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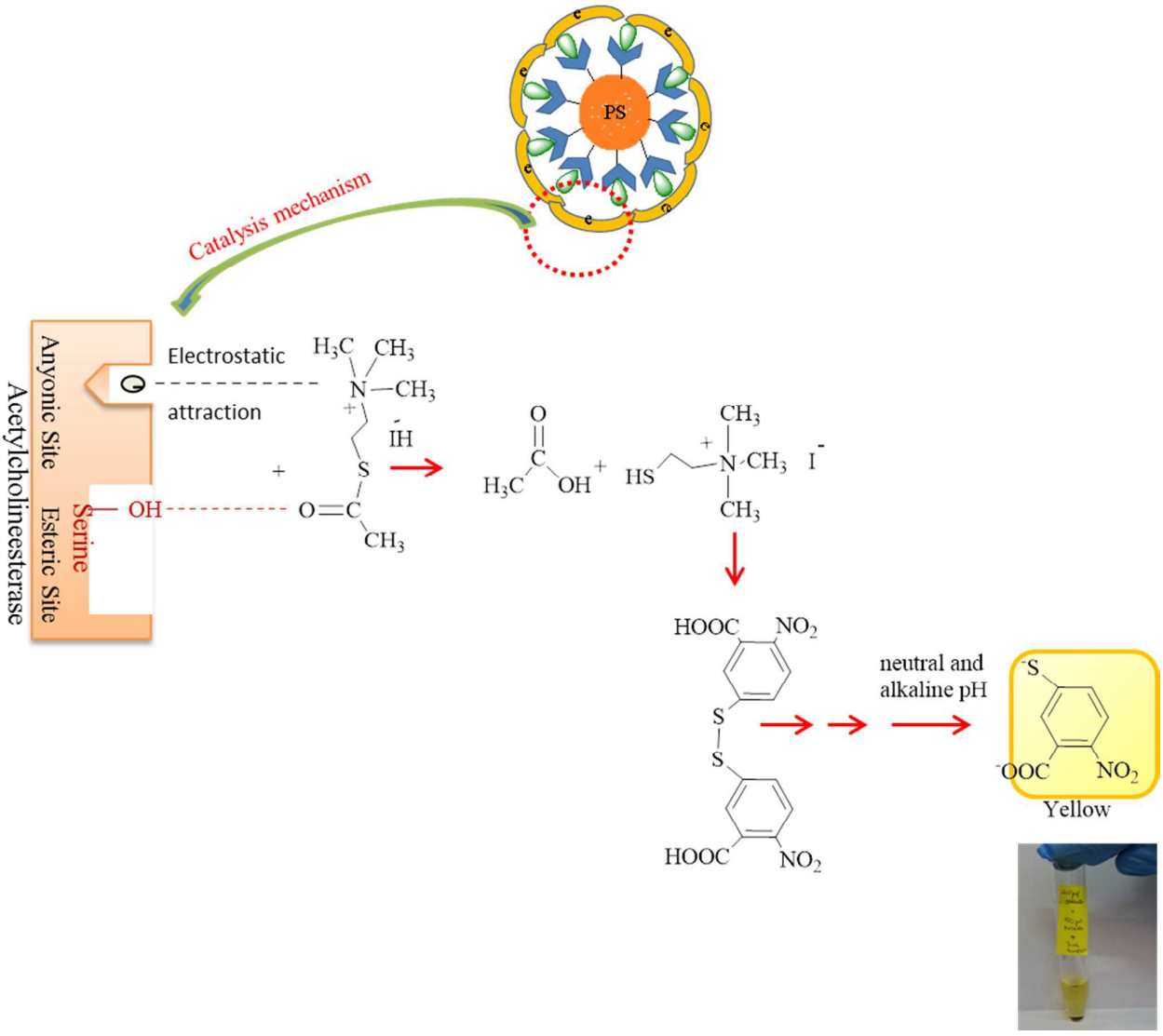

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Immobilization of Acetylcholinesterase for the determination of pesticides, nanoparticules attachhed Pt(II) and Pt(IV)

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Abstract

Ligated Pt(II) and Pt(IV) complexes on nanoparticles have been synthesized for the identification of pesticides according to template method. The morphologies has been investigated using scanning electron microscopy and characterized by means of spectral measurement. Then, Acetylcholinesterase (AChE) was immobilized onto the nanoparticles. Pt(II) and Pt(IV)-tagged nanomaterial-AChE shows high reusability and storage capacity. The catalytic activity of AChE followed Michaelis-Menten kinetics. Assay for enzyme activity measurements demonstrate that the nanosphere ligated Pt(II) have much better performance than Pt(IV). Furthermore, whether or not the interaction between the immobilized enzyme and the 1-Naphthyl-N-methylcarbamate which is carbamate insecticides was examined.

Keywords: Green catalysis, Recycling stability, Acetylcholinesterase, Nanospheres,

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1. Introduction

Very well it is known the importance of acetylcholinesterase (AChE) in the transmission of nerve impulses. Its inactivation is possible by means of some pesticides and heavy metal our around. Inactivation occurs via the hydroxyl groups of serine in the "active site" of acetylcholinesterase (AchE) [1]. For this reason, it is important to develop access method for the rapid detection of some pesticides and heavy metal. In recent years, studies based on immobilization of AChE have been reported by many authors [2, 3] due to its forms the basis to biosensors. The AChE biosensors have been used for detect unknown toxic mixtures, but there are some problems for the identification of toxic mixtures in samples. Therefore, being investigated new methods by authors. One of these methods is immobilization enzyme onto nanospheres. There are an increases in enzyme immobilization on nano-sphere has been studied due to small size and large surface area [4,5]. Nanospheres are the useful strategy to improve the operational stability of immobilization. Therefore the enzyme immobilization into or onto various nanoparticles have been proposed and reported. We can see, various nanomaterials have been investigated for immobilize of acetylcholinesterase (AChE). Especially, immobilization of AChE on nanomaterials of noble metal (such as gold and silver) or carbon nanosphere is common [6,7].

After Norman and et al. [8] pioneered the development of nanomaterial-based polystyrene in 1993, today many researchers have developed immobilization properties of polystyrene nanospheres [9-11]. One of the immobilized species is covalent immobilization. Covalent immobilization of enzyme can protect its conformation, thus lead to better activity and stability [12].

In the present work, by taking the advantages of coordination band in the between enzyme and metal ion, and to improve performance on the recycling stability for

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determination of hazardous materials new a support has been prepared, as illustrated in Fig. 1 and 2. And then, acetylcholinesterase immobilized on nanoparticles attached Schiff bases, Pt(II) and Pt(IV) (Fig. 4A).

The experimental result showed that the prepared nanoparticules attachhed Pt(II) and Pt(IV) had high sensitivity and good operational. We can say, this supports including Platinum ion are a good candidate for immobilization of AChE to detection hazardous materials.

Figure 1 and 2 hereabout

2. Experimental

2.1. Materials and Methods

4-Benzyloxybenzaldehyde, polymer-bound, tryptophan, Acetylcholinesterase (Type C3389, from electric eel, 518 units/mg, 10KU), 5,5′-Dithiobis(2-nitrobenzoic acid), acetylthiocholine iyodide, 1-Naphthyl-N-methylcarbamate were purchased from Sigma (St. Louis, MO). All the other chemicals used in this work were provided by Sigma-Aldrich and used without further purification. IR spectra were recorded on a Nicolet 6700 GA-FTIR instrument in KBr pellets. The GPC measurements were recorded on a Waters 1500 Series Gel permeation chromatography (GPC). Scanning electron microscopy of the Au-Pd-coated compounds was done by using a JEOL JEM 100 CX II scanning electron microscope (JEOL, Peabody, MA) equipped with a Link analytical system. The electron energy used was 20 keV.

2.2. Synthesis of Schiff bases-tagged nanomaterial (4BoBPS-Sch)

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The polymer having Schiff bases were prepared by dropwise with addition of 4-Benzyloxybenzaldehyde, polymer-bound, [(4BoBPS); 50-100 mesh, extent of labeling: 2,5-3.00 mmol/g -CHO loading, 1 % cross-linked with divinylbenzene, (Aldrich)] in hot DMF (30 mL) to a solution of tryptophan (3.75, mmol) in H_2O (10 mL) while stirring. The stirring was continued until the solution was refluxed *ca*. six hours, at 80 ^oC. Then mixture was poured into the acetone (50 mL). The resulting solid was collected from acetone (4BoBPS-Sch). The solid was filtered and dried and kept with desiccator over anhydrous $CaCl_2$.

2.3. Synthesis of Pt(II) / Pt(IV)-tagged nanomaterial {[4BoBPS-Sch-Pt(II)]; [4BoBPS-Sch-Pt(IV)]}

Pt(II) / Pt(IV)-tagged nanomaterial synthesized according to template. All Pt(II) / Pt(IV)-tagged nanomaterial were prepared by following a general method: DMF solutions of the (4-Benzyloxybenzaldehyde), polymer-bound (1 g), platinium ion ($PtCl_2$ and $PtCl_4$) 2.5 mmol in 25 mL DMF were mixed and refluxed for 3 *h*. Then mixture was cooled and was poured into the acetone (50 mL). The resulting solid was collected from acetone (Fig. 2). The solid was filtered and dried and kept in desiccator over anhydrous $CaCl_2$.

2.4. Immobilization of AChE on (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)]

Firstly, enzyme was dissolved in pure water (50 mL, 3.6 x 10^{-4} gL^{-1}). (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)] polymers (0.5 g) were placed to a 2 mL of 3.6 x 10^{-4} gL^{-1} of AchE. This solution was diluted to 10 ml and at room temperature in a shaking water bath for 8 *h*. The immobilized polymer was separated and the free enzyme was removed by washing with phosphate buffer and then stored at 4 °C. Saturation ratio was

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determined as 99.70 %, 87.25 % and 86.40 % for (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)], respectively, from absorbance value in 412 nm.

2.5. Assay for enzyme activity measurement

The catalytic activity of AChE was determined by the Ellman method [13]. Acetylthiocholine iodide used as substrat in this method. Acetylthiocholine (ACH) hydrolysis by acetylcholinesterase (ACHE) and forms acetate and thiocholine. 2-nitro-5-thiobenzoate (TNB) form from reaction of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) with thiocholine. (TNB) ionizes to the TNB^{2-}dianion in water at neutral and alkaline pH. TNB^{2-} gives an intense yellow color with an absorption maximum at 412 nm (Fig. 3). As shown in below, Acetylthiocholine iodide is enzymatically cleaved to TNB and CH_3COOH in the presence of AchE. The DTNB reacts with thiocholine to form yellow coloured TNB^{2-} dianion (at 412 nm). The following reaction was started by adding Acetylthiocholine (0.075M, 20 µL), DTNB (0.01M, 50 µL) and buffer after pre incubating at room temperature (20 ^{o}C) for 30 min. Then, was transferred quartz cuvette for measurement (Fig. 4B).

2.6. Study on carbamate insecticide (1-Naphthyl-N-methylcarbamate)

Firstly; 1-Naphthyl-N-methylcarbamate (carbaryl) was dissolved in Acetonitryl:H_2O (1:4, v/v) and solutions were prepared between 10 µL to 40 µL. After the wavelength scanning for this carbaryl solutions, the absorbance at 291 nm was taken into account. Later, change in absorbance was measured from value in 291 nm, for 60 mg of Schiff bases-tagged nanomaterial ((4BoBPS-Sch)-AchE, [4BoBPS-Sch-Pt(II)]-AchE and [4BoBPS-Sch-Pt(IV)]-AchE) + carbaryl solutions, and then Δ_{abs} was calculated from formule as blown;

($\Delta_{abs} = A_{carbamat} - A_{nanomet + carbamat}$)

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Figure 3 hereabout

2.6. Effect of pH and temperature on activity of free and immobilized AChE

Optimum pH for free and immobilized Acetylcholinesterase were determined by measuring the activity of free and immobilized enzymes in buffers of different pH values ranging from 3.0 to 9.5 The buffers used were: pH: 3.0-4.0 (CH_3COONa/ CH_3COOH); pH:5.0 (NaH_2PO_4/H_3PO_4); pH: 6 (Na_2HPO_4/ NaH_2PO_4) and pH: 7.0-9.0 (NaH_2PO_4/ Na_2HPO_4). In case of temperature studies, free and immobilized enzymes were incubated in the reaction mixtures at different temperatures ranging from 20 °C to 90 °C. The activities of free and immobilized enzyme were plotted against respective temperature. Optimum pH and temperature for immobilized Acetylcholinesterase; the following recipe was used: 4 mL studied buffer (pH 3-9) + 0.075M, 20 µL Acetylthiocholine + 0.01M, 50 µL DTNB + 0.0020 g immobilize acetylcholinesterase. The maximum activity was obtained at pH 5.0 and temperature 40 ^{o}Cfor the free enzyme.

2.7. Effect of substrate

To determine the extent at which immobilization affects the enzyme activity, *K*m and *V*max were determined at optimum pH and 60 ^{o}C temperature. Free and immobilized enzyme was incubated with different substrate concentrations (10 µL - 60 µL / 0.075M) in phosphate buffer of pH 8 (4010 µL -3960 µL), and assayed for enzyme activity at 60 ^{o}C, recommended temperature for enzyme assays.

2.8. Storage stability and reusability of immobilized enzyme

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Storage stability experiments were carried out to determine the stabilities of immobilized enzymes after storage in dry conditions at +4 °C during the 12 months. The enzyme activity was measured every 30 days. Observed results compared to the initial activities. To evaluate the reusability, the acetylcholinesterase immobilized polymeric supports were also washed with buffer solution after any run and reintroduced into a fresh solution. Reaction cycles under the conditions (pH= 8.0, at room temperature) described above were performed. The enzyme activity was measured.

3. Results and discussion

3.1. Characterization of (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)]

The elemental analyses can be considered compatible with the chemical formulas of the compounds. The weight average molecular weight (Mw) was suggested from element analyses. Heterogenlic index and some of the physical properties of all studied polymer were given in Table 1. Molecular weight and molecular weight distribution (Mw/Mn) were determined by gel permeation chromatography (GPC). According to GPC, modified polymers have a very narrow molecular weight distribution (PDI: 0.97, 1.17 and 1.04 for (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)] respectively).

3.2. IR Spectra of (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)]

The characteristic peak of IR spectra, elementel analysis and gel permeation chromatography of (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)] support polymers are given in Table 1.

Three overtone peaks showed in *ca*. 1937, 1870, 1801 cm^{-1} in IR spectra of support nanoplatforms attached Schiff bases / Schiff bases-Pt. IR bands *ca* 3430, 3010, 2922 and 1506 cm^{-1} regions are characteristic of υ(OH) (for adsorbed H_2O or coordination H_2O),

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$\upsilon(CH)_{aromatic}$, $\upsilon(CH)_{aliphatic}$, and $\upsilon(C\text{-}C)_{arom.ring}$, respectively [14,15]. Imine band was observed *ca* 1625 cm^{-1} for (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)].

In the Far IR range spectra of [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)] exhibited one new weak absorption band 479 and 482 cm^{-1} respectively, which can be assigned to Pt–O stretching vibrations.

Table 1 hereabouts

Table 2 presents the elemental compositions of synthesized [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV) obtained from EDX analysis**.** EDX is not the technique of choice for analyzing polymers. Because intensities of the carbon atom is often erratic in modified polymers. However, an EDX-spectrum gives an excellent elemental analysis for all elements in the Periodic Table above beryllium in modified polymer [14]. The combined information from SEM and EDX indicate the genuine modified polymer formation with Pt-Schiff bases.

Table 2 hereabouts

3.3. Studies for biocatalysis

In this study, the amount of loaded enzyme per gram of polymer found according to saturation ratio (s.o.). This ratio was calculated by the following formula, (for (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)]).

$$A_{412} = \varepsilon \times b \times C_{20\ \mu L,\ 0.075\ M}$$

$$As.o._{(412)} = \varepsilon \times b \times C_{(20\ \mu L,\ 0.075\ M\ -\ immobilized\ AChE)}$$

Figure 4 hereabouts

3.4. Influence of pH on the enzyme activity

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The pH is one of the important parameter capable of altering enzymatic activities in aqueous solution. Optimum pH for free and immobilized AChE was determined by measuring the activity of free and immobilized enzymes in buffers of different pH values ranging from 3 to 9. (4BoBPS-Sch), [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)] were observed maximum activities same for each other. Observed maximum activities are illustrated in Fig. 5.

Figure 5 hereabouts

3.5. Influence of temperature on the enzyme activity

Free and immobilized enzymes were incubated in the reaction mixtures at different temperatures ranging from 20 ^{o}C to 90 ^{o}C by measuring the residual activity of enzyme after incubation for 30 minutes. The activities of immobilized enzyme were plotted against respective temperature. The optimum temperature for immobilized AChE on (4BoBPS-Sch) [4BoBPS-Sch-Pt(II)], [4BoBPS-Sch-Pt(IV)] have shown an optimum temperature 40 ^{o}C, 80 ^{o}C and 60 ^{o}C; respectively. Free enzyme has shown an optimum temperature 30 ^{o}C (Fig.5). Higher thermal stability due to immobilization was shown in Fig. 5. The shift of the optimum temperature towards higher temperatures is an indication of the more thermal stability for the enzyme after immobilization process. The increase was observed after at 90 ^{o}C for [4BoBPS-Sch-Pt(II)] due to oxidation-reduction reaction of platinum.

3.6. Kinetic parameters for Free AChE and immobilized AChE

Kinetic parameters of the free and immobilized AChE were investigated at different concentrations of substrate ranging from 75 mM, and the data were plotted as Lineweaver-Burk graphs to calculate *V*max and *K*m values (Table 3). The *V*max value defines the maximum velocity when all of enzyme is saturated with substrate. And also, *K*m, the substrate

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concentration at which an enzyme reaches ½ *V*max reflects the effective characteristic of the enzyme [15].

Figure 5 hereabouts

Kinetic parameters were studied for free AChE and immobilized AChE all at pH=8 and temperature 30 ^{o}C for (4BoBPS-Sch)-AchE, 60 °C for [4BoBPS-Sch-Pt(II)]-AchE and 80 ^{o}C for [4BoBPS-Sch-Pt(IV)]-AchE). The Michaelis-Menten constant (*K*m) and the maximum reaction rate (*V*max) of immobilized AChE were calculated from the Lineweaver-Burk plots (Fig. 6). The determined *K*m/*V*max values which are shows the affinity of the enzyme to substrate, for free and immobilized AChE were found to be about 0.71 / 2.56, 0.13 / 1.70 and 1.35 / 1.90 mM/ mM min^{-1}; respectively. V_{max} value of enzyme was decreased after immobilization onto the on [4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)] except (4BoBPS-Sch) due to due to the platinum-enzyme complex. Because of steric effects, interaction the enzyme of acetylcholine may be prevented. But, in study on [4BoBPS-Sch-Pt(II)]-AchE has increased the interest of the enzyme to substrate.

Table 3 hereabouts

3.7. Storage stability and reusability

The storage stability of enzymes is one of their most important characteristics, as is known; enzymes lose their activity during the storage. In this study, free and immobilized enzymes were stored in a dark bottle at +4 ºC for 12 months [16]. Activity of free and immobilized enzymes was determined every week for 10 months. After the one month, free enzyme activity decreased (retained 96.55 %), however the activity of the immobilized enzyme is

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preserved even after one month (for (4BoBPS-Sch)-AchE, [4BoBPS-Sch-Pt(II)]-AchE and [4BoBPS-Sch-Pt(IV)]-AchE); 97.30 %, 98.21 % and 98.19 %, respectively). The activity of the [4BoBPS-Sch-Pt(II)]-AchE and [4BoBPS-Sch-Pt(IV)]-AchE are better than (4BoBPS-Sch)-AchE . These results indicate that the immobilized AChE retains its high enzymatic activity, which was very important for the preparation of the proposed enzyme support industrial applications. After 10 months, the free AChE, immobilized AChE on to the 4BoBPS-Sch-Pt(II)]-AChE and [4BoBPS-Sch-Pt(IV)]-AChE retained 56.70 %, 67.66 % and 46.25 % for their original activity (in a dry form), respectively.

As shown in Fig. 7, the (4BoBPS-Sch)-AChE, [4BoBPS-Sch-Pt(II)]-AChE and [4BoBPS-Sch-Pt(IV)]-AChE were used repeatedly 15 times and the residual activity was about 20.10 %, 49.11 % and 27.58 % of their initial, respectively. Furthermore, [4BoBPS-Sch-Pt(II)] was used repeatedly 45 times (% 23 residual activity). So, performance on the recycling stability of [4BoBPS-Sch-Pt(II)]-AchE was better than and [4BoBPS-Sch-Pt(IV)]-AchE. And the result is better than the earlier works [17, 18].

3.8. Evaluation on carbamate insecticide

Change in absorbance of Schiff bases-tagged nanomaterials are given in Table 4. UV spectra for carbamate and [4BoBPS-Sch-Pt(II)]-AchE + carbamate are given figure 8 a. The reason for decrease in the absorbance, there is an interaction between enzyme and carbamate insecticide due to enzyme-carbaryl (inhibitor) complex. According to literature, formed the enzyme-inhibitor complex with carbamylation of the serine hydroxyl of enzyme [19] as shown in figure 8 b.

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4. Conclusions

In this work, ligated Pt(II) and Pt(IV) complexes on sphere have been synthesized for the identification of organophosphates. The first time has been presented in this study the catalytic performance on ACh of coordination polymer. ACh Immobilization has been successfully fabricated for the detection of pesticide. The apparent kinetic parameters of the immobilized enzme and free enzyme were compared, and this showed that the Michaelis constant (*Km*) of the immobilized AChE was higher than that of the free AChE, while there was a more pronounced difference in the maximum reaction rates (*V*max).

The activity of AchE immobilization on [4BoBPS-Sch-Pt(II)] is better than studied other polymer. Probably, square-planar structure of [4BoBPS-Sch-Pt(II)] has been protected the three-dimensional structure of the enzyme. Furthermore, operational and storage stabilities of AChE were dramatically improved following immobilization. Especially, as a result of research on carbamate insecticide, our developed immobilization strategy would be great advantages for various industrial applications.

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Table Captions

Table 1 FTIR, GPS, elemental analysis and important physical properties of modified polymer

Table 2

SEM imaging (mag x 1000 for [4BoBPS-Sch-Pt(II)], mag x 1500 for [4BoBPS-Sch-Pt(IV)]) and EDX spectra of studied polymer

Table 3

Kinetic parameters (*K*m/*V*max; mM/ mM min^{-1}) for free AChE and immobilized AChE

Table 4

Change in absorbance of Schiff bases-tagged nanomaterials

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Table 1

FTIR, GPS, elemental analysis and important physical properties of modified polymer

Compound	Chemical formula	IR spectra	
Color, *M_w	(M_w, M_n), PDI	$\nu_{overton}$	νCH=N
		νOH/νC=$C_{a.r}$/$\nu CH_{(ar)}$	νPt-O/Pt-N
(4BoBPS-Sch)	$[(C_8H_8)_6(C_{27}H_{24}N_2O_3)]$	1937, 1869,1801	1623
yellow, 1048	(860, 690), 0.97	3430/1506 / 2928	-
[4BoBPS-Sch-Pt(II)]	$[(C_8H_8)_8(C_{27}H_{24}N_2O_3)ClPtH_2O)]$	1938, 1870,1801	1625
Brown, 1504.5	(840, 670), 1.17	3430/1508 / 2927	479 / no
[4BoBPS-Sch-Pt(IV)]	$[(C_8H_8)_7(C_{27}H_{24}N_2O_3)Cl_3PtH_2O)]$	1938, 1870,1801	1625
Brown, 1471.5	(860, 690), 1.04	3430 / 1508 / 2929	482 / no

*Determined by elemental analyses; no: not observed a.r: aromatic ring

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Table 2

SEM imaging (mag x 1000 for [4BoBPS-Sch-Pt(II)], mag x 1500 for [4BoBPS-Sch-Pt(IV)]) and EDX spectra of studied polymer

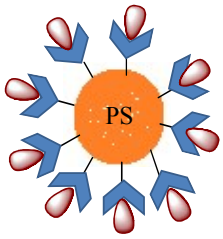
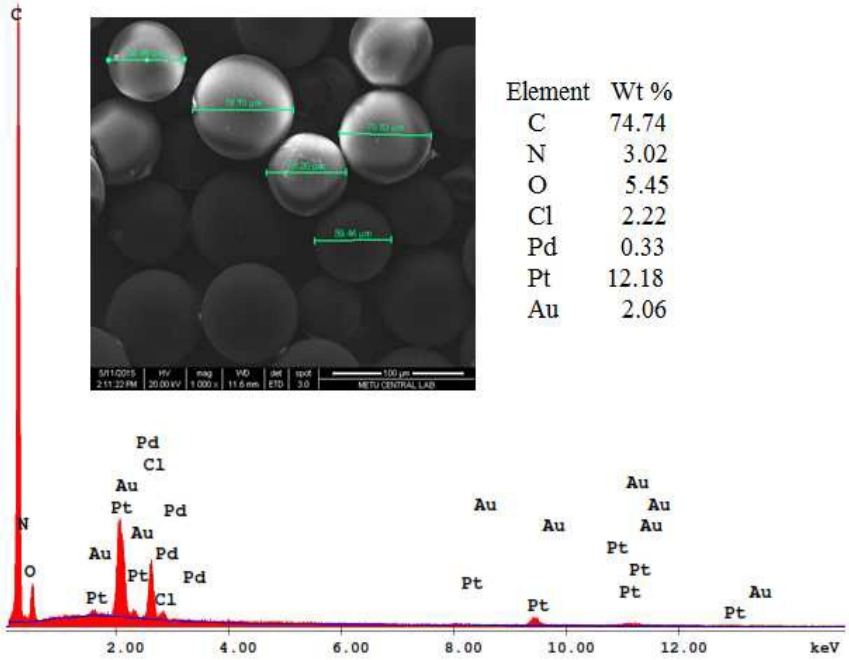
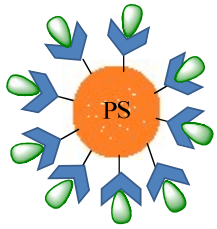
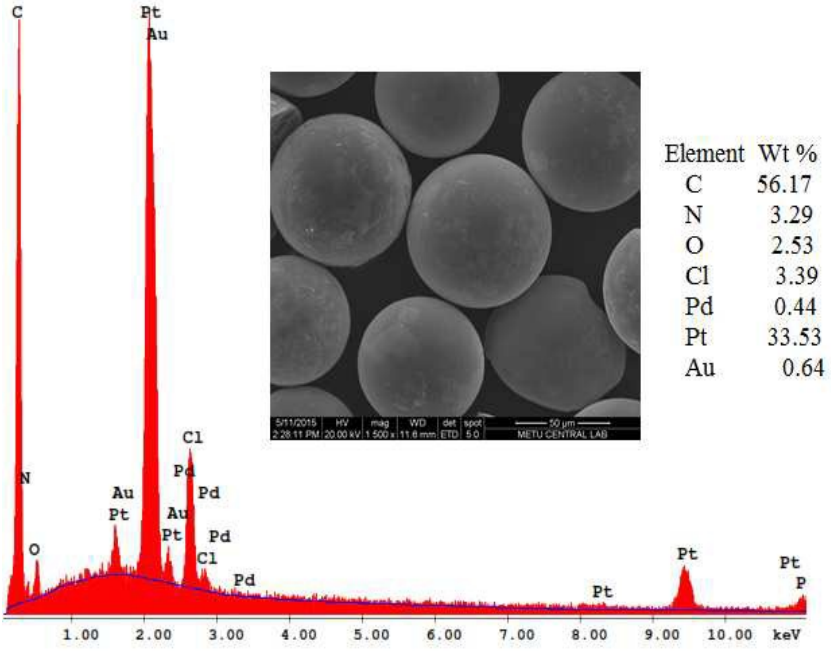
Structure of support	SEM Imaging and EDX
 **[4BoBPS-Sch-Pt(II)]**	 Element Wt % C 74.74 N 3.02 O 5.45 Cl 2.22 Pd 0.33 Pt 12.18 Au 2.06
 **[4BoBPS-Sch-Pt(IV)]**	 Element Wt % C 56.17 N 3.29 O 2.53 Cl 3.39 Pd 0.44 Pt 33.53 Au 0.64

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Table 3

Kinetic parameters (*K*m/*V*max; mM/ mM min^{-1}) for free AChE and immobilized AChE

Working Conditions				Sphere	Supports
pH	t (°C)	Free AChE	(4BoBPS-Sch)-AChE)	[4BoBPS-Sch-Pt(II)]-AChE	[4BoBPS-Sch-Pt(IV)]-AChE
	30	0.07 / 4.32	0.71 / 2.56		
8.0	60	0.58 / 1.82			1.35 / 1.90
	80	0.29 / 1.77		0.13 / 1.70	

Table 4

Change in absorbance of Schiff bases-tagged nanomaterials

Conditions	Absorbance	Δ_{Abs}
Carbamat (10 µL)	0.2376	-
Carbamat (20 µL)	0.2911	-
Pt(II)- Carbamat (10 µL)	0.1880	0.050
Pt(II)- Carbamat (20 µL)	0.2569	0.034
Pt(IV)- Carbamat (10 µL)	0.1697	0.068
Pt(IV)- Carbamat (20 µL)	0.2062	0.085

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Figures

Fig. 1. Sythesis of nanosphere (4BoBPS-Sch).

Fig. 2. Synthesis of nanosphere[4BoBPS-Sch-Pt(II)] and [4BoBPS-Sch-Pt(IV)] according to template.

Fig. 3. Determination mechanism catalytic activity of AChE.

Fig. 4. AChE immobilizaiton on studied polymer (A) and colour of reaction was started by adding Ach (B).

Fig. 5. Effect o

f pH and temperature on enzyme activity.

Fig. 6. Kinetic parameters for free enzyme (yellow point), **(A)**: 4BoBPS-Sch)-AChE, **(B)**: [4BoBPS-Sch-Pt(II)]-AChE, **(C)**: [4BoBPS-Sch-Pt(IV)]-AChE; respectively.

Fig. 7. Storage stability and reusability.

Fig. 8. (A): Spectral cahnge of carbamate insecticide in 10 µL (1) and 20 µL (2), **(B)**: Potential mechanism in between AChE and carbamate.

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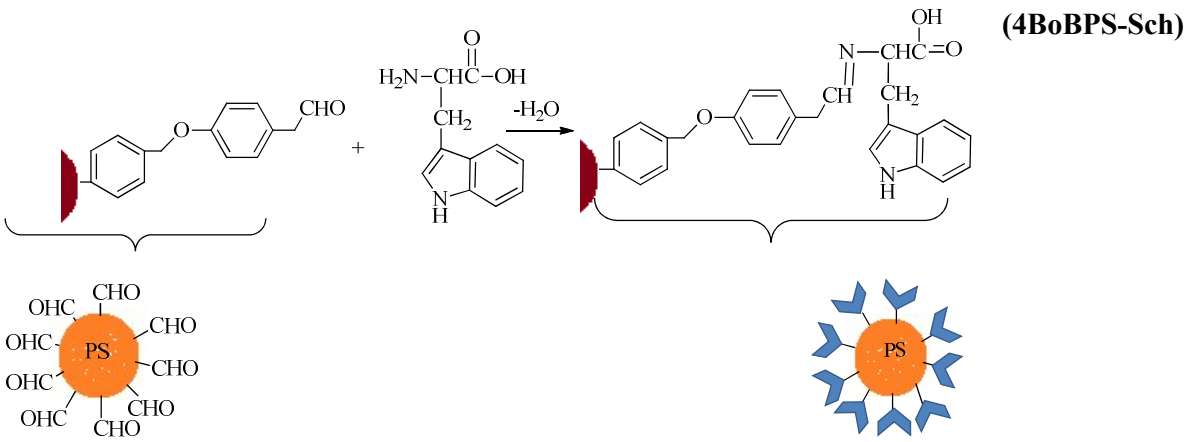

Fig. 1.

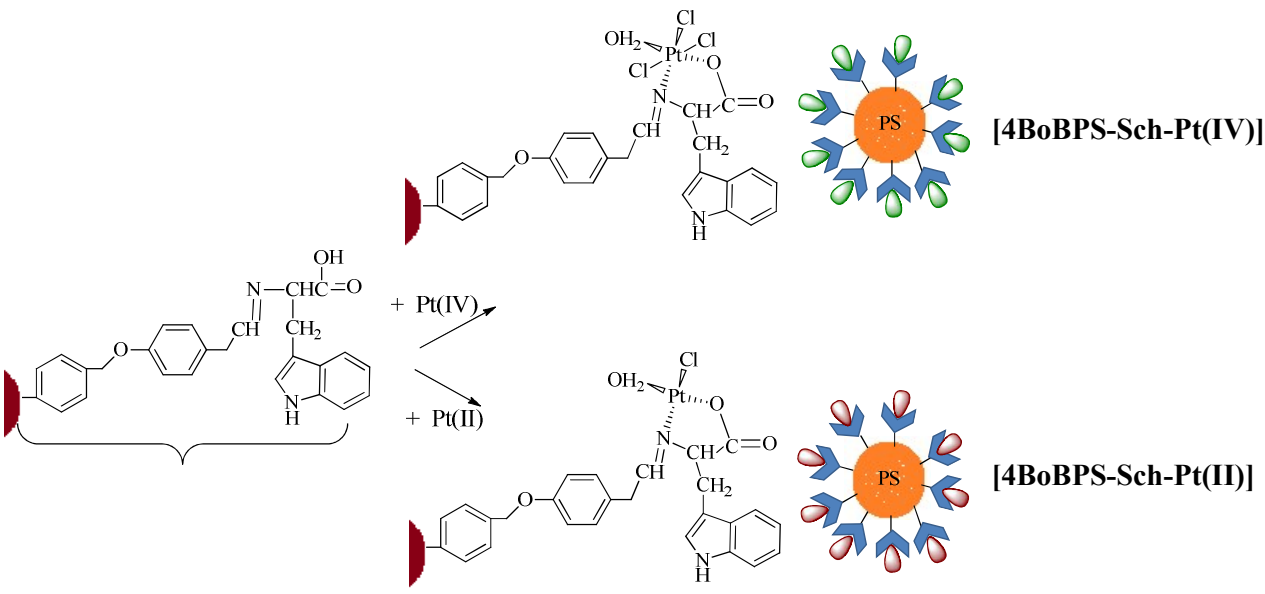

Fig. 2

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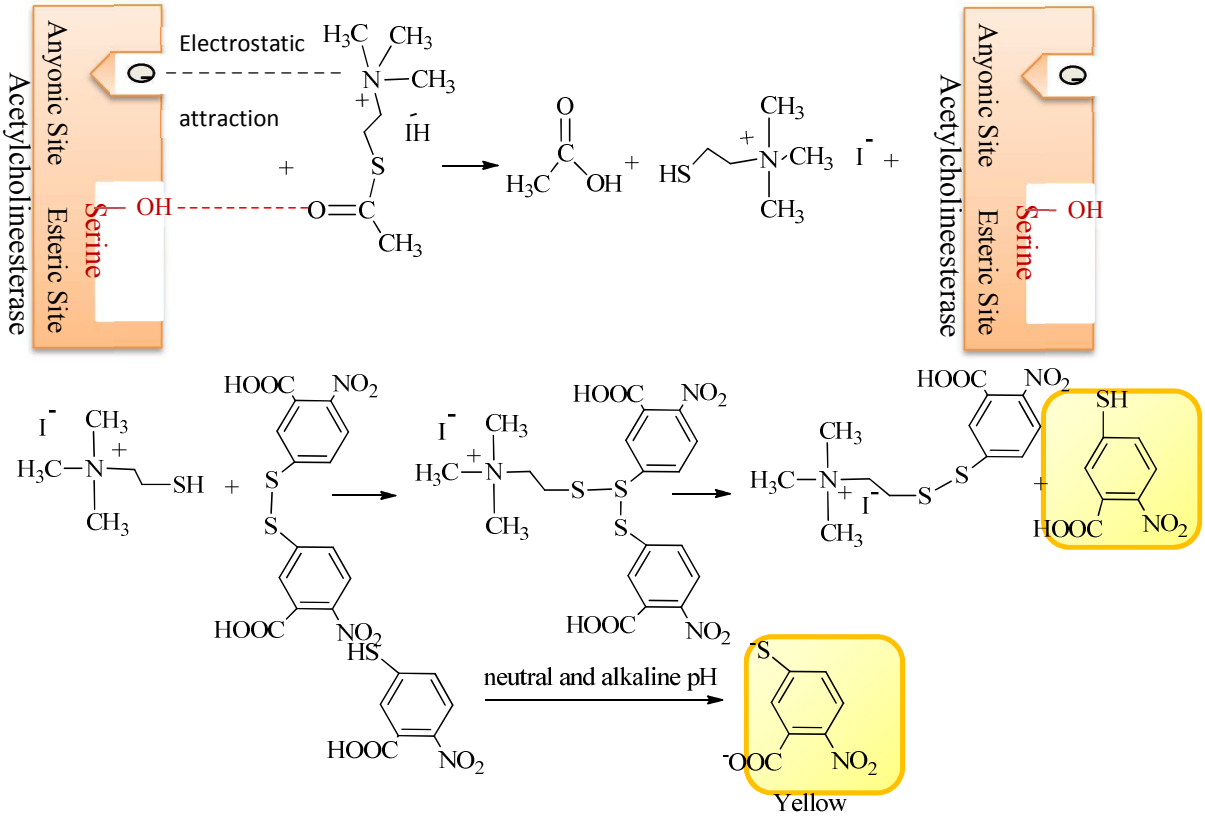

Fig. 3.

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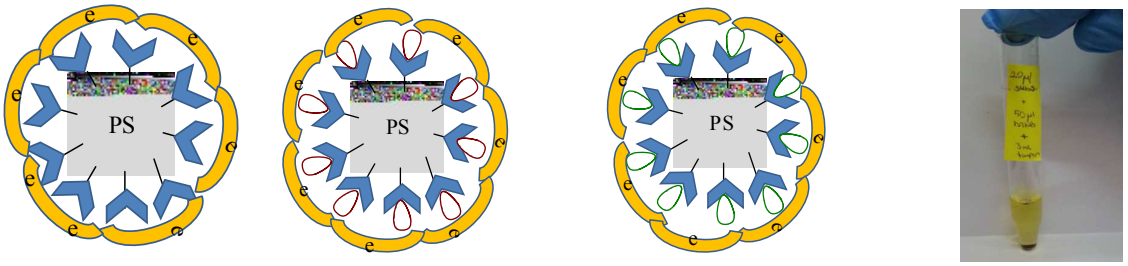

(4BoBPS-Sch)-AChE [4BoBPS-Sch-Pt(II)]-AChE [4BoBPS-Sch-Pt(IV)] -AChE solution media

(A) **(B)**

Fig. 4.

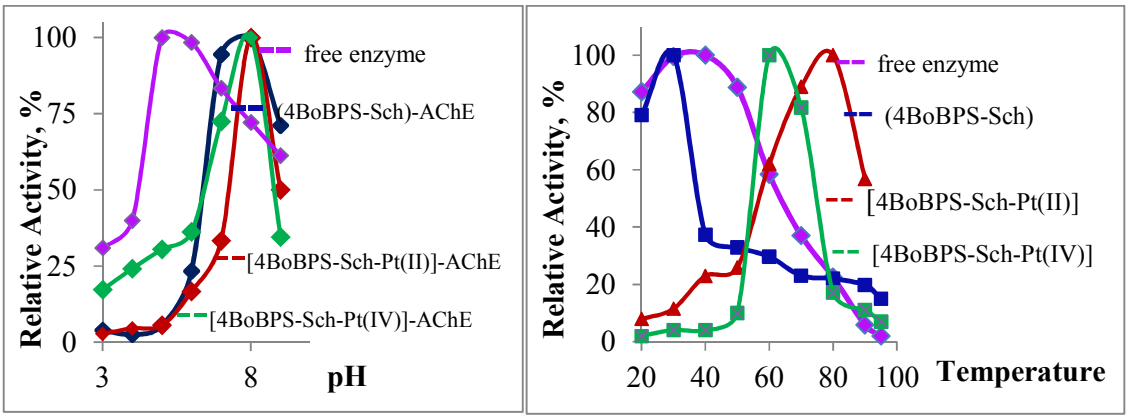

Fig. 5.

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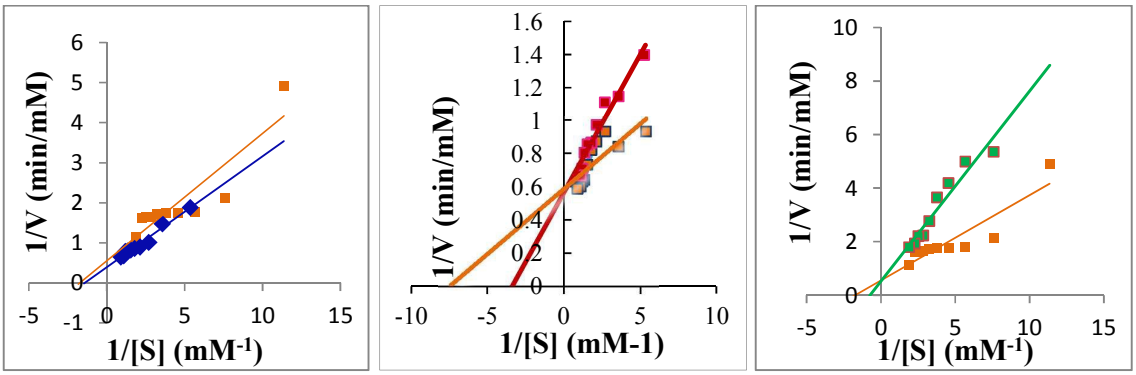

(A) **(B)** **(C)**

Fig. 6. Kinetic parameters for free enzyme (yellow point), **(A)**: 4BoBPS-Sch)-AChE, **(B)**: [4BoBPS-Sch-Pt(II)]-AChE, **(C)**: [4BoBPS-Sch-Pt(IV)]-AChE; respectively.

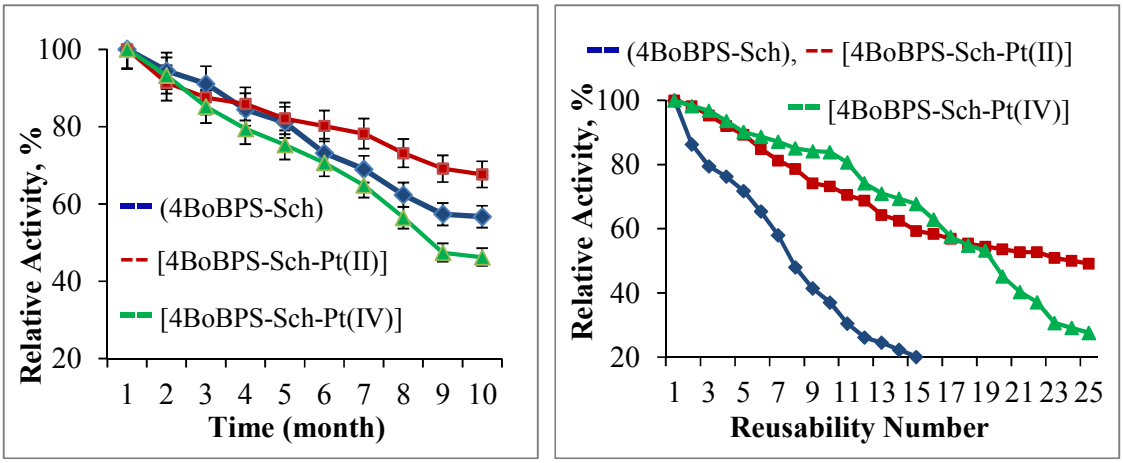

Fig. 7.

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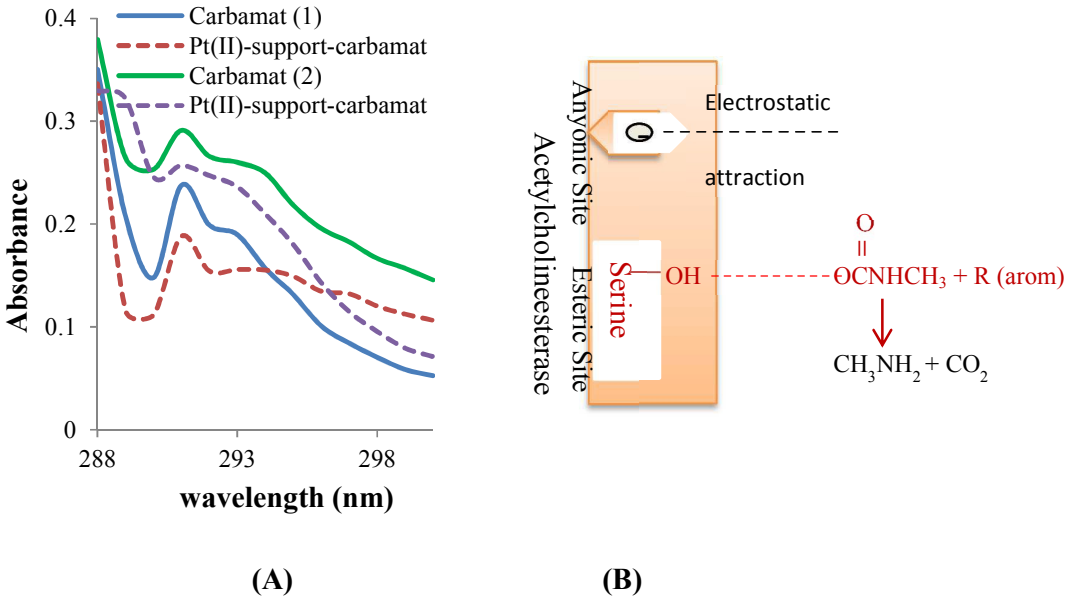

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