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Immobilization of lipase on various acrylic copolymers

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Abstract

Polymer carriers with various degrees of hydrophobicity, porosity, and polarity were studied for immobilization of lipase from *Candida rugosa*. Copolymers and terpolymers were prepared by suspension polymerization of six monomers, ethyl and butyl acrylate, acrylonitrile, methyl methacrylate, 2-hydroxypropyl methacrylate and vinyl acetate, and three crosslinking agents, divinylbenzene, ethylene glycol dimethacrylate and trimethylolpropane triacrylate, in the presence of various diluents. Immobilization of lipase was carried out via glutaraldehyde chemical binding or by adsorption. It was found that the best results were obtained for carriers of butyl acrylate, ethyl acrylate or 2-hydroxypropyl methacrylate crosslinked with ethylene glycol dimethacrylate. © 1997 Elsevier Science S.A.

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1. Introduction

The technological approaches to the modification of fats and oils are the subject of intensive investigations. The conventional non-catalyzed splitting conditions are 250 °C under 50 MPa what makes this process energy consuming. Furthermore, the capital investment for steam splitting is high since a special splitter column is required [1]. Enzymatic hydrolysis takes place in much milder conditions (30–45 °C under atmospheric pressure), capital investments are lower, and such a process usually yields higher quality products [1,2].

The enzyme that catalyzes hydrolysis of triglycerides to fatty acids and glycerol is lipase (triglycerol acylhydrolase, EC 3.1.1.3). The price of lipase is relatively high, so in practical applications reuse of the enzyme is important. It is obvious that lipase should be immobilized in order to use it several times. Immobilization would also facilitate a continuous process.

Many immobilization techniques for lipase have been tried and reviewed recently [1–15]. Authors of some papers have quoted a large number of factors which influenced the performance of the immobilized enzyme [5,6,10,13,15]. Support material and the immobilization strategy were found to be the most important two factors. Most of the carriers were tested for their suitability for the immobilization of lipase and the use of enzyme preparations in a hydrolytic reaction. The protein content, activity, and sometimes stability were determined.

The aim of this work is to examine acrylic carriers polymerized from several monomers and crosslinking agents, displaying various hydrophobicities, porosities, and polarities for lipase immobilization.

2. Materials and methods

2.1. Materials

Lipase from *Candida rugosa*, glutaraldehyde, trihydroxymethylaminomethane (tris) and a lipase activity kit were purchased from Sigma. All other reactants were analytical grade from Fluka and Aldrich.

2.2. Carriers

Acrylic carriers crosslinked with divinylbenzene (DVB), ethylene glycol dimethacrylate (EGDMA) or trimethylolpropane triacrylate (TMPA) were prepared by suspension polymerization [16–18]. The carriers differed also in the

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comonomers. Butyl acrylate (BA), ethyl acrylate (EA), acrylonitrile (AN), methyl methacrylate (MM), 2-hydroxypropyl methacrylate (HPMA) and vinyl acetate (VA) were used. To obtain porous copolymers polymerization was carried out in the presence of inert solvents such as: toluene, octane, heptane, dodecane, hexadecane, butanol, dodecanol, 2-ethylhexanol, and cyclohexanol. Methods used for modification of polymers by aminolysis with ethylenediamine were described elsewhere [19–21]. The apparent and true density were measured pycnometrically. The contents of amino groups were measured using titration method [22].

2.3. Methods

Activation of the carriers with glutaraldehyde and immobilization of the enzyme were performed as described previously [23]. The amount of protein bound to polymer carriers was calculated as the difference between the amount taken for immobilization and the amount eluted from the polymer (mass balance). Protein concentration was determined spectrophotometrically (280 nm, Spectrophotometer Shimadzu 160A) and by the method of Lowry et al. [24].

Activity of immobilized enzyme was determined according to Sigma procedure No. 800 (hydrolysis of olive oil in emulsion system). In the case of high activity preparation, up to a five-fold increase of the volume of all reactants was used. Specific activity of the native enzyme according to the Sigma procedure was 18970 U dm^{-3} mg.

The stability of immobilized preparation was determined by measuring the activity of the sample stored for three months in 0.1 mole dm^{-3} tris-HCl buffer (pH = 7.8; 4 °C).

3. Results and discussion

3.1. Carriers

Since it is well known that so many factors influence efficiency of the enzyme–carrier system it is necessary to screen many carriers in order to find an optimal one for a given enzyme. For porous acrylic copolymers used in this work these factors are: porosity, water regain (amount of water retained in the carrier), surface area, and amino group content.

Tested polymers can be divided into three groups, the first group being polymers crosslinked with the most hydrophobic crosslinking agent — DVB. Next are polymers with EGDMA and the last group consists of polymers with TMPA. Inside each group polymers were differed by using various comonomers such as AN, VA, BA, EA, MM and HPMA. Addition of various comonomers and changes of the amount of crosslinking agent were aimed at modification of hydrophobicity and accessibility of polymers. Additionally, various organic diluents were used as porogens during polymerization resulting in changes of porosity and specific surface area.

Table 1 gives the characteristics of carriers derived from AN/DVB copolymers. As can be seen these polymers retain porosity after modification with ethylenediamine regardless of the type of porogen used during polymerization. A reason for this is the high concentration of AN in the polymerization mixture resulting in early phase separation (AN is a nonsolvent for polyacrylonitrile polymers). Highly ordered polyacrylonitrile regions of polymer serve as additional physical crosslinks. Content of the amino group averages 1.40 mmol g^{-1}. The volume fraction of copolymer in a swollen gel, which is a measure of water content in a swollen gel after taking into account water present in the pores, averages 0.44. Thus, these AN/DVB carriers can be considered highly porous, polar and moderately hydrophilic.

Table 2 contains characteristics of carriers obtained from AN/VA/DVB terpolymers. VA was used to replace part of AN in these terpolymers for two reasons. The first was to incorporate it into polyacrylonitrile sequences, thus breaking the highly ordered structure of polyacrylonitrile copolymers, which should facilitate modification with ethylenediamine. Secondly, during this modification VAmers hydrolyze leaving polymers with hydroxyl groups, which contribute to polymers hydrophilicity. Content of amino groups for these

Table 1
Characteristics of carriers derived from acrylonitrile–divinylbenzene copolymers obtained in the presence of various porogens. All the polymers with 10 wt.% of divinylbenzene. Volume ratio of porogens was 1:9

No.	Porogens	Porosity (%)	Water regain ($g\,g^{-1}$)	V	Specific surface ($m^2\,g^{-1}$)	Amino groupcontent (mmol g^{-1})
1	Heptane Toluene	27.0	2.00	0.41	N/D	1.70
2	Hexadecanee Toluene	48.0	3.05	0.38	16	1.80
3	Heptane Cyclohexanol	43.0	3.48	0.34	42	1.20
4	Hexadecane Cyclohexanol	53.0	2.68	0.51	37	1.10
5	2-Ethylhexanol Cyclohexanol	37.0	2.70	0.48	23	1.40

V, volume fraction of copolymer in swollen gel ($cm^3\,cm^{-3}$).

Table 2
Characteristics of carriers derived from acrylonitrile–vinyl acetate–divinylbenzene terpolymers obtained in the presence of various porogens. All the polymers with 10 wt.% of divinylbenzene. Weight ratio of AN/VA was 3:1. Volume ratio of porogens 1:9

No.	Porogens	Porosity (%)	Water regain ($g\,g^{-1}$)	V	Specific surface ($m^2\,g^{-1}$)	Amino group content ($mmol\,g^{-1}$)
6	Hexadecane Toluene	49.0	2.51	0.48	9.0	1.85
7	Dodecanol Cyclohexanol	39.0	3.59	0.30	25.0	1.79
8	2-Ethylhexanol	30.0	3.32	0.28	18.0	1.78
9	Octane Cyclohexanol	11.0	3.30	0.22	0.7	1.76
10	Butanol Cyclohexanol	10.0	3.63	0.21	0.5	1.52

V, volume fraction of copolymer in swollen gel ($cm^3\,cm^{-3}$).

Table 3
Characteristics of carriers derived from acrylonitrile–butyl(ethyl) acrylate–divinylbenzene terpolymers with various crosslinking degree and obtained in the presence of heptane and toluene (1:1) as porogens. Weight ratio of AN/BA (AN/EA) was 1:1

No.	Monomers	Crosslinking degree (%)	Porosity (%)	Water regain ($g\,g^{-1}$)	V	Specific surface ($m^2\,g^{-1}$)	Amino group content ($mmol\,g^{-1}$)
11	AN/BA	10.0	2.7	2.20	0.23	0.0	1.34
12	AN/BA	12.5	18.0	1.60	0.42	1.3	0.37
13	AN/BA	15.0	10.0	1.60	0.39	1.5	0.21
14	AN/BA	20.0	22.0	1.40	0.99	1.4	0.14
15	AN/EA	15.0	48.0	2.40	0.50	189.0	0.24

V, volume fraction of copolymer in swollen gel ($cm^3\,cm^{-3}$).
AN, acrylonitrile.
BA, butyl acrylate.
EA, ethyl acrylate.

Table 4
Characteristics of carriers derived from butyl acrylate–methyl methacrylate–ethylene glycol dimethacrylate terpolymers obtained in the presence of various porogens. All the polymers with 30 wt.% of EGDMA. Weight ratio of BA/MM was 1:1. Volume ratio of porogens was 1:1

No.	Porogens	Porosity (%)	Water regain ($g\,g^{-1}$)	V	Specific surface ($m^2\,g^{-1}$)	Amino group content ($mmol\,g^{-1}$)
16	Toluene 2-Ethylhexanol	11.0	0.80	0.54	~0.0	0.50
17	Toluene Hexadecane	56.0	1.40	0.89	N/D	0.20
18	Cyclohexanol Hexadecane	3.0	1.40	0.35	1.9	0.40
19	Cyclohexanol 2-Ethylhexanol	6.0	1.05	0.45	~0.0	0.30

V, volume fraction of copolymer in swollen gel ($cm^3\,cm^{-3}$).
N/D, not determined.

polymers is slightly higher, averaging 1.65 mmol g^{-1} and volume fraction of copolymer in swollen gel is about 0.30. Three of these carriers 6, 7 and 8 can be considered highly porous, polar and hydrophilic. another two, 9 and 10, display low porosity. They can be considered as "expanded gels" [22].

In Table 3 properties of AN/BA/DVB and AN/EA/DVB carriers are presented. Replacement of a considerably high amount of AN with alkyl acrylate resulted in decreased porosity (gel structure) as can be seen for polymer 11. When the crosslinking degree was increased (carriers 12–14) porosity was retained unchanged after modification with ethylenediamine. However, chemical modification was limited and so low amino group content and water regain were obtained. These carriers can be considered slightly porous, polar and moderately hydrophobic. Table 4 contains characteristics of carriers obtained from BA/MMA/EGDMA copolymers. These carriers show high porosity when obtained from pol-

Table 5
Characteristics of carriers derived from ethyl acrylate–ethylene glycol dimethacrylate copolymers polymerized in the presence of various porogens. Volume ratio of porogens was 1:1

No.	Porogens	Crosslinking degree (%)	Porosity (%)	Water regain ($g\ g^{-1}$)	V	Specific surface ($m^2\ g^{-1}$)	Amino group content ($mmol\ g^{-1}$)
20	Octane Butanol	30	55.0	7.40	0.70	7.4	1.80
21	Octane Cyclohexanol	30	0.0	2.20	0.26	~0.0	1.60
22	Dodecanol Cyclohexanol	30	0.0	2.80	0.22	~0.0	1.70
23	Hexadecanol Butanol	30	54.0	1.40	0.76	0.2	0.70
24	Hexadecane Cyclohexanol	30	53.0	2.10	0.58	0.7	1.20
25	2-Ethylhexanol Cyclohexanol	30	2.0	2.30	0.25	~0.0	1.40
26	2-Ethylhexanol Butanol	30	2.0	1.90	0.24	0.2	1.50
27	Octane Toluene	30	11.0	2.00	0.30	0.9	1.40
28	Octane Toluene	25	7.0	3.00	0.22	0.2	1.90
29	Octane Toluene	20	0.0	5.10	0.20	~0.0	2.10

V, volume fraction of copolymer in swollen gel ($cm^3\ cm^{-3}$).

ymers formed in the presence of thermodynamically poor solvents such as a mixture of toluene and hexadecane (see carrier 17). Others were obtained in the presence of the mixture of good and poor diluents and displayed low porosity. All of them were difficult to modify with ethylenediamine. Thus, they displayed low amino group content and moderate water regain. These carriers can be considered slightly porous (with the exception of 17), slightly polar and rather hydrophobic.

In Table 5 characteristics of carriers obtained from EA/EGDMA copolymers are presented. Again, they retained porosity when precursors were polymerized in the presence of poor diluents such as long chain alkanes (see carriers 20, 23, 24). Others display small porosity or no porosity at all. Amino group content averages some 1.50 mmol g^{-1}, water regain 2.00 mmol g^{-1} (with the exceptions of polymer 20 and polymers 28 and 29, which are less crosslinked). These polymers can be considered non-porous (except 20, 23, 24), slightly polar and hydrophilic.

The polymers presented in Table 6 contain amino functionality. Carriers were obtained from BA/HPMA/EGDMA terpolymers. In spite of hydroxyl group content the water regain was rather small and volume fraction of polymer in the gel was high, except for carriers 31 and 34. They can be considered highly porous, slightly polar and hydrophobic.

The last group of carriers, characteristics shown in Table 7, was crosslinked with trimethylolpropane triacrylate. They retained porosity, however water regain was moderate and the volume fraction of copolymer in the swollen gel was high. This indicates that almost all water is present in the pores, which implies that modification with ethylenediamine took place mostly on the surface of the polymer and in the pores, whereas the majority of polymeric material remained intact. Thus, as can be expected, amino group content is low. These carriers can be considered highly porous, polar and moderately hydrophobic.

3.2. Enzyme immobilization

It is well known that the immobilization process results in a decrease of enzyme activity. This is due to the changes in the structure of enzyme produced by its covalent linkage to the polymer surface. Additionally, proteins may be partially deposited (adsorbed) on the polymer surface depending on the type and strength of their interactions with the polymer, such as hydrophobic ones or hydrogen bonding. Another contribution to the decrease of enzyme activity may arise from slow kinetics of substrate diffusion into the enzyme–polymer system and slow diffusion of products outside. This overall decrease of activity is usually compensated by better enzyme–polymer stability and ease of handling, which makes these systems suitable for continuous processes and reuse. From this point of view it is important that the enzyme–polymer system should contain as much immobilized enzyme as is possible. In addition, the enzyme should also retain reasonably good activity.

Due to the great number of carriers used for immobilization of lipase, the data are listed in three groups (Tables 8–10) taking into account the kind of crosslinking agents. The carriers are numbered in the same way as in Tables 1–7. The basic characteristics of enzyme–carrier preparation included the amount of bound protein, its activity, and storage stability.

Table 6
Characteristics of carriers derived from butyl acrylate–hydroxypropyl methacrylate–ethylene glycol dimethacrylate terpolymers obtained in the presence of various porogens. All the polymers with 40 wt.% of EGDMA unless indicated otherwise. Weight ratio of BA/HPMA was 1:1; volume ratio of toluene/octane was 1:1 and dodecanol/cyclohexanol 1:9

No.	Porogens	HPMA amount (%)	Porosity (%)	Water regain ($g\,g^{-1}$)	V	Amino group content ($mmol\,g^{-1}$)
30	Toluene Octane	20	53.5	1.70	0.77	0.84
31	Toluene Octane	33	16.1	1.64	0.42	0.38
32	Toluene Octane	50	53.5	1.60	0.94	0.31
33	Dodecanol Cyclohexanol	33	51.9	1.08	0.96	0.68
34	Dodecanol Cyclohexanol	50	2.0	0.74	0.58	0.42
35	Dodecanol Cyclohexanol	33	42.5	1.15	0.98	0.53
36	Toluene Octane [a]	50	56.5	1.30	0.90	none
37	Toluene Octane [b]	50	53.5	1.13	0.91	none
38	Toluene Octane [c]	50	53.0	1.16	0.89	none

V, volume fraction of copolymer in swollen gel ($cm^3\,cm^{-3}$).
HPMA amount, calculated as: mole of HPMA/mole of (BA + HPMA) 100%.
[a,b,c] crosslinking degree, respectively: 50 wt.%, 60 wt.%, 80 wt.%.

Table 7
Characteristics of carriers derived from acrylonitrile–trimethylolpropane triacrylate copolymers polymerized in the presence of various porogens volume ratio of porogens was 1:1

No.	Porogens	Crosslinking degree (wt.%)	Porosity (%)	Water regain ($g\,g^{-1}$)	V	Amino group content ($mmol\,g^{-1}$)
39	Cyclohexanol Hexadecane	40	48.5	0.94	0.99	0.60
40	Cyclohexanol 2-Ethylhexanol	40	53.0	1.11	0.98	0.51
41	Cyclohexanol Dodecanol	40	55.2	1.23	0.99	0.46
42	Toluene	40	54.5	1.21	0.93	0.39
43	Toluene	50	59.0	1.18	0.99	0.38
44	Toluene	60	55.2	1.03	0.98	0.35

V, volume fraction of copolymer in swollen gel ($cm^3\,cm^{-3}$).

Additional information is expressed by yield of immobilization, specific activity of protein as well as expressed activity. For example, in experiment no. 1 the carrier bound 1.12 mg of protein per 1 cm^3 which is 45.3% of the initial amount of protein (III). The activity of immobilised preparation was 2422 U cm^{-3}, which is equivalent to 2163 U dm^{-3} mg^{-1} protein (this is specific activity). This value is 11.4% of the native's enzyme specific activity (II). The activity after 3 month's storage was equal to 84.4% of the initial activity (storage stability).

The comparison of enzyme activity after three months of storage reveals an unusual feature: fifteen enzyme preparations did not lose their activities. Moreover, in some cases (carriers 17, 33, 42–44) activity after three months was greater than that immediately after immobilization. Such a phenomenon was observed earlier [22]. Also, in our unpublished work on amylase and trypsin immobilization, activity of enzymes upon storage was increased [25]. Since in this work it happened only when amino group content was low (0.20–0.68 mmol g^{-1}) and carriers displayed high porosity ($\sim$50%), it can be concluded that upon storage for a longer period of time the enzyme linked to the carrier only by few covalent bonds might have assumed a configuration closer to that of the native enzyme. Such motion would be impossible if many amino groups were involved in immobilization of single molecules of lipase.

Another unusual feature is the change of carrier volume upon enzyme immobilization. In some cases (for example

Table 8
Characteristics of the enzyme immobilized on carriers crosslinked with divinylbenzene. Specific activity of native enzyme 18970 U dm $^{-3}$ mg $^{-1}$

No.	I (%)	Bound protein (mg cm^{-3})	Activity (U l^{-1} cm^{-3})	Specific activity (U l^{-1} mg^{-1})	II (%)	III (%)	Storage stability (%)
1	+2	1.12	2422	2163	11.4	45.3	84.4
2	0	1.21	966	798	4.2	48.0	73.9
3	−18	2.05	2814	1373	7.3	66.7	94.5
4	0	1.65	2926	1773	9.4	65.5	74.6
5	−32	2.52	3472	1378	7.3	68.0	67.7
6	+2	1.87	994	783	4.1	78.1	105.6
7	−12	1.22	868	711	3.8	64.8	74.2
8	−4	1.07	2254	2107	11.1	62.0	65.8
9	−12	1.32	1554	1177	6.2	70.1	16.2
10	−26	1.68	952	567	2.0	75.0	77.5
11	−8	2.36	3948	1673	8.9	67.0	85.1
12	−2	1.68	2128	1267	6.7	66.0	65.1
13	0	0.59	266	451	2.4	23.6	63.2
14	−4	0.69	252	365	1.9	26.5	49.9
15	−22	1.71	2623	1534	8.1	53.4	60.3

I, (volume of carrier before immobilization) / (volume of carrier after immobilization) × 100%.
II, active enzyme in bound protein calculated as: (active enzyme) / (specific activity of native enzyme × protein attached to carrier) × 100%.
III, Immobilization yield based on total protein attached to carrier.

Table 9
Characteristics of the enzyme immobilized on carriers crosslinked with ethylene glycol dimethacrylate. Specific activity of native enzyme 18 970 U dm^{-3} mg^{-1}

No.	I (%)	Bound protein (mg cm^{-3})	Activity (U l^{-1} cm^{-3}])	Specific activity (U l^{-1} mg^{-1})	II (%)	III (%)	Storage stability (%)
16	0	1.21	3906	3228	17.1	48.0	92.8
17	−16	0.80	2030	2538	13.4	26.7	191.7
18	−14	0.81	2184	2696	14.3	27.6	82.1
19	−12	1.35	5950	4407	23.3	47.1	52.7
20	−4	N/D	3374	N/D	N/D	N/D	67.6
21	−14	1.36	4144	3047	16.1	46.9	53.4
22	−15	1.68	3798	2261	12.0	56.5	57.9
23	+4	1.27	2464	1940	10.3	40.8	85.2
24	−8	1.43	1764	1234	6.5	40.6	84.1
25	−50	2.53	2940	1162	6.2	53.2	93.5
26	−32	3.15	2548	809	4.3	66.1	85.7
27	−18	0.82	3290	4012	21.2	26.7	110.2
28	−6	0.61	1862	3052	16.2	23.0	109.0
29	−44	2.34	5336	2280	12.1	52.0	89.3
30	−2	1.37	5292	3836	20.4	42.8	66.9
31	−12	1.41	3696	2621	13.9	39.6	86.0
32	−20	1.31	4382	3345	17.7	33.5	102.6
33	−4	2.05	4046	1974	10.4	62.9	140.5
34	+2	1.59	2884	1814	9.6	51.8	108.7
35	+4	1.99	2450	1231	6.5	66.1	103.4
36	−24	1.19	4324	3634	19.2	28.9	94.9
37	−4	0.77	3192	4145	21.9	23.6	120.2
38	−10	1.45	3416	2356	12.5	41.7	117.2

Symbols are indicated in Tables 8, 9 and 8.
N/D, not determined.

carriers 25, 26, 29) these changes can be as high as 50%. Usually the carrier–enzyme system has a smaller volume than the starting polymer and it is likely to happen in the case of carriers with higher amino group content and for non-porous polymers. These two facts can lead to the conclusion that shrinkage is due to crosslinking during activation with glutaraldehyde (an aldehyde with two reactive groups can react with two amino groups on the polymer) or such crosslinking can occur during contact between an aldehyde-activated polymer and the enzyme. Carriers which retained porosity during

Table 10
Characteristics of the enzyme immobilized on carriers crosslinked with trimethylolpropane triacrylate. Specific activity of native enzyme 18 970 U dm^{-3} mg^{-1}

No.	I (%)	Bound protein (mg cm^{-3})	Activity (U l^{-1} cm^{-3})	Specific activity (U l^{-1} mg^{-1})	II (%)	III (%)	Storage stability (%)
39	−8	1.26	2296	1822	9.6	36.1	61.0
40	−6	N/D	1974	N/D	N/D	N/D	68.1
41	−2	0.99	1526	1541	8.2	30.2	67.9
42	0	1.20	728	607	3.2	36.4	217.3
43	+6	1.09	560	514	2.7	36.0	135.0
44	0	1.22	616	505	2.7	38.0	136.4

Symbols are indicated in Table 8.
N/D, not determined.

modification with ethylenediamine also maintained structural integrity during immobilization of lipase.

In this study of lipase immobilization it was found that excellent activity is displayed by several polymers — 19, 21, 29, 30, 32, 33, and 36. All of them have activity above 4000 U l^{-1} per 1 cm^3 (see Table 9) and all are polymers crosslinked with EGDMA. The worst activity have enzyme preparations crosslinked with DVB or TMPA (see carriers 13, 14, 42, 43, and 44 in Tables 8 and 10).

When the enzyme is located in the suitable microenvironment its specific activity and content of active enzyme in bound protein should be relatively high. Three polymers (19, 27, and 37) may be considered as such carriers. All of them are crosslinked with EGDMA. One of the above (37) is a system in which lipase was immobilized by non-covalent bonding (presumably hydrophobic) since the polymer did not contain amino groups. This particular method seems to be efficient but such a system lacks stability in organic media releasing enzymes to the solution. The remaining two preparations (19 and 27) contain 30 wt.% of crosslinking agent, both are slightly porous. The major difference between them is amino group content: 19 contains a small amount, 0.30 mmol g^{-1}, whereas 27 has 1.90 mmol g^{-1}. This difference also produces higher water regain and lower volume fraction of polymer in the swollen gel (V). These two preparations display very different stability. For 19 only 52.7% and in the case of 27 as much as 110.2% of the immobilized lipase activity is retained after three months. However, from only two preparations no conclusion can be drawn.

In order to increase the number of potentially useful enzyme–carrier systems we next selected a group of preparations with a specific activity of enzyme in the range 3000–4000 U l^{-1} mg^{-1} (16, 20, 21, 28, 30, 32 and 36). Here again all of the above are polymers with EGDMA as the crosslinking agent. One of carrier–enzyme system (36) is obtained by non-covalent bonding of lipase to the polymer. Its activity is high and its stability is very good, which shows that a lack of covalent bonding allows the "native" configuration of enzyme even inside polymeric beads. Among the rest of the preparations there are three which preserve their activity during three month storage (16, 28, 32, see Table 8) and three (20, 21, 30) with activity falling to approximately 60% of the original value. However, after comparing polymer properties, there is no apparent correlation between activity and stability of immobilized lipase and polymer characteristic.

4. Conclusions

It is concluded that a large amount of various factors, which cannot be changed independently, influence the efficiency of lipase immobilization and make drawing a detailed conclusion impossible. However, it is possible to make a general statement based on the obtained results.

1. The effect of carrier porosity on the properties of immobilized lipase may be neglected.
2. There is no evidence of correlation between either the carrier parameters or the kind of porogens and amount of immobilized protein.
3. Considering the activity of all enzyme–carrier preparations it can be concluded that the best polymer matrices are these with EGDMA, which have well established hydrophobicity. Among them, terpolymers of BA/MM/EGDMA with higher polarity seem to be the best.
4. The carriers with well established hydrophobicity and medium polarity with EGDMA crosslinker seem to create an appropriate microenvironment for lipase. Carriers with DVB or TMPA, both with medium polarity and hydrophobicity, do not provide the proper environment for the studied enzyme.
5. The most stable enzyme–carrier preparations were obtained from polymers crosslinked with TMPA or EGDMA. Generally, the best carriers were synthesized from BA/HPMA/EGDMA terpolymers in the presence of such porogens as octane and toluene or dodecanol and cyclohexanol. The common feature of stable enzyme preparations for the other groups of carriers was the use of toluene or cyclohexanol as co-diluents.
6. Enzyme preparations were poorly stable for carriers synthesized with either heptane and toluene or octane-(hexadecane) and cyclohexanol as diluents.
7. Considering the effects of the polymer matrix on the amount and activity of bound lipase, the authors selected carrier 33 (terpolymer BA/HPMA/EGDMA) as the best for the immobilization of lipase. This carrier is character-

ized by low porosity and water regain, and medium content of $-NH_2$ groups. It is a polar carrier with well established hydrophobic character. It immobilizes a large amount of protein with high activity (more than 4000 U l^{-1} per 1 cm^3 of carrier). Specific activity and content of active lipase in bound protein reach the moderate level but stability of this preparation shifted carrier 33 to the top of our interest. The more detailed study on optimisation of the immobilisation procedure, the enzyme–carrier activity characteristics in a wide range of pH and temperature as well as its use in the process of colza-oil hydrolysis, will be the subject of future research.

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