The effect of PAMAM dendrimer concentration, generation size and surface functional group on the aqueous solubility of candesartan cilexetil

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General Experimental

IR (ATR) spectra ($4000\text{--}650\text{ cm}^{-1}$, resolution 4 cm^{-1}) were recorded on PerkinElmer Spectrum 100 FT-IR Spectrometer (Serial No:LR64912C). ^1H NMR and ^{13}C NMR spectra were recorded in CDCl_3 using Bruker Avance 500 MHz Spectrometer and TMS as an internal reference. Thermo Scientific Flash EA 2000 Series (Organic Elemental Analyzer) CHN/S was used for the determination of main organics. Microwave-irradiated reactions were carried out with a CEM Focused MicrowaveTM Synthesis System, Model Discover (CEM Corporation, North Carolina, USA) with a continuous microwave power delivery system with operator selectable power output from 0 to 300W ($\pm 30\text{ W}$) programmable in 1-watt increments, infrared temperature control system programmable from 25 to 250 °C, pressure controlled and 5 to 125 mL vessel capacity was used as microwave reactor (Serial Number DU9472). Spectroscopic titrations were carried out automatically by using TitroLine® 7000 ((SI Analytics GmbH, Hattenbergstrabe, 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.

Experimental procedures for the synthesis of PAMAMs

Synthesis of PAMAMs

Microwave-assisted synthesis (MAS) of PAMAM- NH_2 (**Gn.NH₂**) and PAMAM-TRIS (**Gn.TRIS**) dendrimers, with ethylene diamine core, from half generation PAMAM- OCH_3 (**Gn.5**) dendrimers were performed according to our previous studies (1), and (2), respectively. On the other hand, PAMAM- COOH dendrimers (**Gn.COOH**) were prepared by the slight modification of literature procedure (3) and our previous study (4). Purity of PAMAMs was characterized by ATR-FTIR, ^1H -NMR, ^{13}C -NMR, EA and supported by spectroscopic titrations. In this way, starting from ethylenediamine (EDA) core, different generations (G2-G4) PAMAMs with

different surface groups (NH_2 , TRIS, and COOH) were synthesized by repeating the cycle in

Scheme 1.

Scheme 1. Synthetic route for PAMAM- NH_2 , PAMAM-TRIS and PAMAM- COOH dendrimers.

General procedure for the synthesis of PAMAM-OCH₃ and PAMAM-NH₂ dendrimers

Synthesis of PAMAM-OCH₃ and PAMAM-NH₂ dendrimers involve two consecutive reactions, the alkylation (i) and the amidation (ii). In the alkylation steps, excess methyl acrylate (2.5 M eq. per terminal amine) was added to the methanolic solution of dendrimer core EDA or full generation PAMAM-NH₂ dendrimers (Gn.NH₂). 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 liquid phase polymer retention (LPR) technique. The resulting PAMAM-OCH₃ dendrimers (Gn.5) G1.5, G2.5, G3.5 were colorless oil. Yields were between 92-95 % (Table S1).

Table S1. Preparation of EDA core ester terminated PAMAMs (Gn.5).

Gn	R-Amine g (mmol)	MA g, (mmol)	MeOH (mL)	Time (h)	Yield (%)
G1.5	10.99 (21.26)	18.30 (212)	40	24	92
G2.5	15.64 (10.93)	18.82 (218)	40	24	95
G3.5	14.60 (4.48)	15.43 (179)	40	24	93

In the amidations, excess EDA (10 M eq. of EDA per ester branched half generation) was added to the stirred methanolic solution of PAMAM-OCH₃ dendrimers (Gn.5). The final mixture was irradiated with microwave (MW) at 200W for 60 min. Final traces of EDA were 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 PAMAM-NH₂ dendrimers G2.NH₂, G3.NH₂, G4.NH₂. Yields were between the ranges of 90-95% (Table S2)

Table S2. Preparation of EDA core **PAMAM-NH₂** dendrimers (**G_n.NH₂**) by MAS.

G _n	R-Ester g (mmol)	EDA g (mol)	MeOH ^a (mL)	MW (watt)	Time (min)	Yield (%)
G2.NH ₂	19.12 (15.85)	76.25 (1.26)	4.0	200	60	95
G3.NH ₂	20.05 (7.14)	68.66 (1.14)	3.5	200	60	90
G4.NH ₂	15.85 (2.63)	50.70 (0.84)	4.0	200	60	90

^a Methanol as dielectric constant enhancer.

General procedure for the synthesis of PAMAM-TRIS dendrimers

PAMAM-TRIS dendrimers were synthesized by following the method we described in our recent study (2), and summarized briefly hereafter. Methanolic solution of PAMAM-OCH₃ dendrimers (**G1.5- G3.5**) 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 °C. 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 (N₂) gas pressure for 24 hours by using Millipore ultrafiltration disks having the molecular cut of size 1 kDA. Final methanolic solution of retentate product **PAMAM-TRIS dendrimers** (**G_n.TRIS**) (**G2.TRIS- G4.TRIS**) was removed under vacuum below the bath temperature of 65 °C. Yields were between the ranges of 93-96% (Table S3.)

Table S3. Preparation of EDA core **PAMAM-TRIS dendrimers** (*Gn.TRIS*) by MAS.

Gn	R-Ester	Tris	K ₂ CO ₃	MeOH	MW	Time	Yield (%)
	g (mmol)	g (mmol)	g (mmol)	(mL)	(watt)	(min)	
G3.TRIS	1.72 (0.61)	1.43 (11.77)	2.03 (14.71)	10.0	200	120	96
G4.TRIS	0.99 (0.16)	0.76 (6.29)	1.08 (7.85)	10.0	200	140	93

General procedure for the synthesis of PAMAM-COOH dendrimers

PAMAM-COOH dendrimers were prepared by slight modification of the literature (3) procedure and our previous study (4). Briefly, a methanolic solution of **PAMAM-OCH₃ dendrimers** (**G.1.5-3.5**) was mixed with 1.5 M equiv. of NaOH per terminal ester. The final mixture was stirred for 24 h. Excess amount of solvent was removed under vacuum at bath temperature 65 °C. The remaining oil was dissolved in methanol and again evaporated in vacuo. Drying under vacuum resulted in a white powder product. Yields were 100% (Table S4.)

Table S4. Preparation of EDA core **PAMAM-COOH dendrimers** (*Gn.COOH*).

Generation	R-Ester	NaOH	MeOH	Time	Yield (%)
	g (mmol)	g (mmol)	(mL)	(h)	
E3.COOH	0.79 (0.28)	0.27 (6.80)	5.0	24	100
E4.COOH	0.65 (0.10)	0.21 (5.20)	4.0	24	100

Experimental procedures for the spectroscopic titrations of PAMAM-COOH and PAMAM-TRIS dendrimers with Cu²⁺ ions

Spectroscopic titration of PAMAMs with Cu²⁺ ions

Spectroscopic titrations were conducted on the synthesized PAMAM-TRIS and PAMAM-COOH dendrimers to characterize and show the structural monodispersity or homogeneity by using a

PG-70 batch type UV-Vis instrument. UV measurements were conducted within the wavelength range of 400-900 nm with a 10 mm quartz UV cells. The spectroscopic titrations experiments were carried out according to our previous work (2). In summary, Table S5 presents the concentrations of dendrimer and CuSO₄ solutions used in the spectroscopic titration.

Table S5. Concentrations used to titrate aqueous solutions of PAMAM-TRIS and PAMAM-COOH dendrimers with Cu²⁺ ions.

Dendrimer	Dendrimer. Conc. (mM)	Conc. of CuSO ₄ (mM)	CuSO ₄ (mM) Increment (μL)
G3.TRIS	0.441	80.14	43.00
G4.TRIS	0.228	81.54	37.00
G3.COOH	0.637	80.14	44.00
G4.COOH	0.321	80.14	44.00

Spectroscopic characterization and the determination of the number of tertiary amine groups present in PAMAM-COOH and PAMAM-TRIS dendrimers

Crooks et al. (5, 6) have shown that Cu²⁺ ions coordinate with the maximum number of four tertiary amine numbers. Addition of Cu²⁺ ions to TRIS and COOH-terminated PAMAM dendrimers in aqueous solutions immediately resulted in a deep-blue color change. This color change due to intra-dendrimer metal complexes was characterized by UV-Vis spectroscopy with a broad absorption band at λ_{max} = 680 nm for TRIS (Fig. S1) and COOH (Fig. S2) terminated PAMAMs. This band belonging to the copper *d-d* transition indicates the complex formation between the two species at 1:4 dendrimer to tertiary amine molar ratio (7). The maximum number of internal tertiary amine numbers of dendrimers evaluated in this study were shown in Table 1.

Figure S1. Absorption spectra of G4.TRIS dendrimer (0.228 mM) solution titrated with Cu^{2+} (81.54 mM) at 680 nm.

Figure S2. Absorption spectra of G4.COOH dendrimer (0.321 mM) solution titrated with Cu^{2+} (80.14 mM) at 680 nm.

Absorbance at $\lambda_{\text{max}}=680$ nm versus Cu^{2+} /DENs molar ratio plots were used to determine experimental end points at where dendrimer could bind the maximum number of Cu^{2+} ions. That is, maximum molar excess of Cu^{2+} that can be loaded to dendrimer was calculated from these plots. The maximum molar excess of Cu^{2+} ions, TRIS and COOH-terminated PAMAMs can bind were shown on the spectroscopic titration curves (Fig. S3, S4.), and also summarized in Table S6. Results revealed that the number of tertiary amine numbers observed from spectroscopic titration data were in good agreement with the calculated ones (Table S6). TRIS and COOH ended dendrimers evaluated in this study absorb the number of Cu^{2+} ions equivalent the number of tertiary amine numbers. This correlation also indicates the structure of PAMAMs is almost monodisperse and highly pure. Thus, it could be concluded that each Cu^{2+} ion is coordinated by four tertiary amine groups.

Figure S3. Spectroscopic titration curve of (a) G3.TRIS, (b) G4.TRIS with Cu^{2+} ions.

Figure S4. Spectroscopic titration curve of the (a) G3.COOH and (b) G4.COOH with Cu^{2+} ions.

Table S6. Number of tertiary amine groups of PAMAM-TRIS and PAMAM-COOH dendrimers available for binding with Cu²⁺ ions.

Dendrimer	Calculated end point ^a	Experimentally obtained end point
G3.TRIS	14	13.83
G4.TRIS	30	29.50
G3.COOH	14	14.00
G4.COOH	30	28.28

^aCalculated end point refers the number of theoretical tertiary amine number present in dendrimer structure (see also Table 1). Each end point has been calculated as an average value of three experimental runs.

Characterization data of the PAMAMs

G1.5

Yellowish gel (23.58 g, 92%). Elemental analysis C₅₄H₉₆N₁₀O₂₀: Found: C, 54.10; H, 8.07; N, 11.78. Calc.: C, 53.81; H, 8.03; N, 11.62%. ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3296 (NH), 1730 (C=O), 1653 (HNC=O), 1535 (HNC=O). ¹H NMR δ H(300 MHz; D₂O) 2.34 (16H, bm, NR₂CH₂CH₂COOCH₃), 2.49 (16H, t, NR₂CH₂CH₂COOCH₃), 2.69 (8H, bm, CONHCH₂CH₂NR₂), 3.19 (8H, bm, CONHCH₂CH₂NR₂), 3.65 (24H, s, COOCH₃). ¹³C NMR δ C(400 MHz; D₂O) 31.42 (CH₂CH₂COOCH₃), 47.50 (CH₂CH₂COOCH₃), 48.7 (CH₂CH₂NR₂), 50.01 (COOCH₃), 171.1 (NCH₂CH₂CONH), 171.8 (COOCH₃).

G2.5

Yellowish gel (29.16 g, 95%). Elemental analysis C₁₂₆H₂₂₄N₂₆O₄₄: Found: C, 54.4; H, 8.17; N, 13.12. Calc.: C, 53.91; H, 8.04; N, 12.97%. ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3294 (NH), 1731 (C=O), 1644(HNC=O), 1544 (HNC=O). ¹H NMR δ H(400 MHz; D₂O) 2.37 (32H, bm,

NR₂CH₂CH₂COOCH₃), 2.45 (32H, t, NR₂CH₂CH₂COOCH₃), 2.64 (16H, bm, CONHCH₂CH₂NR₂), 3.15 (16H, bm, CONHCH₂CH₂NR₂), 3.61 (48H, s, COOCH₃). ¹³C NMR δC(400 MHz; D₂O) 31.96 (CH₂CH₂COOCH₃), 47.67 (CH₂CH₂COOCH₃), 48.18 (CH₂CH₂NR₂), 49.54 (COOCH₃), 170.56, 170.87 (NCH₂CH₂CONH), 171.56 (COOCH₃).

G3.5

Yellowish gel (25.06 g, 93%). Elemental analysis C₂₇₀H₄₈₀N₅₈O₉₂: 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). ¹H NMR δH(400 MHz; D₂O) 2.40 (64H, bm, NR₂CH₂CH₂COOCH₃), 2.52 (64H, t, NR₂CH₂CH₂COOCH₃), 2.67 (32H, bm, CONHCH₂CH₂NR₂), 3.17 (32H, bm, CONHCH₂CH₂NR₂), 3.62 (96H, s, COOCH₃). ¹³C NMR δC(400 MHz; D₂O) 31.32 (CH₂CH₂COOCH₃), 47.41 (CH₂CH₂COOCH₃), 48.47 (CH₂CH₂NR₂), 50.35 (COOCH₃), 171.02, 171.15, 171.43 (NCH₂CH₂CONH), 172.01 (COOCH₃).

G2.NH₂

Yellowish gel (23.97 g, 95%). Elemental analysis C₆₂H₁₂₈N₂₆O₁₂: Found: C, 52.31; H, 9.14; N, 25.68. Calc.: C, 52.08; H, 9.02; N, 25.47%. ATR-IR ν_{max}/cm⁻¹ 3280 (NH), 1635 (HNC=O), 1549 (HNC=O). ¹H NMR δH(400 MHz; D₂O) 2.25 (16H, bm, NR₂CH₂CH₂CONH), 2.55 (16H, bm, NR₂CH₂CH₂CONH), 2.66 (16H, bm, CONHCH₂CH₂NH₂), 3.12 (16H, bm, CONHCH₂CH₂NH₂). ¹³C NMR δC(400 MHz; D₂O) 33.64 (NCH₂CH₂CONH), 40.12 (CONHCH₂CH₂NH₂), 42.32 (CONHCH₂CH₂NH₂), 50.10 (NCH₂CH₂CONH), 170.8, 171.12 (NCH₂CH₂CONH).

G3.NH₂

Yellowish gel (20.93 g, 90%). Elemental analysis C₁₄₂H₂₈₈N₅₈O₂₈: Found: C, 52.65; H, 9.05; N, 25.24. Calc.: C, 52.38; H, 8.91; N, 24.95%. ATR-IR ν_{max}/cm⁻¹ 3283 (NH), 1639 (HNC=O), 1546

(HNC=O). ^1H NMR δH (400 MHz; D_2O) 2.26 (32H, brm, $\text{NR}_2\text{CH}_2\text{CH}_2\text{CONH}$), 2.58 (32H, brm, $\text{NR}_2\text{CH}_2\text{CH}_2\text{CONH}$), 2.64 (32H, t, $\text{CONHCH}_2\text{CH}_2\text{NH}_2$), 3.12 (32H, brm, $\text{CONHCH}_2\text{CH}_2\text{NH}_2$). ^{13}C NMR δC (400 MHz; D_2O) 32.23 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 39.54 ($\text{CONHCH}_2\text{CH}_2\text{NH}_2$), 41.02 ($\text{CONHCH}_2\text{CH}_2\text{NH}_2$), 49.67 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 171.42, 171.54, 171.69 ($\text{NCH}_2\text{CH}_2\text{CONH}$).

G4.NH₂

Yellowish gel (16.39 g, 90%). Elemental analysis $\text{C}_{302}\text{H}_{608}\text{N}_{122}\text{O}_{60}$: Found: C, 52.85; H, 9.02; N, 24.93. Calc.: C, 52.50; H, 8.87; N, 24.73%. ATR-IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3270 (NH), 1643 (HNC=O), 1550 (HNC=O). ^1H NMR δH (400 MHz; D_2O) 2.24 (64H, brm, $\text{NR}_2\text{CH}_2\text{CH}_2\text{CONH}$), 2.52 (64H, t, $\text{NR}_2\text{CH}_2\text{CH}_2\text{CONH}$), 2.63 (64H, t, $\text{CONHCH}_2\text{CH}_2\text{NH}_2$), 3.11 (64H, brm, $\text{CONHCH}_2\text{CH}_2\text{NH}_2$). ^{13}C NMR δC (400 MHz; D_2O) 32.02 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 39.43 ($\text{CONHCH}_2\text{CH}_2\text{NH}_2$), 41.89 ($\text{CONHCH}_2\text{CH}_2\text{NH}_2$), 48.22 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 170.44, 170.52, 170.58, 170.67 ($\text{NCH}_2\text{CH}_2\text{CONH}$).

G3.COOH

White powder (0.73 g, 100%). Elemental analysis $\text{C}_{110}\text{H}_{192}\text{N}_{26}\text{O}_{44}$: Found: C, 51.62; H, 7.62; N, 14.26. Calc.: C, 51.15; H, 7.49; N, 14.10%. ATR-IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3292 (COOH), 1650 (HNC=O), 1569 (HNC=O), 1404 (O-H). ^1H NMR δH (400 MHz; D_2O) 2.71 (32H, t, $\text{CH}_2\text{CH}_2\text{COOH}$), 2.89 (32H, m, $\text{CH}_2\text{CH}_2\text{COOH}$). ^{13}C NMR δC (400 MHz; D_2O) 35.31 ($\text{CH}_2\text{CH}_2\text{COOH}$), 47.61 ($\text{CH}_2\text{CH}_2\text{COOH}$), 48.32 ($\text{CH}_2\text{CH}_2\text{NR}_2$), 177.68 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 183.75, 183.81, 184.21, 184.25 (COOH).

G4.COOH

White powder (0.70 g, 100%). Elemental analysis $\text{C}_{238}\text{H}_{416}\text{N}_{58}\text{O}_{92}$: Found: C, 52.45; H, 7.69; N, 14.90. Calc.: C, 51.39; H, 7.54; N, 14.61%. ATR-IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3282 (COOH), 1644 (HNC=O),

1564 (HNC=O), 1403 (O-H). ^1H NMR δH (400 MHz; D_2O) 2.41 (64H, bm, $\text{CH}_2\text{CH}_2\text{COOH}$), 2.76 (64H, m, $\text{CH}_2\text{CH}_2\text{COOH}$). ^{13}C NMR δC (400 MHz; D_2O) 35.19 ($\text{CH}_2\text{CH}_2\text{COOH}$), 47.86 ($\text{CH}_2\text{CH}_2\text{COOH}$), 48.09 ($\text{CH}_2\text{CH}_2\text{NR}_2$), 177.50 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 183.86, 183.98, 184.01, 184.05 (COOH).

G3.TRIS

Opaque oil (2.49 g, 96%). Elemental analysis $\text{C}_{174}\text{H}_{336}\text{N}_{42}\text{O}_{76}$: Found: C, 49.75; H, 8.14; N, 14.10. Calc.: C, 49.37; H, 8.00; N, 13.90%. ATR-IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3263 (COH), 1635 (HNC=O), 1558 (HNC=O), 1392 (O-H). ^1H NMR δH (400 MHz; CD_3OD) 2.56 (32H, bm, $\text{CH}_2\text{CH}_2\text{CONHCR}_3$), 2.76 (32H, bm, $\text{CH}_2\text{CH}_2\text{CONHCR}_3$), 3.57 (96H, bs, CH_2OH). ^{13}C NMR δC (400 MHz; CD_3OD) 32.22 ($\text{CH}_2\text{CH}_2\text{CONHCR}_3$), 48.70 ($\text{CH}_2\text{CH}_2\text{CONHCR}_3$), 49.24 ($\text{CH}_2\text{CH}_2\text{NR}_2$), 56.51 ($\text{CONHCR}_2\text{CH}_2\text{OH}$), 63.26 ($\text{CONHCR}_2\text{CH}_2\text{OH}$), 174.66, 174.82, 180.73, 181.01 ($\text{NCH}_2\text{CH}_2\text{CONH}$).

G4.TRIS

Opaque oil (1.35 g, 93%). Elemental analysis $\text{C}_{366}\text{H}_{704}\text{N}_{90}\text{O}_{156}$: Found: C, 49.98; H, 8.10; N, 14.40. Calc.: C, 49.60; H, 8.01; N, 14.22%. ATR-IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3263 (COH), 1641 (HNC=O), 1558 (HNC=O), 1394 (O-H). ^1H NMR δH (400 MHz; CD_3OD) 2.52 (64H, bm, $\text{CH}_2\text{CH}_2\text{CONHCR}_3$), 2.70 (64H, bm, $\text{CH}_2\text{CH}_2\text{CONHCR}_3$), 3.65 (192H, bs, CH_2OH). ^{13}C NMR δC (400 MHz; CD_3OD) 32.62 ($\text{CH}_2\text{CH}_2\text{CONHCR}_3$), 48.88 ($\text{CH}_2\text{CH}_2\text{CONHCR}_3$), 49.02 ($\text{CH}_2\text{CH}_2\text{NR}_2$), 56.37 ($\text{CONHCR}_2\text{CH}_2\text{OH}$), 63.06 ($\text{CONHCR}_2\text{CH}_2\text{OH}$), 174.62, 174.92, 180.93, 181.21 ($\text{NCH}_2\text{CH}_2\text{CONH}$).

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