Investigations on Vinylene Carbonate. VI. Immobilization of Alkaline Phosphatase onto Poly(vinylene carbonate)–Jeffamine® Hydrogel Beads

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SYNOPSIS

Hydrogel-like polymers with reactive cyclic carbonate (CCA) groups have been obtained by the cross-linking reaction of poly(vinylene carbonate) (PVCA) with Jeffamines®. By this reaction, microspheric hydrogel beads with a high water content and a high concentration of reactive CCA groups were prepared. The beads were used as a matrix for the immobilization of the enzyme alkaline phosphatase (ALP) and showed a considerable capacity to couple ALP and a reasonable retention of activity for the immobilized ALP, depending on the reaction conditions. The immobilized ALP exhibited a better thermal stability than did native ALP and a high residual activity was found after repeated use.

INTRODUCTION

In a previous paper,[1] the immobilization of alkaline phosphatase (ALP) onto vinylene carbonate (VCA)–*N*-vinyl-*N*-methylacetamide (VIMA) grafted low-density polyethylene (LDPE) films was reported. It was found that the films were suitable for immobilization of ALP and that the surface concentration of active ALP bound onto the films could be increased by inserting spacers or bovine serum albumin between the support and the enzyme.

The VCA–VIMA–LDPE grafts were quite hydrophilic and had a reasonable amount of reactive VCA groups. However, because most enzymes and antibodies function optimally in aqueous systems, very hydrophilic polymeric materials seem to be the preferred supports for immobilization of biologically active compounds. A hydrophilic support will increase the compatibility with the enzyme molecules, leading to an easier approach for the enzymes to the support[2] and could also make the environment for the immobilized enzymes more "comfortable."[3] Highly hydrophilic and swellable polysaccharides, e.g., chemically modified cellulose,[4] starch,[5] and agarose,[6] or other synthetic hydrogels, such as acrylic gels[7,8] and hydroxyalkyl methacrylate gels,[9] have proved to be suitable carriers for coupling of enzymes.

Poly(vinylene carbonate) (PVCA) is an interesting polymer because of a high concentration of VCA groups available for coupling. However, PVCA is very hydrophobic and it was assumed that an increase of the hydrophilicity might be possible from a reaction of part of the cyclic carbonate (CCA) groups in PVCA with hydrophilic or water-soluble compounds containing amino groups. Therefore, cross-linking of PVCA was studied with copolymers of ethylene oxide and propylene oxide, end-capped with amino groups (Jeffamines®) to investigate the effect on the hydrophilicity. A preliminary study on the PVCA–Jeffamine cross-linked films showed that hydrogels containing high concentrations of CCA groups could be obtained.

Because for the immobilization of enzymes microspheric beads are often used due to their easy handling, possible preparation of columns, and facilitating rapid flow of the substrate through the matrix,[10] the cross-linking of PVCA with Jeffamines

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in emulsion was attempted to make microspheric hydrogel beads.

In this paper, reaction conditions for the preparation of PVCA–Jeffamine hydrogel beads will be discussed as well as the immobilization of the enzyme ALP onto the beads and the properties of the immobilized ALP.

EXPERIMENTAL

Materials

Poly(vinylene carbonate) (PVCA) was prepared as described in Ref. 11 (molecular weight, 4.2×10^4); Jeffamines with molecular weights of 600, 900, and 2000 were purchased from Texaco Chemical Co. (Bellaire, TX). Silicon oil M-100 ($d^{25} = 0.97$ (g/mL), $h^{25} = 100$ mPa S) was purchased from Bayer Nederland (Mijdrecht, The Netherlands). Alkaline phosphatase (ALP, EC.3.1.3.1 from bovine intestinal mucosa, Type 1-S), and *p*-nitrophenyl phosphate disodium salt (pNPP) were products from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of analytical grade.

Preparation of Cross-linked Films by Reaction of PVCA with Jeffamines

Since the cross-linking reaction of PVCA with Jeffamines is very fast even at room temperature, the PVCA solutions were mixed with Jeffamines at a low temperature. PVCA, 0.3 g, was dissolved in 4 mL of *N,N*-dimethylformamide (DMF) and cooled at 0°C for 10 min. Jeffamine solution, 0.5 mL, in DMF (0°C) was added to the PVCA solution with stirring. The resulting solution was cast on a glass plate with a doctor's knife. Conditions during the cross-linking reaction and the evaporation of the solvent (DMF) were nitrogen, room temperature for 24 h, and 40°C for another 24 h. The films were dried in vacuum at 40°C for 24 h.

Preparation of Microspheric Hydrogel Beads from PVCA and Jeffamines

The microspheric hydrogel beads were prepared by cross-linking of PVCA with Jeffamines in emulsion using Silicon oil M-100 as the continuous phase and DMF as the solvent for both PVCA and Jeffamines. For a typical experiment, 500 mL of silicon oil was added to a flat-bottomed vessel equipped with four baffles to eliminate the strong vortex formation as reported in the literature[12,13] and cooled at 5°C for 15 min with vigorous stirring (600 rpm). To this oil, 4.0 mL of a PVCA solution in DMF mixed with the Jeffamine in DMF at 5°C (NH_2/CCA, 20/100, mol/mol, final PVCA concentration in DMF, 8% w/w) was added dropwise from a syringe. The resulting DMF-in-oil emulsion was kept stirring at 5°C for 30 min to obtain a fine dispersion, and further cross-linking between PVCA and the Jeffamine was effected by heating (1 h at room temperature and 2 h at 40°C). The beads were isolated by centrifugation (1000 rpm), washed thoroughly with cyclohexane to free them from silicon oil, further washed with ethanol, and dried in vacuum at 40°C for 24 h. Yields were 67–70% (w/w). The size of the beads was measured by scanning electron microscopy (SEM) using a JEOL JSM 35CF.

Measurement of the Water Content of the Films and Beads

The films were placed in deionized water for 24 h with stirring. The samples were then weighed wet after blotting to remove the surface water. The equilibrium water content of the films was calculated as follows:

$$\text{Water content \% (w/w)} = \frac{W_w - W_d}{W_w} \times 100\%$$

where W_w = wet weight of the films (g) and W_d = dry weight of the films (g). The equilibrium water content of the beads are the values, measured for films[14] prepared using the same ratios of PVCA and Jeffamines as for the beads. The equilibrium swelling volume of the beads was measured after swelling in water for 24 h.

Immobilization of ALP onto PVCA–Jeffamine Beads

The beads (50 mg) were swollen in 5 mL of a 0.05 *M* glycine buffer at pH 9 for 24 h, washed three times with the same buffer, and suspended in 5.0 mL of an ALP solution in the same buffer. The suspension was incubated at 4°C for 16 h except where otherwise mentioned. The resulting mixture was filtered and the beads were washed with the buffer solution. The supernatant and the washings were made up to 100 mL with the buffer solution and the enzyme content was determined by measuring the absorbance at 280 nm. The immobilized ALP content was calculated from the difference of the absorbance of the ALP solution before and after the reaction.[15–17] Reference curves were used for the absorption (280 nm) as a

function of the enzyme concentrations. Absorption spectra were recorded on a UVIKON 930 spectrophotometer. The remaining cyclic carbonate groups were blocked with ethanolamine (10% w/v, pH 9)[10] or with glycine (10% w/v, pH 9) at 4°C for 24 h.

Activity Assays on ALP

The activity of the native and the immobilized ALP was determined spectrophotometrically using *p*-nitrophenyl phosphate as the substrate in 0.1 *M* glycine buffer, pH 10.4, with 1 m*M* $MgCl_2$ and 1 m*M* $ZnCl_2$, at 25°C according to Bessey.[18,19] The activity was calculated as follows[19]

$$\text{Activity (unit/g sample)} = \frac{V}{\varepsilon \cdot l \cdot w} \times \Delta E$$

where V is the final volume of the assay solution; e, the molar extinction coefficient ($e_{405nm} = 18.5$ [cm^2/μ mol] for *p*-nitrophenol[19]); l, the cuvette length (1 cm), ω, the sample size (g), and ΔE, the increment of the absorbance at 405 nm per min. The active enzyme coupled onto the beads was calculated as

$$\text{Active ALP coupled (mg/g dry support)} = \frac{\text{activity/g dry support}}{\text{activity/mg native ALP}}$$

Estimation on pH Optima of Native and Immobilized ALP

The activity of native and immobilized ALP in media with different pH's were measured at 25°C with a pNPP concentration of 0.45 m*M*/L.

Thermal Stability of Native and Immobilized ALP

Native ALP and beads containing immobilized ALP were incubated at different temperatures for 15 min or at 50°C for different times, and samples were taken and kept at 25°C (5–10 min). The activity was then determined as described earlier. The first-order inactivation rate constant k_i was evaluated by the equation

$$\ln A = \ln A_0 - k_i t \qquad (1)$$

where A_0 is the initial activity and A is the activity after heat treatment for t min.[20]

Determination of the Michaelis Constant

The Michaelis–Menten constant K_m of native and immobilized ALP was evaluated using pNPP solutions (ranging from 1.25 to 45×10^{-2} m*M*) in 0.1 *M* glycine buffer at pH 10.4, with 1 m*M* $MgCl_2$ and 1 m*M* $ZnCl_2$ at 25°C, on the basis of the Lineweaver–Burk plot:

$$\frac{1}{v(\Delta[S])} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

where v is the reaction rate, i.e., the change of the concentration of the substrate with time (ΔS); $[S]$, the concentration of the substrate; V_{max}, the maximum reaction rate; and K_m, the Michaelis–Menten constant.

Reusability of Immobilized ALP Beads

The activity of 10 mg beads containing immobilized ALP was determined first. The suspension of the beads in the substrate solution (pNPP) was filtered and the beads were washed thoroughly with 0.05 *M* glycine buffer at pH 9 and used for the next activity determination. The process was repeated 17 times and the activity was determined in each case.

Storage Stability of Immobilized ALP

Immobilized ALP beads were stored in 0.05 *M* Tris–HCl buffer, pH 8, at 4°C for certain days and the ALP activity was determined as a function of time as described earlier.

RESULTS AND DISCUSSION

Hydrophilicity of the Cross-linked PVCA–Jeffamine Films

Data for the Jeffamines, which were used for studying the hydrophilicity of cross-linked PVCA–Jeffamine films, are shown in Table I. For the cross-linking of PVCA with the Jeffamines, the cyclic carbonate groups will react with the amino groups, as illustrated in Figure 1.

The water contents of the cross-linked films are given in Figure 2 and it is evident that the hydrophilicity of the hydrophobic PVCA could be markedly increased by cross-linking with the Jeffamines. An increase of the water content with increasing amino group concentration was found for all the Jeffamines. A dependence of the water content on the

Table I Structure and Characteristics of Jeffamines (According to the Manufacturer):

$$[H_2N]_{2/a}-[\underset{CH_3}{\underset{|}{C}}HCH_2]_b-[O\underset{CH_3}{\underset{|}{C}}HCH_2]_c-[OCH_2CH_2]_d-[OCH_2\underset{CH_3}{\underset{|}{C}}H]_e-[NH_2]_{a/2}$$

	Approximate Values (Molar Fractions)				
Jeffamine	*a*	*b*	*c* + *e*	*d*	Approximate Molecular Weight
ED-600	0.05	0.07	0.24	0.62	600
ED-900	0.04	0.05	0.16	0.76	900
ED-2001	0.02	0.02	0.07	0.89	2000

molecular weight of the Jeffamines was observed for NH_2/CCA molar ratios ≤ 0.02; however, with higher ratios, the different Jeffamines showed the same behavior. Cross-linking of PVCA with the Jeffamines resulted in the formation of hydrogels with high water contents (ca. 90% w/w) and with a high concentration of unreacted CCA groups.

The increase of the hydrophilicity can be explained by the effect of the very hydrophilic ethylene oxide groups, which are present in the Jeffamines. The dependence of the water content on the molecular weight of the Jeffamines at lower NH_2/CCA ratios seems to indicate the effect of the difference in concentration of ethylene oxide groups in the Jeffamines (Table I). Obviously, at a certain concentration of ethylene oxide groups, an increase of the amount of ethylene oxide groups does not lead to higher water contents and no differences in the effect of the various Jeffamines are observed.

Hydrogel Beads of PVCA–Jeffamines

Preparation of the beads was performed in an emulsion.[12,13] Based on the study on PVCA–Jeffamine films, the molar ratio of the NH_2 groups in the Jeffamines to the VCA groups in PVCA was chosen as 20% to obtain hydrogel beads with a high water content as well as to achieve a fast gel formation. After preliminary experiments, it was concluded that the best conditions were silicon oil M-100 as the continuous phase, a 1 L baffled vessel, a maximum volume of silicon oil of 500 mL containing 4 mL of a polymer solution with a PVCA concentration in DMF of 8% (w/v), and a stirring speed of 600 rpm (Janke & Kunkel, RW 20, DZM, The Netherlands). The shape and the size of the resulting beads was studied by SEM (Fig. 3). The equilibrium water content and the equilibrium swelling volume of the beads are both given in Table II.

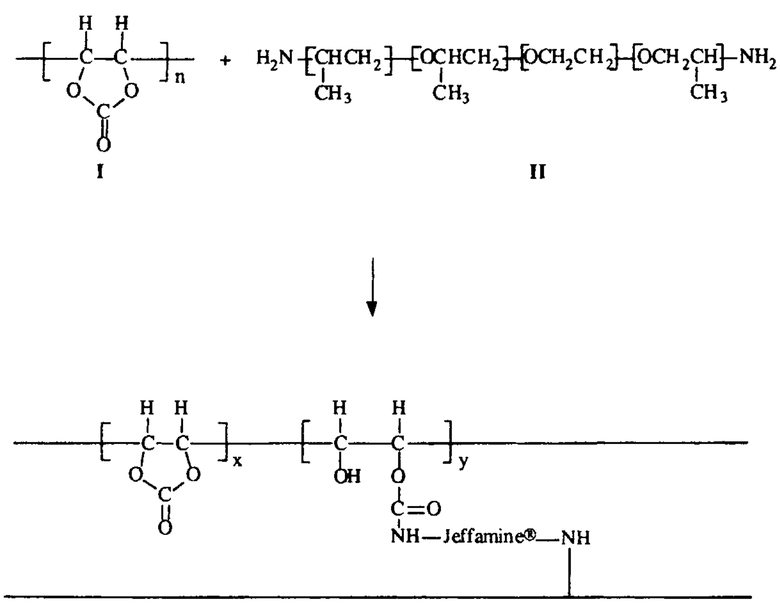

Figure 1 Scheme of the cross-linking reaction of PVCA (**I**) with Jeffamines (**II**).

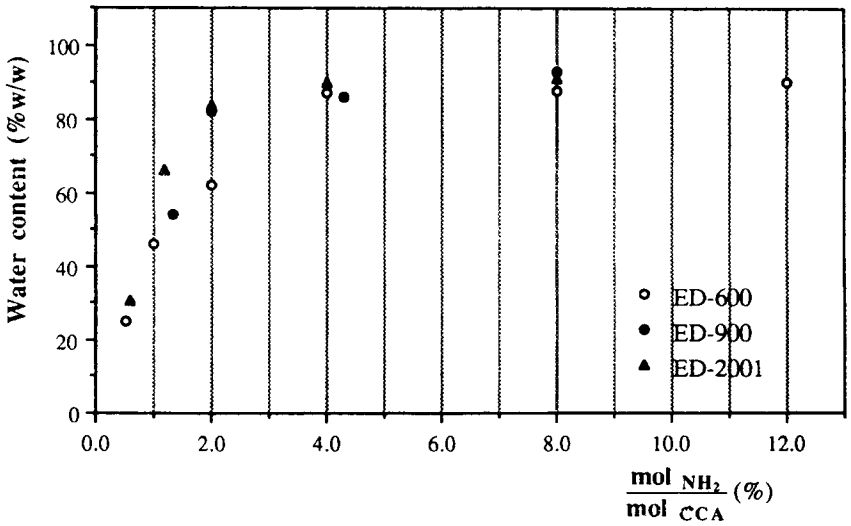

Figure 2 Effect of the molar ratio of amino to CCA groups and of the molecular weight of the Jeffamines on the water content of cross-linked PVCA–Jeffamine films.

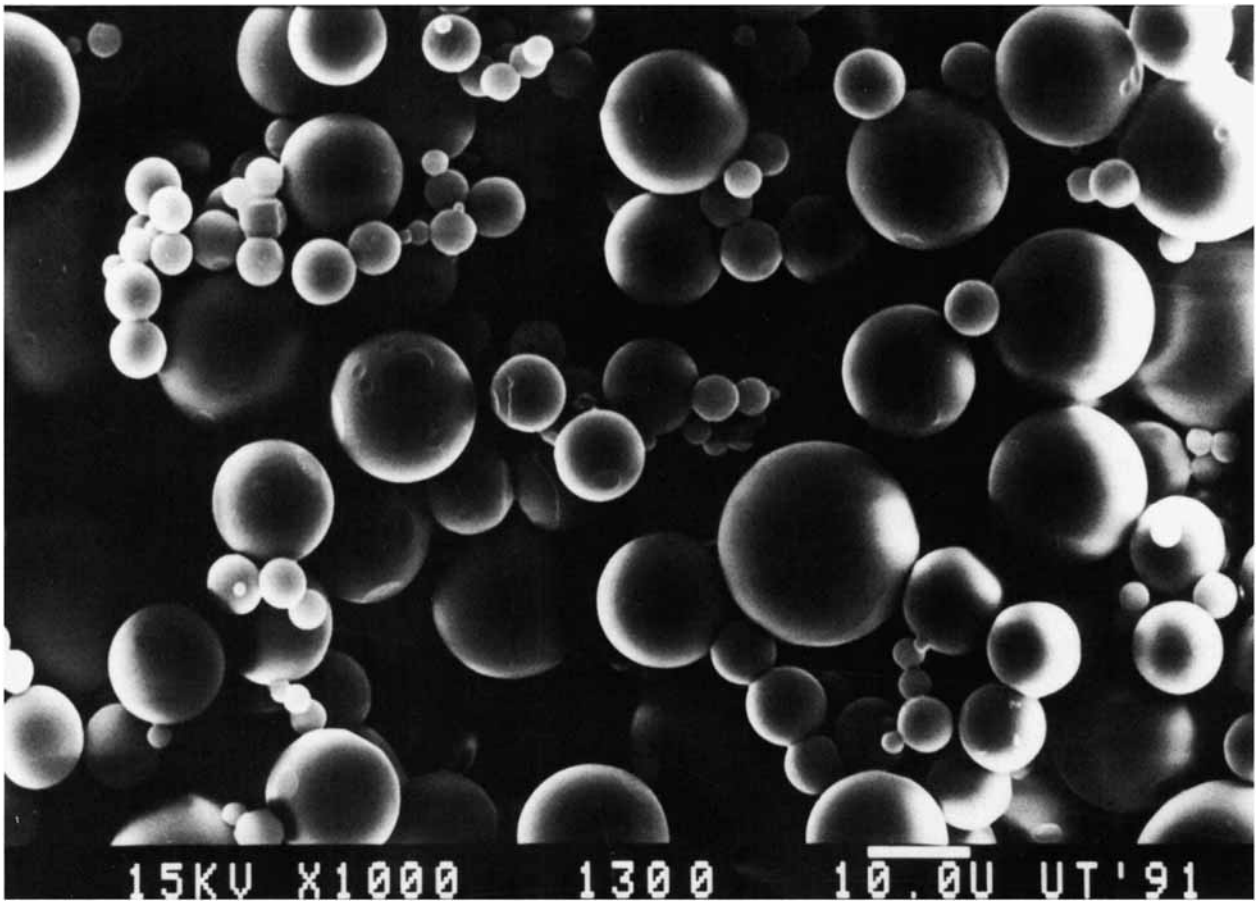

Figure 3 Scanning electron micrograph of PVCA–Jeffamine microspheres (sample S-1).

Table II Properties of Microspheric PVCA–Jeffamine Hydrogel Beads

Sample	Jeffamine	(%) $\frac{Mol_{NH_2}}{Mol_{CCA}}$	Water Content (% w/w)	Size (μm)	Swelling in Water (mL/g dry beads)	[CCA] (mmol/g dry beads)
S-1	ED-600	20	90	4–20	16	6.8
S-2	ED-900	20	90	4–20	18	5.7
S-3	ED-2001	20	90	4–20	20	3.5

From the data shown in Table II, it can be concluded that by the emulsion cross-linking reaction of PVCA with the Jeffamines in silicon oil microspheric hydrogel beads with high water contents and a high concentration of functionally reactive CCA groups could be made, and, therefore, these beads might be suitable for application as a support for the coupling of bioactive components, e.g., enzymes.

Immobilization of Alkaline Phosphatase (ALP) onto PVCA–Jeffamine Hydrogel Beads

The microspheric PVCA–Jeffamine hydrogel beads were used for the immobilization of ALP under very mild conditions without activation. All the experiments were performed at 4°C. After the immobilization reaction, the remaining carbonate groups were blocked with ethanolamine or with glycine. The properties of the PVCA–Jeffamine hydrogel beads obtained by cross-linking PVCA with Jeffamine ED-600 (sample S-1) were studied in more detail because of a high concentration of CCA groups (Table II) and because these beads could be easily prepared. Some parameters such as the pH of the reaction medium, the reaction time, and the ALP concentration were varied and the effects on the amount and the activity of ALP attached to the beads were investigated. In addition, the immobilization of ALP onto the other hydrogel beads prepared by cross-linking of PVCA with Jeffamines ED-900 (S-2) and ED-2001 (S-3) was investigated.

Effect of pH of the Reaction Medium on the Immobilization

Table III shows the effect of the pH of the reaction medium on the immobilization of ALP and on the activity of ALP coupled to the matrix. It is evident that the coupling yield as well as the retention of the activity depended on the pH, with an optimum at pH 9. The results of the control experiment (1) indicate adsorption of ALP onto the beads, because the carbonate groups had been blocked by ethanolamine before contact with ALP. However, the adsorption is quite low and most of the ALP in the other experiments will be present in an immobilized form onto the support by a covalent linkage through the carbonate groups.

Table III Effect of pH of the Medium on the Coupling of ALP and the Activity of Immobilized ALP[a]

Exp.	pH	Enzyme Coupled (mg/g dry support)	Coupling Yield (%)	Retention of Activity[b] (%)
1	7.1	51.4	25.7	3.1
2	8.0	72.6	36.3	6.5
3	8.5	80.8	40.4	7.0
4	9.0	106.0	53.0	16.3
5	9.5	73.6	36.8	6.8
6	10.0	21.0	10.5	0.6
Control-1[c]	8.5	12.0	6.0	2.5

[a] Immobilization conditions: dry beads (sample S-1) 50.0 mg; enzyme solution 5.0 mL; enzyme concentration 2.0 mg/mL in 0.05*M* Tris–HCl buffer (pH 7.1–8.5) and glycine buffer (pH 9–10), respectively; temperature 4°C; reaction time 16 h.

[b] The activity of native ALP was 1.37 U/mg.

[c] The control was made by treating the beads (sample S-1) with 10% (w/v) of ethanolamine at room temperature for 24 h to block the cyclic carbonate groups.

Table IV Effect of Reaction Time on the Immobilization of ALP onto the Support[a]

Exp.	Coupling Time (h)	Enzyme Coupled (mg/g dry support)	Coupling Yield (%)	Retention of Activity[b] (%)
7	2	99.6	49.8	15.1
8	4	102.2	51.1	13.2
9	8	104.2	52.1	12.5
4	16	106.0	53.0	16.3
10	24	107.0	53.5	12.3

[a] Immobilization conditions: dry beads (sample S-1) 50.0 mg; enzyme solution 5.0 mL in 0.05*M* glycine buffer at pH 9; enzyme concentration 2.0 mg/mL; temperature 4°C.
[b] As indicated in Table III.

The maximum amount and activity of ALP coupled to the beads at pH 9 may be explained in the following way: At lower pH values, the amino groups on the surface of the protein are protonated to a considerable extent and are therefore not effective as nucleophiles. At higher pH values, hydrolysis of carbonate groups will compete increasingly with the attack of the nucleophilic groups on the protein molecule.[21,22] In addition, it is possible that the enzyme is less stable at higher pH values, resulting in a very low retention of activity after 16 h.

Effect of Reaction Time on the Immobilization

Table IV shows the effect of the reaction time on the amount and activity of ALP coupled to the beads (pH 9, see Table III). The immobilization nearly reaches a maximum already after 2 h, and the amount of ALP coupled to the matrix has become reasonably high as compared with the amounts of ALP immobilized onto other matrices.[3,23] Probably, the coupling with ALP is favored due to the reactive carbonate groups in the matrix as well as to the high concentration of these groups. Because the reaction time had nearly no influence on the activity of the coupled ALP, in most experiments, a reaction time of 16 h was chosen for practical reasons.

Effect of the ALP Concentration on the Immobilization

The effect of the protein concentration initially present in the coupling mixture (pH 9, see Table III) on the immobilization of ALP onto the PVCA–Jeffamine hydrogel beads is illustrated in Table V. With increasing ALP concentration in the immobilization mixture, the amount of the protein coupled onto the matrix was raised and, simultaneously, the coupling yield decreased. The activity of the coupled enzyme on the matrix, however, was significantly influenced by increasing the enzyme concentration. Possibly, the loss of activity in exp. 11 (Table V) is less than in the other experiments because of a much lower amount of enzyme coupled onto the matrix. A dramatic decreasing activity at high enzyme loading indicates that the enzyme becomes less efficient. This inefficiency may be due to a crowding of protein molecules on the support, which reduces the acces-

Table V Effect of ALP Concentration on the Immobilization onto the Support[a]

Exp.	[*E*] (mg/mL)	Enzyme Coupled (mg/g dry support)	Coupling Yield (%)	Active Enzyme[b] (mg/g dry support)	Retention of Activity (%)
11	1.0	49.0	49.0	33.4	68.2
4	2.0	106.0	53.0	17.3	16.3
12	3.0	149.4	49.8	9.0	6.0
13	4.0	170.6	42.7	8.2	5.5
14	5.0	198.4	39.7	15.7	7.9

[a] Immobilization conditions: dry beads (sample S-1) 50.0 mg; enzyme solution 5.0 mL in 0.05*M* glycine buffer at pH 9; temperature 4°C; reaction time 16 h.
[b] As indicated in Table III.

sibility of the enzyme to the matrix.[24,25] It may also be caused by multiple attachments of the enzyme onto the matrix because a high enzyme concentration in the mixture favors such an intermolecular reticulation reaction of the proteins with the matrix.[26,27]

Effect of the Type of the Matrix on the Immobilization of ALP

By using Jeffamines with different molecular weights for the cross-linking of PVCA, different matrices could be obtained (Table II). The immobilization of ALP onto these matrices at pH 9 (Table III) was also investigated and the results are shown in Table VI. It can be seen that the highest retention of activity after immobilization was obtained with the beads formed by the cross-linking of PVCA with Jeffamine ED-900 (S-2), whereas the extent of ALP attached onto the beads as well as the coupling yield were about the same for the different supports. However, it is not clear why ALP coupled onto S-2 showed the high retention of activity.

Properties of the Immobilized ALP

Information about the effect on the enzymatic properties by the immobilization of enzymes is important not only for the application of immobilized enzyme systems but also for the elucidation of structure–function relationships and the mechanisms of enzyme reactions. Therefore, properties of the immobilized ALP (on matrix S-1) were studied in relationship to pH optima, thermal stability, reaction kinetics (Michaelis–Menten constants), reusability, and storage stability.

Table VI Effect of the Molecular Weight of the Jeffamine in the PVCA–Jeffamine Beads on the Coupling of Alkaline Phosphatase[a]

Sample	Enzyme Coupled (mg/g dry beads)	Coupling Yield (%)	Retention of Activity[b] (%)
S-1	106.0	53.0	16.3
S-2	111.8	55.9	44.5
S-3	96.2	48.1	15.3

[a] Immobilization conditions: polymeric support 50.0 mg; enzyme solution 5.0 mL in 0.05*M* glycine buffer at pH 9; enzyme concentration 2.0 mg/mL; temperature 4°C; reaction time 16 h.
[b] As indicated in Table III.

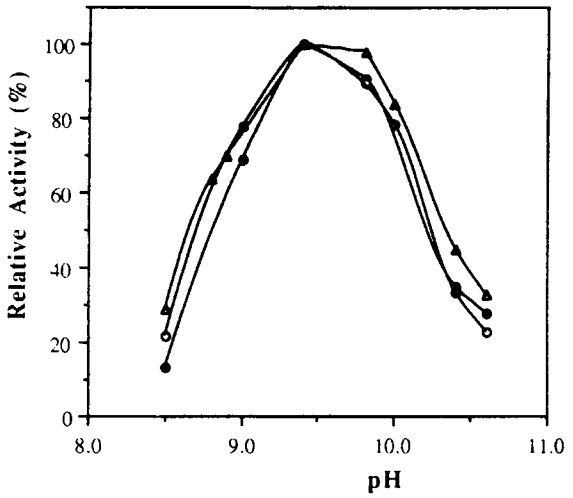

Figure 4 Activity as a function of pH: (●) native ALP; (○) immobilized ALP, blocked with ethanolamine; (△) immobilized ALP, blocked with glycine.

Activity as a Function of pH

The pH profile for the ALP immobilized PVCA–Jeffamine beads in which the remaining carbonate groups after the immobilization reaction were blocked with ethanolamine or with glycine is shown in Figure 4, together with results obtained with the native enzyme. Both native and immobilized ALP are very sensitive to the pH of the reaction medium. Immobilization did not affect the optimum pH, although some negative charges were introduced into the matrix by blocking the remaining carbonate groups with glycine. It has been reported that the pH profile of ALP immobilized onto other supports was also similar to that of the native enzyme.[1,28]

Kinetic Constants

Conformational changes of the enzyme protein may occur on immobilization, and the affinity between enzyme and substrate may be changed, possibly resulting in an effect on the kinetic constants for the immobilized enzymes. Therefore, determination of the apparent Michaelis–Menten constant (K_m) was performed for the native and the immobilized ALP using pNPP as a substrate at pH 10.4. The native enzyme showed a K_m value of 0.75 mmol/L, whereas immobilized ALP exhibited higher K_m values, which depended on the reagent used to block the remaining CCA groups. The increase in K_m may be due to diffusion limitation of the substrate to the water-insoluble matrix.[22,29–32] The apparent K_m of the im-

mobilized ALP blocked with glycine was higher (3.16 mmol/L) than that of the immobilized ALP blocked with ethanolamine (2.34 mmol/L), probably because of a repulsion between the negatively charged glycine group on the matrix and the negatively charged substrate pNPP.[29]

Thermal Stability

The thermal stability of immobilized enzymes is one of the most important criteria with regard to their applications. As is well known, immobilized enzymes, especially in covalently bound systems, are more resistant against heat and denaturing agents than are the soluble forms.[17] The effect of a heat treatment on the activity of the immobilized ALP in 0.05 *M* glycine buffer, pH 9, is shown in Figure 5, and it is evident that the immobilized ALP is more stable than is the native ALP at higher temperatures. It was calculated that the thermoinactivation at 50°C for the immobilized ALP reveals a process characterized by the following constants: $k_1 = 8.44 \times 10^{-2}$ min^{-1} and $k_2 = 4.17 \times 10^{-2}$ min^{-1}. The native ALP loses 90% of its initial activity by a heat treatment at 50°C for 25 min. It is clear that immobilized ALP manifests an increase in thermal stability when compared to the native enzyme, possibly due to the covalent binding of ALP onto the matrix leading to a reduction in mobility.[33]

Reusability

The reusability of supports with immobilized enzymes is also important, and Figure 6 shows the effect of repeated use on the activity of immobilized ALP. A residual activity of about 90% was found after reacting 17 times with pNPP, illustrating the stability of the enzyme–matrix linkage.

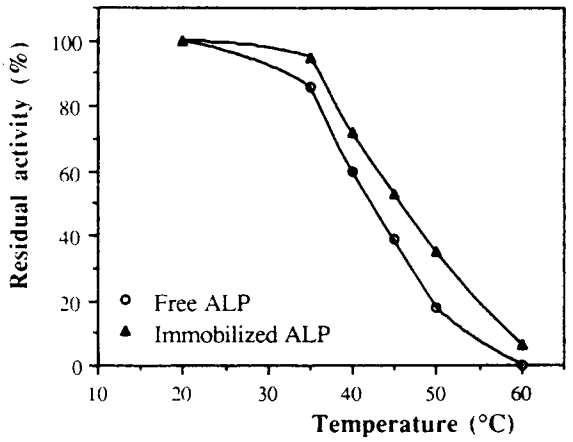

Figure 5 Effect of heat treatment on the activity of ALP (pH 9, 15 min).

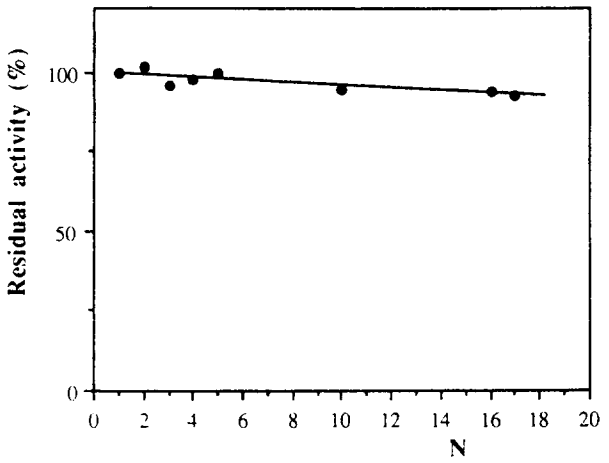

Figure 6 Effect of repeated use (N) on the activity of immobilized ALP.

Storage Stability

Suspensions of the immobilized ALP in 0.05 *M* glycine buffer at pH 9 were stored at 4°C for a certain time, and the activity was measured. It was found that the residual activity was about 50% after 1 month.

CONCLUSION

The results in this study demonstrate that hydrogel-like polymers with reactive cyclic carbonate groups could be made by reaction of part of the carbonate groups in PVCA with Jeffamines. Based on this reaction, microspheric hydrogel beads of PVCA–Jeffamine were prepared with a high water content and a high concentration of carbonate groups and, therefore, these beads might be used as a suitable matrix for the immobilization of enzymes.

The immobilization of alkaline phosphatase onto the PVCA–Jeffamine beads strongly depended on the pH of the reaction medium and the enzyme concentration, but only slightly on the reaction time. The beads had a considerable capacity to couple ALP and a reasonable retention of activity was found for the immobilized ALP. The immobilized ALP showed a higher thermal stability than did native ALP and a high residual activity was observed after repeated use.

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