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Short Communication

β-Cyclodextrin polymer as the immobilization matrix for peroxidase and mediator in the fabrication of a sensor for hydrogen peroxide

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Abstract

A novel immobilization approach based on the supramolecular function between β-cyclodextrin polymer (β-CDP) and cationic dyes, and the condensation polymerization among β-CDP, glutaric dialdehyde and horseradish peroxidase (HRP) in the fabrication of a hydrogen peroxide sensor is described. IR and UV–vis spectroscopy were employed to characterize the structure of the composite membrane modified on the surface of the electrode. AFM was used to visualize the morphology of the composite membrane. The characteristics of supramolecular inclusion compounds between β-CDP and cationic dyes were studied. The fabricated H_2O_2 sensor responded rapidly to H_2O_2 in the linear range from 1.0 to 1.1 mmol l^{-1} with a detection limit of 0.5 mol l^{-1}.

Keywords: Supramolecular inclusion complex; Cationic dye mediator; Biosensor; Hydrogen peroxide

1. Introduction

The fabrication of H_2O_2 sensors has attracted great interest because the measurement of H_2O_2 is the basis of detecting many biologically active materials, such as glucose, cholesterol and uric acid [1,2]. The immobilization of an enzyme and an electron transfer mediator is one of the critical steps in the construction of a H_2O_2 mediator bioelectrode, a kind of second-generation amperometric biosensor. The mediators have to be immobilized firmly on the electrode in case the soluble mediating species might diffuse away from the electrode surface into the bulk solution. On the other hand, sufficient mobility of the applied mediator is also vital to the sensor when the mediator is employed to facilitate electron communication between the redox center of the enzyme and the electrode. Some inorganic porous materials, such as montmorillonite [3], zeolite [4] and clay [5,6] have been proven to be promising as the immobilization materials, due to their good mechanical, thermal and chemical stability, ion-exchanger and electrocatalytic properties, hydrophobic property and existing mesopores. Many researchers suggested that the absorption and cationic exchange function of the enzyme and the mediator be attributed to the excellent electrochemical response using these porous materials. However, in the view of supramolecular chemical theory, some natural inorganic materials, such as montmorillonite and zeolite are typical cage compounds. The type of inorganic solid particles are suitable for the host, which includes the guest molecule to form a cage compound. So the excellent immobilization characteristics of the enzyme and the mediator using montmorillonite and zeolite depend partly on their special supramolecular structures, in which the cage compound acted as a host, and a mediator as a guest. With this in mind, the purpose of this work is to illustrate that the supramolecular action could be a novel immobilization approach in the fabrication of a biosensor.

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β-CD is a cyclic oligomer comprised of seven α-D-glucopyranose units linked 1→4, as in an amylose. The interior cavity of the doughnut-shaped molecule provides a relatively hydrophobic environment into which various organic substrates, especially hydrophobic substances can be trapped selectively. β-CD cross-linked polymer was selected as the immobilization matrix in this work due to its special structural features. Such a porous three-dimension network provides not only a large hydrophobic surface for enzyme and mediator loading, but also a desirable microenvironment to transform enzymatically-produced hydrogen peroxide and proton more efficiently. The inclusion of β-CDP and heteroanthracene ring cationic dyes as the mediator, eg phenazine, phenoxazine and phenoxthine dyes, such as methylene blue (MB), azure A (AA), toluidine blue (TB), resorcinol blue (RB), neutral red (NR), safranine T (ST) and indigo carmine (IC) were investigated. The inclusion formation constants were determined. Then the cationic dyes and the enzyme were co-immobilized on the electrode by supramolecular function between β-CDP and the guest molecule, as well as the condensation polymerization among β-CDP, glutaric dialdehyde and HRP [7]. The fabricated H_2O_2 sensor exhibited excellent characteristics on the firm immobilization and a fair electronic mobility. So a novel immobilization approach to the enzyme and the mediator was provided, and its attractive properties would find various practical applications.

2. Experimental

2.1. Material

Peroxidase from horseradish (HRP) (Type IV) was purchased from Sigma. The cross-linked polymer of β-CD and epoxy chloropropane was synthesized according to the literature [8] and milled to a fine resin with a grain of 150 mesh. Cellulose triacetate (average degree of polymerization 200–400) was obtained from Beijing Chemical Reagent Co. Britton–Robinson buffer solution (pH 7.0) served as the supporting electrolyte. All aqueous solutions were prepared in twice distilled water.

2.2. Apparatus

Cyclic voltammetric and amperometric signals were obtained with a PAR polarographic analyzer, Model 174 (EG&G, Princeton Applied Research Corporation, USA), equipped with a glassy carbon electrode (GCE) as a working electrode, a saturated calomel electrode as the reference electrode, and a platinum wire as the auxiliary electrode. Spectral measurements were made on a Shimadzu UV-240 spectrophotometer with a 10 mm pathlength fused silica cuvette (Tokyo, Japan). IR spectra were recorded on a FT-JR 5DX spectrometer (Nicolet). Atomic force microscope (AFM) images were obtained with a Benyuan 930 b scanning probe microscope (Benyuan Co., China).

2.3. Construction of H_2O_2 sensor

The dye solution, buffer solution and ionic strength conditioning agent were mixed with β-CDP, and the mixture was incubated at 25°C for 40 min. Then the supramolecular inclusion compound of β-CDP and the mediator molecule was obtained. Firstly, 5 μl of 40 g l^{-1} β-CDP-mediator inclusion compound colloid was mixed carefully and thoroughly with 10 μl of 5% cellulose triacetate which acted as a supporting carrier in acetone. Then 5.0 mg HRP and 5.0 mg BSA were dissolved into this colloid and subsequently 5 μl of 5% glutaric aldehyde solution was mixed completely with it as soon as possible. The composite membrane coated GCE was prepared by spin-coating the colloid on the GCE surface at 3000 rpm and allowed to dry under ambient conditions for 3 h. A 5 μl aliquot of such a mixture was pipetted onto the surface of mica and allowed to dry for AFM observation. The sensor was kept in 0.1 mol l^{-1} phosphate buffer (pH 7.0) at 4°C.

2.4. Evaluation of EC_{50} and absorption and the amount included (Q)

The experiments were performed on a vibrator by treating for 40 min 0.1000 g of β-CDP, a certain amount of the cationic dye, and buffer solutions under different pH values at an ionic strength of 0.1 mol l^{-1}. After equilibrating the guest concentration in the liquid phase, the equilibrium concentration of guest ($[G]_{eq}$) was determined at its maximum absorption wavelength by observing the supernatant liquid. EC_{50}, i.e. the dye concentration that gives 50% maximal saturation of β-CDP, as well as absorption and the amount included (Q) was calculated from the these experimental results.

2.5. The measurement of H_2O_2

All experiments were conducted in a cell containing 10 ml B-R buffer solution as the supporting electrolyte at 30.0 ± 0.2°C. All experimental solutions were deoxygenated thoroughly by bubbling N_2 through the solution for at least 6 min prior to use. In the potentiostatic experiment, successive additions of stock H_2O_2 solution in the buffer were made and current–time data were recorded after a constant residual current had been established under stirring. The sensor response was measured by the difference between the total and the residual current.

3. Results and discussion

3.1. The surface characteristics of β-CDP-cationic dye-HRP modified electrode

The IR absorption bands of β-CDP and its absorption and inclusion compounds with MB, glutaric aldehyde and HRP are presented in Table 1. After the formation of an inclusion compound with MB, the absorption band of the hydroxyl group in β-CDP split into peaks at 3422 and 3179 cm^{-1}. The strength of the absorption band decreased significantly. β-CDP-MB had absorption bands at 1368 and 1346 cm^{-1}; a shift to lower wavenumbers in comparison with those of β-CDP, which was attributed to the formation of a hydrogen bond between a $-CH_2-$ group and other relative groups. β-CDP-MB also had absorption bands at smaller wavenumbers at 1643 and 1137 cm^{-1}, and the strength of the absorption band decreased greatly. These facts indicated that intermolecular interaction existed between them. The original association of hydrogen bonds in β-CDP molecules was destroyed and a new hydrogen bond appeared between β-CDP and the guest, MB. In the IR spectrum of β-CD-MB, the appearance of some absorption bands (1592, 1176 cm^{-1}) was attributed to the characteristic absorption of MB. However, the fact that the absorption band of MB at $2834 \sim 2917$ cm^{-1} could not be observed, indicated that the stretching vibration of $-CH_2-$ was being hindered by the formation of the inclusion compound. It was seen from the IR spectrum of the β-CDP-glutaric aldehyde that a new absorption band at 1703 cm^{-1} was attributed to the formation of a carboxyl group in the complex, probably due to the condensation between the hydroxyl group in β-CDP and the aldehyde group in glutaric aldehyde. β-CDP-glutaric aldehyde possessed an absorption band at 1600 cm^{-1} or so, indicating that a part of the carbonyl group in glutaric aldehyde did not participate in the reaction during condensation, and eventually became a group hanging on the chain of the polymer. These groups can be used in the immobilization of HRP. So it is possible to immobilize HRP on the β-CDP resin through glutaric aldehyde. This has been illustrated by IR spectra of β-CDP-MB-glutaric aldehyde-HRP with the mixture exhibiting the weaker absorption at 1600 cm^{-1} or so. As a result, β-CDP resin would incorporate together with MB, glutaric aldehyde and the enzyme by supramolecular and covalent interaction. It is an excellent immobilization matrix for a mediator and an enzyme.

Table 1
IR absorption bands of β-CDP and its absorption and inclusion compound

Samples	IR absorption band/cm^{-1}
β-CDP	3382 ~ 3190(ν-OH, s), 2917 ~ 2834(ν-C–H, s), 1643(ν-CH=O, s), 1396 ~ 1368(δCH_2, m), 1137(ν_aC–O–C, s), 1039(ν_aR-C–OH, s)
β-CDP-MB	3422, 3179(ν-OH, s), 1624(νCH=O, s), 1592(νC=N–CH), 1381, 1346(δCH_2, m), 1176($\nu(CH_2)_3N$, m), 1098(ν_aC–O–C, s), 1014(ν_aR-C–OH, s)
β-CDP-glutaric aldehyde	3402, 3201(ν-OH, s), 2881(ν-C–H, s), 1703(νC=O, vs), 1640 (νC=O, 1598(νCH=O, s), 1375, 1336(δCH_2, m), 1078(ν_aC–O–C, s), 1029(ν_aR-C–OH, s)
β-CDP-HRP	3376(ν-OH, s), 2898(ν-C–H, s), 1601 (νCH=O, s), 1383,1359 (δCH_2, m), 997(ν_aR-C–OH, s)
β-CDP-MB-glutaric aldehyde-HRP	3422, 3181 (ν-OH, s), 2908(ν-C–H, s), 1699(νC=O, vs), 1601(νC=O, vs), 1592(νC=N–C, m), 1372, 1331(δCH_2, m), 1232($\nu(CH_2)_3N$, m), 1147, 1008(ν_aR-C–OH)

AFM is becoming increasingly useful in studies of electrode surface characteristics. AFM images, obtained in the present study as shown in Fig. 1, show the morphology of the composite film modified on the electrode surface. A porous structure was observed in the cellulose triacetate membrane (Fig. 1(1)), with a pore diameter of 0.60 (A) to 1.2 μm (B). When β-CDP was mixed with cellulose triacetate acetone solution before membrane formation, most of the holes in the membrane were covered (Fig. 1(2)), and the pore diameter was reduced to 0.34 μm (A). However, there still existed about 10% pores in the composite membrane of β-CDP and cellulose triacetate. The molar mass of the inclusion compounds increased greatly when a dye, such as MB, was included by β-CDP. Moreover, the aggregation of inclusion compounds was attributed to a larger granularity of inclusion compounds than that of β-CDP itself. So a rough and irregular topography of the composite membrane of β-CDP-MB-glutaric aldehyde-HRP was observed (Fig. 1(3)).

3.2. Characteristics of β-CDP-cationic dye mediator supramolecular inclusion compounds

3.2.1. Absorption spectra of inclusion compounds of β-CDP and cationic dyes

The incorporation of the dye and the polymer resin was identified by resin phase absorption spectra with β-CDP resin as the background, since no apparent absorption band of β-CDP blank resin was observed in the range of 350–750 nm. The absorption band of MB showed a red shift of 10 and 5 nm at 605 and 610 nm, respectively, when it was included and absorbed on the resin from the aqueous solution. Similarly, the absorption bands of AA, TB, RB, NR, ST and IC exhibited shifts to 620, 607, 620, 502, 504 and 613 nm from 648, 629, 611, 544, 521 and 604 nm, respectively, when incorporated in the β-CDP resin. The absorption strength of the solid phase increased greatly compared with the dye solution, probably due to the enrichment

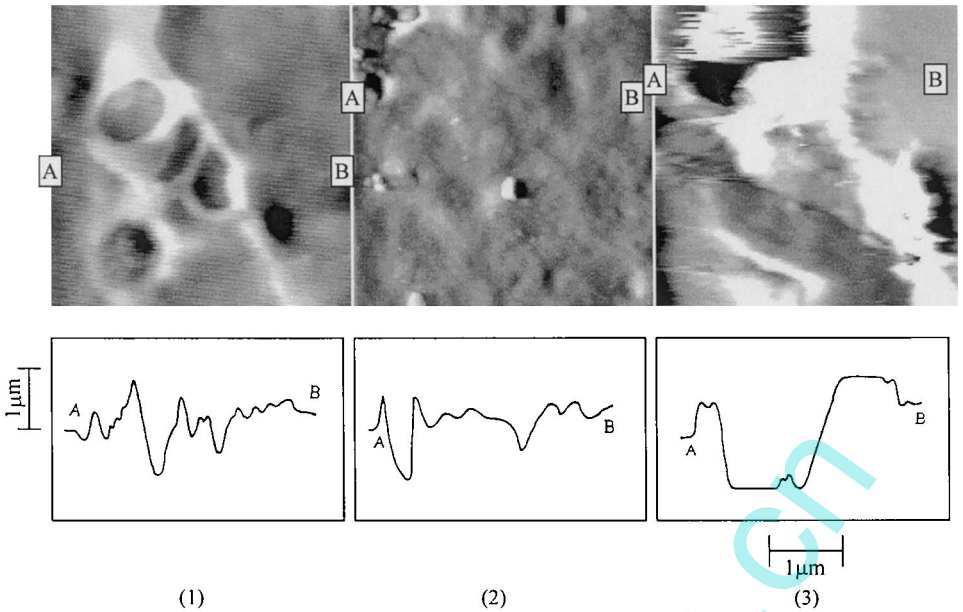

Fig. 1. A series of AFM images of cellulose triacetate membrane (1) and composite membranes of β-CDP (2), or β-CDP-MB-glutaric aldehyde-HRP (3) and cellulose triacetate membrane.

of β-CDP resin suggesting the formation of inclusion compounds, consistent with the results from the IR spectrum.

3.2.2. Effect of pH on EC_{50} and adsorption and included amount (Q)

As noted earlier, β-CD exhibited a peculiar structure with the interior hydrophobic, and the exterior hydrophilic. The stability of the inclusion compound of β-CDP was associated with the hydrophobicity of the guest molecule, so it depended greatly on the pH of the solution. EC_{50} values were measured in the solution of different pH, with the results shown in Table 2.

The absorption of the guest per gram of β-CDP is given by the following equation:

$$Q = ([G]_0 - [G]_{eq})V/m_A$$

where $[G]_{eq}$, $[G]_0$ are equilibrium and initial concentration of a guest, cationic dye compound, respectively, V is the volume of the dye solution in liters, and m_A is the mass of β-CDP (host) in grams. The effect of pH on the adsorption and inclusion amount of azo molecule, Q was studied, with the results shown in Table 2.

It was concluded from Table 2 that, when RB or IC were the guest of β-CDP, EC_{50} increased with pH, probably due to increasing ionization of the guest molecule as a result of the formation of the ion $R\text{-}SO_3^-$. Conversely, when AA, TB or ST was the guest of β-CDP, EC_{50} values decreased with the increase of pH, because the positive charge the tertiary amine possessed in acidic or neutral conditions was neutralized at high pH and the electroneutral product formed. For the same reason, when MB or NR was the guest, EC_{50} values first decreased with the increase of pH in acidic or neutral solution, but finally increased slowly in a strong basic condition, probably resulting from the increasing ionization with excessive OH^-.

3.3. Electrochemical characteristic of the biosensor

From the cyclic voltammograms of 5.85×10^{-4} mol l^{-1} MB, 7.80×10^{-5} mol l^{-1} AA, 1.11×10^{-4} mol l^{-1} TB, 2.25×10^{-4} mol l^{-1} RB, 1.04×10^{-4} mol l^{-1} NR, 1.00×10^{-4} mol l^{-1} ST and 2.57×10^{-5} mol l^{-1} IC in 0.1 mol l^{-1} phosphate buffer at GCE and that of the cationic dye incorporated in β-CDP-modified membrane, two rather different quasi-reversible waves were observed for the free and membrane-incorporated dye with scan potential between 0 and −1.00 V. The β-CDP inclusion compound modified electrode displayed more negative peak potentials but larger ΔE_p values resulting from the interaction of the dyes with β-CDP resin. However, almost all of ΔE_p values were less than 100 mV, except for that of RB less than 120 mV under the optimal conditions, which indicated that there was relatively rapid charge transfer through the modified membrane as well as the charge transfer from the membrane to the electrode. In addition, NR and ST displayed two pairs of quasi-reversible waves, which were attributed to two consecutive reactions. The linear relationship of peak current to the square root of scan rates indicated that the electrode reactions initiated by dyes in the working buffers were controlled mainly by

Table 2
The effects of pH on EC_{50} and Q of cationic azo dyes

pH	MB [a]		AA [b]		TB [c]		RB [d]		NR [e]		ST [f]		IC [g]	
	10^5 EC_{50}/mol l^{-1}	Q/μmol b^{-1}	10^5 EC_{50}/mol l^{-1}	Q/μmol b^{-1}	10^5 EC_{50}/mol l^{-1}	Q/μmol b^{-1}	10^5 EC_{50}/mol l^{-1}	Q/μmol b^{-1}	10^5 EC_{50}/mol l^{-1}	Q/μmol b^{-1}	10^5 EC_{50}/mol l^{-1}	Q/μmol b^{-1}	10^5 EC_{50}/mol l^{-1}	Q/μmol b^{-1}
2.56	9.39	7.42	7.03	5.94	1.27	19.1	6.15	10.5	11.2	7.69	7.94	4.05	4.75	4.80
3.78	9.34	7.52	6.25	7.49	1.26	19.2	7.49	9.82	9.55	10.9	7.82	4.29	4.89	4.52
4.56	9.19	7.82	6.16	7.68	1.28	18.9	7.54	7.72	9.35	11.3	7.66	4.62	4.96	4.37
5.75	8.82	8.56	6.14	7.72	1.30	18.3	7.98	6.84	8.85	12.3	7.39	5.16	5.03	4.24
6.80	8.58	9.04	6.03	7.93	1.28	18.7	8.17	6.46	8.70	12.6	6.65	6.63	5.06	4.19
7.40	8.34	9.52	5.85	8.29	1.25	19.4	8.34	6.12	8.60	12.8	6.38	7.18	5.08	4.13
8.69	7.35	11.5	5.79	8.42	1.24	19.5	8.61	5.58	8.40	13.2	6.23	7.47	5.14	4.02
9.37	8.35	9.14	5.72	8.56	1.23	19.7	8.93	4.94	8.70	12.6	6.14	7.65	5.26	3.77
10.38	8.78	8.63	5.59	8.81	1.20	20.3	10.7	1.45	8.80	12.4	5.95	8.04	5.27	3.75
11.20	8.83	8.54	5.35	9.29	1.15	21.4	11.0	0.739	8.95	12.1	5.90	8.13	5.39	3.51

[a] $[MB]_0 = 1.31 \times 10^{-4}$ mol l^{-1}.
[b] $[AA]_0 = 1.00 \times 10^{-4}$ mol l^{-1}.
[c] $[TB]_0 = 2.22 \times 10^{-4}$ mol l^{-1}.
[d] $[RB]_0 = 1.14 \times 10^{-4}$ mol l^{-1}.
[e] $[NR]_0 = 1.50 \times 10^{-4}$ mol l^{-1}.
[f] $[ST]_0 = 9.97 \times 10^{-5}$ mol l^{-1}.
[g] $[IC]_0 = 7.15 \times 10^{-5}$ mol l^{-1}.

the diffusion of dyes in the solutions, whereas that by the dye incorporated in β-CDP membrane might be under the control of diffusion of protons in the solution.

The formal potentials $E°$ of β-CDP-HRP-dye modified electrodes versus pH at a scan rate of 20 mV s^{-1} are indicated in Table 3. Formal potentials for adsorbed MB as a function of pH gave a straight line from pH 1.8 to 5.3 with a slope of approximately 60 mV/pH unit. Another straight line with a slope of approximately 30 mV/pH unit was obtained from 5.3 to 10.38. Such a pH-dependence was in accordance with the results in the literature [3], which suggested the following reaction schemes:

$pH > 5.3\ MB^+ + H^+ + 2e^- \rightarrow MBH$

$pH < 5.3\ MB^+ + 2H^+ + 2e^- \rightarrow MBH^+$

Similar results were obtained from AA, TB, RB, NR, ST and IC, which suggested the following schemes:

AA: $pH\ 2.5 \sim 10.4\ AA^+ + H^+ + 2e^- \rightarrow AAH$

TB: $pH > 7.3\ TB^+ + H^+ + 2e^- \rightarrow TBH$

$pH < 7.3\ TB^+ + 2H^+ + 2e^- \rightarrow TBH^+$

RB: $pH > 2.5 \sim 10.4\ RB^+ + H^+ + 2e^- \rightarrow RBH$

NR: $pH > 7.2\ NR^+ + 2H^+ + 2e^- \rightarrow NRH^+$

$pH < 7.2\ NR^+ + H^+ + 2e^- \rightarrow NBH$

ST: $pH\ 2.5 \sim 10.4\ ST^+ + H^+ + 2e^- \rightarrow STH$

IC: $pH > 7.6\ IC^+ + H^+ + 2e^- \rightarrow ICH$

A novel mediated H_2O_2 sensor was then constructed with cationic dye mediator and HRP incorporated in the β-CD polymers immobilized on a GCE surface. In the absence of H_2O_2, a typical reversible voltammogram of MB included and absorbed to the β-CDP was observed. When H_2O_2 was added to the solution, the voltammetric behavior changed dramatically with an enhanced reduction current and a decreased oxidation current with MB as the mediator, showing that a catalytic reaction occurred at the H_2O_2 sensor. This demonstrated that MB incorporated in β-CD polymer could effectively shuttle electrons between the enzyme redox center and the base electrode. The sensor exhibited excellent bioelectrocatalytic activity toward the reduction of hydrogen peroxide. A subsequent addition of hydrogen peroxide to the solution provoked a sharp increase in the reduction current and the time required to reach 95% of maximum response was less than 60 s. In contrast, electron transfer via a mediator was more effective in the bioelectrocatalytic reduction of hydrogen peroxide at the H_2O_2 enzyme electrode The calibration plot for hydrogen peroxide showed very linear behavior from 1.0 to 1.1 mmol l^{-1} at the operational potential -0.21 V, with slope, intercept and correlation coefficient of $(5.64 \pm 0.11) \times 10^3$, $(-5.01 \pm 0.25) \times 10^{-3}$ and 0.992, respectively with MB as the electron transfer shuttle. A fairly low detection limit of 0.5 μmol l^{-1} H_2O_2 was estimated at a signal-to-noise radio of 3. The sensitivity of the cationic dyes incorporated in β-CDP should be attributed to the specific supramolecular interaction as the immobilization method resulting from its mobility in the host cavity, in addition to their stability in inclusion complexes.

The long-term stability was examined by measuring the response to various concentrations of hydrogen peroxide periodically for 6 months. The activity of the sensor remained at 94.7% for a month when stored dry at 4°C, and 90.1% after storage for 2 months. The lifetime of the sensor is not less than 6 months when stored at 4°C. The H_2O_2 sensor could be used continuously for eight assays and indicated 2.9% relative standard deviations for 12 successive assays of 0.1 mmol l^{-1} H_2O_2.

Table 3
(Formal potentials for adsorbed dyes as a function of pH)/V

Dye	pH	1.81	2.56	3.78	4.56	5.72	6.80	7.40	8.69	9.62	10.38
MB	E_{pc}/V	−0.015	−0.068	−0.178	−0.239	−0.291	−0.321	−0.346	−0.389	−0.436	−0.457
	E_{pa}/V	0.068	−0.021	−0.113	−0.172	−0.242	−0.278	−0.304	−0.352	−0.311	−0.412
AA	E_{pc}/V	−0.201	−0.221	−0.263	−0.275	−0.314	−0.330	−0.358	−0.406	−0.435	−0.442
	E_{pa}/V	−0.116	−0.138	−0.171	−0.193	−0.225	−0.259	−0.271	−0.300	−0.325	−0.332
TB	E_{pc}/V	−0.040	−0.087	−0.143	−0.201	−0.270	−0.323	−0.367	−0.383	−0.383	−0.385
	E_{pa}/V	0.018	−0.046	−0.092	−0.166	−0.216	−0.272	−0.326	−0.322	−0.328	−0.331
RB	E_{pc}/V	−0.100	−0.222	−0.269	−0.302	−0.352	−0.365	−0.410	−0.452	−0.487	−0.491
	E_{pa}/V	0.045	−0.140	−0.185	−0.214	−0.270	−0.269	−0.297	−0.330	−0.363	−0.375
NR	E_{pc}/V	−0.330	−0.450	−0.525	−0.576	−0.660	−0.712	−0.754	−0.809	−0.828	−0.862
	E_{pa}/V	−0.201	−0.342	−0.440	−0.498	−0.572	−0.643	−0.669	−0.721	−0.741	−0.780
ST	E_{pc}/V	−0.426	−0.501	−0.515	−0.558	−0.597	−0.608	−0.646	−0.675	−0.695	−0.715
	E_{pa}/V	−0.331	−0.425	−0.458	−0.483	−0.518	−0.544	−0.580	−0.599	−0.621	−0.644
IC	E_{pc}/V	−0.198	−0.210	−0.259	−0.282	−0.320	−0.375	−0.404	−0.414	−0.414	−0.407
	E_{pa}/V	−0.134	−0.149	−0.194	−0.219	−0.256	−0.313	−0.334	−0.327	−0.328	−0.322

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References

[1] G.G. Guilbaut, Handbook of Enzymatic Methods of Analysis, Marcel Dekker, New York, 1976.
[2] T. Nakaminani, K. Kuwabata, H. Yoneyama, Anal. Chem. 69 (1997) 2367.
[3] C. Lei, Z. Zhang, H. Liu, J. Kong, J. Deng, Anal. Chim. Acta. 73 (1996) 332.
[4] B. Liu, R. Hu, J. Deng, Anal. Chem. 69 (1997) 2343.
[5] Y. Liu, H. Liu, J. Qian, J. Geng, T. Yu, J. Chem. Tech. Biotechnol. 64 (1995) 269.
[6] T. Asakura, M. Demura, Biotechnol. Bioeng. 33 (1989) 598.
[7] H. Wu, B. Wu, S. Zhou, Chem. J. Chin. Univ. 15 (1994) 1026.
[8] M. Komiyama, H. Hirai, Polymer J. 19 (1987) 773.