Macedonian Journal of Chemistry and Chemical Engineering, Vol. **35**, No. 2, pp. 263–270 (2016)

MJCCA9 – 719

ISSN 1857-5552
e-ISSN 1857-5625

Received: March 25, 2016
Accepted: June 15, 2016

DOI: doi.org/10.20450/mjcce.2016.895
Original scientific paper

CHARACTERIZATION OF Co(II)-PAMAM DENDRIMER COMPLEXES WITH POLYPROPYLENE OXIDE CORE BY USING UV-VIS SPECTROSCOPY

Ali Serol Ertürk[1,*], Mustafa Ulvi Gürbüz[2], Metin Tülü[2], Abdürrezzak Emin Bozdoğan[2]

[1]*Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, 02040, Adıyaman University, Adıyaman, Turkey*
[2]*Department of Chemistry, Yıldız Technical University, 34210, Istanbul, Turkey*
aserturk@adiyaman.edu.tr; aserturk@gmail.com

The aim of this study was to characterize poly(amidoamine) (PAMAM) dendrimer complexes of Co(II) ions by using UV-Vis spectroscopy. For this aim, a generation-4 polypropylene oxide (Jeffamine® T-403) cored and amine-terminated PAMAM dendrimer (P_4) was used as a model complexation agent. Optimum complexation condition for P_4 was determined by potentiometric and spectroscopic titration studies. Extent of protonation (EOP) of the amino groups of P_4 was determined from the potentiometric titration data and validated with spectroscopic titration data. The results indicated that pH 8 was the optimum dendrimer aqueous solution media, where the available number of tertiary amines present in P_4 was the highest for possible metal complexation. At the optimized conditions, UV-Vis characterization of the Co(II)-P_4 complexes was performed and 585–635 nm *d-d* transition bands were observed as the characteristic complexation bands of the tertiary amine groups of P_4 with Co(II) ions. Obtained Co(II)-P_4 complexes might be considered for the development of magnetic resonance image (MRI) improvers or MRI contrast agents.

Keywords: PAMAM dendrimer; Jeffamine; complexation; UV-Vis spectroscopy; MRI contrast agents

КАРАКТЕРИЗАЦИЈА НА РАМАМ-ДЕНДРИМЕРНИ КОМПЛЕКСИ НА Co(II) СО ПРОПИЛЕН-ОКСИДЕН КОСТУР СО КОРИСТЕЊЕ НА UV-VIS СПЕКТРОСКОПИЈА

Целта на ова истражување е да се карактеризираат поли(амидоаминski) (PAMAM) дендримерни комплекси на јоните на Co(II) со помош на UV-Vis спектроскопија. За таа цел беа користени полипропилено оксиден костур од генерацијата-4 (Jeffamine® T-403) и аминski терминиран PAMAM дендример (P_4) како модел за средство за комплексирање. Оптималните услови за P_4 беа определени со помош на потенциометриски и спектроскопски титрациски испитувања. Степенот на протонирање (EOP) на аминогрупите на P_4 беше определен со потенциометриски титрации, кои потоа беа валидирани со спектроскопски титрации. Резултатите укажуваат дека оптимален медиум се водни раствори со pH 8 кога достапниот број на терцијарни амини присутни во P_4 беше највисок за можно комплексирање на металот. Во оптимизираните услови беше извршена UV-Vis карактеризација на комплексите на Co(II)-P_4. Беа регистрирани 585–635 nm *d-d* транзициски ленти како карактеристични комплексирачки ленти на терцијарните аминski групи на P_4 со јоните на Co(II). Добиените комплекси на Co(II)-P_4 можат да се земат предвид за развивање на подобрувачи на магнетно резонатни слики (MRI) и како контрастни средства за MRI.

Клучни зборови: PAMAM дендримери; комплексирање; UV-Vis спектроскопија; контрастни средства за MRI

1. INTRODUCTION

Cobalt is a heavy metal that is found rarely in small quantities (25 μg/g) in the earth's crust [1]. It is biodegradable and accumulated in living cells [2]. There are both benefits and harms of cobalt for living organisms. While there are proofs of benefits of cobalt for the growth of plants [3], high concentrations of Co(II) cations in aqueous solutions are toxic for livings on land and in water, and can cause DNA strand breakage [4, 5]. On the other hand, cobalt as a B12 complex vitamin component is essential to human beings, in trace amounts to other mammals, and Co-60 radioactive isotope is used in nuclear medicine in the treatment of cancer as a tracer [6]. In addition, extended cobalt complexes with an amine ligand, carboxylate ligand, and a multidentate thiol-containing organic ligand can be used as magnetic resonance imaging (MRI) improvers [7]. Thus, the characterization of Co(II) complexes is important.

Poly(amidoamine) (PAMAM) dendrimers are three dimensional, highly branched, monodispersive synthetic polymers. Their unique structures give them the ability of behaving like a chelating agent by binding metal ions from interior tertiary amine groups and surface primary amines [8]. Tertiary and primary amine groups of PAMAM dendrimers are Lewis bases, and they can bind most metals simultaneously. However, primary amines of dendrimers cannot bind metal ions depending on the pH characteristic of the media. When the extent of protonation (EOP) of amine groups is out of the pH range of metal binding or all amine groups are protonated, they are not available for metal ion coordination [9].

Polymeric core centered or dendronized dendrimers come to denser packets as their generation size increases. They have more active functional groups and can exhibit distinctive physical and chemical properties, and find a wide range of applications [10]. Jeffamine® T-403 is a commercially available molecule and has a trifunctional primary amine with a molecular average weight of approximately 440.

In the present study, starting from a Jeffamine® T-403 polymer based molecule, a generation-4 Jeffamine® T-403 cored PAMAM dendrimer (P_4) was synthesized by the successive addition of the repeating units of monomers, methyl acrylate and ethylenediamine to successive generations of dendrimers. Metal binding abilities of P_4 in aqueous solutions were investigated by UV-Vis spectroscopy. While investigating the EOP of P_4 in the pH range of 2–12 with potentiometric titrations, it was observed that protonation of the tertiary amine groups of P_4 was correlated with the maximum absorption band range of 288–292 nm. Finally, depending on this observation, binding ability of P_4 was studied and absorption bands of Co(II)-dendrimer complexes where the complexation occurs with different dendrimer to Co(II) metal ion ratios were shown.

2. EXPERIMENTAL

2.1. *Materials*

Jeffamine® T-403 and ethylenediamine were purchased from Sigma-Aldrich. Methyl acrylate, *n*-butanol, sodium hydroxide, 37% hydrochloric acid, cobalt(II) chloride hexahydrate, and potassium hydrogen phthalate were purchased from Merck. 18.2 MΩ Millipore Milli-Q deionized water was used in the experiments. All chemicals used in experiments were of analytical grade and used without further purification unless otherwise stated. Dendrimer solutions were stored at 4ºC. Liquid-phase polymer-based retention (LPR) ultrafiltration membranes, Amicon 8000 Stirred Cell and dialysis membranes having the molecular cut of size (MWCO) 500, 1000, and 3000 Da were supplied from Millipore.

2.2. *Instrumentation and software*

The CEM Focused Microwave™ Synthesis System, Model Discover (CEM Corporation, North Carolina, USA) with a continuous microwave power delivery system with the operator selectable power output from 0–300 watts (± 30 watts) programmable in 1-watt increments, infrared temperature control system programmable from 25–250ºC, and 5–125 ml vessel capacity was used as a microwave reactor during the synthesis of PAMAM dendrimers.

Potentiometric titrations were carried out automatically by using TitroLine® 7000 (SI Analytics GmbH, Hattenbergstraße, Germany) autotitrator and thermostated titration vessel under nitrogen media. Temperature was kept at room temperature (25 ± 0.1ºC) using a Polysceince® digital temperature controller circulating bath (Polysceince, Illinois, USA). The titrator was controlled by a personal computer with Schoot Instruments, Titrisoft® 2.73 software. pH data were collected with IoLine ultra precise glass electrode with iodine/iodide

iodine/iodide reference system. Glass electrode was calibrated with Merck pH 4.0, 7.0, and 11.0 buffer solutions. The IR spectra (4000–400 cm^{-1}, resolution 4 cm^{-1}) were recorded with a Perkin Elmer Spectrum One (Serial No: C68739) in ATR. NMR spectra were recorded on a Bruker Avance 400 MHz Spectrometer.

Spectroscopic titrations were carried out automatically by using a TitroLine® 7000 (SI Analytics GmbH, Hattenbergstraße, Germany) autotitrator equipped with thermostated titration vessel under nitrogen media and PG TG 70 UV-Vis spectrophotometer equipped with UVWin5 Software v5.0.5, together.

2.3. *Synthesis of Jeffamine@ T-403 cored PAMAM dendrimers (JCPDs)*

JCPDs were synthesized by following the procedures reported in our recent studies [11, 12]. The synthesis involves two consecutive reactions, which are Michael addition and the amidation reaction, respectively. Michael addition of excess methyl acrylate (2.5 M eq. per terminal amine) to Jeffamine@ T-403 core in methanol gives the ester-terminated half-generation PAMAM dendrimers (PAMAM-OCH_3), $P_{n.5.}$ The successive amidation reaction of PAMAM-OCH_3 with excess ethylenediamine (EDA) (10 M eq. of EDA per ester branched half-generation) in methanol under appropriate microwave irradiation produces amine-terminated full generation PAMAM dendrimers (PAMAM-NH_2) referred to $P_{n.}$ Repetition of Michael addition and amidation reactions gives next higher generation. By repeating the Michael addition and amidation reactions, we synthesized both PAMAM-OCH_3 ($P_{0.5}$-$P_{3.5}$) and PAMAM-NH_2 (P_1-P_4) dendrimers. Purification of the synthesized dendrimers were succeeded by using 1-3 kDa dialysis membranes in methanol-water (1:1) depending on the relative molecular weight of $P_{n.5}$ and P_n by LPR technique. While resulting pure PAMAM-OCH_3 dendrimers were water insoluble, PAMAM-NH_2 dendrimers were water-soluble. Thus, the highest generation P_4, which has the highest number of available amino groups for binding with Co(II) ions, was used in complexation studies (Table 1).

2.4. *Potentiometric titrations*

Acid base titrations were conducted according to the literature [13, 14]. Here, 56.10 mg P_4 was weighted and diluted to 60.0 ml with 0.10 M NaCl solution. The resulting solution was transferred into a 100 ml thermostated vessel and temperature was kept at 25 ± 0.1°C. Titrations were carried out by using 0.258 N HCl (forward titration) and 0.248 N NaOH (back titration) (standardized against to KHP). First of all, amine groups of P_4 were fully protonated by forward titrations up to pH ~2.0. Afterwards, investigation of the deprotonation pH ranges of the tertiary and primary amine groups of P_4 was performed by back titrations up to pH ~12.0.

2.5. *Spectroscopic titrations*

Spectroscopic titrations were carried out according to our previous study [11]. All of the UV-Vis measurements were taken between the wavelengths of 250–350 nm with 10 mm quartz UV cells. Precisions of titrations were increased ± 0.01 pH unit by using a TitroLine® 7000 (SI Analytics GmbH, Hattenbergstraße, Germany) autotitrator supported with Titrisoft® 2.73 software. The pH meter was calibrated with at least three buffer solutions at pH 4.0, 7.0 and 10.0 before each experiment. The titrant was 0.1015 N HCl. All the spectra were obtained using a 1.00 cm quartz cuvette and all were referenced to water. After each pH adjustment, dendrimer solution was transferred into a cuvette and the absorption spectrum was recorded.

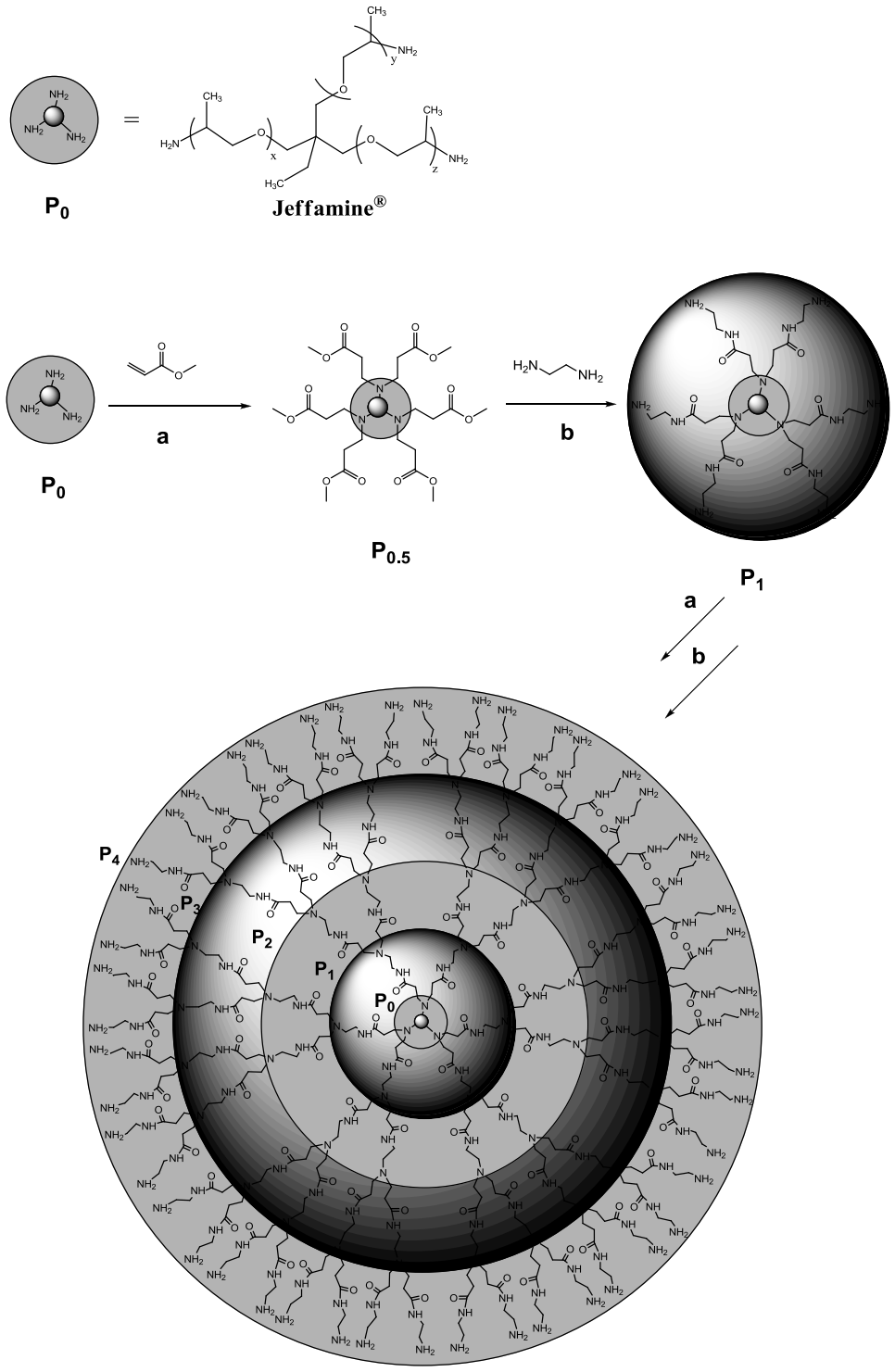
2.6. *Preparation of Co(II)-PAMAM dendrimer complexes*

The JCPDs have many metal binding sites (amino groups) (Table 1). P_4, which has the highest number of available amino groups for metal binding, was evaluated as the model dendrimer for complexation with Co(II) ions. All of the synthesized dendrimer samples used in this study were stored in 10 wt% stock solutions in methanol at ±4°C. After dilution of the dendrimer stock solutions with deionized water to prepare 100 μM solutions, the methanol of the solutions was removed by evaporation. These solutions were then subsequently used to prepare aqueous P_4 and Co(II) solutions with the desired concentrations. All of the dendrimer solutions were diluted to 100 μM by adjusting the final pH of the solutions to 8.0 with reagent grade 37% hydrochloric acid and 50% sodium hydroxide. Then, the proper amount of 25 mM cobalt(II) chloride hexahydrate solutions was added over dendrimer solutions to obtain desired concentrations where the final Co(II) to dendrimer molar concentration ratios were 15:1, 20:1, 30:1 and 60:1.

3. RESULTS AND DISCUSSION

3.1. *Preparation and characterization of JCPDs*

The JCPDs were synthesized via divergent approach (Scheme 1) and characterized with ^{1}H NMR, ^{13}C NMR, FTIR-ATR, and GPC, and the results were in good agreement with the literature [11, 12]. The prepared dendrimers were stored in methanolic solution and stored at ±4ºC. Some characteristic and characterization data of the different generation JCPDs are listed in Table 1.



Scheme 1. Synthesis and structure development of P_1-P_4 JCPDs, (a) Michael addition step and (b) amidation step [11]

T a b l e 1

Selected physicochemical properties of P_1–P_4 [11][a]

Gn.	Mw	Mn (SEC)	M_W (SEC)	PDI	Number of tertiary amines	Number of primary amines	Number of total amino groups
P_1	1124	960	980	1.02	3	6	15
P_2	2492	2300	2500	1.09	9	12	39
P_3	5228	4300	4400	1.02	21	24	87
P_4	10700	9200	9600	1.04	45	48	183

[a]Gn.: generation; Mw: theoretical molecular weight (g/mol); Mn (SEC): nominal molecular weight measured by size exclusion chromatography; Mw (SEC): molecular weight measured by size exclusion chromatography; PDI: polydispersity index.

3.2. *Potentiometric measurements*

Tertiary and primary amine groups of PAMAMs are Lewis bases and they can bind most metals simultaneously (Table 1) [8]. However, the primary amines of PAMAMs cannot bind metal ions depending on the pH characteristic of the media when the EOP of amine groups is out of the pH range of metal binding or all amine groups are protonated. Therefore, they are not available for metal ion coordination. Figure 1 shows the titration curves obtained from direct titration with 0.258 N HCl volumetric solution and back titration with 0.248 N NaOH. Titration curves exhibited three end points. The first is for excess acid added to dendrimer solution during direct titration, while the other two are for tertiary and primary amines, respectively. The first derivative of the back potentiometric titration curve of P_4 was overlapped to show end points. Diallo et al. [9] accepted the maximum points in the second derivative curve as the inflection points referring to p*K*a values of the tertiary and primary amines of PAMAM dendrimers. By using the same approach, we calculated the pK_a values of tertiary and primary amine groups of P_4 to be 6.71 and 9.84, respectively (Table 2). Inspection of Table 2 shows that the pK_a values we observed were in good agreement with the literature.

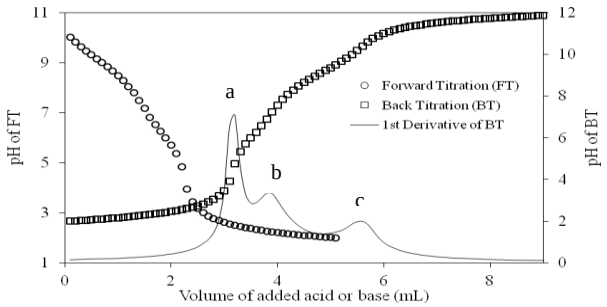


Fig. 1. Illustration of forward and back potentiometric titration curves of P_4 with the second derivative curve of back titration curve. a is the end point for excess HCl, b and c are the end points for tertiary and primary amines

T a b l e 2

pKa values for the tertiary and primary amine groups of P_4

Dendrimer	pK_a of tertiary N groups	pK_a of terminal amine groups	pK_a of tertiary N groups (literature)	pK_a of terminal amine groups (literature)
P_4	6.71	9.84	6.85[a]–6.70[b]–6.30[c]	10.29[a]–9.00[b]–9.23[c]

[a]Data taken from Diallo et al. [9]. [b]Data taken from Cakara et al. [15]. [c]Data taken from Niu et al. [16]. p*K*a values were calculated by taking the inflection points of the second derivative of titration curves as it is stated in the study of Diallo et al. [9]. Acid base titration of the aqueous solutions of the P_4 can be seen in Fig.1.

3.3. *Effect of extent of protonation (EOP) on metal binding ability of PAMAM dendrimers*

Figure 2 shows the predicted extent of protonation (α) values of tertiary and primary amine groups of P_4 dendrimer in aqueous solutions within the pH range of 2–12. These values were calculated by using Hendersen-Hasselbech equation (1) with the calculated p*K*a values on Table 2 [17].

$$\log\frac{\alpha}{1-\alpha} = \mathrm{p}K\mathrm{a} - \mathrm{pH} \qquad (1)$$

where α is extent of protonation, pK_a is the acidity constants for tertiary and primary amine groups, and pH is the measurement of the hydronium ion concentration of the aqueous media.

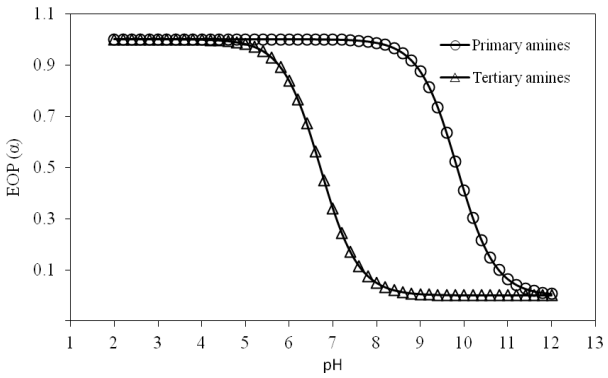


Fig. 2. Extent of protonation (α) of primary and tertiary amine groups of P_4

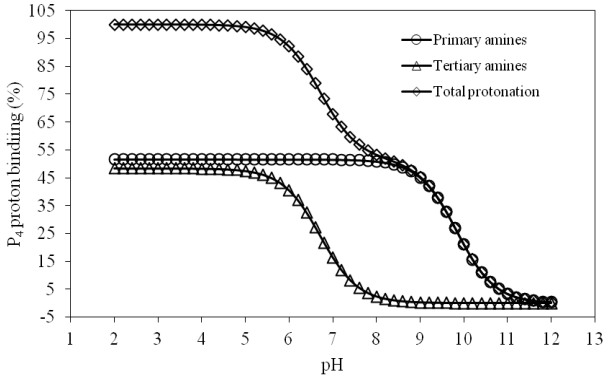


Fig. 3. Proton binding (%) curve for P_4

Some of the selected predicted EOP values of the amino groups of the P_4 dendrimer are given in Table 3 in order to look closely at the available metal binding sites and behavior of P_4 at low, neutral and high pH values. Results indicated that all amine groups of P_4 became almost protonated at low pH (≤5) while at neutral pH 7, only the tertiary amine groups remained unprotonated (α = 0.339). On the other hand, at higher pH 9, the tertiary amine groups just remained deprotonated (α = 0.05). These results are correlated with those reported by Diallo et al. [9] (Table 2).

T a b l e 3

Extent of protonation (EOP) values of tertiary and primary amine groups of P_4 as a function of pH[a]

A. Fraction of protonated tertiary amine groups				
Dendrimer	pH 3	pH 5	pH 7	pH 9
P_4	0.999	0.980	0.339	0.005
B. Fraction of protonated primary amine groups				
Dendrimer	pH 3	pH 5	pH 7	pH 9
P_4	0.999	0.999	0.998	0.873

[a]Data obtained from the EOP graph of P_4 (Fig. 2)

Structures of dendrimers can be controllable and the mass of dendrimers increases as the generation (Gn.) increases (Table 1). Tertiary and primary amine groups of P_4 are the possible proton binding sites. Figure 3 shows the proton binding percent values of P_4 in the pH range of 2–12. These values were driven from EOP values presented in Figure 2. During the derivation of these values, it was benefited from core shell protonation methodology [15, 18, 19]. This methodology asserts that dendrimers start to protonate at higher pH and continue to protonate as the pH decreases. During this protonation, amino groups present in each core protonate together (Fig. 3).

Taking into consideration Figure 2 and Figures 3 together, it could be easily seen that the protonation of the tertiary amine groups lasts approximately in the pH range of 5–8, while primary amine groups are found to be in the deprotonated conformation over pH ~8. On the other hand, all of the amino groups are protonated under pH ~5. Almost 55% of the primary amine groups are protonated with a change in pH 5 (99.07%) to pH 8 (53.23%). That is, at a pH of about 8, most primary, but few tertiary, amines are protonated and such information is difficult to obtain from titration data alone.

The pH of the aqueous dendrimer solutions are desired to be at the pH at which all primary amine groups are in the protonated conformation and all of the tertiary amine groups are deprotonated, in other words, free for binding in terms of coordination chemistry [9]. Investigation of Figure 3 reveals that pH 8 is the most desired pH, where the metal binding ability of P_4 is expected to be highest and P_4 behaves like hosts to many metal ions with the deprotonated inner tertiary amine groups. For this reason, to attain maximum complexation, we evaluated the pH of the aqueous dendrimer solutions at pH 8 to gain maximum complexation between P_4 and Co(II) ions.

3.4. *Spectroscopic titrations and UV-Vis characterization of PAMAM dendrimers*

Figure 4 shows the change in the UV-Vis absorption spectra of unbuffered 100 μM P_4 dendrimer during spectroscopic titrations. A maximum absorption band during the spectroscopic titrations of P_1–P_4 was observed in the wavelength range of 280–285 nm with a change in pH from 2.03 to 9.97. This band is attributed to internal tertiary amines of PAMAMs as an indication of the monodispersity and purity [20] (Fig. 4). The inset of Figure 4 is the pH versus absorbance at λ_{max}= 280 nm of P_4 unbuffered aqueous solution. The investi-

gation of the inset of Figure 4 also indicates that P_4 dendrimer can exhibit interesting ligand properties as chelating agents, especially in the pH range of 6.0–8.0. Interestingly, spectroscopic titration results validated the potentiometric titration studies where pH 8 is the most favorable pH for tertiary amine groups of P_4 to have the highest opportunity to bind Co(II) ions.

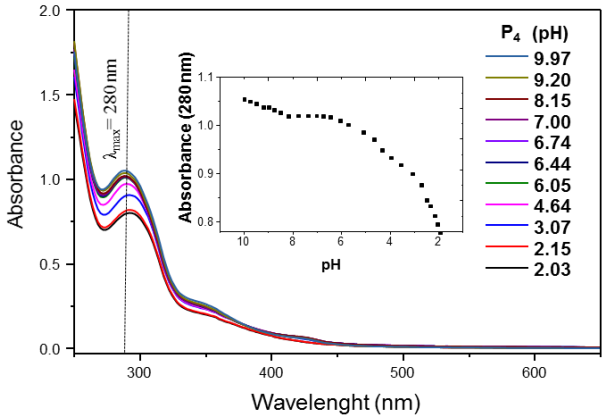


Fig. 4. Representative absorption spectra of P_4 as a function of pH. The inset graph shows how the band at 280 nm changes as a function of pH..

3.5. *UV-Vis characterization of Co(II)-P_4 complex*

Characterization of the Co(II) binding ability of PAMAMs in aqueous solutions was performed by UV-Vis spectroscopy. Normally, hexa-coordinated Co(II) aqua complexes in aqueous solutions are observed at λ_{max} = 510–512 nm [21]. UV-Vis measurements were performed between the wavelength ranges of 200–750 nm with 1.00 nm interval against the dendrimer solutions. Displacement of the 512 nm band with 585–635 nm *d-d* transition bands, confirmed the formation of Co(II)-P_4 complexes (Fig. 5).

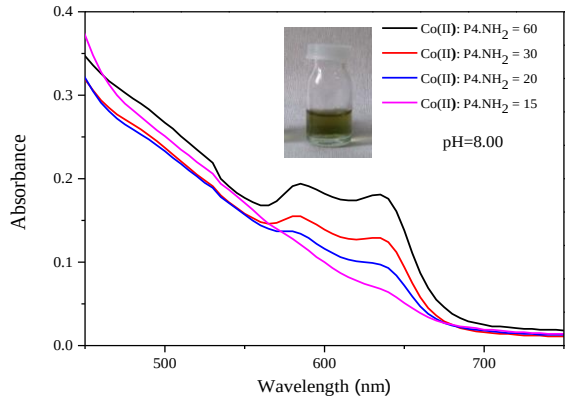


Fig. 5. UV-Vis spectra of Co(II)-P_4 complexation bands at 585–635 nm

Complexation studies were conducted in the pH range of 6–8 and best observations were performed at pH 8, as predicted by potentiometric and spectroscopic studies conducted on the P_4 dendrimer. In addition, the color of dendrimer solution, when the pH was adjusted to 8, immediately turned dark green after the addition of Co(II) ions. This color change and absorption bands could be attributed to the formation of tetra amine complexes of Co(II) with dendrimer internal tertiary amine groups (Fig. 5).

4. CONCLUSION

In the present study, Co(II) complexes of a model generation-4 Jeffamine® cored PAMAM dendrimer (P_4) were synthesized. The optimum complexation conditions for the P_4 was tried to determine with potentiometric and spectroscopic titration studies. pH data driven from potentiometric titration were used to form EOP profiles in order to investigate the protonation behavior of P_4. Investigation of EOP and proton binding profiles showed that pH 8 was the optimum dendrimer aqueous solution media, where the available number of tertiary amines present in P_4 was highest for possible metal complexation. The new Co(II)-P_4 complexes obtained could be used in possible future applications as MRI improvers or MRI contrast agents.

Acknowledgements. This research has been supported by Yıldız Technical University Scientific Research Projects Coordination Department. Project Numbers (2011-01-02-KAP04, 2011-01-02-KAP05, 2011-01-02- KAP06 and 2012-01-02-DOP05).

REFERENCES

[1] J. H. Kim, H. J. Gibb, P. D. Howe, M. Sheffer, U. N. E. Programme, I. L. Organisation, W. H. Organization, I.-O.P.f.t.S.M.o. Chemicals, Cobalt and Inorganic Cobalt Compounds, World Health Organization (2006).

[2] S. E. Bailey, T. J. Olin, R. M. Bricka, D. D. Adrian, A review of potentially low-cost sorbents for heavy metals, *Water Res.*, **33**, 2469–2479 (1999). DOI: 10.1016/s0043-1354(98)00475-8.

[3] A. Kabata-Pendias, *Trace Elements in Soils and Plants*, Third Edition, Taylor & Francis, (2010). DOI: 10.1201/9781420039900.ch5.

[4] J. G. Hengstler, U. Bolm-Audorff, A. Faldum, K. Janssen, M. Reifenrath, W. Götte, D. Jung, O. Mayer-Popken, J. Fuchs, S. Gebhard, H. G. Bienfait, K. Schlink, C. Dietrich, D. Faust, B. Epe, F. Oesch, Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected, *Carcinogenesis*, **24**, 63–73 (2003). DOI: 10.1093/carcin/24.1.63.

[5] K. Yamamoto, S. Inoue, A. Yamazaki, T. Yoshinaga, S. Kawanishi, Site-specific DNA damage induced by cobalt(II) ion and hydrogen peroxide: role of singlet oxygen, *Chem. Res. Toxicol.*, **2**, 234–239 (1989). DOI: 10.1021/tx00010a004.

[6] A. Sigel, H. Sigel, R. K. O. Sigel, *Organometallics in Environment and Toxicology*, RSC Publishing, (2010). DOI: 10.1039/9781849730822..

[7] J. F. Hainfeld, Extended organic cobalt and nickel magnetic complexes, in, Google Patents (2003).

[8] F. Zeng, S. C. Zimmerman, Dendrimers in supramolecular chemistry: From molecular recognition to self-assembly, *Chem. Rev. (Washington, D. C.)*, **97**, 1681–1712 (1997). DOI: 10.1021/CR9603892.

[9] M. S. Diallo, S. Christie, P. Swaminathan, L. Balogh, X. Shi, W. Um, C. Papelis, W. A. Goddard, III, J. H. Johnson, Jr., Dendritic Chelating Agents. 1. Cu(II) Binding to Ethylene Diamine Core Poly(amidoamine) Dendrimers in Aqueous Solutions, *Langmuir*, **20**, 2640–2651 (2004). DOI: 10.1021/la036108k.

[10] A. D. Schluter, J. P. Rabe, Dendronized polymers: synthesis, characterization, assembly at interfaces, and manipulation, *Angew. Chem., Int. Ed.*, **39**, 864–883 (2000). DOI: 10.1002/(SICI)1521-3773(20000303)39:5<864::AID-ANIE864>3.0.CO;2-E.

[11] A. S. Ertürk, M. Tülü, A. E. Bozdoğan, T. Parali, Microwave assisted synthesis of Jeffamine cored PAMAM dendrimers, *Eur. Polym. J.*, **52**, 218–226 (2014). DOI: 10.1016/j.eurpolymj.2013.12.018.

[12] A. S. Erturk, M. U. Gurbuz, M. Tulu, A. E. Bozdogan, Water-soluble TRIS-terminated PAMAM dendrimers: microwave-assisted synthesis, characterization and Cu(ii) intradendrimer complexes, *RSC Adv.*, **5**, 60581–60595 (2015). DOI: 10.1039/C5RA11157A.

[13] I. J. Majoros, B. Keszler, S. Woehler, T. Bull, J. R. Baker, Acetylation of Poly(amidoamine) Dendrimers, *Macromolecules*, **36**, 5526–5529 (2003). DOI: 10.1021/ma021540e.

[14] I. J. Majoros, T. P. Thomas, C. B. Mehta, J. R. Baker, Poly(amidoamine) Dendrimer-Based Multifunctional Engineered Nanodevice for Cancer Therapy, *J. Med. Chem.*, **48**, 5892–5899 (2005). DOI: 10.1021/jm0401863.

[15] D. Cakara, J. Kleimann, M. Borkovec, Microscopic Protonation Equilibria of Poly(amidoamine) Dendrimers from Macroscopic Titrations, *Macromolecules*, **36**, 4201–4207 (2003). DOI: 10.1021/ma0300241.

[16] Y. Niu, L. Sun, R. M. Crooks, Determination of the Intrinsic Proton Binding Constants for Poly (amidoamine) Dendrimers via Potentiometric pH Titration, *Macromolecules*, **36**, 5725–5731 (2003). DOI: 10.1021/ma034276d.

[17] V. Kabanov, A. Zezin, V. Rogacheva, Z. G. Gulyaeva, M. Zansochova, J. Joosten, J. Brackman, Polyelectrolyte behavior of astramol poly(propyleneimine) dendrimers, *Macromolecules*, **31**, 5142–5144 (1998). DOI: 10.1021/ma971643a.

[18] L. Sun, R. M. Crooks, Interactions between Dendrimers and Charged Probe Molecules. 1. Theoretical Methods for Simulating Proton and Metal Ion Binding to Symmetric Polydentate Ligands, *J. Phys. Chem. B*, **106**, 5864–5872 (2002). DOI: 10.1021/jp020189w.

[19] M. H. Kleinman, J. H. Flory, D. A. Tomalia, N. J. Turro, Effect of Protonation and PAMAM Dendrimer Size on the Complexation and Dynamic Mobility of 2-Naphthol, *J. Phys. Chem. B*, **104**, 11472–11479 (2000). DOI: 10.1021/jp001882r.

[20] S. Pande, R. M. Crooks, Analysis of poly(amidoamine) dendrimer structure by UV–Vis spectroscopy, *Langmuir*, **27**, 9609–9613 (2011). DOI: 10.1021/la201882t.

[21] F. A. Cotton, *Advanced Inorganic Chemistry*, Wiley, (1999). DOI: 10.5860/choice.37-0940.