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Original scientific paper

PREPARATION OF FERROCENE CORE DENDRIMERS AND IMMOBILIZATION OF AChE FOR DETECTION OF DICLOFOP-METHYL HERBICIDE

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The aim of this work was to investigate diclofop-methyl detection (found in a variety of herbicides) using novel dendrimers that have ferrocene cores, as well as their Pt(II) and Pt(IV) complexes. Novel dendrimer structures were synthesized and characterized by molar conductivity, magnetic susceptibility, FTIR, UV-Vis, ^{1}H-NMR and LC-MS methods. Then, the AChE (acetylcholine esterase) enzyme was immobilized on the novel dendrimers, and the optimal parameters (pH, temperature, repeated use, storage stability, substrate concentration) were determined for immobilized AChE. Lastly, changes in absorbance intensity were measured, and calibration graphs were plotted that reflect the inhibition reaction of immobilized AChE with diclofop-methyl. The inhibition interaction of the enzyme immobilized to the Pt(II) ion containing dendrimers was higher than that of the Pt(IV) ion containing dendrimers.

Keywords: ferrocene; dendrimers; diclofop-methyl; acetylcholinesterase; immobilization

ПОДГОТОВКА НА ДЕНДРИМЕРИ СО ЈАДРО ОД ФЕРОЦЕН И ИМОБИЛИЗАЦИЈА НА AChE ЗА ДЕТЕКЦИЈА НА ХЕРБИЦИДОТ ДИКЛОФОП-МЕТИЛ

Целта на ова истражување беше да се испита детекцијата на диклофоп-метил (што се наоѓа во повеќе хербициди) со употреба на нови дендримери што имаат јадро од фероцен, како и нивните комплекси што содржат јони на Pt(II) и Pt(IV). Беше извршена синтеза на нови дендримерски структури што беа карактеризирани преку нивната моларна спроводливост, магнетната чувствителност, FTIR, UV-Vis, ^{1}H-NMR и LC-MS методи. Потоа ензимот ацетилхолин естераза (AChE) беше имобилизиран на новите дендримери и беа определени оптималните параметри (pH, температура, повторувана употреба, стабилност при складирање, концентрација на супстратот) за имобилизираниот AChE. Најпосле, беа измерени промените во интензитетот на апсорпција и беа подготвени калибрациски криви што ја одразуваат реакцијата на инхибиција на имобилизираниот AChE со диклофоп-метил. Реакцијата на инхибиција на ензимот имобилизиран на дендримерите што содржат јони на Pt(II) беше повисока во споредба со дендримерите што содржат јони на Pt(IV).

Клучни зборови: фероцен; дендримери; диклофоп-метил; ацетилхолинестераза имобилизација

1. INTRODUCTION

Dendrimers are monodisperse macromolecules in a spherical branched structure. Their formation starts from a nuclear atom and is shaped through the repeated chemical reactions. Dendrimers can be expanded to the desired size by adding successive layers [1–3]. The gaps in the dendrimers enable the transference of the molecules and/or nanoparticles contained in them into any environment. Non-covalent interaction between the guest-host is effective in the occurrence of such a transfer [4]. Also, thanks to their donor-acceptor groups and large surface areas, the enzyme allows them to be used as a support material in immobilization [5, 6]. One of the most important

events in the field of dendrimer chemistry is the incorporation of transition metals into the dendritic structures as a functional material with electrochemical and catalytic properties. Gaps exist between dendrimer arms and surface groups, which indicates that dendrimers are suitable candidates for catalysis applications. In particular, placing the controlled number of organometallic redox active groups at the centre of the branched structures helps the functionalization of the dendrimers [7, 8]. Among metallodendrimers, those containing ferrocene show high potential in electrochemistry, particularly in relation to the development of non-reactive biosensors [9]. Lee and colleagues prepared a biosensor that contains an AChE/ChOx binary enzyme system for the electrochemical determination of the diazone assay, used for pest removal. Ferrocene was used as a mediator, thus indicating that ferrocene was involved in a bioelectrocatalysis [10].

Today, pesticide-related poisonings are frequently encountered in humans and animals [11, 12]. Pesticides, especially agrochemicals, are used in farming to kill weeds and insects. Herbicides are used to control unwanted plants [13]. Herbicides disrupt the hormonal balance that regulates plant metabolism, such as cell division, cell elongation, protein synthesis and respiration [14]. Therefore, determination of these substances is vital [15, 16]. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are common methods used in the detection of diclofop-methyl. Liu and colleagues have previously used gas chromatography to detect the residual diclofop-methyl in both soil and plant samples. The amount of residual diclofop-methyl was found to be between 0.01 and 0.05 mg/kg for the investigated samples [17]. Özhan and colleagues developed a simple and precise method to simultaneously analyze the herbicides (chlorsulfuron, diuron, bentazone, linuron, chlorpropham, fenoxoprop-ethyl, MCPA, diclofop-methyl, fluazifop-butyl and trifluraline) in water samples. The group used the high-performance liquid chromatography with a diode-array detector (HPLC-DAD) method and determined the amount of pesticides to be between 0.012 and 0.035 μg l^{-1} for the investigated samples [18]. Baali and colleagues produced impedimetric organic biosensors where the *Candida rugosa* lipase enzyme was added to the bovine serum albumin (BSA) and glutaraldehyde organic matrix to detect the organochloride pesticides in aqueous solutions [19]. The AChE enzyme may be used to determine these substances, due to AChE enzyme inhibition in pesticide media. The AChE enzyme reacts with acetylthiocholine iodide. A yellow-colored solution (5-mercapto-2-nitrobenzoic acid) is formed in the presence of the colorant 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), which was produced when DTNB was added to the reaction medium. If the AChE enzyme is inhibited, no yellow-colored product occurs. By immobilizing the enzyme, the aforementioned reactions may be used several times.

In this study, our motivation is to detect diclofop-methyl, which is widely used in pesticide control, using an immobilized AChE enzyme. For this purpose, metal-containing dendrimers were synthesized (Fig. 1). Enzyme immobilization studies of the first- and second-generation dendrimers have previously been reported [20]. In our previous work, the AChE enzyme was immobilized to the support material. The applicability of the immobilized enzyme in pesticide determination was investigated. The inhibition interaction of the enzyme immobilized to the dendrimers containing the Pt(II) ion with herbicide was found to be higher than the inhibition interaction with immobilized dendrimers containing the Pt(IV) ion. The reason for this is that Pt(II) dendrimers have a planar structure and are effective in maintaining the three-dimensional structure of the enzyme as a result of the interaction with the enzyme, thus allowing for competition of the substrate and pesticide.

2. EXPERIMENTAL SECTION

2.1. *Materials and methods*

1,1'-Ferrocenedicarboxaldehyde; melamine; glutaraldehyde; 2-amino-5-nitrophenol; platinum(II) chloride; platinum(IV) chloride; toluene; acetone; DMSO; acetylcholinesterase (type C3389, from electric eel, 518 units per mg, 10 KU); 5,5′-dithiobis(2-nitrobenzoic acid); acetylthiocholine iodide and diclofop-methyl (methyl 2-[4-(2,4-dichlorophenoxy) phenoxy]propanoate) were purchased from Sigma (St. Louis, MO). All other chemicals used in this work were provided by Sigma-Aldrich and were used without further purification.

Elemental analysis (C, H, N) was carried out using a LECO-932 CHNS analyzer, and the Fe concentrations were determined on an Atomic Absorption Spectrophotometer Perkin-Elmer 2380. Infrared spectra were recorded on a Bruker Vertex-80 FTIR-ATR spectrometer between 4000 and 450 cm^{-1}. ^1H Nuclear Magnetic Resonance (NMR) spectra was recorded between 0 and 13 ppm on Jeol JNM-LA 500 FTNMR. ^1H NMR spectra were recorded with a Bruker DPX-300 MHz and 100 MHz using tetramethylsilane (TMS) as an internal standard and DMSO-d_6 as the solvent. Electronic spectra were recorded on a UV-1800 ENG240V

spectrophotometer in dimethylformamide (DMF). Melting points were determined with a Barnstead-Electrothermal-9200 melting point apparatus. The molar conductivities were measured with an inoLab Cond 730 conductivity meter ($5 \cdot 10^{-4}$ $moll^{-1}$ in DMF solution). Magnetic measurements were performed with a Sherwood Scientific Magnetic Susceptibility Balance (Model No: MK 1) at 26 ± 0.1 °C with $Hg[Co(NCS)_4]$ as a calibration.

2.2. *Synthesis of extended second-generation dendrimer: [2G-NO_2-Pt(II)] and [2G- NO_2-Pt(IV)]*

First-generation (1G) and second-generation (2G) dendrimers were previously synthesized by our group [20, 21]. In this work, 1G and 2G dendrimers were reacted with 2-amino-5-nitrophenol and Pt(II)/Pt(IV) metal salts, and extended second-generation [2G-NO_2-Pt(II)] and [2G- NO_2-Pt(IV)] dendrimers were obtained (see Fig. 1). A solution of the 2G is dissolved in 20 ml DMF/CH_3OH (1:5 v/v). The solution was stirred magnetically at room temperature. $8 \cdot 10^{-3}$ mol (1.233 g) of 2-amino-5-nitrophenol was dissolved in CH_3OH (10 ml), added dropwise to the solution of the 2G, and the resulting solution was stirred and heated again at room temperature for an hour. An $8 \cdot 10^{-3}$ mol quantity of metal(II) chloride ($PtCl_2$: 2.128 g, $PtCl_4$: 2.695 g) dissolved in DMF (10 ml) was added drop by drop to the mixture solution. The mixture was magnetically stirred for 1 day and heated to 85 ºC. The complexes were precipitated from the solution using toluene and were filtered and washed with 1:1 methanol/toluene. They were then dried in a vacuum oven.

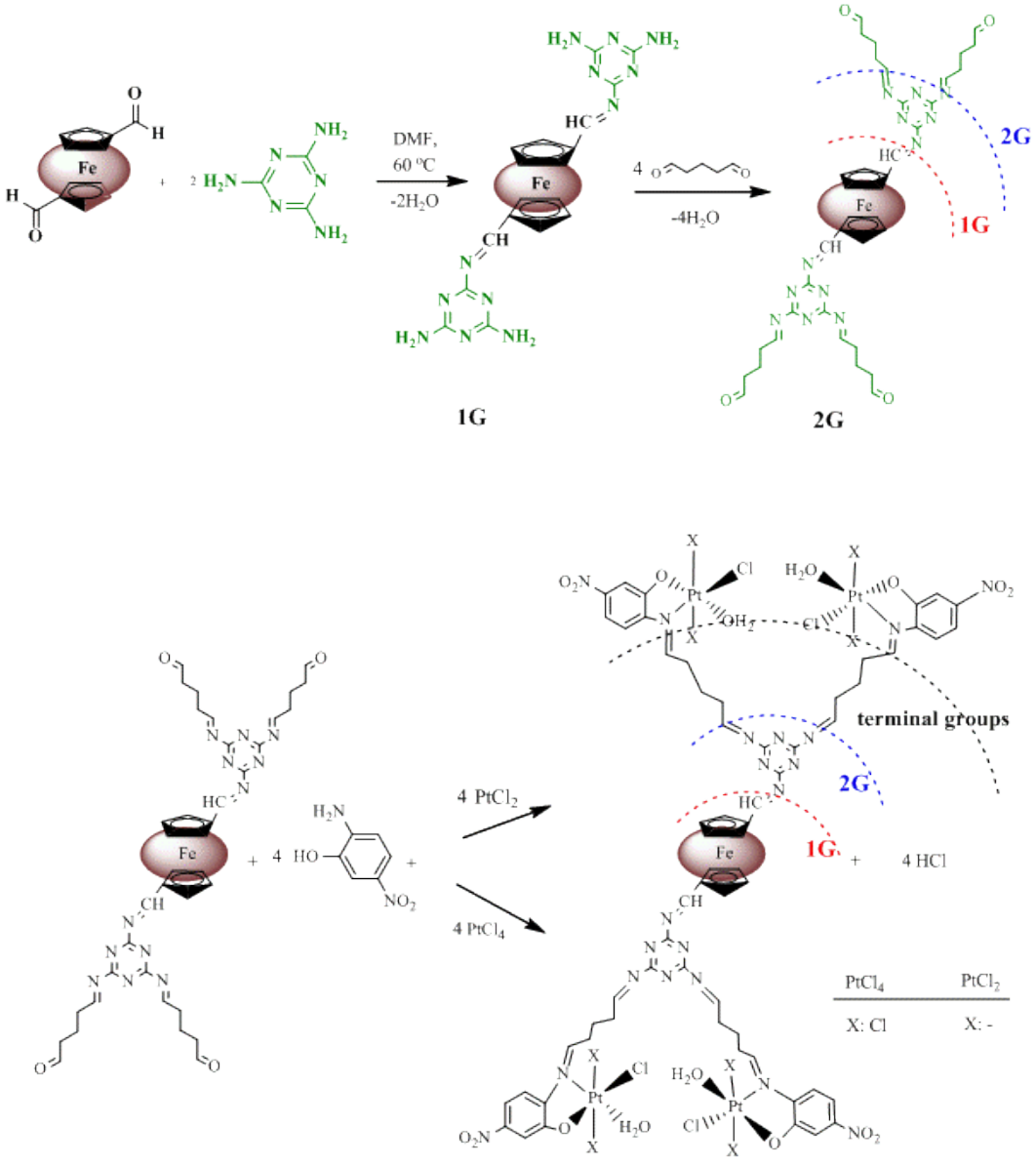

Fig. 1. Synthesis schematics of the first, second and extended second-generation dendrimers

2.3. *Enzyme immobilization and optimization studies*

2.3.1. *Immobilization of AChE on 2G, [2G-NO_2-Pt(II)] and [2G-NO_2-Pt(IV)]*

Firstly, the AChE enzyme was dissolved in deionized water (50 ml, $3.6 \cdot 10^{-4}$ g l^{-1}), and then, 2 ml of AChE enzyme solution was added into 0.5 g of 2G, 2G-NO_2-Pt(II) and 2G-NO_2-Pt(IV) dendrimers. The mixture was stirred for 1 day on a magnetic stirrer at room temperature to allow immobilization. At the end of the reaction, the immobilized dendrimers were removed from the solution medium by filtration and stored at +4 °C (Fig. 2). The saturation ratio was determined to be 98.60 %, 85.75 % and 80.45 % for 2G, [2G-NO_2-Pt(II)] and [2G-NO_2-Pt(IV)], respectively, from an absorbance value in 412 nm.

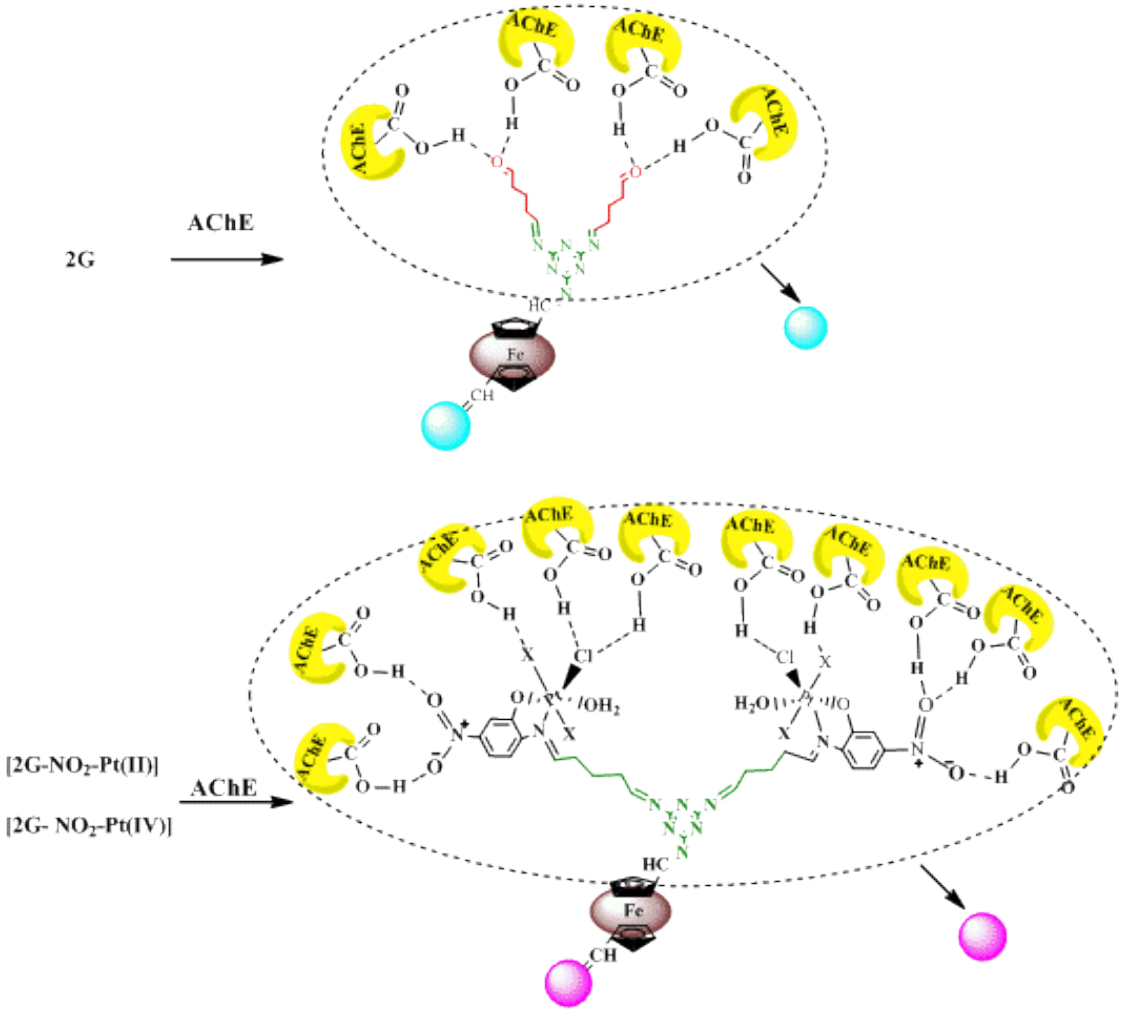

Fig. 2. Schematic representation of enzyme immobilization to synthesized dendrimers

2.3.2. *Assay for enzyme activity measurement*

Ellman's spectrophotometric method was used to determine the activity of the enzyme acetylcholinesterase [22]. The AChE enzyme gives a reaction with the substrate acetylthiocholine iodide. The thiocholine iodide reacts with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to form a yellow compound (5-mercapto-2-nitrobenzoic acid). The maximum absorbance of this compound is 412 nm. The enzyme activity was determined by monitoring the absorbance change in this wavelength.

2.3.3. *Effect of pH and temperature on activity of free and immobilized AChE*

Seven different tubes were prepared, and 2.0 mg of an AChE enzyme immobilized dendrimer was placed into each tube. The following recipe

was used for the free and immobilized AChE assay: 4 ml of the studied buffer (pH 3–9) + 20 µl of acetylthiocholine (0.075 M) + 50 µl of DTNB (0.01 M) were mixed and added to each tube. Absorbance change was monitored using a ultraviolet–visible (UV-Vis) spectrophotometer.

To examine the effect of temperature on immobilized enzyme activity, immobilized dendrimers were put on buffer solutions at optimum pH. Incubation was performed at temperatures ranging from 20–90 ºC. Subsequently, the enzyme activity was measured.

2.3.4. *Effect of substrate*

To investigate the effect of the substrate concentration on the activity of the immobilized enzyme, immobilized dendrimers were added to the substrate solution (acetylthiocholine iodide) at different concentrations (10–50 µl/0.075 M). The enzyme activity was measured by providing the incubation reaction. A Lineweaver-Burk calibration graph was drawn, and K_m and V_{max} values were determined.

2.3.5. *Storage stability and reusability of immobilized enzyme*

Storage stability experiments were carried out to determine the stabilities of the immobilized enzymes after storage in dry conditions at +4 °C over 8 months. The enzyme activity was measured every 30 days. The observed results were compared to the initial activities. To evaluate the reusability, the acetylcholinesterase immobilized dendrimers were also washed with a buffer solution after any run and were reintroduced into a fresh solution. Reaction cycles were performed under the conditions (pH = 8.0, at room temperature) described above. The enzyme activity was measured.

2.3.6. *Qualitative determination of pesticides by immobilized AChE enzyme*

Pesticides inhibit the enzyme by covalent bonding to the hydroxyl group of the serine amino acid in the active site of the enzyme acetylcholinesterase. In Figure 3, the mechanism of inhibition that is possible between the enzyme and diclofop-methyl is given.

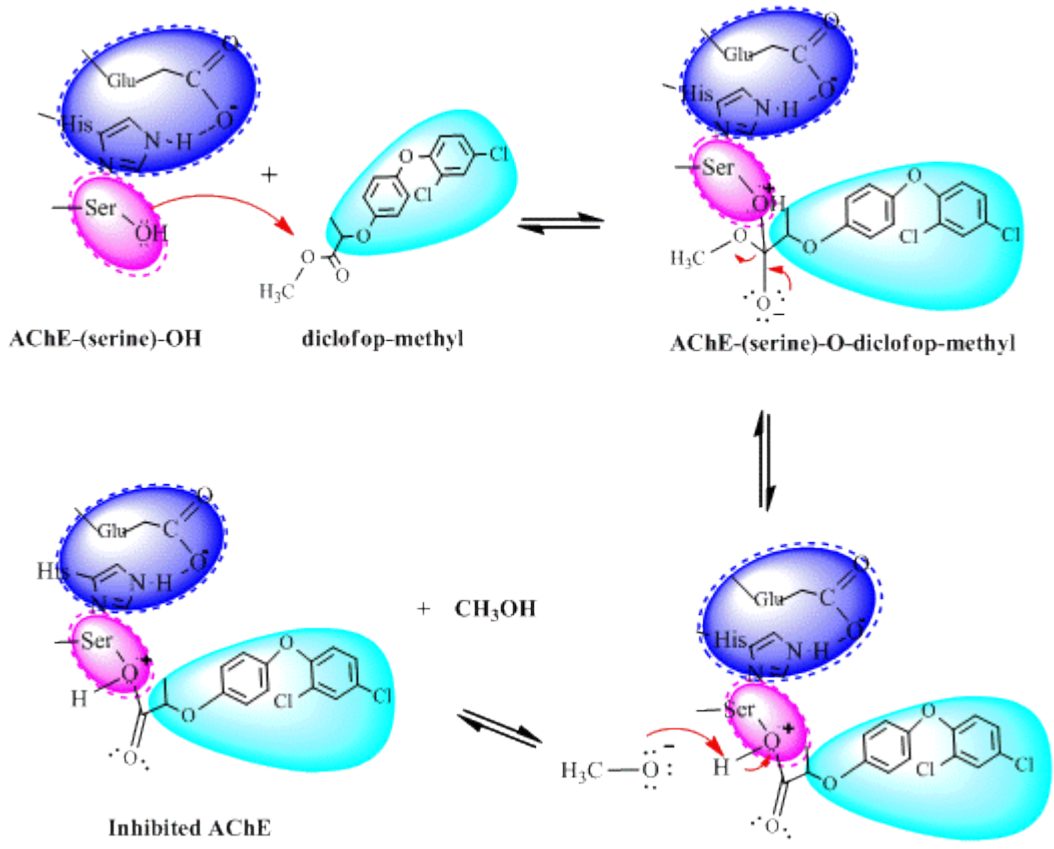

Fig. 3. Mechanism of inhibition that is possible between the enzyme and diclofop-methyl

For the herbicide detection, firstly, a $5.86 \cdot 10^{-8}$ M solution of diclofop-methyl was prepared in a water/acetonitrile mixture (19 : 1 v/v). Then, varying volumes of diclofop-methyl solution in the range of 10 to 50 µl were added to the immobilized dendrimers (2G-AChE, 2G-NO_2-Pt(II)-AChE and 2G-NO_2-Pt(IV)-AChE). Absorbance values of 412 nm wavelength were measured by the spectrophotometer. Then, Δ_{abs} was calculated from the formula below:

$$\Delta_{abs} = A_{herbicide} - A_{dendrimer+herbicide}$$

3. RESULTS AND DISCUSSION

Novel dendrimers with ferrocene cores and their Pt(II) and Pt(IV) complexes were synthesized and characterized by different physicochemical methods, including molar conductivity measurements and spectroscopic (IR, UV–Vis, LC-MS and ^{1}H NMR) techniques. The products obtained ranged from black to brown and are air-stable in the solid state. The dendrimers are only soluble in C_2H_5OH, DMF and DMSO and are insoluble in an apolar solvent such as CCl_4 and benzene. The molar conductance values of dendrimers containing Pt(II) and Pt(IV) are found to be 28 Ω^{-1} cm^2 mol^{-1} and 68.4 Ω^{-1} cm^2 mol^{-1} in $5 \cdot 10^{-4}$ M DMF solutions, respectively (Table 1). According to these results, dendrimers are nonelectrolytes [23]. The magnetic moment of dendrimers containing Pt(II) and Pt(IV) indicates that these complexes are diamagnetic. Dendrimers containing metal have a square planar and octahedral geometry for dendrimers including Pt(II) and dendrimers including Pt(IV), respectively.

T a b l e 1

Colors, partial elemental analyses and molar conductivity measurements of the synthesized dendrimers

Compound	Color	λ°_{M*}	Found (Calc.) %				
			C	H	N	Pt	Fe
2G-NO_2-Pt(II) $C_{62}H_{62}Cl_4FeN_{20}O_{16}Pt_4$ (2321.2)	Brown	28	32.79 (32.08)	3.06 (2.69)	12.35 (12.07)	34.10 (33.62)	1.99 (2.41)
2G-NO_2-Pt(IV) $C_{62}H_{62}Cl_{12}FeN_{20}O_{16}Pt_4$ (2604.9)	Dark brown	68.4	29.94 (28.59)	2.61 (2.40)	11.11 (10.75)	30.40 (29.96)	2.01 (2.14)

* Ω^{-1} cm^2 mol^{-1}

3.1. *IR, UV-visible, LC-MS and NMR spectra of second and expanded second-generation dendrimers including azomethine*

Table 2 summarizes the main infrared (IR) spectra results of the extended second-generation dendrimers. For the IR bands in extended second-generation dendrimers including azomethine [2G-NO_2-Pt(II)] and [2G-NO_2-Pt(IV)], the azomethine bands in the IR spectra of the extended second-generation dendrimer appear in the range of 1600–1650 cm^{-1}. The bands in the 2915–2914 cm^{-1}, 3055–3053 cm^{-1} and 1245–1242 cm^{-1}, 1458–1456 cm^{-1} regions are ascribed to $\nu CH_{aliphatic}$, $\nu CH_{aromatic}$ vibrations and ferrocene vibrations, respectively [24–26]. The IR spectra of all extended second-generation dendrimers exhibit characteristic bands of coordination water at ca. 3300 cm^{-1} and 1600 cm^{-1} assigned to ν_{H2O} and δ_{H2O} vibrations, respectively [26, 27]. These observations clearly suggest that the water molecules are coordinated to the metal ion (Fig. 1). The appearance of bands in the 486–483 and 563–761 cm^{-1} regions are due to ν(Pt-N) and ν(Pt-O), respectively [24]. On the basis of the IR spectral results, it may be deduced that the anion of the Schiff base is coordinated to the metal ion as a bidentate ligand (Fig. 1).

Electronic absorption spectra of the [2G-NO_2-Pt(II)] and [2G-NO_2-Pt(IV)] complexes were recorded in spectroscopic grade DMF, and their tentative assignments are given in Table 3. The electronic spectra of dendrimers in DMF show that bands ca. 300 nm are attributed to the azomethine $n \rightarrow \pi^*_{(-C=N)}$ transition. The bands at higher energies (201–207, 296–300, 253–257 nm) are associated with the benzene $\sigma \rightarrow \sigma^*$, $n \rightarrow \pi^*_{(C=N)}$ and $\pi \rightarrow \pi^*$ transition [25]. The d-d bands from the spectra of the Fc molecule having low intensities appeared at 389–392 nm [25]. Dendrimers including Pt(IV) observed d-d bands ca, 540 nm [27].

T a b l e 2

Infrared spectroscopic assignments (cm^{-1}) for the synthesized dendrimers

Compound	ν(C=N)	$\nu(NH_2)$	νCH (ald.)	$\nu(C–H)_{aliph.}$	$\nu(C–H)_{arom.}$	ν M–N	ν M–O
2G-NO_2-Pt(II)	1638 1630 1622	–	–	2915	3053	483	561
2G-NO_2-Pt(IV)	1640 1625 1620	–	–	2914	3055	486	563

T a b l e 3

UV-Vis spectrum values (nm) of synthesized dendrimers

Compound	λ_{max} (10^{-4} M DMF)				
	σ→ σ*	π →π*$_{(bnz)}$	π →π*$_{(C=N)}$	n→ π*$_{(C=N)}$	d→d$_{(Fc)}$
2G-NO_2-Pt(II)	201	234	253	300	389
2G-NO_2-Pt(IV)	207	231	257	296	392

3.2. *Studies for biocatalysis*

In this study, the amount of loaded enzyme per gram of dendrimers was defined as the saturation ratio (s.r.). This ratio was calculated by the following formula (for 2G, [2G-NO_2-Pt(II)] and [2G-NO_2-Pt(IV)]):

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

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

3.3. *Influence of pH on enzyme activity*

The optimum pH for the activity of 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. Both the free enzyme and the immobilized dendrimers have the highest relative activity at pH 8.

3.4. *Influence of temperature on enzyme activity*

Free and immobilized enzymes were incubated in the reaction mixtures at different temperatures ranging from 20 ºC to 90 ºC by measuring the residual activity of the enzyme after incubation for 30 minutes. The activities of the immobilized enzyme were plotted against respective temperature. The optimum temperature for immobilized AChE on 2G, [2G-NO_2-Pt(II)] and [2G-NO_2-Pt(IV)] was determined as 60 ºC, 40 ºC and 60 ºC, respectively. The free enzyme has shown an optimum temperature as 30 ºC. The shift of the optimum temperature toward higher temperatures is an indication of greater thermal stability for the enzyme after the immobilization process.

3.5. *Kinetic parameters for free AChE and immobilized AChE*

The effect of the substrate concentration was examined at the optimum pH and at the optimum temperature that we had previously determined. For this purpose, absorbance values of substrate solutions were determined, and various graphs (1/*S* versus 1/*V*) were drawn. The K_m and V_{max} values calculated from these graphs are given in Table 4 [28].

T a b l e 4

Kinetic parameters (K_m/V_{max}; mM/mM min^{-1}) for free AChE and immobilized AChE

Symbol of dendrimers	pH	T (°C)	Dendrimer-AChE		Free enzyme	
			K_m	V_{max}	K_m	V_{max}
2G-AChE	8	60	0.20	0.44	0.07	4.32
2G-NO_2-Pt(II)-AChE	8	40	0.30	0.38	0.99	2.06
2G-NO_2-Pt(IV)-AChE	8	60	0.33	0.33	0.07	4.32

Kinetic parameters were studied for free AChE and immobilized AChE at pH = 8 and at a temperature of 60 ºC for 2G-AChE, 40 ºC for [2G-NO_2-Pt(II)]-AchE and 60 ºC for [2G-NO_2-Pt(IV)]-AChE). The determined K_m/V_{max} values, which show the affinity of the enzyme to substrate, for free and immobilized AChE were found to be about 0.20/0.44, 0.30/0.38 and 0.33/0.33 mM/ mM min^{-1}, respectively. The V_{max} value of the enzyme decreased after immobilization onto the [2G-NO_2-Pt(II)] and [2G-NO_2-Pt(IV)], but not upon the immobilization to the 2G due to the platinum-enzyme complex. Due to the steric effects, interaction of acetylcholine may be prevented. According to K_m, the affinity of the AChE immobilized on 2G to the substrate is greater than the [2G-NO_2-Pt(II)] and [2G-NO_2-Pt(IV)].

3.6. *Storage stability and reusability*

The storage stability of enzymes is one of their most important characteristics: it is common knowledge that enzymes halt their activity during storage. In this study, free and immobilized enzymes were stored in a dark bottle at +4 ºC for 8 months (Fig. 4). When the studies on storage stability were evaluated, it was observed that the relative activities were maintained at a 95 % level at the end of 1 month. After 6 months, approximately 80 % of the relative activity of 2G-AChE is maintained, while the Pt(II) and Pt(IV) complexes are maintained at about a 75 % level. The relative activity of 2G-NO_2-Pt (IV)-AChE between the immobilized dendrimers was found to be the lowest, at a 65.9 % level after 8 months.

2G-AChE, [2G-NO_2-Pt(II)]-AChE and [2G-NO_2-Pt(IV)]-AChE were used repeatedly 10 times, and the residual activity was about 70.69 %, 73.55 % and 71.67 % of their initial levels, respectively. Furthermore, 2G-AChE was used repeatedly 20 times (50.18 % residual activity). Therefore, performance on the recycling stability of [2G-NO_2-Pt(II)]-AChE was better than [2G-NO_2-Pt(IV)]-AChE.

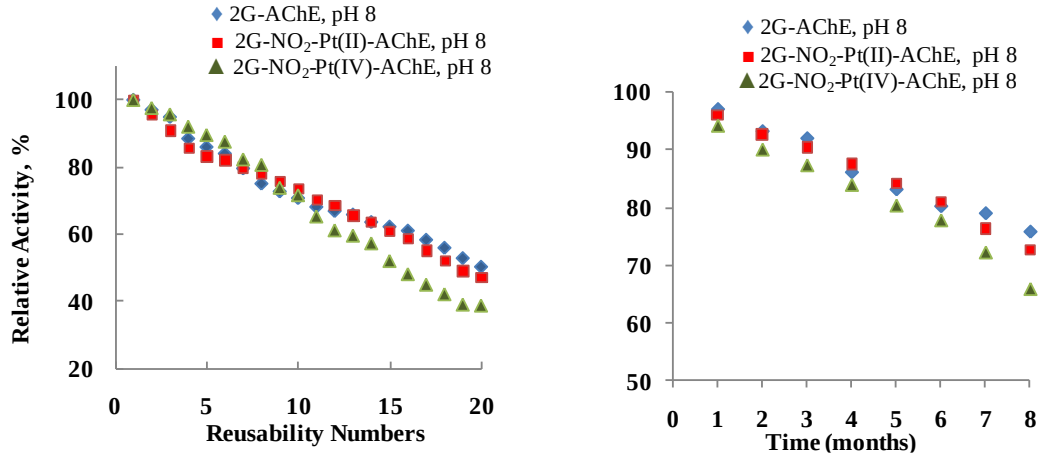

Fig. 4. Storage stability and reusability for immobilized enzymes

3.7. *Evaluation of the results of the inhibition of immobilized dendrimers with diclofop-methyl*

Changes in absorbance intensity were recorded as a result of the addition of the pesticide solution, ranging between 10 and 50 µl in the environment of immobilized dendrimers (Fig. 5). The amount of pesticides required to halve the enzyme activity is shown in Figure 6. When the immobilized dendrimers were compared, it was seen that less herbicide was needed to reduce the enzyme activity of Pt(II) ion containing dendrimers to 50 %, whereas, it was found that this value was significantly higher for the 2G. Calibration graphs were investigated, and it was seen that the required amounts of pesticide to reduce the enzyme activity were found to be 90.69 µl, 40.01 µl and 45.60 µl for 2G-AChE, [2G-NO_2-Pt(II)]-AChE and [2G-NO_2-Pt(IV)]-AChE, respectively (Fig. 6). Therefore, it was concluded that the use of the 2G-AChE ligand in the detection of diclofop-methyl would not be economical.

The inhibition interaction of the enzyme immobilized to the dendrimers containing the Pt(II) ion with herbicide was higher than the inhibition interaction with immobilized dendrimers containing the Pt(IV) ion. The reason for this behaviour is that Pt(II) dendrimers have a planar structure and are effective in maintaining the three-dimensional structure of the enzyme.

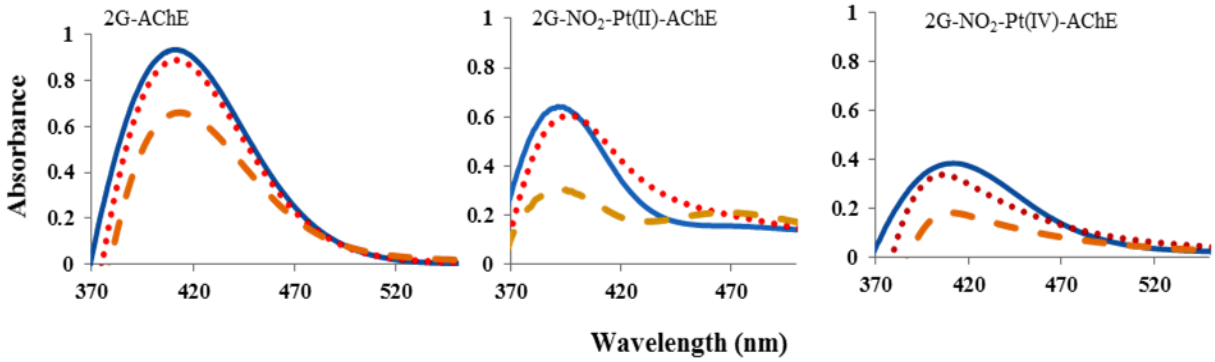

Fig. 5. Spectral change of diclofop-methyl herbicide interacting with immobilized dendrimer is given (10 µl diclofop-methyl+immobilized dendrimers (red line); 50 µl diclofop-methyl+immobilized dendrimers (brown line)).

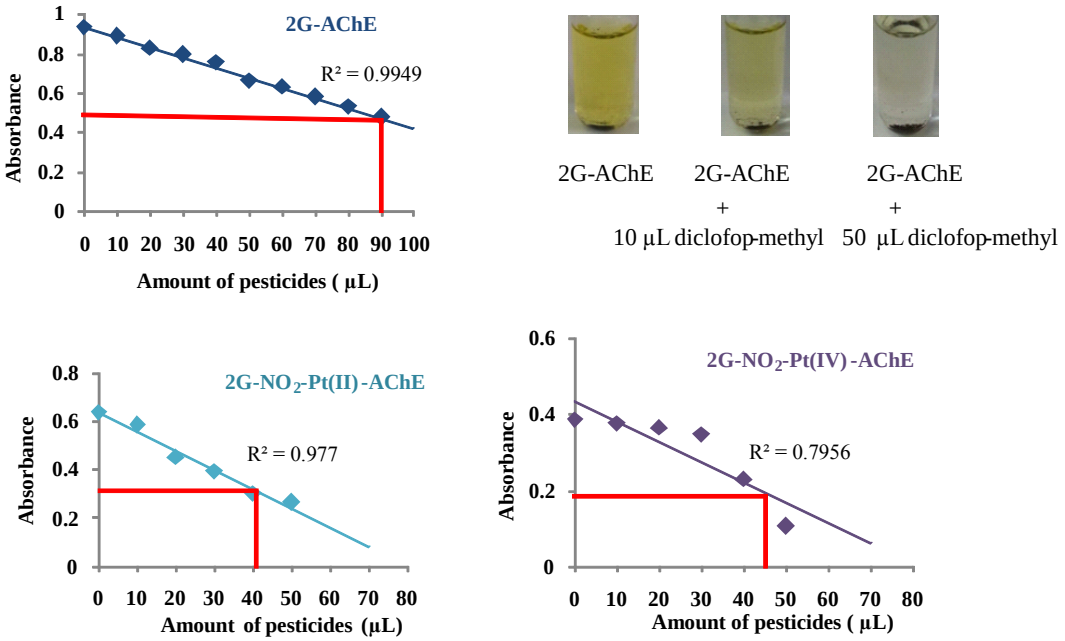

Fig. 6. The calibration graphs of the pesticide amounts that are necessary to reduce the enzyme activity by 50 %

4. CONCLUSIONS

Acetylcholinesterase is a classical biomarker used to monitor the contamination and poisoning of organophosphate (OP) and carbamate pesticides. The use of the AChE enzyme for the determination of herbicides was much less studied then for the organophosphate and carbamate pesticides [29, 30]. For this purpose, ferrocene containing dendrimers were prepared for the determination of herbicides, and the immobilization of the AChE enzyme was performed. The kinetic parameters were calculated at the end of the immobilization, and it was observed that the K_m value of the 2G-NO_2-Pt (II) immobilized dendrimer was significantly lower than that of the free enzyme. The result showed that the immobilized enzyme was more related to the substrate than the free enzyme.

When the studies on the herbicide detection of the immobilized enzyme were examined, the inhibition interaction of the enzyme immobilized to the dendrimers containing the Pt(II) ion with the herbicide was higher than the inhibition interaction with immobilized dendrimers containing the Pt(IV) ion. The Pt(II) ion containing immobilized dendrimers should be used to determine herbicides in low concentrations. Taken together, these results suggest that enzymes with a high cost of purification can be immobilized on functional dendrimers. Hence, they can contribute to the preparation of affordable and reliable sensors with high levels of sensitivity.

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