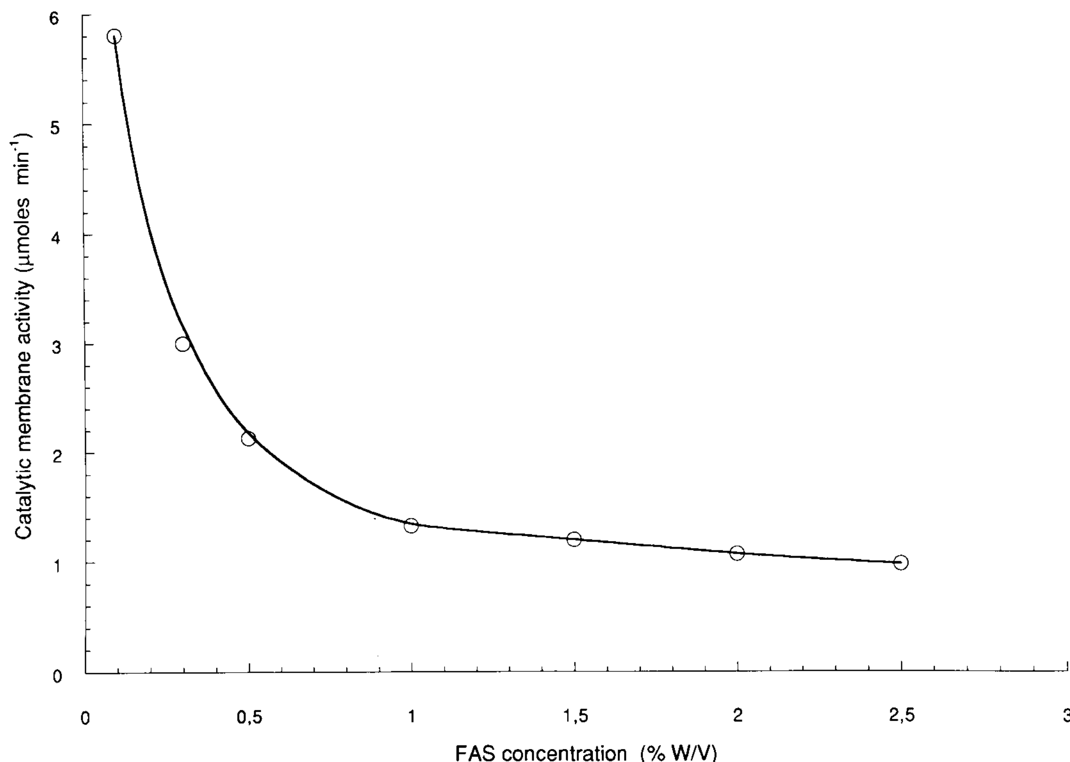


Figure 4 Catalytic membrane activity as a function of FAS concentration. Experimental conditions used are reported in the footnote to Table V.

mopolymer formation, and therefore more monomer units are available to attach the free radicals formed on the Teflon membrane. As a result, the number of grafted branches, that is the density of AA branches on the membrane surface, is increased. Consequently, when the same AA grafting degree occurs at different FAS concentrations, the length of the grafted branches must decrease where the FAS concentration is higher. The synergetic occurrence of the increase of the branch density and of the decrease of the grafted branch length create protein–protein interaction and formation of hydrogen bonds between functional groups of the enzyme and grafted branches. Both of these two circumstances make the membrane less active, thus deactivating some enzymes and changing the microenvironment near the catalytic site.

Table VI Effect of AA Concentration

AA Concentration (%)	Irradiation Time Used During First Grafting (h)	AA Grafting Degree (%)	Irradiation Time Used During Second Grafting (h)	HEMA Grafting Degree (%)	Catalytic Membrane Activity (μmol min^{-1})
5	10	21.5	16	30.0	0.97
10	10	31.5	16	39.6	3.50
15	10	32.5	16	43.0	5.80
20	10	30.0	16	41.2	4.60
25	10	27.0	16	39.4	2.51

Results reported herein were obtained under the following experimental conditions: a = 5, 10, 15, 20, and 25% AA concentration; b = 0.1% FAS concentration; $c = t_1$ = 10 h; d = 10% HEMA cocnentration; e = 45 mg mL^{-1} enzyme concentration; and $f = t_2$ = 16 h.

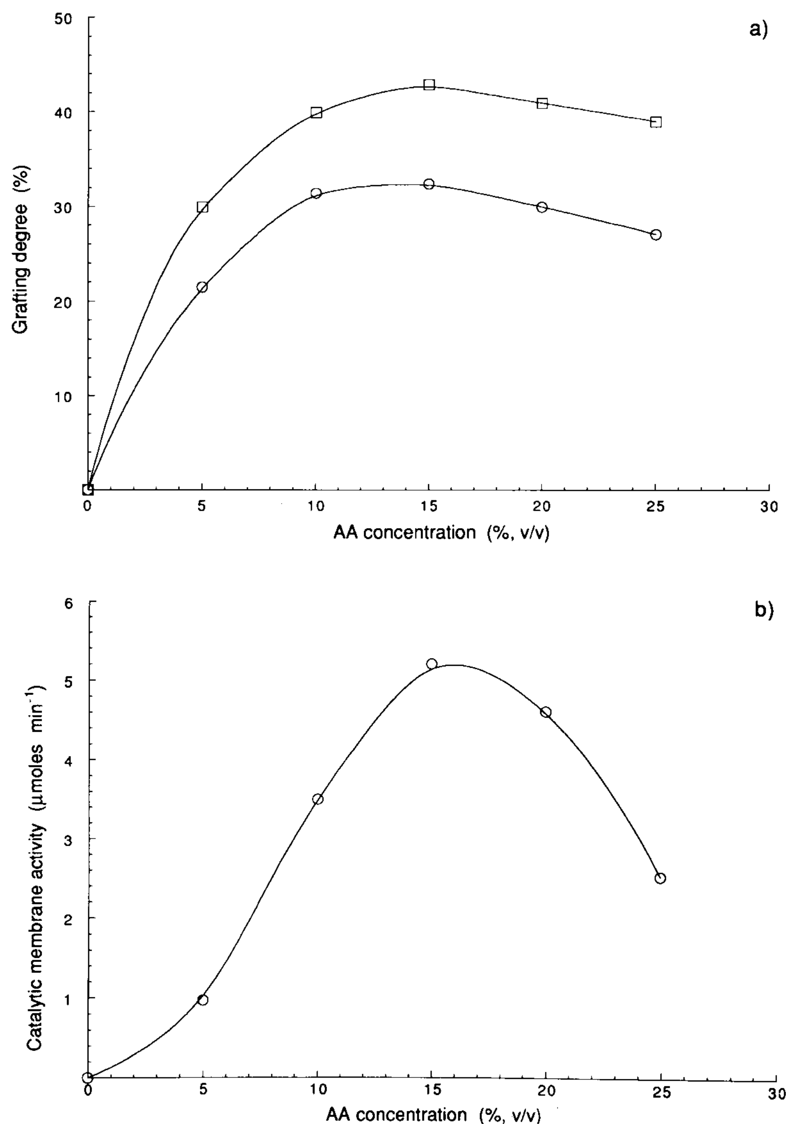


Figure 5 (a) AA (○) and HEMA (⊡) grafting degrees as a function of AA concentration. (b) Catalytic membrane activity as a function of AA concentration. Experimental conditions used are reported in the footnote to Table VI.

Dependence on AA Concentration

Let us examine the dependence of the catalytic membrane activity on AA concentration, remembering that the AA chains constitute the most probable attachment sites for HEMA grafting. To this aim, we have performed experiments under the same conditions used in the previous section, with the exception that FAS concentration was kept constant at 0.1%, whereas AA concentration ranged from 5 to 25%. The results, reported in Table VI and in Figures 5(a,b), show a maximum at 15% AA concentration. To understand these results, it is sufficient to explain the behavior of the first grafting degree, which controls the second grafting degree and the catalytic membrane activity. FAS affects the production of the grafted branches on the surface of the Teflon membrane and inhibits AA homopolymerization. These two roles are in competition and dependent on the AA and FAS concentrations. Keeping the FAS concentration constant, at low AA concentrations, the rate of grafting is higher than the rate of homopolymerization, whereas at high AA concentration the reverse occurs. In our experiments, the AA concentrations giving the best conditions for obtaining the maximum of grafting degree occur in a range centered about the 15%. The same behav-

Table VII Effect of HEMA Concentration

HEMA Concentration (%)	Irradiation Time Used During First Grafting (h)	AA Grafting Degree (%)	Irradiation Time Used During Second Grafting (h)	HEMA Grafting Degree (%)	Catalytic Membrane Activity (μmol min^{-1})
2	10	30.0	16	11.0	1.00
4	10	30.6	16	13.0	1.38
6	10	31.0	16	24.0	1.46
8	10	29.7	16	46.5	2.10
10	10	32.0	16	43.0	5.20
12	10	30.5	16	72.0	4.32
15	10	30.5	16	79.0	3.32

Results reported herein were obtained under the following experimental conditions: a = 15% AA concentration; b = 0.1% FAS concentration; $c = t_1 = 10$ h; d = 2, 4, 6, 8, 10, 12, and 15% HEMA concentration; e = 45 mg mL^{-1} enzyme concentration; and $f = t_2 = 16$ h.

ior of Figure 5(a) was found by other authors in the case of styrene monomer.[5,8]

Dependence on HEMA Concentration

This study has been performed under the same experimental conditions used in the previous paragraph, with the exception that now the AA concentration was kept constant at 15%, whereas HEMA concentration ranged from 2 to 15%. The results of these experiments are reported in Table VII, where it is possible to observe how the AA grafting degree remains constant with the same conditions for first grafting, whereas HEMA–en-

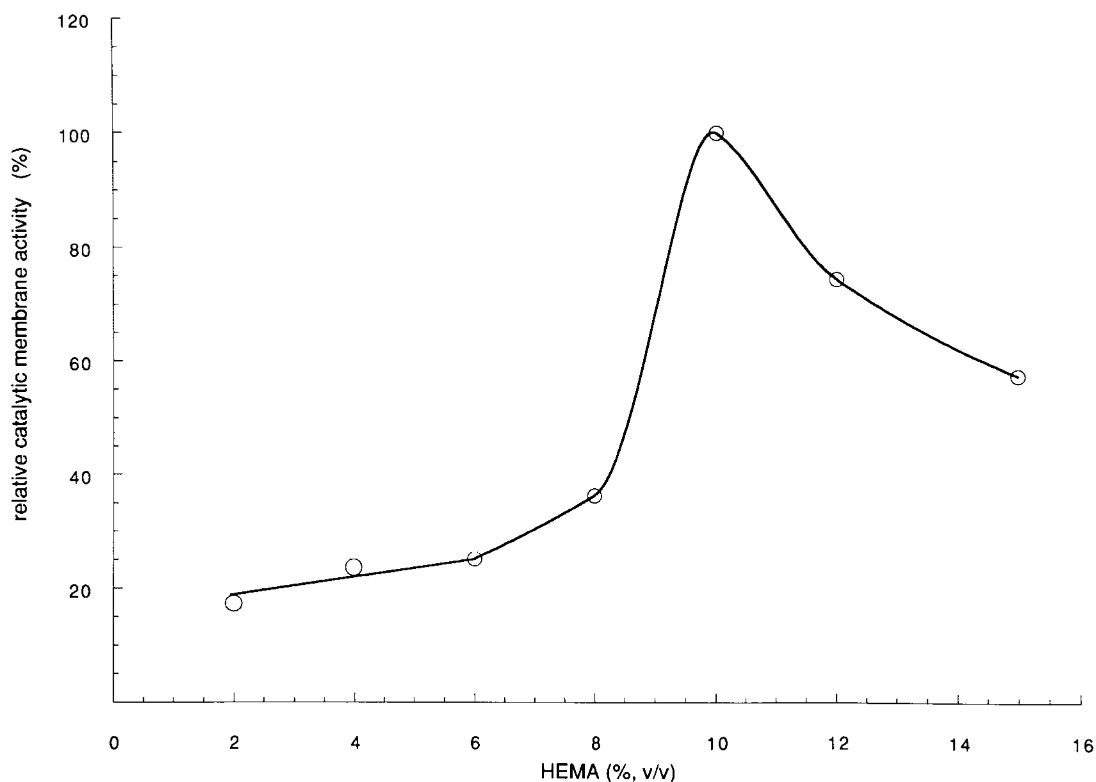


Figure 6 Relative catalytic membrane activity as a function of HEMA concentration. Experimental conditions used are reported in the footnote to Table VII.

zyme grafting linearly increases with HEMA concentration and the activity of the catalytic membrane has a maximum. This behavior is more evident in Figure 6, where the relative activity of the catalytic membrane is reported as a function of HEMA concentration.

CONCLUSIONS

In concluding this work, we want to observe how the double grafting technique improved the activity of the biocatalytic membrane. The direct grafting of HEMA and enzyme indeed given catalytic membrane some five times less active than ones obtained with the double grafting technique as it is possible to see in Figure 1 by comparing the results under the symbols ○ and □.

The conditions for obtaining the best activity of the double-grafted membranes are: $a = 15\%$ AA concentration (v/v); $b = 0.1\%$ FAS concentration (w/v); $c = t_1 = 10$ h for the irradiation time during first grafting; $d = 10\%$ HEMA concentration (v/v); $e = 45$ mg mL^{-1} for enzyme concentration; and $f = t_2 = 32$ h for irradiation time during second grafting.

One of the aims of this work was to obtain a Teflon catalytic membrane to be used in nonisothermal bioreactors, which have been found to provide higher efficiencies with respect to the same bioreactors operating under comparable isothermal conditions.[21–26] Having obtained by the grafting technique such a membrane, we now have the possibility of improving the functioning of nonisothermal bioreactors. Experiments in this direction are presently planned in our laboratory.

Out of the aim of this work came the biochemical characterization of the biocatalytic membranes now obtained by studying the dependence of their activity on substrate concentration, pH, and temperature. These results are reported elsewhere,[32] together with the ones relative to the same enzyme immobilized on Teflon membranes grafted with other monomers, such as acrylamide or methacrylic acid.

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REFERENCES

1. G. Box, A. Chapiro, M. Huglin, A. M. Jendrychowska Bonamour, and T. O'Neill, *J. Polym. Sci.,* **22,** 493 (1968).
2. C. McCormick and L. Park, *J. Polym. Sci.,* **22,** 49 (1984).
3. T. S. Godjevargova, A. R. Dimov, and N. Vasileva, *J. Appl. Polym. Sci.,* **54,** 355 (1994).
4. T. S. Godjevargova and A. R. Dimov, *J. Appl. Polym. Sci.,* **57,** 487 (1995).
5. J. L. Garnett, S. V. Jankiewicz, R. Levot, and D. F. Sangster, *Rad. Phys. Chem.,* **25,** 509 (1985).
6. I. Kaetsu, M. Kumakura, T. Fujimura, M. Yoscida, M. Asano, N. Kasai, and M. Tamada, *Rad. Phys. Chem.,* **27,** 245 (1986).
7. A. S. Hoffman, W. R. Gombotz, S. Uoenoyama, L. C. Dong, and G. Schmer, *Rad. Phys. Chem.,* **27,** 265 (1986).
8. J. L. Garnett, S. V. Jankiewicz, R. Levot, and D. F. Sangster, *Rad. Phys. Chem.,* **27,** 301 (1986).
9. Y. Arica and V. N. Hasirci, *Biomaterials,* **8,** 489 (1987).
10. M. Carenza and G. Palma, *Ann. N.Y. Acad. Sci.,* **542,** 115 (1988).
11. M. Alves da Silva, C. G. Beddows, M. H. Gill, J. T. Guthrie, A. J. Guiomar, S. Kotov, and A. P. Piedade, *Rad. Phys. Chem.,* **35,** 98 (1990).
12. E. H. Docters, E. E. Smolko, and C. E. Suarez, *Rad. Phys. Chem.,* **35,** 102 (1990).
13. K. Hajizadhe, H. B. Halsall, and W. R. Heinemann, *Anal. Chim. Acta,* **243,** 23 (1991).
14. I. Gursel and V. N. Hasirci, *Biomaterials,* **13,** 150 (1992).
15. L. Doretti, D. Ferrara, and S. Sora, *Biosensors and Bioelectronics,* **8,** 443 (1993).
16. T. S. Godjevargova, *J. Appl. Polym. Sci.,* **61,** 334 (1996).
17. J. H. Bentvelzen, F. Kimura-Yih, H. B. Hopfemberg, and V. Stannet, *J. Appl. Polym. Sci.,* **17,** 809 (1973).
18. G. C. Tealdo, P. Canepa, and S. Munari, *J. Membr. Sci.,* **9,** 191 (1981).
19. P. Aptel, N. Challard, J. Cuny, and J. Neel, *J. Membr. Sci.,* **1,** 271 (1976).
20. G. Morel, J. Jozefowicz, and P. Aptel, *J. Appl. Polym. Sci.,* **23,** 2397 (1979).
21. D. G. Mita, M. A. Pecorella, P. Russo, S. Rossi, U. Bencivenga, P. Canciglia, and F. S. Gaeta, *J. Membr. Sci.,* **78,** 69 (1993).
22. D. G. Mita, M. Portaccio, P. Russo, S. Stellato, G. Toscano, U. Bencivenga, P. Canciglia, A. D'Acunto, N. Pagliuca, S. Rossi, and F. S. Gaeta, *Biotechnol. Appl. Biochem.,* **22,** 281 (1995).
23. P. Russo, A. Garofalo, U. Bencivenga, R. Rossi, D. Castagnolo, A. D'Acunzo, F. S. Gaeta, and D. G. Mita, *Biotechnol. Appl. Biochem.,* **23,** 141 (1996).
24. M. Portaccio, S. Stellato, S. Rossi, U. Bencivenga, P. Canciglia, F. Palumbo, F. S. Gaeta, and D. G.

Mita, *Biotechnol., Biotechnol. Appl. Biochem.,* **24,** 25 (1996).
25. P. Russo, A. De Maio, A. D'Acunzo, A. Garofalo, U. Bencivenga, S. Rossi, R. Annicchiarico, F. S. Gaeta, and D. G. Mita, *Res. Microbiol.,* **148,** 271 (1997).
26. S. Stellato, M. Portaccio, S. Rossi, U. Bencivenga, G. La Sala, G. Mazza, F. S. Gaeta, and D. G. Mita, *J. Membr. Sci.,* **129,** 175 (1997).
27. F. S. Gaeta and D. G. Mita, *J. Membr. Sci.,* **3,** 191 (1978).
28. F. Bellucci, E. Drioli, F. S. Gaeta, D. G. Mita, N. Pagliuca, and F. G. Summa, *Trans. Farad. Soc. II.,* **75,** 247 (1979).
29. N. Pagliuca, G. Perna, D. G. Mita, F. S. Gaeta, B. Karamanlis, and F. Bellucci, *J. Membr. Sci.,* **16,** 91 (1983).
30. D. G. Mita, U. Bencivenga, A. D'Acunto, N. Pagliuca, G. Perna, S. Rossi, and F. S. Gaeta, *Gazzetta Chim. Ital.,* **118,** 79 (1988).
31. F. S. Gaeta, E. Ascolese, U. Bencivenga, J. M. Ortiz de Zarate, N. Pagliuca, G. Perna, S. Rossi, and D. G. Mita, *J. Phys. Chem.,* **96,** 6342 (1992).
32. M. S. Mohy Eldin, U. Bencivenga, M. Portaccio, S. Stellato, S. Rossi, M. Santucci, P. Canciglia, F. S. Gaeta, and D. G. Mita, *J. Appl. Polym. Sci.* **68,** 613 (1998).