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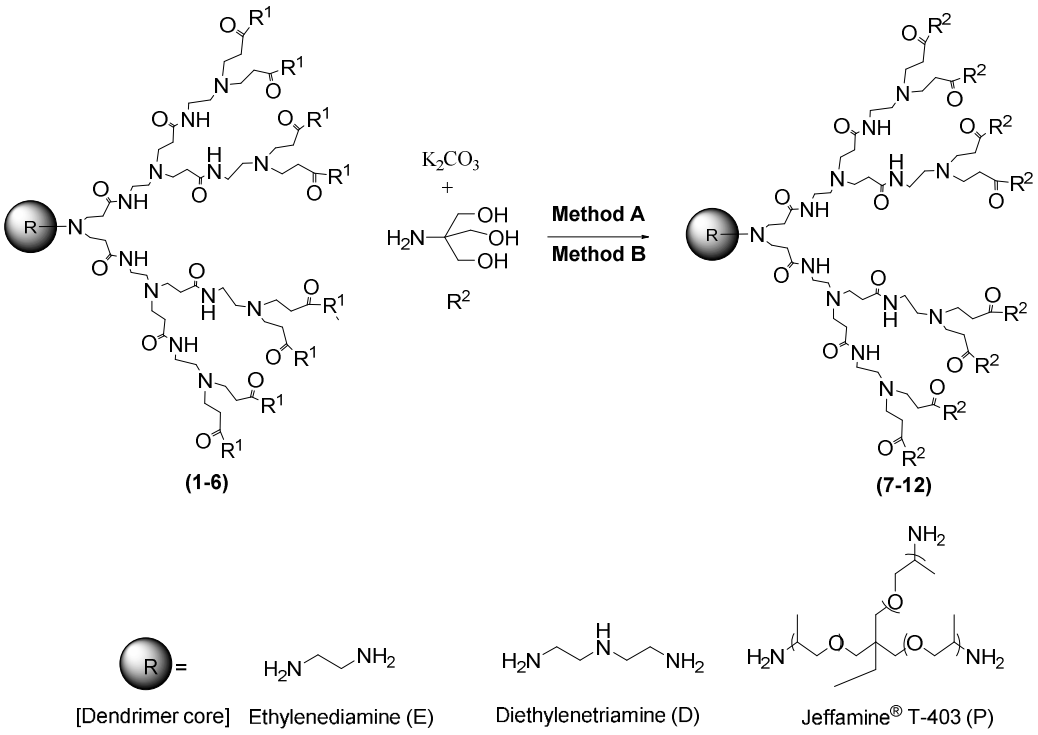
Graphical abstract

Microwave-assisted synthesis, characterization and Cu(II) complexes of water-soluble TRIS-terminated PAMAM dendrimers as novel potential drug carriers and templates for nanomaterials.



Water-soluble TRIS-terminated PAMAM dendrimers: Microwave-assisted synthesis, characterization and Cu(II) intradendrimer complexes

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Abstract: This study is the first report describing the microwave-assisted synthesis (MAS) of poly (amido amine) (PAMAM) dendrimers with TRIS surface functional groups (PAMAM-TRIS). Six PAMAM-TRIS dendrimers were synthesized using both new developed conventional and microwave methods. Five of them are novel. Three different cores, one polymeric Jeffamine® T-403 and two monomeric, ethylenediamine and diethylenetriamine, were used in the syntheses. All the reactions were monitored by attenuated total reflectance (ATR). It was observed that microwave reactions proceeded 3.5 to 4.0 times faster than conventional reactions. Therefore, fast, easy and one-pot MAS of six different water-soluble PAMAM-TRIS dendrimers was accomplished with 90-96% higher yields in 110-140 min short reaction times and under mild reaction conditions, methanol as solvent. The other ester terminated half generation precursor PAMAM (PAMAM-OCH_3) dendrimers used for the synthesis of the PAMAM-TRIS dendrimers were obtained by utilizing conventional and microwave methods together. In purification of all the PAMAM dendrimers, liquid phase polymer-based retention (LPR) technique was used. The PAMAM-TRIS dendrimers were characterized by ^{1}H NMR, ^{13}C NMR, ATR (IR), EA, potentiometric and spectroscopic titrations. Furthermore, Cu(II)-PAMAM-TRIS dendrimer complexes were prepared and characterized by UV-VIS spectroscopy. The synthesized PAMAM-TRIS dendrimers can be considered as new drug carrier systems and should find use in widespread application fields, especially in the future pharmaceutical and catalytic study but not limited to be used in the other fields.

Keywords: dendrimer, PAMAM, microwave synthesis, surface modification, TRIS; complexation

Introduction

Poly(amido amine) (PAMAM) dendrimers are the class of most widely studied Starburst macromolecules. They can be distinguishable from linear polymers with their unusual chemical and physical properties [1]. These properties can alter due to construction units, which are core, repeating branches and surface groups. While the segment of PAMAM dendrimers comprising from core and repeating branch units is called as internal cavity, the segment, including surface groups is called as outer periphery. Control of these segments allows PAMAM dendrimers to be used as proper target materials in a wide range of applications involving catalysis [2, 3], antibacterial agents [4-6], gene therapy [7-9], and drug-delivery [10, 11].

Aqueous solubility of PAMAM dendrimers is substantially important. For example, water-soluble PAMAM dendrimers can be a host for guest-small hydrophobic acidic molecules with their internal cavities. Several amides and tertiary amine groups exist in these cavities. These groups can entrap small molecules as drugs to the cavity of dendrimer by hydrogen bonding, electrostatic interaction, or both. Thus, these cavities can play the role of a container for various hydrophobic drugs. On the other hand, outer periphery with hydrophilic groups can attain PAMAM dendrimers to the proper water solubility [12]. As a result, they can be used as potential drug carriers and delivery systems in physiological environments. Moreover, PAMAM dendrimers are used as templates to control size, stability, and solubility of nanoparticles in the range of 1 nm to 4-5 nm [2]. Crooks et al. [2] announced dendrimers to be good hosting metal nanoparticles. These well-defined nanoparticles have the uniform structures. Therefore, they can take part in catalytic reactions, resist to agglomeration, and be selective to control encapsulation of small substrate molecules [13, 14]. Furthermore, stable host-guest interaction is desired for both intradendrimer metal complexes, and drug-drug carrier conjugates. In particular, synthesis of water-soluble derivatives of drugs with different chemical formulations is generally tried while developing efficient drug-delivery systems. However, even small structural changes to improve water solubility can often lead to dramatic decrease in the efficacy of drug. For this reason, drug carrier systems can be helpful to increase the water solubility of drugs by encapsulation since the therapeutic efficacy and the abilities of accessing to the target sites of a drug are increased.

Modifying the structure of PAMAM dendrimers is important to reach desired water solubility. TRIS is the abbreviation of the common known organic compound, Tris

(hydroxymethyl) aminomethane, with the formula $(HOCH_2)_3CNH_2$. It is widely used in biochemistry, molecular biology, and is highly water-soluble. Ester-terminated PAMAM dendrimers (PAMAM-OCH_3) are generally water insoluble. Dendrimers are known as designable polymers and can be used as building blocks. Surface modification of water insoluble PAMAM-OCH_3 dendrimers with TRIS makes them water-soluble. Not only are TRIS-terminated PAMAM dendrimers (PAMAM-TRIS) water-soluble drug-delivery agents, but also synthetic precursors for dendrimer-encapsulated metal nanoparticles. However, preparation of these PAMAM dendrimers requires three to four days under conventional heating and DMSO as solvent [12, 15]. So far, a little attention has been paid to develop fast; easy and green synthesis of the PAMAM-TRIS dendrimers. Therefore, alternative approaches are necessary.

Microwave-assisted synthesis (MAS) of PAMAM dendrimers is an attractive subject field on the agenda and a challenging research topic. A few studies have been conducted up to now for fast, facile and efficient synthesis of PAMAM dendrimers by using hyphenated synthesis and advanced purification techniques such as liquid phase polymer retention (LPR) or membrane filtration (MF) together [16, 17]. When reaction times, chemical wastages of solvents, reaction conditions, and environmental effects are taken into consideration, MAS can shorten reaction times [18], convert hours to minutes, enhance reaction rate, prevent side product formation and give higher yields when compared with conventional methods. In addition, it can stop wastage of solvents, present neat and sustainable reaction conditions and protocols [19], and stop global warming. Hence, MAS is a helpful technique and can be a good alternative for the synthesis of the water-soluble PAMAM-TRIS dendrimers.

This paper presents fast, efficient and one-pot synthesis of six PAMAM-TRIS dendrimers, five of which is novel, from PAMAM-OCH_3 dendrimer precursors using new developed conventional and microwave-assisted methods. By using these methods, starting from two different monomeric core, which are ethylenediamine (E), diethylenetriamine (D), and one polymeric core, which is Jeffamine® T-403 (P), MAS of third and fourth-generation PAMAM-TRIS dendrimers was performed. The synthesized PAMAM-TRIS dendrimers were characterized by ^{1}H NMR, ^{13}C NMR, ATR(IR), EA and UV-VIS spectroscopy in addition to potentiometric and spectroscopic titrations. Finally, Cu(II)-PAMAM-TRIS dendrimers complexes were prepared and structural defects in the

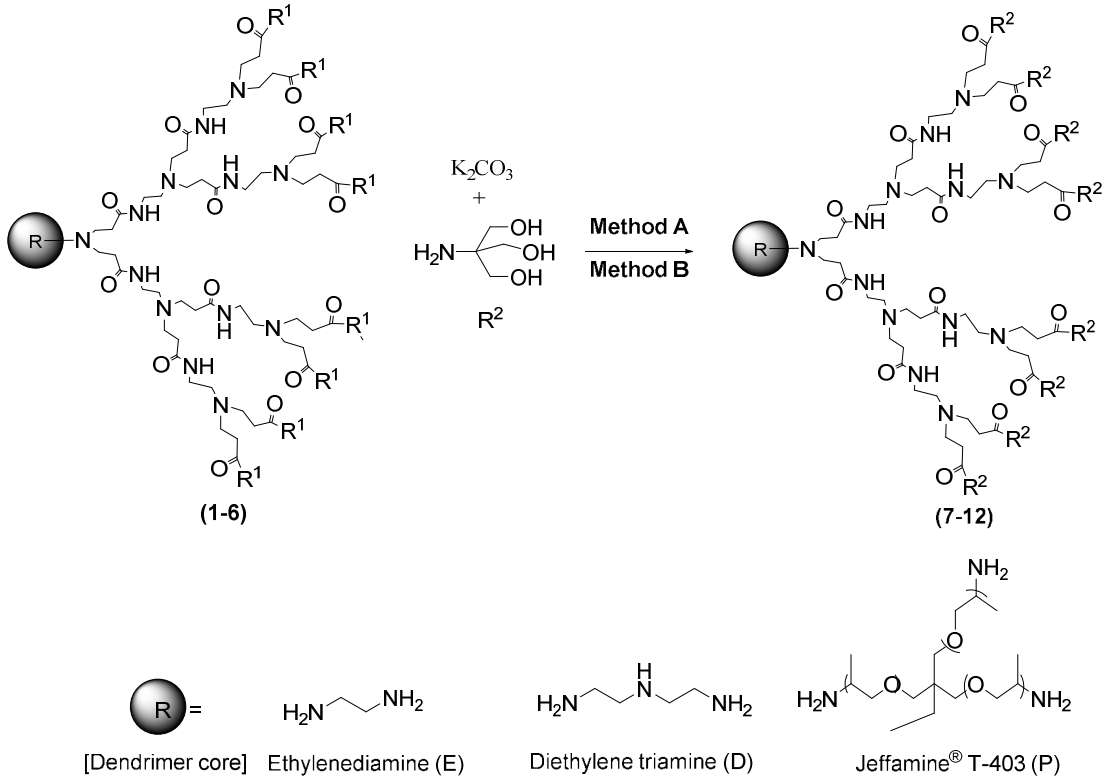
internal cavities of the PAMAM-TRIS dendrimers were investigated to show the purity and monodispesity of them by spectroscopic Cu(II) titrations.

Results and discussion

Synthesis of PAMAM-TRIS dendrimers

Terminal groups of PAMAM dendrimers attain them unique properties. Amine-terminated PAMAM dendrimers (PAMAM-NH_2) are water-soluble and mostly at protonated conformation at the pH media of the living cells' physiological functions [20]. Thus, when they come in contact with negatively charged cells, hemolysis of cells occurs. This phenomenon makes a negative effect on the future development of the clinical applications of amine terminated dendrimers [21].

On the other hand, drug delivery and toxicity studies on the surface modified derivatives of PAMAM dendrimers revealed that carboxyl terminated PAMAM dendrimers (PAMAM-COOH) are less toxic, and their negative charge on the periphery prohibits them to bind or interact with the negatively charged surface of the cells [22, 23]. Ideally, it is expected to be an interaction between a drug carrier or delivery system and cell. Thus, we decided to synthesize a series of water-soluble TRIS-terminated water-soluble PAMAM dendrimers (Scheme 1.). Syntheses were performed by both conventional (Method A) and microwave-assisted (Method B) methods. As a result, we have developed a new microwave-assisted method for the surface modification of PAMAM dendrimers with TRIS functional groups to improve their water solubility.



Comp.	R	R^1	Comp.	R	R^2
1	E	OCH_3	**7**	E	$NHC(CH_2OH)_3$
2	D	OCH_3	**8**	D	$NHC(CH_2OH)_3$
3	P	OCH_3	**9**	P	$NHC(CH_2OH)_3$
4	E	$NH(CH_2)_2N((CH_2)_2CO_2CH_3)_2$	**10**	E	$NH(CH_2)_2N((CH_2)_2CONHC(CH_2OH)_3)_2$
5	D	$NH(CH_2)_2N((CH_2)_2CO_2CH_3)_2$	**11**	D	$NH(CH_2)_2N((CH_2)_2CONHC(CH_2OH)_3)_2$
6	P	$NH(CH_2)_2N((CH_2)_2CO_2CH_3)_2$	**12**	P	$NH(CH_2)_2N((CH_2)_2CONHC(CH_2OH)_3)_2$

Scheme 1. Synthesis of PAMAM-TRIS dendrimers (**Cn.TRIS**) (**7-12**) from PAMAM-OCH_3 dendrimers (**Cn.5**) (**1-6**)

As it can be seen from Scheme 1, six different PAMAM-OCH_3 dendrimer precursors (**1-6**) were used in the synthesis of PAMAM-TRIS dendrimers (**7-12).** PAMAM-OCH_3 dendrimers (**1-6)** were synthesized according to our recent developed microwave-assisted divergent synthesis method [17]. In surface modification, three different types of dendrimer core, which are ethylenediamine (E), diethylenetriamine (D), and Jeffamine T-403 (P), were used. Therefore, a series of water-soluble PAMAM-TRIS dendrimers (**7-12**) possessing various numbers of terminal hydroxyl groups and properties were obtained. The coded generations and the physico-chemical properties of these PAMAM dendrimers were summarized in Table 1.

Table 1. The theoretical characteristic data of PAMAM-OCH_3 (**1-6**) and PAMAM-TRIS dendrimers (**7-12**)[a]

Dendrimer	Generation	Molecular formula	MW (g/mol)	Number of tertiary amines (^3N)	Number of terminal esters (OCH_3)	Number of terminal hydroxyls (OH)
1	E2.5	$C_{126}H_{224}N_{26}O_{44}$	2808	14	16	-
2	D2.5	$C_{159}H_{283}N_{33}O_{55}$	3537	18	20	-
3	P2.5	$C_{207}H_{377}N_{39}O_{71}$	4562	21	24	-
4	E3.5	$C_{270}H_{480}N_{58}O_{92}$	6012	30	32	-
5	D3.5	$C_{339}H_{603}N_{73}O_{115}$	7543	38	40	-
6	P3.5	$C_{423}H_{761}N_{87}O_{143}$	9368	45	48	-
7	E3.TRIS	$C_{174}H_{336}N_{42}O_{76}$	4234	14	-	48
8	D3.TRIS	$C_{199}H_{383}N_{53}O_{95}$	5230	18	-	60
9	P3.TRIS	$C_{279}H_{545}N_{63}O_{119}$	6700	21	-	72
10	E4.TRIS	$C_{366}H_{704}N_{90}O_{156}$	8865	30	-	96
11	D4.TRIS	$C_{459}H_{883}N_{113}O_{195}$	11109	38	-	120
12	P4.TRIS	$C_{567}H_{1097}N_{135}O_{239}$	13647	45	-	144

[a]The theoretical characteristic data including the masses, the number of tertiary amines [24], terminal esters and hydroxyl groups (three folds of the number of terminal ester groups) of dendrimers were calculated according to literature [20, 24] by using Scheme 1.

When TRIS is attached to PAMAM-OCH_3 dendrimers, the number of resulting terminal hydroxyl groups are three folded (Table 1). Thus, PAMAM-TRIS dendrimers could gain infinitively water-soluble properties and can be used as drug carrier systems in many applications [12]. Newkome et al. [15, 25] reported the synthesis of PAMAM-TRIS dendrimers from commercially available ethylenediamine cored dendrimers previously using conventional synthesis methods. By applying the same procedure, Beezer et al. [12] synthesized three water-soluble PAMAM-TRIS dendrimers from PAMAM-OCH_3 dendrimers. However, no other alternative conventional or microwave-assisted methods have been reported for the fast, one-pot synthesis of water-soluble TRIS surface modified PAMAM dendrimers up to now.

We herein, as a new approach, showed the synthesis of third (C3.TRIS) and fourth (C4.TRIS) generation PAMAM-TRIS dendrimers with E, D and P cores using MAS method. Monitoring the reaction conditions, deciding the amount of solvents, and where

to stop reactions were determined with a similar approach used in our recent study [17]. In ATR monitoring, disappearance of the esteric 1730 cm^{-1} peak, and formation of ~3260 cm^{-1} broad hydroxyl peak, and ~1635 and ~1554 cm^{-1} amide I and amide II peaks were used as the indicators of the completion of reactions (Fig. 1). In Table 2, MAS conditions for the TRIS surface modification of PAMAM-OCH_3 dendrimers with Method B (**1-6**) were presented. First of all, before obtaining the optimum conditions reported in Table 2, a series of trial experiments with different power (watt), time (min), and various molar ratios of PAMAM-OCH_3 dendrimer precursors to TRIS or K_2CO_3 were performed. Afterwards, the best reaction conditions were determined as shown in Table 2. Furthermore, the ideal molar ratios of TRIS and K_2CO_3 to PAMAM-OCH_3 dendrimers (**1-6**) were determined to be 1.20 and 1.50 times molar equivalence of the number of terminal ester groups of PAMAM-OCH_3 dendrimers (1-6), respectively. Finally, the syntheses of PAMAM-TRIS products (**7-12**) were performed within 110-140 min when compared to three [12] and four days [15, 25] in conventional methods (Table 2).

Table 2. Preparation of PAMAM-TRIS dendrimers (**7-12)** from PAMAM-OCH_3 dendrimers (**1-6**) with Method B

Prdct.	Prc.	Precursor g (mmol)	TRIS g (mmol)	K_2CO_3 g (mmol)	MeOH (mL)	MW (watt)	Time (min)	Yield[a] (%)
7	**1**	1.72 (0.61)	1.43 (11.77)	2.03 (14.71)	10	200	120	96
8	**2**	0.98 (0.27)	0.80 (6.64)	1.15 (8.29)	15	200	110	95
9	**3**	0.68 (0.15)	0.53 (4.33)	0.75 (5.42)	10	200	120	91
10	**4**	0.99 (0.16)	0.76 (6.29)	1.08 (7.85)	10	200	140	93
11	**5**	1.12 (0.14)	0.81 (6.70)	1.16 (8.40)	13	200	125	94
12	**6**	0.84 (0.09)	0.62 (5.16)	0.89 (6.46)	10	200	135	93

[a]Isolated percent yield after LPR purification

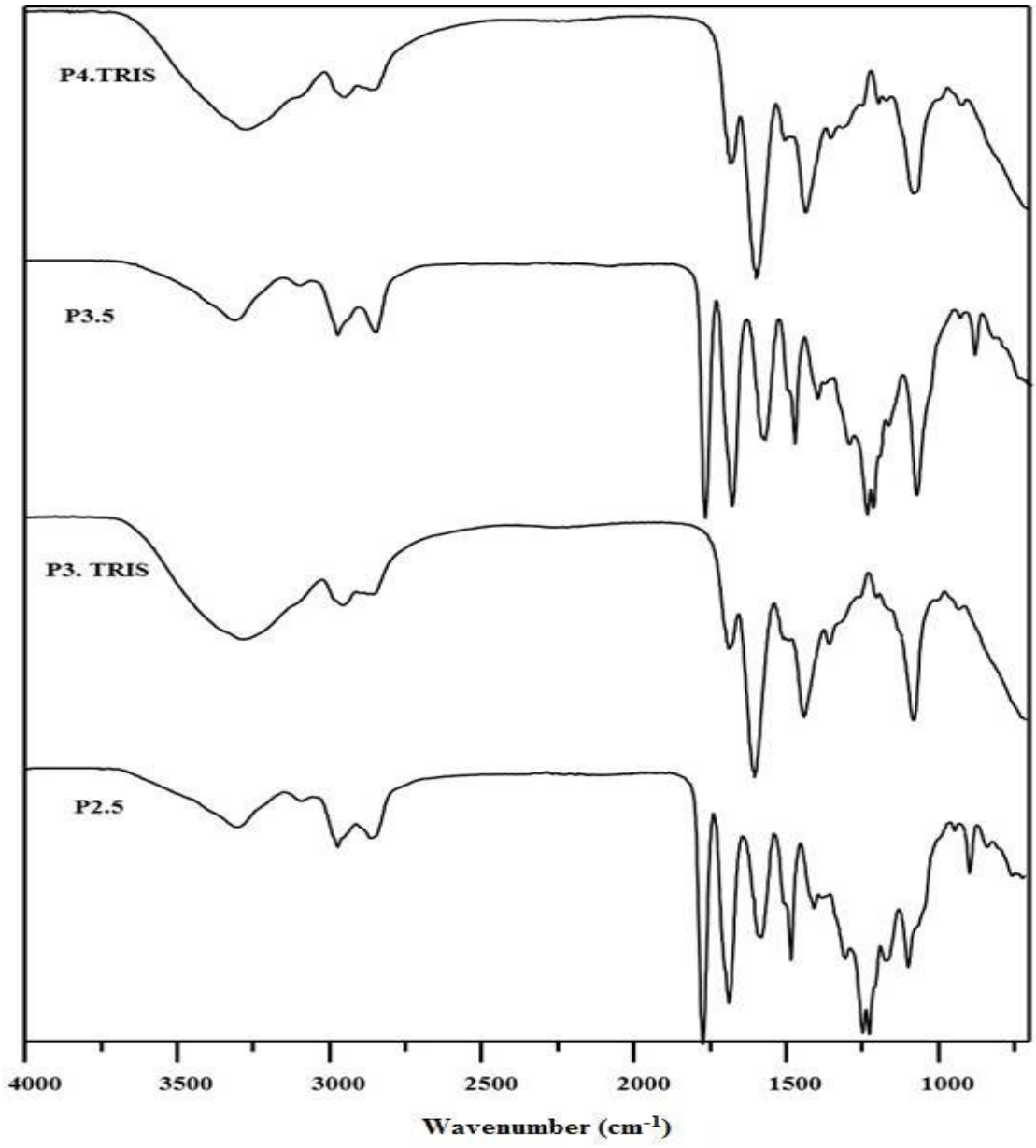


Figure 1. A representative ATR (IR) spectra of pure ester (**Cn.5**) and TRIS-terminated (**Cn.TRIS**) for Jeffamine® T-403 cored PAMAM dendrimers

The synthesized PAMAM-TRIS dendrimers (**7-12**) could be easily characterized via ^{1}H NMR and ^{13}C NMR. All the expected signals are at the correct intensity and position (Fig. 2 and Fig. 3). The resonances from methyl ester of **6** at 3.59 ppm are no longer visible and confirms the complete conversion of ester functional groups to TRIS groups with a new singlet at 3.63 ppm (Fig. 2). This singlet resulting from the resonances of new methylene protons adjacent to the terminal hydroxyl groups also indicates the fully conversion to **12**. Moreover, ^{13}C NMR spectrum of **12** shows the right number of carbon signals. 181. 09 from exterior amides, and 175.5, 174.65 ppm from interior amides

correspond to TRIS, and interior amides prove the fully conversion of **6** to **12** (Fig. 3). Likewise to ^{1}H NMR, the strong resonance corresponding to the methyl groups of terminal methyl group at 172.43 (C=O), 51.15 ($COOCH_3$) ppm in the ^{13}C NMR is no more available (Fig. 3). This is also an indication of good purity. In ^{13}C NMR of TRIS terminated conversions, the formation of 56.38 (NHCR3), 63.6 ppm (CH_2OH) bands were indicated the fully formation of TRIS-terminated PAMAM dendrimers. Therefore, ^{1}H NMR and ^{13}C NMR spectroscopy evaluations prove a good purity.

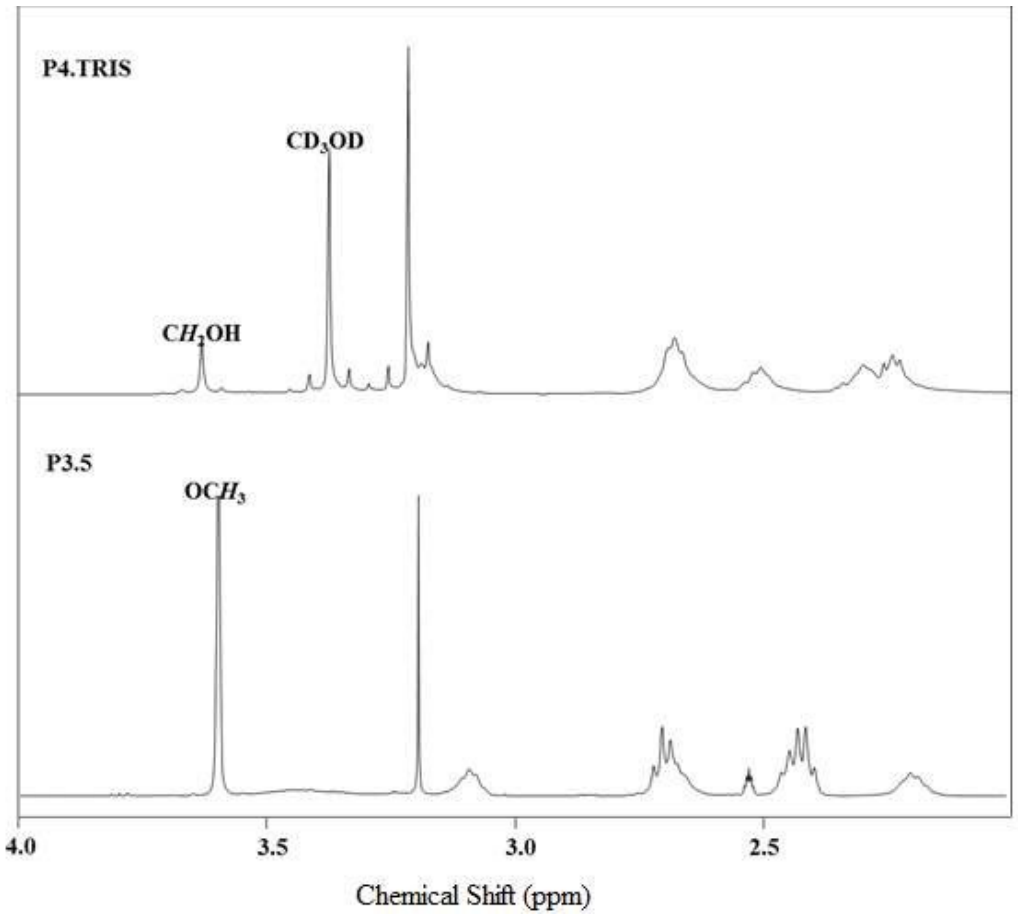


Figure 2. ^{1}H NMR spectrum monitoring of the conversion of **6** (bottom, in DMSO-d6) to **12** (top, in CD_3OD)

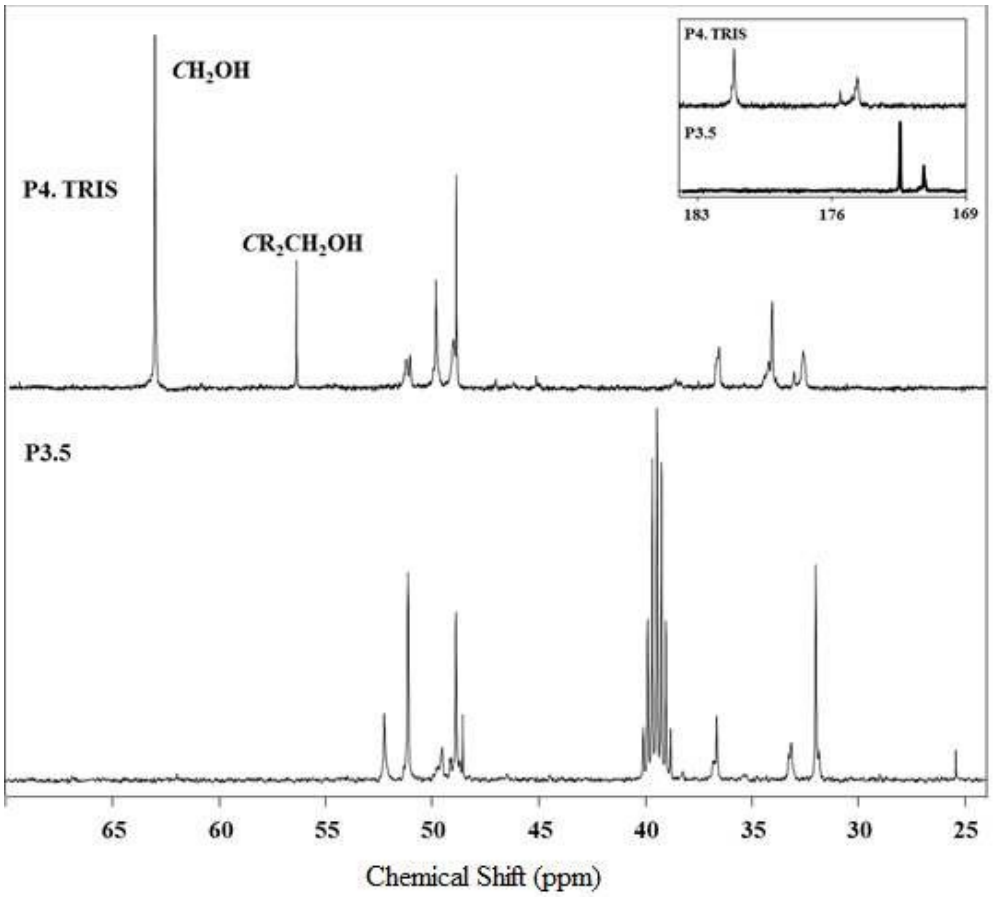


Figure 3. ^{13}C NMR spectrum monitoring of the conversion of **6** (bottom, in DMSO-d6) to **12** (top, in CD_3OD)

Comparison of microwave-assisted method (Method B) with conventional method (Method A)

Surface modification of **3** with TRIS to synthesize **9** was selected as a model representative reaction for the conversion of PAMAM-OCH_3 dendrimers to water-soluble PAMAM dendrimers with terminal hydrophilic hydroxyls. This conversion reaction was compared by Method A (preheated oil bath) and Method B (CEM discovery Labmade-Open Vessel mode). Conversions were determined with ATR (IR) monitoring of the disappearance of esteric 1730 cm^{-1} and formation of ~1635 cm^{-1} and ~1554 cm^{-1} amide I and amide II peaks, and ~3260 cm^{-1} broad hydroxyl peaks by taking identical aliquots at specific time intervals (Fig. 4). The conversions achieved by Method B were after 30 min (91%), 60 min (93%), 120 min (100%) while 60 min (56%), 120 min (61%), 180 min (80%), 240 min (92%), 300 min (97%), 360 min (99%) and 420 min (100%) by Method A (Fig. 5). Comparison of the observed conversion values revealed that Method B is 3 to

4 times faster than Method A when compared under same reaction conditions (Table 3). Microwave reactions were performed under a series of different power and time trial experiments by refluxing at 70-90°C bulk temperature methanol as solvent. The optimum conditions were summarized comparatively to conventional synthesis in Table 3. As it can be seen from Table 3, syntheses of PAMAM-TRIS dendrimers (**7-12**) were accomplished not only in shorter reaction times but also with the higher product yields 91-96%. Compounds **8-12** are the novel water-soluble PAMAM-TRIS dendrimers with hydroxyl end groups. We have increased the product yield of compound **7** from 54% (96 h) [12], 83% (72 h)[26] to 96% (120 min). In fact, we have observed that the reaction time required for **7** under the conventional methods shortened up to 36-38 times. It is also important to state in terms of green chemistry that this is the first report where the methanol is solvent (Table 2).

Table 3. Optimization of the reaction conditions for the synthesis of PAMAM-TRIS dendrimers (**7-12**)

Core (**Cn.5**)	Product (**Cn.TRIS**)	Method A (Conv.)		Method B (MW)		Literature	
		Time (h)	Yield (%)	Time (min.)	Yield (%)	Time (days)	Yield (%)
1	**7**	7.5	95	120	96	4[a],3[b]	83[a], 54[b]
2	**8**	7.5	95	110	95	-	-
3	**9**	7.5	90	120	91	-	-
4	**10**	8	93	140	93	-	-
5	**11**	8.5	94	125	94	-	-
6	**12**	8	92	135	93	-	-

[a]data taken from [26]. Reaction was carried out at 40 °C in DMSO under conventional conditions.
[b]data taken from [12]. Reaction was carried out at 50 °C in DMSO under conventional conditions

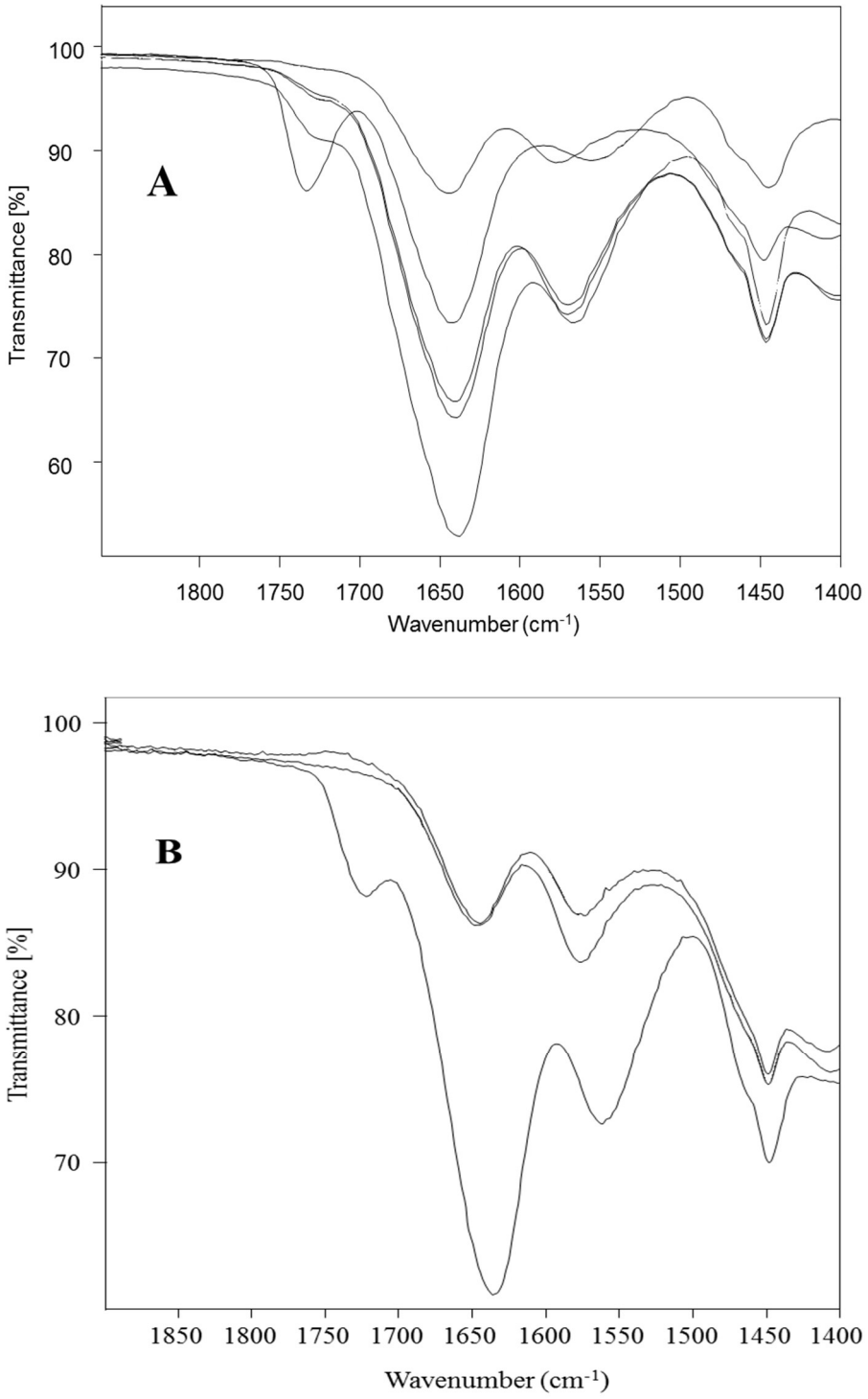


Figure 4. ATR(IR) monitored conversion spectrum from **3** to **9**. **(A)** Method A conditions: Oil bath, reflux, 7.5 h. **3** to TRIS and K_2CO_3 molar ratio 1:1.20 and 1:1.50 respectively **(B)** Method B conditions: Open vessel, 200 W, reflux, 120 min., **3** to TRIS and K_2CO_3 molar ratio 1:1.20 and 1:1.50 respectively

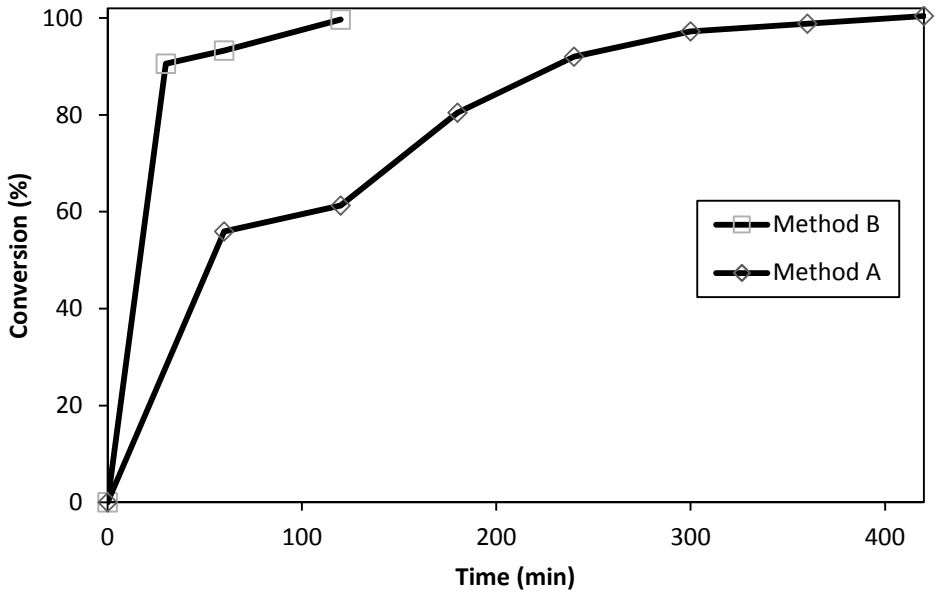


Figure 5. Conversion of **3** to **9**. Reaction time: 420 min for Method A in oil bath, and 120 min. for Method B. MW Open vessel mode at 200 W. (Conversion (%) was calculated from the ATR (IR) spectra in Fig. 4)

Potentiometric titrations of PAMAM-TRIS dendrimers

All potentiometric titrations of PAMAM-TRIS dendrimers were carried out by using TitroLine® 7000 autotitrator with high precise IoLine glass electrode with an iodine/iodide reference system. Titrisoft® 2.73 software was used to manage the titration processes over a personal computer. Secure mode was used to collect pH data reducing the pH drift error to a minimum on the pH reading error of 0.002 and volume reading error of 0.001 mL. Hence, it was further verified that all titration curves were fully reversible and independent of the dendrimer concentration within experimental error, indicating that dendrimer-dendrimer interactions are negligible.

Potentiometric acid-base titrations allow us to determine the average number of primary and tertiary amine groups of PAMAM-TRIS dendrimers. Initial pH of the aqueous PAMAM-TRIS dendrimer solutions was in the pH range of 9.7-10.0. According to back titration procedure, initial pH of the solutions was adjusted to pH ~2.0 and back titrated with standardized NaOH. In potentiometric titrations, two distinctive end points were observed (Fig. 6). One of these end points was for back titration of excess acid added to initial dendrimer solution while the second one was for the total number of mmoles of

the observed tertiary amine groups. Sample potentiometric titration curves of 1**0-12** can be seen in Figure 6. Second derivatives of the potentiometric titration curves were overlapped to show end points obviously. These points are accepted as the inflection points. Diallo et al. [27] accept these inflection points as to be pKa values of TRIS ended dendrimers. By using the same approach, pKa values of the tertiary amine groups of PAMAM-TRIS dendrimers **7-12** were presented in Table 4. It can be seen that the pKa values increases as the basicity of PAMAM-TRIS dendrimers increase in aqueous solutions with increasing number of surface hydroxyl numbers (Table 1 and Table 4).

Table 4. pKa values of the PAMAM-TRIS dendrimers at the corresponding inflection point[a]

PAMAM-TRIS dendrimers	**7**	**8**	**9**	**10**	**11**	**12**
pKa (^{3}N)	9.71	9.75	9.63	9.81	9.79	9.78

[a]Inflection points in Fig.6 used for pKa (^{3}N)values [27].

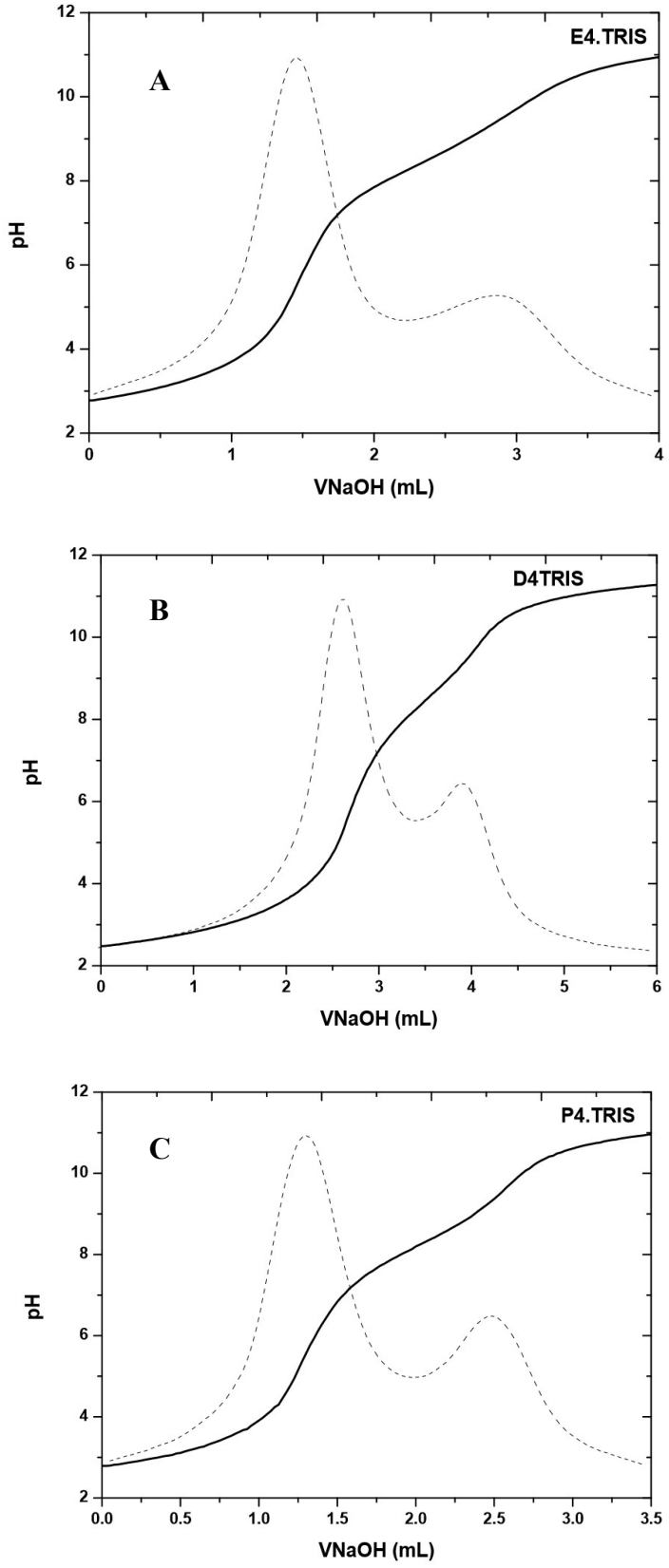


Figure 6. Potentiometric pH titration curve for (A) E4.TRIS (**10**), (B) D4.TRIS (**11**), and (C) P4.TRIS (**12**) at 100 mM ionic strength

PAMAM-TRIS dendrimers (**7-12**) have only the tertiary amine groups (Table 1). For this reason, the number of experimentally observed tertiary amine groups were used as supportive to explain potentiometric titrations results of PAMAM-TRIS dendrimers. The difference between theoretical and experimental tertiary amine numbers gives us the information about the structural monodispersity. As it can be seen from Figure 6, only the tertiary amine groups can be observed from potentiometric studies of PAMAM-TRIS dendrimers **10-12**. Calculations of present tertiary amine (^{3}N) numbers of PAMAM-TRIS dendrimers (**7-12)** were presented in Table 5. Results revealed that there exists a good correlation between the theoretical and practical number of tertiary amine numbers of PAMAM-TRIS dendrimers (**7-12)**. These results are also important that synthesized dendrimers are almost pure and have ideal monodispersity and characteristics.

Table 5. Practical and theoretical tertiary amine (^{3}N) values of PAMAM-TRIS dendrimers (**7-12**)[a]

PAMAM-TRIS dendrimers	^{3}N Theoretical Values	^{3}N Practical Values	%Correlation
7	14	13.67 ± 0.32	97.64
8	18	17.80 ± 0.64	98.88
9	21	20.74 ± 0.30	98.76
10	30	30.82 ± 1.08	102.73
11	38	37.07 ± 0.67	97.55
12	45	42.99 ± 1.57	95.53

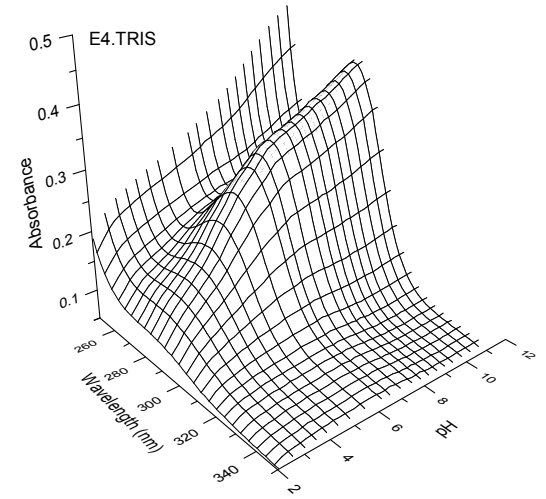
[a] Results were calculated from potentiometric titrations for five repeated experiments

Spectroscopic titrations of PAMAM-TRIS dendrimers

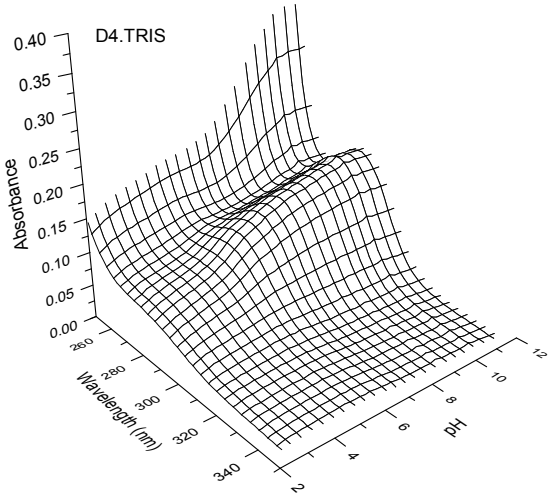
Spectroscopic titrations of PAMAM-TRIS dendrimers (**7-12**) in the pH range of 2-12 were revealed a prominent absorption band in 280-286 nm (Fig. 7). In Figure 7, It could be observable that the intensity of the absorption band at λ_{max} 280-286 nm decreases as the pH decreases, indicating the protonation of tertiary amine groups [28]. We have observed this band for all the spectroscopic titrations of PAMAM-TRIS dendrimers. Titrations were carried out between the pH 2-12 at a constant ionic strength in order to prevent the shielding effect of the amino groups of the dendrimers. Between this pH range, PAMAM-TRIS dendrimers solutions displayed a characteristic λ_{max} absorption

band between 280-286 nm. It could be easily seen that this band does not disappear even low pH<3. All the PAMAM-TRIS dendrimers (**7-12**) have different number of absorbing cites which are tertiary amine groups (Table 1). That is, depending on the pH of the media, PAMAM-TRIS dendrimers can be found in different protonated conformations (absorbing species).

A



B



C

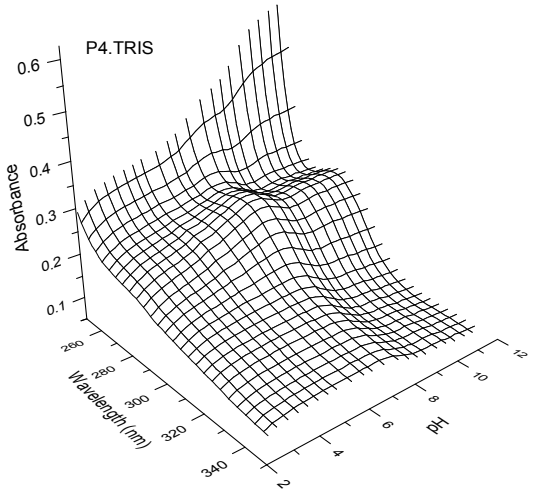


Figure 7. 3D plots of UV spectroscopic titration of E4.TRIS (**10**) (7.08 x 10^{-4} M)) (A), D4.TRIS (**11**) (3.70 x 10^{-4} M) (B), P4.TRIS (**12**) (6.06 x 10^{-4} M) (C) solution with 0.098 N HCl at 25 ± 0.1 ^{o}C, *I*=100 mM NaCl

Synthesis and Characterization of Cu(II)-PAMAM-TRIS dendrimers intradendrimer complexes

Spectroscopic Titrations of PAMAM-TRIS dendrimers with Cu^{2+} ions

Crooks et al. [29, 30] have shown that Cu^{2+} ions coordinate with the maximum number of four tertiary amine numbers. Addition of Cu^{2+} ions to PAMAM-TRIS dendrimers (**7-12**) in aqueous solutions immediately resulted in a deep blue color change. This color change due to Cu(II)-PAMAM-TRIS dendrimer complexes (**7-12**) was characterized with UV-VIS spectroscopy with a broad absorption band at λ_{max} = 680 nm (Fig.8). In order to learn about Cu^{2+} binding capacity of PAMAM-TRIS dendrimers (**7-12**), spectroscopic titration studies were performed. Representative spectroscopic titration spectra of PAMAM-TRIS dendrimers (**10-12**) with Cu^{2+} ions were shown in Figure 8. 680 nm band belonging the copper *d-d* transition indicates the Cu(II)-PAMAM-TRIS dendrimers complexation between the two species at 1:4 dendrimer to tertiary amine molar ratio [31].

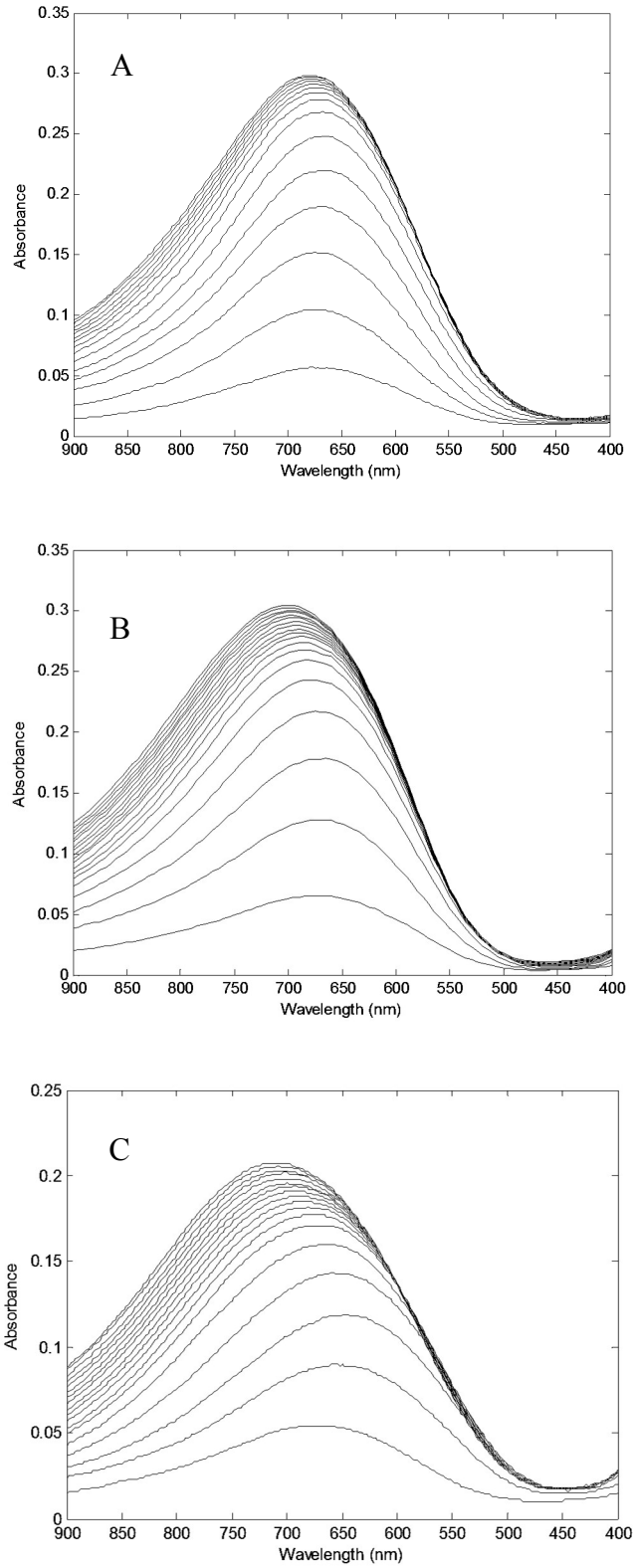


Figure 8. Absorption spectra of PAMAM-TRIS dendrimers at 680 nm (A) **10** (0.228 mM) solution titrated with Cu^{2+} (81.54 mM); (B) **11** (0.202 mM) solution titrated with Cu^{2+} (80.14 mM) (C) **12** (0.148 mM) solution titrated with Cu^{2+} (81.54 mM)

Absorbance at λ_{max}= 680 nm versus Cu^{2+}/PAMAM-TRIS dendrimers molar ratio plots were used to determine experimental end points at where PAMAM-TRIS dendrimers (**7-12)** could bind the maximum number of Cu^{2+} ions. That is, maximum molar excess of Cu^{2+} that can be loaded to PAMAM-TRIS dendrimers was calculated from these plots (Fig. 9). The maximum molar excess of Cu^{2+} ions, PAMAM-TRIS dendrimers (**10-12)** can bind were shown on the spectroscopic titration curves (Fig. 9) and summarized in Table 6. Results revealed that the number of tertiary amine numbers observed from spectroscopic titration data were in good agreement with the calculated ones. It can be concluded that PAMAM-TRIS dendrimers (**7-12**) absorb the number of Cu^{2+} ions equivalent the number of tertiary amine numbers. This correlation also indicates that the structure of PAMAM-TRIS dendrimers is at the desired monodispersity and highly pure. Thus, it could be also concluded that each Cu^{2+} ion is coordinated by four tertiary amine groups.

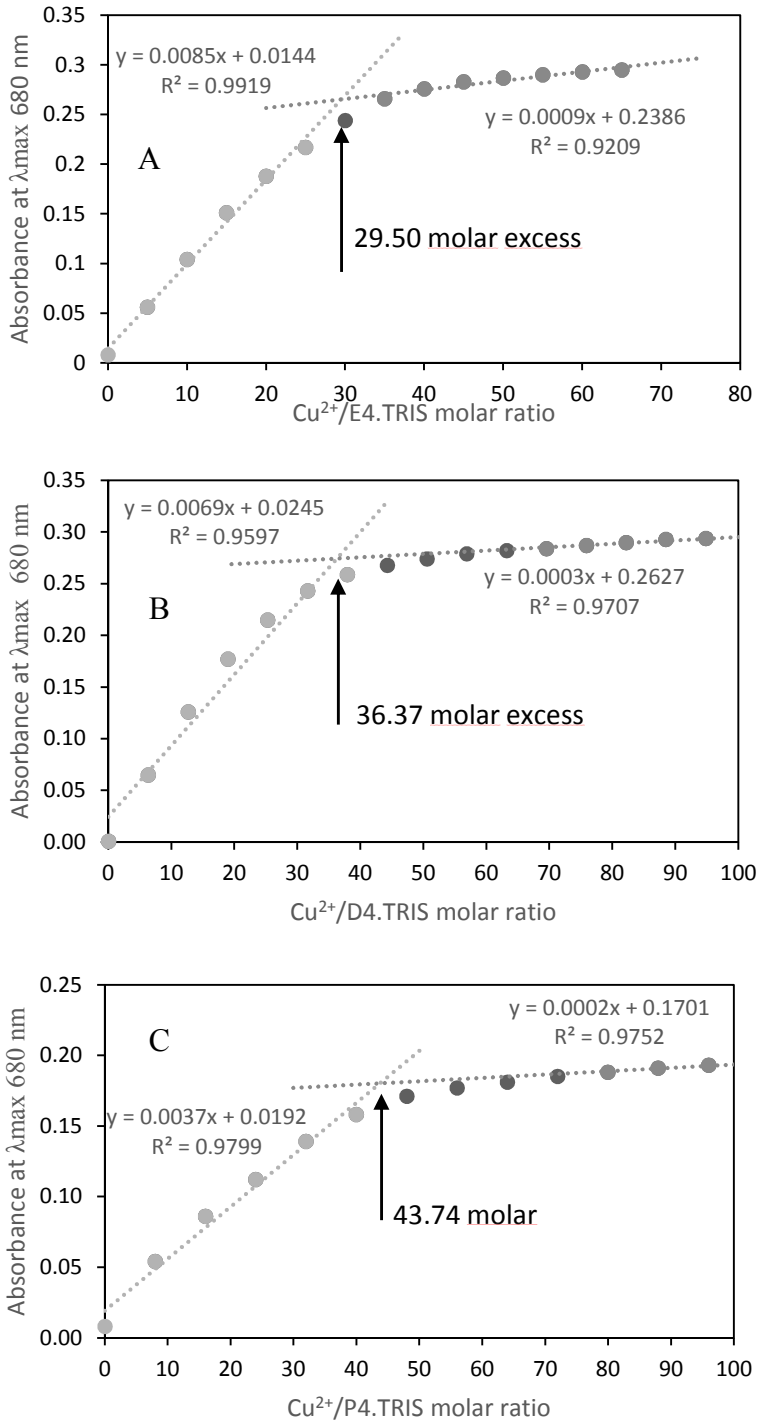


Figure 9. Spectroscopic titration curve of PAMAM-TRIS dendrimers (A) **10**; (B) **11**; (C) **12** with Cu^{2+} ions

Table 6. Number of tertiary amine groups on PAMAM-TRIS dendrimers (**7-12**) available for binding with Cu^{2+} ions

PAMAM-TRIS dendrimers	Calculated end point	Experimentally obtained end point[a]
7	14	13.83 ± 0.52
8	18	17.08 ± 0.43
9	21	20.88 ± 0.35
10	30	29.50 ± 0.48
11	38	36.37 ± 0.52
12	45	43.74 ± 1.02

[a]Each end point has been calculated as an average value of three experimental runs

Synthesis and UV-VIS Characterization of Cu(II)-PAMAM-TRIS intradendrimer complexes

After the number of Cu^{2+} ions that can be loaded to PAMAM-TRIS dendrimers (**7-12)** were determined from binding and spectroscopic Cu^{2+} titrations (Table 6), similar synthetic procedures were applied for all the evaluated dendrimers as the followings. Generally, 10.0 mL of 5.0 μM aqueous PAMAM-TRIS dendrimer solutions were prepared. This concentration was kept constant for all the dendrimer solutions. The pH of the solutions was adjusted to pH ~8. Correct stoichiometric amount of aqueous $CuSO_4$ (pH ~4.62) that can PAMAM-TRIS dendrimers bind calculated from Table 6 was added to each solution. The final pH of the solutions was about pH ~5. The solutions were then stirred for 30 min to allow the complexation of Cu^{2+} ions with the inner tertiary amines of PAMAM-TRIS dendrimers.

Transition *d-d* complex band resulting from the coordination of internal amine groups of ethanol amine terminated PAMAM dendrimers were reported at λmax at 605 nm [30]. This band was reported in the range of 600-800 nm depending on surface modification with TRIS [31] and could not be observable at low concentrations. Upon the addition of the appropriate molar ratio of calculated $CuSO_4$ solutions to PAMAM-TRIS dendrimers solutions, a strong band at around 270-280 nm for Cu(II)-PAMAM-TRIS dendrimers (**7-12)** complexes was occurred. This band is assigned to ligand to metal charge transfer (LMCT) bands [32-34]. In addition, *d-d* copper transition band at around 680 nm for all

Cu(II)-PAMAM-TRIS complex solutions were observed. Sample spectra of PAMAM-TRIS dendrimers (**10-12)** evidencing the complexation by color and UV-VIS spectra change can be seen in Figure 10.

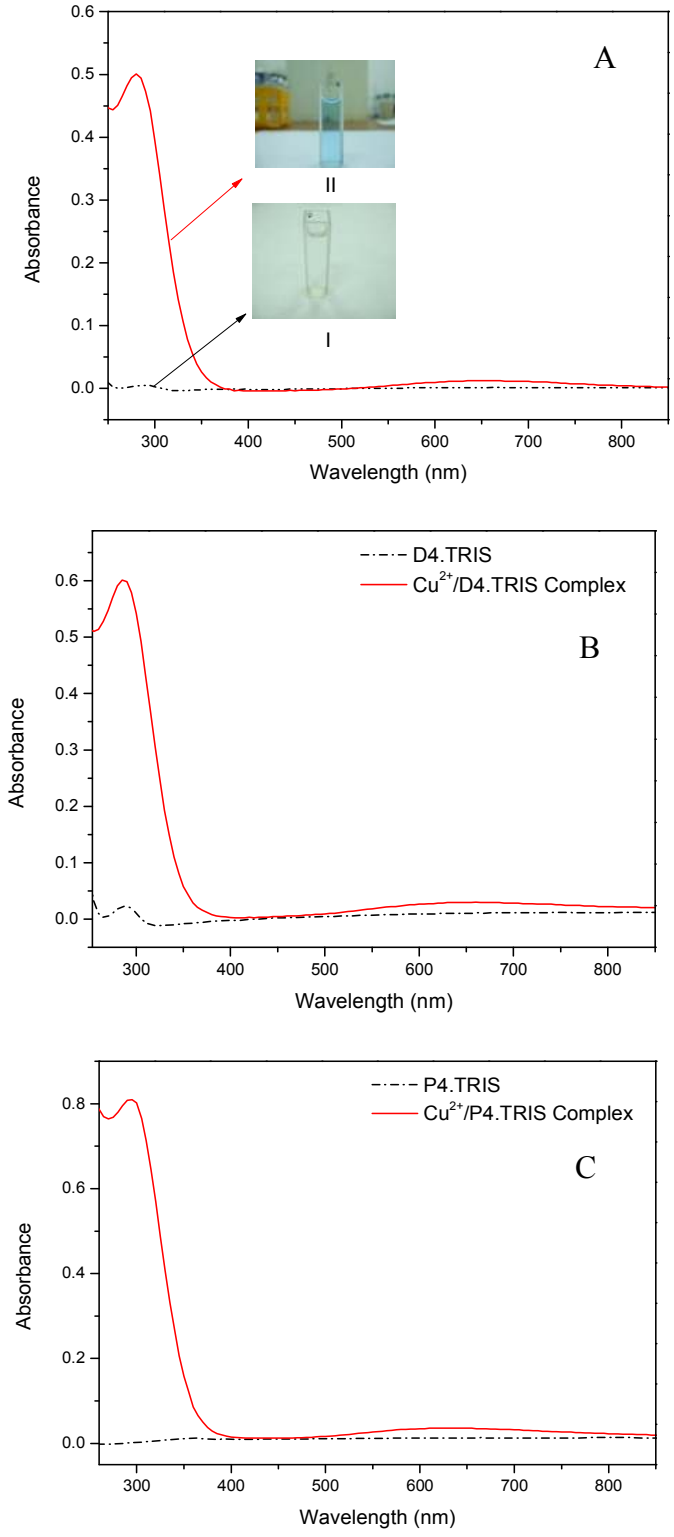


Figure 10. Change in color and UV-VIS absorption spectra during the complexation of Cu^{2+}-E4.TRIS: (I) aqueous solution of E4.TRIS, (II) Cu^{2+} dendrimer complex solution. (A); Cu^{2+}-D4.TRIS (B); Cu^{2+}-P4.TRIS (C). Color change was observed similarly in all complexations.

Conclusion

This article has reported the surface modification of PAMAM-OCH_3 dendrimers with TRIS. PAMAM-TRIS dendrimers were synthesized by both conventional and microwave methods and best reaction conditions were optimized. The new developed microwave method (Method B) allows the synthesis of PAMAM-TRIS dendrimers in 110-140 minutes rather than three to four days [15, 25] as an alternative to Method A.

With the use of new developed MAS method, the fast, efficient, easy, and one-pot synthesis of six water-soluble PAMAM-TRIS dendrimers, five are novel, with different cores, which are ethylenediamine, diethylenetriamine and Jeffamine® T-403 were performed. The other PAMAM-OCH_3 dendrimers (**2, 5**), which are precursors for PAMAM-TRIS dendrimers (**8, 11**), show the characteristic of being literaturally novel molecules. Furthermore, the synthesized molecules are water-soluble except PAMAM-OCH_3 dendrimers (**1-6**). Synthesized water-soluble PAMAM-TRIS dendrimers (**7-12**) were purified by LPR technique and characterized by ^{1}H-NMR, ^{13}C-NMR, ATR (IR), EA, UV-VIS spectroscopy, potentiometric and spectroscopic titrations. Spectroscopic titration studies of the PAMAM-TRIS dendrimers with Cu^{2+} ions revealed that all of the PAMAM-TRIS dendrimers are in good purity and high yield. As a result, they could be used as the possible potential drug carriers and templates for the synthesis of dendrimer-encapsulated metal nanoparticles as catalysts in the future studies at the desired purity and monodispersity.

Experimental

Materials

Jeffamine® T-403 Mn 440 was purchased from Aldrich. Methyl acrylate, ethylenediamine, diethylenetriamine, methanol, n-butanol, tris(hydroxymethyl) aminomethane were purchased from Merck. NaOH, 37 % HCl, NaH_2PO_4, NaCl, KHP, $NaBH_4$, were supplied from Merck. All solutions were prepared by 18.2 MΩ Millipore Milli-Q deionized. NaOH solutions were used as titrant after standardized with a primary grade KHP. Standardized HCl against KHP was used as excess acid in order to adjust initial pH of dendrimer solutions. pH 4.0, 7.0, 11.0 buffer solutions for the calibration were supplied from Merck. Dendrimer solutions were stored at 4 °C. Unless otherwise stated all chemicals were in analytical grade and used without further purification. Liquid-

phase polymer-based retention (LPR) ultrafiltration membranes, Amicon 8000 Stirred Cell and dialysis membranes having the molecular cut of size (MWCO) 1-3 kDa were supplied from Millipore.

Instrumentation and software

The CEM Focused Microwave™ synthesis system, model, Discover (CEM Corporation, North Carolina, USA) with a continuous microwave power output from 0-300 watts (± 30 watts) programmable in 1-watt increments, infrared temperature control system programmable from 25- 250 ºC, and 5-125 mL vessel capacity, was used as microwave reactor.

The IR spectra (4000–400 cm^{-1}, resolution 4 cm^{-1}) were recorded with a Perkin Elmer Spectrum One (Serial No: C68739) in ATR. The NMR spectra were recorded on a Bruker Avance 500 MHz Spectrometer. Thermo Scientific Flash EA 2000 Series (Organic Elemental Analyzer) CHN/S was used for the determination of main organics. The UV-VIS absorbance spectra were obtained using a PG T 70 Spectrometer (PG Instruments, England) and a quartz cuvette having an optical path length of 1.00 cm.

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 ± 0.1 ºC) using Polysceince® digital temperature controller circulating bath (Polysceince, Illinois, USA). 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 reference system. Glass electrode was calibrated with Merck pH 4.0, 7.0, 11.0 buffer solutions.

Spectroscopic titrations were carried out automatically by using TitroLine® 7000 autotitrator equipped with thermostated titration vessel under nitrogen media and PG TG 70 UV-VIS spectrophotometer equipped with UVWin5 Software v5.0.5, together.

General procedure for the synthesis of PAMAM-OCH_3 dendrimers (1-6)

PAMAM-OCH_3 dendrimers (**1-6**) were synthesized by following the procedure reported in our recent study[17] and briefly summarized hereafter. This method involves the alkylation and amidation steps. In the alkylation steps, excess methyl acrylate (2.5 M eq. per terminal amine) was added to the methanolic solution of dendrimer core (**C**)

(ethylenediamine (E), diethylenetriamine (D) and Jeffamine® T-403 (P)) or full generation PAMAM dendrimers (**Cn**). The reaction mixture was stirred for 24 h at room temperature and excess reagents and solvents were removed under vacuum at 65 °C bath temperatures and purified by means of LPR. The resulting PAMAM-OCH_3 dendrimers (**Cn.5**) E0.5, D0.5 and P0.5 were colorless oil.

In the amidations, excess E (10 M eq. of E per ester branched half generation) was added to the stirred methanolic solution of PAMAM-OCH_3 dendrimers (**Cn.5**). The final mixture was irradiated with MW at 200W for 60 min. Final traces of E was firstly removed under vacuum below the bath temperature of 65 °C by using 50 mL of n-butanol as hydrogen competitive reagent for three times. The resulting product was purified by means of LPR. Final methanolic solution of retentate product was removed under vacuum below the bath temperature of 65 °C. The final products were full generation amine terminated PAMAM dendrimers (PAMAM-NH_2 dendrimers) (**Cn**) E1, D1, and P1. By repeating the above cycle, E2.5, E3.5, D2.5, D3.5, P2.5 and P3.5 were synthesized. Yields were between the ranges of 92-96% (Table 7.)

Table 7. Preparation of **E**, **D** and **P** cored ester terminated PAMAM dendrimers (**Cn.5**)

PAMAM-OCH_3 dendrimers (**Cn.5**)	R-Amine[a] (**Cn**) g (mmol)	MA g (mmol)	MeOH (mL)	Time (h)	Yield (%)
E2.5 (**1**)	15.64 (10.93)	18.82 (218)	40	24	95
E3.5 (**2**)	14.60 (4.48)	15.43 (179)	40	24	93
D2.5 (**3**)	6.76 (3.72)	8.02 (93)	40	24	96
D3.5 (**4**)	8.12 (1.98)	8.53 (100)	40	24	95
P2.5 (**5**)	13.48 (5.40)	13.95 (162)	40	24	96
P3.5 (**6**)	12.00 (2.29)	11.84 (138)	40	24	92

[a]R-amine refers to full generation precursor [17]

General procedure for the conventional synthesis of PAMAM-TRIS dendrimers (7-12) with Method A

Methanolic solution of PAMAM-OCH_3 dendrimers (C2.5, C3.5) was added to a stirred suspension of TRIS (1.2 M equiv. per terminal ester) and anhydrous potassium carbonate (1.5 M equiv. of per terminal ester) in 10-15 mL of MeOH. The resulting mixture was vigorously stirred at 90 °C preheated oil bath for 7-8 hours. The final reaction mixture

was filtered to remove excess potassium carbonate and filtrate collected. Then product was purified with LPR by dialyzing against 50% aqueous methanol solution under 15 psi nitrogen (N_2) gas pressure for 24 hours by using Millipore ultrafiltration disks having the molecular cut of size 1 kDA. Final methanolic solution of retentate product (**Cn.TRIS**) was removed under vacuum below the bath temperature of 65 ^oC. Yields were between the ranges of 90-95%.

General procedure for the microwave-assisted synthesis of PAMAM-TRIS dendrimers (7-12) with Method B

Methanolic solution of PAMAM-OCH_3 dendrimers (C2.5, C3.5) was added to a stirred suspension of TRIS (1.2 M equiv. per terminal ester) and anhydrous potassium carbonate (1.5 M equiv. of per terminal ester) in 10-15 mL of MeOH. The resulting mixture was irradiated at 200 W for 110-140 min by refluxing at bulk temperature of 70-90 ^oC. The final reaction mixture was filtered to remove excess solid reagents, and filtrate collected. Then product was purified with LPR method. In other words, it was continuously dialyzed with 50% aqueous methanol solution under 15 psi nitrogen (N_2) gas pressure for 24 hours by using Millipore ultrafiltration disks having the molecular cut of size 1 kDA. Final methanolic solution of retentate product (**Cn.TRIS**) was removed under vacuum below the bath temperature of 65 ^oC. Yields were between the ranges of 93-96%.

LPR experiments

Appropriate Millipore ultrafiltration membrane disk was equipped with Amicon 8000 stirred cell was used for LPR method. (1:1) MeOH: Methanolic aqueous solution of crude product was transferred into the cell. Depending on the expected size of the product, membrane disks were selected in the range of MWCO 1-3 kDA. The solution was diluted to 200 mL inside the cell. Methanol-water mixture was used as feeding solvent. Continuous dialysis was performed under 15 psi nitrogen pressure for 24 hours. Finally, methanol water mixture was evaporated under vacuum.

Potentiometric titrations of PAMAM-TRIS dendrimers

Acid base titration of PAMAM-TRIS dendrimers were conducted according to literature [35, 36]. Analytical grade NaCl was added to a precisely weighed portion of PAMAM-TRIS

dendrimers solutions to prevent shielding of amine group each other because of interactions, and keep the ionic strength (I) constant at 100 mM. Titrations were carried out automatically by using autotitrator and thermostated titration vessel under continuous N_2 stream. Temperature was kept at room temperature (25 ± 0.1 °C). PAMAM-TRIS dendrimers (18-22 mg) were dissolved in 20 mL of 0.10 M NaCl solution to give a final concentration of 0.9-1.1 mg/mL the PAMAM-TRIS dendrimers solutions were titrated with standard HCl solution and back titrated with standard NaOH solution, respectively (Table 8).

Table 8. Experimental potentiometric titration conditions for different PAMAM-TRIS dendrimers[a]

PAMAM-TRIS dendrimers (**Cn.TRIS**)	Total weight (mg)	pH range	Initial Volume (mL)	Excess acid (mL)	HCl (mol/L)	NaOH (mol/L)	Data points
E3.TRIS (**7**)	19.53	2.78-11.00	20.0	1.325	0.098	0.050	148
D3.TRIS (**8**)	19.76	2.47-11.20	20.0	1.125	0.098	0.050	248
P3.TRIS (**9**)	18.46	2.81-11.00	20.0	1.425	0.099	0.048	152
E4.TRIS (**10**)	21.00	2.77-11.00	20.0	1.700	0.098	0.050	167
D4.TRIS (**11**)	19.76	2.47-11.30	20.0	1.950	0.098	0.050	246
P4.TRIS (**12**)	21.10	2.79-11.00	20.0	1.300	0.099	0.048	146

[a]25 µL increments, error in reading is 1 µL, *I*=100 mm at 25 ± 0.1 °C, error in pH reading is 0.002

Spectroscopic titrations of PAMAM-TRIS dendrimers

All of the UV-VIS measurements were taken between the wavelength ranges of 250-350 nm with 10 mm quartz UV cells. Precision of titration was increased ± 0.01 pH unit by using a TitroLine® 7000 (SI Analytics GmbH, Hattenbergstraße, Germany) autotitrator supported with Titrisoft® 2.73 software. After each pH adjustment, dendrimer solution transferred into the cuvette and the absorption spectrum is recorded. The pH meter was calibrated with at least three buffer solutions at pH 4.0, 7.0 and 10.0 before each experiment. Ionic strength was maintained by adding appropriate amount of 0.10 M NaCl.

Spectroscopic titrations of PAMAM-TRIS dendrimers with Cu^{2+} ions and synthesis of Cu(II)-PAMAM-TRIS complexes

E, **D** and **P** cored PAMAM-TRIS dendrimers have many metal binding cites. In order to determine the maximum metal loading capacity of each dendrimer, the number of metal ions that can be coordinated with the tertiary amine groups of PAMAM-TRIS dendrimers were determined by spectroscopic Cu^{2+} titrations. Spectroscopic titrations of PAMAM-TRIS dendrimers with Cu^{2+} ions and synthesis of Cu(II)-PAMAM-TRIS dendrimer complexes were adapted from the literature [29, 30]. Before each titration, the pH of the aqueous unbuffered PAMAM-TRIS dendrimers solutions was adjusted to pH ~8.0 and then unbuffered $CuSO_4$ (pH~4.6) was used to titrate dendrimer solutions (Table 9).

In General, spectroscopic titrations were carried out by the addition of a 10.0 mL aqueous PAMAM-TRIS dendrimer solution to a thermostated vessel at 25 ± 0.1 ºC. Then, identical aliquots of $CuSO_4$ solution were added to the vessel each time while a stir bar vigorously stirring the solution. 15~20 seconds were usually allowed to provide sufficient time for Cu^{2+} to bind the dendrimer before an absorbance measurement was acquired. UV-VIS spectrum of the solution was recorded in the wavelength range of 400-900 nm with 5.00 nm intervals. λ_{max} = 680 nm was the wavelength, which was associated with the complexation of Cu^{2+} ions with the internal tertiary amines of PAMAM-TRIS dendrimers. When the excess of Cu^{2+} ions was added after the equivalence point, the increase in the absorbance is levelled off indicating the maximum metal Cu^{2+} ions loading capacity of dendrimer. Finally, spectroscopic titration plots, on which absorbance at peak maximum of 680 nm as a function of the Cu^{2+} ions per PAMAM-TRIS dendrimers, were plotted. In order to determine the maximum metal loading capacity of each PAMAM-TRIS dendrimers, titration end point was estimated as the extrapolated intersection of the linear regions of the curve before and after the equivalence point. The small absorbance beyond the equivalence point is due to small absorbance contributed by the titrant.

Table 9.Concentrations used to titrate aqueous solutions of PAMAM-TRIS dendrimers (**7-12**)

PAMAM-TRIS dendrimers (**Cn.TRIS**)	Dendrimer. Conc. (mM)	Conc. of $CuSO_4$ (mM)	$CuSO_4$ (mM) Increment (μL)
E3.TRIS (**7**)	0.441	80.14	43.00
D3.TRIS (**8**)	0.351	93.12	20.00
P3.TRIS (**9**)	0.269	81.54	35.00
E4.TRIS (**10**)	0.228	81.54	37.00
D4.TRIS (**11**)	0.202	80.14	42.00
P4.TRIS (**12**)	0.148	81.54	38.00

Characterization

*E2.5 (**1**)*

Yellowish gel (29.16 g, 95%). Elemental analysis $C_{126}H_{224}N_{26}O_{44}$: Found: C, 54.4; H, 8.17; N, 13.12. Calc.: C, 53.91; H, 8.04; N, 12.97%. ATR-IR ν_{max}/cm^{-1} 3294 (NH), 1731 (C=O), 1644(HNC=O), 1544 (HNC=O). ^{1}H NMR δH(400 MHz; D_2O) 2.37 (32H, bm, $NR_2CH_2CH_2COOCH_3$), 2.45 (32H, t, $NR_2CH_2CH_2COOCH_3$), 2.64 (16H, bm, $CONHCH_2CH_2NR_2$), 3.15 (16H, bm, $CONHCH_2CH_2NR_2$), 3.61 (48H, s, $COOCH_3$). ^{13}C NMR δC(100 MHz; D_2O) 31.96 ($CH_2CH_2COOCH_3$), 47.67 ($CH_2CH_2COOCH_3$), 48.18 ($CH_2CH_2NR_2$), 49.54 ($COOCH_3$), 170.56, 170.87 (NCH_2CH_2CONH), 171.56 ($COOCH_3$).

*D2.5 (**2**)*

Yellowish gel (12.64 g, 96%). Elemental analysis $C_{159}H_{283}N_{33}O_{55}$: Found: C, 54.49; H, 8.22; N, 13.19. Calc.: 53.99; H, 8.06; N, 13.07%. ATR-IR ν_{max}/cm^{-1} 1730 (C=O). ^{1}H NMR δH(300 MHz; DMSO-d_6) 2.62 (40H, m, $NR_2CH_2CH_2COOCH_3$), 2.85 (40H, m, $NR_2CH_2CH_2COOCH_3$), 2.87 (40H, m, $CONHCH_2CH_2NR_2$), 3.29 (40H, brm, $CONHCH_2CH_2NR_2$), 3.78 (60H, s, $COOCH_3$). ^{13}C NMR δC(75 MHz; DMSO-d_6) 32.66 (20C, $NR_2CH_2CH_2COOCH_3$), 37.28 (10C, $CONHCH_2CH_2NR_2$), 50.48 (20C, $NR_2CH_2CH_2COOCH_3$), 51.83 (10C, $CONHCH_2CH_2NR_2$), 51.87 (20C, $COOCH_3$), 172.89, 173.13, 173.19 (10C, $NR_2CH_2CH_2CONH$), 174.84 (20C, $COOCH_3$).

*P2.5 (**3**)*

Yellowish gel (23.65 g, 96%). Elemental analysis $C_{207}H_{377}N_{39}O_{71}$: Found: C, 55.50; H, 8.78; N, 13.25. Calc.: C, 55.00; H, 8.57, N, 13.12%. ATR-IR ν_{max}/cm^{-1} 3291 (NH), 1732 (C=O), 1644(HNC=O), 1535 (HNC=O). ^{1}H-NMR δH(400 MHz; DMSO) 2.42 (48H, t, $CH_2CH_2COOCH_3$), 2.74 (48H, t, $CH_2CH_2COOCH_3$), 3.65 (72H, s, $COOCH_3$). ^{13}C NMR δC(100 MHz; DMSO) 172.86 ($COO\ CH_3$), 75.41 ($COOCH_3$), 51.48 ($CH_2CH_2NR_2$), 49.15 ($CH_2CH_2COOCH_3$), 32.63 ($CH_2CH_2COOCH_3$).

*E3.5 (**4**)*

Yellowish gel (25.06 g, 93%). Elemental analysis $C_{270}H_{480}N_{58}O_{92}$: Found: C, 55.21; H, 8.21; N, 13.76. Calc.: C, 53.95; H, 8.05; N, 13.51%. ATR-IR ν_{max}/cm^{-1} 3271 (NH), 1726 (C=O), 1638 (HNC=O), 1557 (HNC=O). ^{1}H NMR δH(400 MHz; D_2O) 2.40 (64H, bm, $NR_2CH_2CH_2COOCH_3$), 2.52 (64H, t, $NR_2CH_2CH_2COOCH_3$), 2.67 (32H, bm, $CONHCH_2CH_2NR_2$), 3.17 (32H, bm, $CONHCH_2CH_2NR_2$), 3.62 (96H, s, $COOCH_3$). ^{13}C NMR δC(100 MHz; D_2O) 31.32 ($CH_2CH_2COOCH_3$), 47.41 ($CH_2CH_2COOCH_3$), 48.47 ($CH_2CH_2NR_2$) 50.35 ($COOCH_3$), 171.02, 171.15, 171.43 (NCH_2CH_2CONH), 172.01 ($COOCH_3$).

*D3.5 (**5**)*

Yellowish gel (14.20 g, 95%). Elemental analysis $C_{339}H_{603}N_{73}O_{115}$: Found: C, 55.12; H, 8.27; N, 13.81. Calc.: C, 53.99; H, 8.06; N, 13.56%. ATR-IR ν_{max}/cm^{-1} 1730 (C=O). ^{1}H NMR δH(300 MHz; DMSO-d_6) 2.60 (80H, m, $NR_2CH_2CH_2COOCH_3$), 2.86 (80H, brm, $NR_2CH_2CH_2COOCH_3$), 2.89 (80H, brm, $CONHCH_2CH_2NR_2$), 3.27 (80H, brm, $CONHCH_2CH_2NR_2$), 3.78 (120H, s, $COOCH_3$). ^{13}C NMR δC(75 MHz; DMSO-d_6) 32.66 (40C, $NR_2CH_2CH_2COOCH_3$), 37.31 (20C, $CONHCH_2CH_2NR_2$), 51.83 (40C, $NR_2CH_2CH_2COOCH_3$), 51.87 (40C, $COOCH_3$), 52.92 (20C, $CONHCH_2CH_2NR_2$), 171.71, 171.77, 171.85 (20C, NCH_2CH_2CONH), 173.13 (40C, $COOCH_3$).

*P3.5 (**6**)*

Yellowish gel (19.75 g, 92%). Elemental analysis $C_{423}H_{761}N_{87}O_{143}$: Found: C, 56.17; H, 8.65; N, 13.81. Calc.: C, 54.54; H, 8.33; N, 13.52%. ATR-IR ν_{max}/cm^{-1} 3291 (NH), 1732 (C=O), 1642 (HNC=O), 1535 (HNC=O). ^{1}H-NMR δH(400 MHz; DMSO) 2.42 (96H, t,

$CH_2CH_2COOCH_3$), 2.68 (96H, t, $CH_2CH_2COOCH_3$), 3.59 (144H s, $CH_2CH_2COOCH_3$). ^{13}C NMR δC(100 MHz; DMSO) 172.43 ($COOCH_3$), 51.15 ($CH_2CH_2COOCH_3$), 48.87 ($CH_2CH_2COOCH_3$), 32.00 ($CH_2CH_2COOCH_3$).

*E3.TRIS (**7**)*

Opaque oil (2.49 g, 96%). Elemental analysis $C_{174}H_{336}N_{42}O_{76}$: Found: C, 49.75; H, 8.14; N, 14.10. Calc.: C, 49.37; H, 8.00; N, 13.90%. ATR-IR ν_{max}/cm^{-1} 3263 (COH), 1635 (HNC=O), 1558 (HNC=O), 1392 (O-H). ^{1}H NMR δH(400 MHz; CD_3OD) 2.56 (32H, bm, $CH_2CH_2CONHCR_3$), 2.76 (32H, bm, $CH_2CH_2CONHCR_3$), 3.57 (96H, bs, CH_2OH). ^{13}C NMR δC(100 MHz; CD_3OD) 32.22 ($CH_2CH_2CONHCR_3$), 48.70 ($CH_2CH_2CONHCR_3$), 49.24 ($CH_2CH_2NR_2$), 56.51 ($CONHCR_2CH_2OH$), 63.26 ($CONHCR_2CH_2OH$), 174.66, 174.82, 180.73, 181.01 (NCH_2CH_2CONH).

*D3.TRIS (**8**)*

Opaque oil (1.40 g, 95%). Elemental analysis $C_{199}H_{383}N_{53}O_{95}$: C, 47.86; H, 7.77; N, 14.92. Calc.: C, 47.44; H, 7.66; N, 14.73%. ATR-IR ν_{max}/cm^{-1} 3266 (COH), 1635 (HNC=O), 1554 (HNC=O), 1392 (O-H). ^{1}H NMR δH(300 MHz; CD_3OD) 2.61 (40H, m, $CH_2CH_2CONHR_3$), 2.78 (40H, bm, $CH_2CH_2CONHR_3$), 2.77 (40H, bm, $CONHCH_2CH_2NR_2$), 3.23 (40H, bm, $CONHCH_2CH_2NR_2$), 3.65 (120H, s, CH_2OH). ^{13}C NMR δC(75 MHz; CD_3OD) 33.67 (20C, $NR_2CH_2CH_2COOCH_3$), 38.23 (10C, $CONHCH_2CH_2NR_2$), 50.33 (20C, $NR_2CH_2CH_2COOCH_3$), 51.22 (10C, $CONHCH_2CH_2NR_2$), 56.47 (15C, $CONHCCH_2OH$), 63.69 (48C, $CONHCCH_2OH$), 174.83, 175.35, 180.48, 181.12 (20C, NCH_2CH_2CONH).

*P3.TRIS (**9**)*

Opaque oil (0.91 g, 91%). Elemental analysis $C_{279}H_{545}N_{63}O_{119}$: Found: C, 50.00; H, 7.04; N, 14.66. Calc.: C, 49.52; H, 6.95; N, 14.44%. ATR-IR ν_{max}/cm^{-1} 3275 (COH), 1645 (HNC=O), 1563 (HNC=O), 1393 (O-H). ^{1}H NMR δH(400 MHz; CD_3OD) 2.58 (48H, bm, $CH_2CH_2CONHCR_3$), 2.77 (48H, t, $CH_2CH_2CONHCR_3$), 3.54 (192H, bs, CH_2OH). ^{13}C NMR δC(100 MHz; CD_3OD) 32.64 ($CH_2CH_2CONHCR_3$), 48.91 ($CH_2CH_2CONHCR_3$), 52.08 ($CH_2CH_2NR_2$), 56.30 ($CONHCR_2CH_2OH$), 63.12 ($CONHCR_2CH_2OH$) 174.61, 175.53, 181.12 (NCH_2CH_2CONH).

*E4.TRIS (**10**)*

Opaque oil (1.35 g, 93%). Elemental analysis $C_{366}H_{704}N_{90}O_{156}$: Found: C, 49.98; H, 8.10; N, 14.40. Calc.: C, 49.60; H, 8.01; N, 14.22%. ATR-IR ν_{max}/cm^{-1} 3263 (COH), 1641 (HNC=O), 1558 (HNC=O), 1394 (O-H). ^{1}H NMR δH(400 MHz; CD_3OD) 2.52 (64H, bm, $CH_2CH_2CONHCR_3$), 2.70 (64H, bm, $CH_2CH_2CONHCR_3$), 3.65 (192H, bs, CH_2OH). ^{13}C NMR δC(100 MHz; CD_3OD) 32.62 ($CH_2CH_2CONHCR_3$), 48.88 ($CH_2CH_2CONHCR_3$), 49.02 ($CH_2CH_2NR_2$), 56.37 ($CONHCR_2CH_2OH$), 63.06 ($CONHCR_2CH_2OH$), 174.62, 174.92, 180.93, 181.21 (NCH_2CH_2CONH).

*D4.TRIS (**11**)*

Opaque oil (1.46 g, 94%). Elemental analysis $C_{459}H_{883}N_{113}O_{195}$: Found: C, 49.96; H, 8.08; N, 14.42. Calc.: C, 49.64; H, 8.01; N, 14.25%. ATR-IR ν_{max}/cm^{-1} 3261 (COH), 1640 (HNC=O), 1554 (HNC=O), 1388 (O-H). ^{1}H NMR δH(300 MHz; CD_3OD) 2.55 (80H, bm, $CH_2CH_2CONHR_3$), 2.70 (80H, bm, $CH_2CH_2CONHR_3$), 2.82 (80H, bm, $CONHCH_2CH_2NR_2$), 3.29 (80H, bm, $CONHCH_2CH_2NR_2$), 3.71 (240H, s, CH_2OH). ^{13}C NMR δC(75 MHz; CD_3OD) 33.55 (40C, $CH_2CH_2CONHR_3$), 38.60 (20C, $CONHCH_2CH_2NR_2$), 50.03 (40C, $CH_2CH_2CONHR_3$), 51.10 (20C, $CONHCH_2CH_2NR_2$), 56.37 (30C, $CONHCR_2CH_2OH$), 63.6 (96C, $CONHCR_2CH_2OH$), 174.93, 175.44, 180.4, 181.04 (40C, NCH_2CH_2CONH).

*P4.TRIS (**12**)*

Opaque oil (1.17 g, 93%). Elemental analysis $C_{567}H_{1097}N_{135}O_{239}$: Found: C, 49.62; H, 6.86; N, 14.85. Calc.: C, 48.98; H, 6.73; N, 14.73%. ATR-IR ν_{max}/cm^{-1} 3263 (COH), 1641 (HNC=O), 1558 (HNC=O), 1392 (O-H). ^{1}H NMR δH(400 MHz; CD_3OD) 2.5 (96H, bm, CH_2CH_2CONHC), 2.68 (96H, t, CH_2CH_2CONHC), 3.63 (288H, bs, CH_2OH). ^{13}C NMR δC(100 MHz; CD_3OD) 32.59 (CH_2CH_2CONHC), 48.97 (CH_2CH_2CONHC), 51.18 ($CH_2CH_2NR_2$), 56.38 ($CONHCR_2CH_2OH$), 63.02 ($CONHCR_2CH_2OH$) 174.65, 175.55, 181.09 (NCH_2CH_2CONH).

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