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 H₂O₂ sensor responded rapidly to H₂O₂ in the linear range from 1.0 to 1.1 mmol l⁻¹ with a detection limit of 0.5 mol l⁻¹. © 2000 Elsevier Science S.A. All rights reserved.

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

1. Introduction

The fabrication of H₂O₂ sensors has attracted great interest because the measurement of H₂O₂ 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 H₂O₂ 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.
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 phenothine 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 inclusion compound of β-CDP and the guest molecule, as well as the condensation polymerization among β-CDP, glutaric dialdehyde and HRP [7]. The fabricated H$_2$O$_2$ 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 voltammometric and amperometric signals were obtained with a PAR polargraphic 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$_2$O$_2$ 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-CDP-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 ~ 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.

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.

<table>
<thead>
<tr>
<th>Samples</th>
<th>IR absorption band/cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-CDP</td>
<td>3382<del>3190(ν−OH, s), 2917</del>2834(ν−C−H, s), 1643(ν−CH−O, s), 1396~1368(δCH$_3$, m), 1137(ν$\times$−O−C, s), 1039(ν$\times$R−C−OH, s)</td>
</tr>
<tr>
<td>β-CDP-MB</td>
<td>3422, 3179(ν−OH, s), 1624(ν−CH−O, s), 1592(ν$\times$C−N−CH), 1381, 1346(δCH$_3$, m), 1176(ν(CH$_3$)$_2$N, m), 1098(ν$\times$C−O−C, s), 1014(ν$\times$R−C−OH, s)</td>
</tr>
<tr>
<td>β-CDP-glutaric aldehyde</td>
<td>3402, 3201(ν−OH, s), 2881(ν−C−H, s), 1703(νC−O, vs), 1640(νC−O, 1598(νC−O−H, s), 1375, 1336(δCH$_3$, m), 1078(νC−O−C−s, s), 1029(ν$\times$R−C−OH, s)</td>
</tr>
<tr>
<td>β-CDP-HRP</td>
<td>3376(ν−OH, s), 2898(ν−C−H, s), 1601(ν−CH−O, s), 1383,1359 (δCH$_3$, m), 997(ν$\times$R−C−OH, s)</td>
</tr>
<tr>
<td>β-CDP-MB-glutaric aldehyde-HRP</td>
<td>3422, 3181(ν−OH, s), 2908(ν−C−H, s), 1699(νC−O, vs), 1601(νC−O−H, s), 1592(νC−N−C, m), 1372, 1311(δCH$_3$, m), 1232(ν(CH$_3$)$_2$N, m), 1147, 1008(ν$\times$R−C−OH)</td>
</tr>
</tbody>
</table>
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 $5.85 \times 10^{-4}$ mol $1^{-1}$ MB, $7.80 \times 10^{-5}$ mol $1^{-1}$ AA, $1.11 \times 10^{-4}$ mol $1^{-1}$ TB, $2.25 \times 10^{-4}$ mol $1^{-1}$ RB, $1.04 \times 10^{-4}$ mol $1^{-1}$ NR, $1.00 \times 10^{-4}$ mol $1^{-1}$ ST and $2.57 \times 10^{-5}$ mol $1^{-1}$ IC in 0.1 mol $1^{-1}$ 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 $\Delta E_p$ values resulting from the interaction of the dyes with β-CDP resin. However, almost all of $\Delta E_p$ 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

<table>
<thead>
<tr>
<th>pH</th>
<th>MB (^a)</th>
<th>AA (^b)</th>
<th>TB (^c)</th>
<th>RB (^d)</th>
<th>NR (^e)</th>
<th>ST (^f)</th>
<th>IC (^g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^5 EC_{50}/mol \text{l}^{-1}</td>
<td>Q/\mu\text{mol} \text{b}^{-1}</td>
<td>10^5 EC_{50}/mol \text{l}^{-1}</td>
<td>Q/\mu\text{mol} \text{b}^{-1}</td>
<td>10^5 EC_{50}/mol \text{l}^{-1}</td>
<td>Q/\mu\text{mol} \text{b}^{-1}</td>
<td>10^5 EC_{50}/mol \text{l}^{-1}</td>
</tr>
<tr>
<td>2.56</td>
<td>9.39</td>
<td>7.42</td>
<td>7.03</td>
<td>5.94</td>
<td>1.27</td>
<td>19.1</td>
<td>6.15</td>
</tr>
<tr>
<td>3.78</td>
<td>9.34</td>
<td>7.52</td>
<td>6.25</td>
<td>7.49</td>
<td>1.26</td>
<td>19.2</td>
<td>7.49</td>
</tr>
<tr>
<td>4.56</td>
<td>9.19</td>
<td>7.82</td>
<td>6.16</td>
<td>7.68</td>
<td>1.28</td>
<td>18.9</td>
<td>7.54</td>
</tr>
<tr>
<td>5.75</td>
<td>8.82</td>
<td>8.56</td>
<td>6.14</td>
<td>7.72</td>
<td>1.30</td>
<td>18.3</td>
<td>7.98</td>
</tr>
<tr>
<td>6.80</td>
<td>8.58</td>
<td>9.04</td>
<td>6.03</td>
<td>7.93</td>
<td>1.28</td>
<td>18.7</td>
<td>8.17</td>
</tr>
<tr>
<td>7.40</td>
<td>8.34</td>
<td>9.52</td>
<td>5.85</td>
<td>8.29</td>
<td>1.25</td>
<td>19.4</td>
<td>8.34</td>
</tr>
<tr>
<td>8.69</td>
<td>7.35</td>
<td>11.5</td>
<td>5.79</td>
<td>8.42</td>
<td>1.24</td>
<td>19.5</td>
<td>8.61</td>
</tr>
<tr>
<td>9.37</td>
<td>8.35</td>
<td>9.14</td>
<td>5.72</td>
<td>8.56</td>
<td>1.23</td>
<td>19.7</td>
<td>8.93</td>
</tr>
<tr>
<td>10.38</td>
<td>8.78</td>
<td>8.63</td>
<td>5.59</td>
<td>8.81</td>
<td>1.20</td>
<td>20.3</td>
<td>10.7</td>
</tr>
<tr>
<td>11.20</td>
<td>8.83</td>
<td>8.54</td>
<td>5.35</td>
<td>9.29</td>
<td>1.15</td>
<td>21.4</td>
<td>11.0</td>
</tr>
</tbody>
</table>

\(^a\) [MB]_0 = 1.31 \times 10^{-4} \text{ mol l}^{-1}.
\(^b\) [AA]_0 = 1.00 \times 10^{-4} \text{ mol l}^{-1}.
\(^c\) [TB]_0 = 2.22 \times 10^{-4} \text{ mol l}^{-1}.
\(^d\) [RB]_0 = 1.14 \times 10^{-4} \text{ mol l}^{-1}.
\(^e\) [NR]_0 = 1.50 \times 10^{-4} \text{ mol l}^{-1}.
\(^f\) [ST]_0 = 9.97 \times 10^{-5} \text{ mol l}^{-1}.
\(^g\) [IC]_0 = 7.15 \times 10^{-5} \text{ 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 \( E^0 \) 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:

\[
\begin{align*}
\text{pH} &> 5.3 \quad \text{MB}^+ + \text{H}^+ + 2e^- \rightarrow \text{MBH} \\
\text{pH} &< 5.3 \quad \text{MB}^+ + 2\text{H}^+ + 2e^- \rightarrow \text{MBH}^+ 
\end{align*}
\]

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\(_2\)O\(_2\) 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\(_2\)O\(_2\), a typical reversible voltam-
mogram of MB included and absorbed to the β-CDP
was observed. When H\(_2\)O\(_2\) was added to the solution,
the voltammetric behavior changed dramatically with
an enhanced reduction current and a decreased oxida-
tion current with MB as the mediator, showing that a
catalytic reaction occurred at the H\(_2\)O\(_2\) 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 exhib-
it excellent bioelectrocatalytic activity toward the re-
duction 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 hydro-
gen peroxide at the H\(_2\)O\(_2\) enzyme electrode. The calibra-
tion 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 correla-
tion 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 \(\mu\)mol l\(^{-1}\) H\(_2\)O\(_2\) was estimated at a signal-to-noise
radio of 3. The sensitivity of the cationic dyes incorpo-
rated 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\(_2\)O\(_2\) sensor could be used continu-
ously for eight assays and indicated 2.9% relative stan-
dard deviations for 12 successive assays of 0.1 mmol
l\(^{-1}\) H\(_2\)O\(_2\).

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

<table>
<thead>
<tr>
<th>Dye</th>
<th>pH</th>
<th>1.81</th>
<th>2.56</th>
<th>3.78</th>
<th>4.56</th>
<th>5.72</th>
<th>6.80</th>
<th>7.40</th>
<th>8.69</th>
<th>9.62</th>
<th>10.38</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td>( E_{pc}/V )</td>
<td>-0.015</td>
<td>-0.068</td>
<td>-0.178</td>
<td>-0.239</td>
<td>-0.291</td>
<td>-0.321</td>
<td>-0.346</td>
<td>-0.389</td>
<td>-0.436</td>
<td>-0.457</td>
</tr>
<tr>
<td></td>
<td>( E_{pa}/V )</td>
<td>0.068</td>
<td>-0.021</td>
<td>-0.113</td>
<td>-0.172</td>
<td>-0.242</td>
<td>-0.278</td>
<td>-0.304</td>
<td>-0.352</td>
<td>-0.311</td>
<td>-0.412</td>
</tr>
<tr>
<td>AA</td>
<td>( E_{pc}/V )</td>
<td>-0.201</td>
<td>-0.221</td>
<td>-0.263</td>
<td>-0.275</td>
<td>-0.314</td>
<td>-0.330</td>
<td>-0.358</td>
<td>-0.406</td>
<td>-0.435</td>
<td>-0.442</td>
</tr>
<tr>
<td></td>
<td>( E_{pa}/V )</td>
<td>-0.116</td>
<td>-0.138</td>
<td>-0.171</td>
<td>-0.193</td>
<td>-0.225</td>
<td>-0.259</td>
<td>-0.271</td>
<td>-0.300</td>
<td>-0.325</td>
<td>-0.332</td>
</tr>
<tr>
<td>TB</td>
<td>( E_{pc}/V )</td>
<td>-0.040</td>
<td>-0.087</td>
<td>-0.143</td>
<td>-0.201</td>
<td>-0.270</td>
<td>-0.323</td>
<td>-0.367</td>
<td>-0.383</td>
<td>-0.383</td>
<td>-0.385</td>
</tr>
<tr>
<td></td>
<td>( E_{pa}/V )</td>
<td>0.018</td>
<td>-0.046</td>
<td>-0.092</td>
<td>-0.166</td>
<td>-0.216</td>
<td>-0.272</td>
<td>-0.326</td>
<td>-0.322</td>
<td>-0.328</td>
<td>-0.331</td>
</tr>
<tr>
<td>RB</td>
<td>( E_{pc}/V )</td>
<td>-0.100</td>
<td>-0.222</td>
<td>-0.269</td>
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Acknowledgements

The authors thank the National Natural Foundation Commission (China) for financial support.

References
