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RESEARCH PAPER

New-generation Jeffamine® D230 core amine, TRIS and carboxyl-terminated PAMAM dendrimers: Synthesis, characterization and the solubility application for a model NSAID drug Ibuprofen

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ABSTRACT

Many therapeutically active drugs are poor water soluble and, therefore, bioavailability of these molecules in the living cells is low and a major problem. In this study, new-generation Jeffamine® D230 core, amine (NH_2), Tris(hydroxymethyl) aminomethane (TRIS), and carboxyl (COOH) terminated poly(amidoamine) PAMAM dendrimers (PAMAMs) were synthesized. Synthesized new-generation PAMAMs were characterized by ^{1}H NMR, ^{13}C NMR, ATR-FTIR, and investigated as solubility enhancer of a sample non-steroidal anti-inflammatory drug (NSAID) Ibuprofen (IBU). The effect of generation size (D2-D4), concentration (0-2.0 mM), and surface functional group (NH_2, COOH, TRIS) of the synthesized new-generation PAMAMs on the aqueous solubility of IBU was also investigated. The observed solubility enhancement of IBU was in the order of D4.COOH (18.21 mg/mL)> D3.COOH (13.21 mg/mL)> D4.TRIS (10.30 mg/mL)> D2.COOH (8.55 mg/mL)> D3.TRIS (6.04 mg/mL)> D4.NH_2 (4.56 mg/mL)> D3.NH_2 (3.36 mg/mL)> D2.TRIS (2.42 mg/mL)> D2.NH_2 (1.86 mg/mL). Results showed that synthesized PAMAMs improved the solubility of IBU significantly (30 to 247-fold) with an increasing generation size, and concentration.

Keywords: Dendrimers, poly (amidoamine) PAMAM, Jeffamine, drug carrier, NSAID, ibuprofen

1. Introduction

The therapeutic efficacy of a drug in an appropriate dose might decrease due to its inadequate access to the target site of action in the body. To overcome this problem, drug is usually given high doses to the body to show the desired impact. Disappearance of the remedial effectiveness of drug substances stems from the low solubility of drug pharmaceutical ingredients in the aqueous environment of the human body. Results of the studies conducted in medicinal chemistry have shown that water-soluble derivatives of the chemical formulations of drugs can be prepared successfully; however, even small constructional changes in the structure may lead to the dramatic decreases in their efficacy (1).

Drug carriers are defined as any substances incorporating to improve the delivery and the effectiveness of drugs (2). Various drug carrier systems such as liposomes, micelles, nanoparticles, and nanorods are used to perform the desired release of the drugs having weak solubility and therefore, low bioavailability in the body, and to minimize the interaction of the drugs with the healthy tissues (3). Regarding these systems, the main encountered drawbacks

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are instability within the body, interaction with the healthy tissues, allowing absorption in the kidneys due to nano sizes, and thus, expelling out the blood circulation (4, 5). To avoid these handicaps, dendrimers can take over as potential drug-delivery systems (6).

Among drug carriers systems, poly(amidoamine) (PAMAM) dendrimers (PAMAMs) take an important role not only with their controllable size, structures, and surface functional groups, but also with the presence of large internal cavities, which serve as hosts for many poor soluble active pharmaceutical ingredients (APIs) (7). Core, repeated branches, and type of surface functional groups of PAMAMs are the main factors besides their generation, concentration, and pH media to improve the solubility of drugs. Due to these properties of PAMAMs, they have great advantages in the solubilization and controlled release of many poor soluble pharmaceuticals such as anticancer, antimalarial, antiviral, antitubercular, antimicrobials, non-steroidal anti-inflammatory (NSAIDs) and anti-hypertensive (8-12).

Over the last decades, although PAMAMs have gained much interest in new applications in pharmaceutical chemistry, there still exists reports about the cytoxicity and hemolotic toxicity of amine-terminated PAMAMs (PAMAM-NH_2) (8, 13). To get rid of the mentioned toxicity and gain bio-compatibility, surface modification of cationic PAMAM-NH_2 dendrimers with anionic or neutral functional groups have been presented (14, 15). Results of these studies have shown that carboxyl-terminated PAMAMs (PAMAM-COOH), in particular, have been found to be less toxic than cationic PAMAM-NH_2 dendrimers (16). On the other hand, Twyman et al. (17) were synthesized Tris(hydroxymethyl) aminomethane (TRIS) terminated PAMAMs (PAMAM-TRIS) as an alternative to PAMAM-COOH in order to use them as drug-delivery systems. The result of their study has showed that PAMAM-TRIS dendrimers increased the solubility of small hydrophobic acidic drugs. However, commercial unavailability, laborious synthesis with three to four-day reaction times, and tedious purification steps when DMSO used as solvent limited the potential use and investigation of PAMAM-TRIS dendrimers as drug carriers for a wide spectrum of poor soluble drugs. In our recent study (18), we have developed a fast, easy and one-pot microwave-assisted synthesis method for the synthesis of PAMAM-TRIS dendrimers. During synthesis, higher product yields (90-96%) and short reaction times (110-140 min) were observed under mild reaction conditions using methanol as solvent. Therefore, higher opportunity of using of PAMAM-TRIS dendrimers as potential drug-delivery systems for the future studies and clinical applications has been provided (18). Here, we focus on using neutral PAMAM-TRIS dendrimers as potential drug carriers for NSAIDs by using Ibuprofen (IBU) as a sample model drug, and comparing their solubilization properties with cationic PAMAM-NH_2 and anionic PAMAM-COOH dendrimers.

In the current study, new-generation Jeffamine® D230 (**D**) core NH_2, TRIS, and COOH-terminated D2-D4 PAMAMs were synthesized. Synthesized new types of PAMAMs were characterized by ^{1}H NMR, ^{13}C NMR, ATR-FTIR, spectroscopic titrations, and investigated solubility enhancer of a sample NSAIDs group member drug IBU. On the basis of this aim, the effect of concentration, generation size (D2-D4), and surface functional group (NH_2, COOH, TRIS) of Jeffamine® D230 (**D**) cored PAMAMs on the aqueous solubility of IBU (Fig. 1) was also in investigated.

2. Materials and methods

2.1. Materials and apparatus

Jeffamine® D230 (**D**), methyl acrylate, EDA, TRIS, methanol, n-butanol, NaOH, $CuSO_4$ and IBU were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals were of analytical grade and used without further purification. All solutions were prepared by using Milli-Q deionized water (Merk Millipore Corporation, Billerica, Massachusetts, USA). The ATR-FTIR spectra (4000–400 cm^{-1}) were recorded with a Bruker spectrometer (Ettlingen, Germany). UV-Vis spectra were recorded with PG TG 70 ((Leicestershire, UK). NMR measurements were performed by using a Bruker 500 MHz spectrometer (Ettlingen, Germany). Synthesis System, model Discover (CEM Corporation, North Carolina, USA) with a continuous microwave power delivery system with operator selectable power output from 0 to 300 W (±30 W) programmable in 1-watt increments, infrared temperature control system programmable from 25 to 250 °C, and 5 to 125 mL vessel capacity was used as microwave reactor. Spectroscopic titrations were carried out automatically by using TitroLine® 7000 ((SI Analytics GmbH, Hattenbergstrabe, Germany)) autotitrator equipped with thermostated titration vessel under nitrogen media.

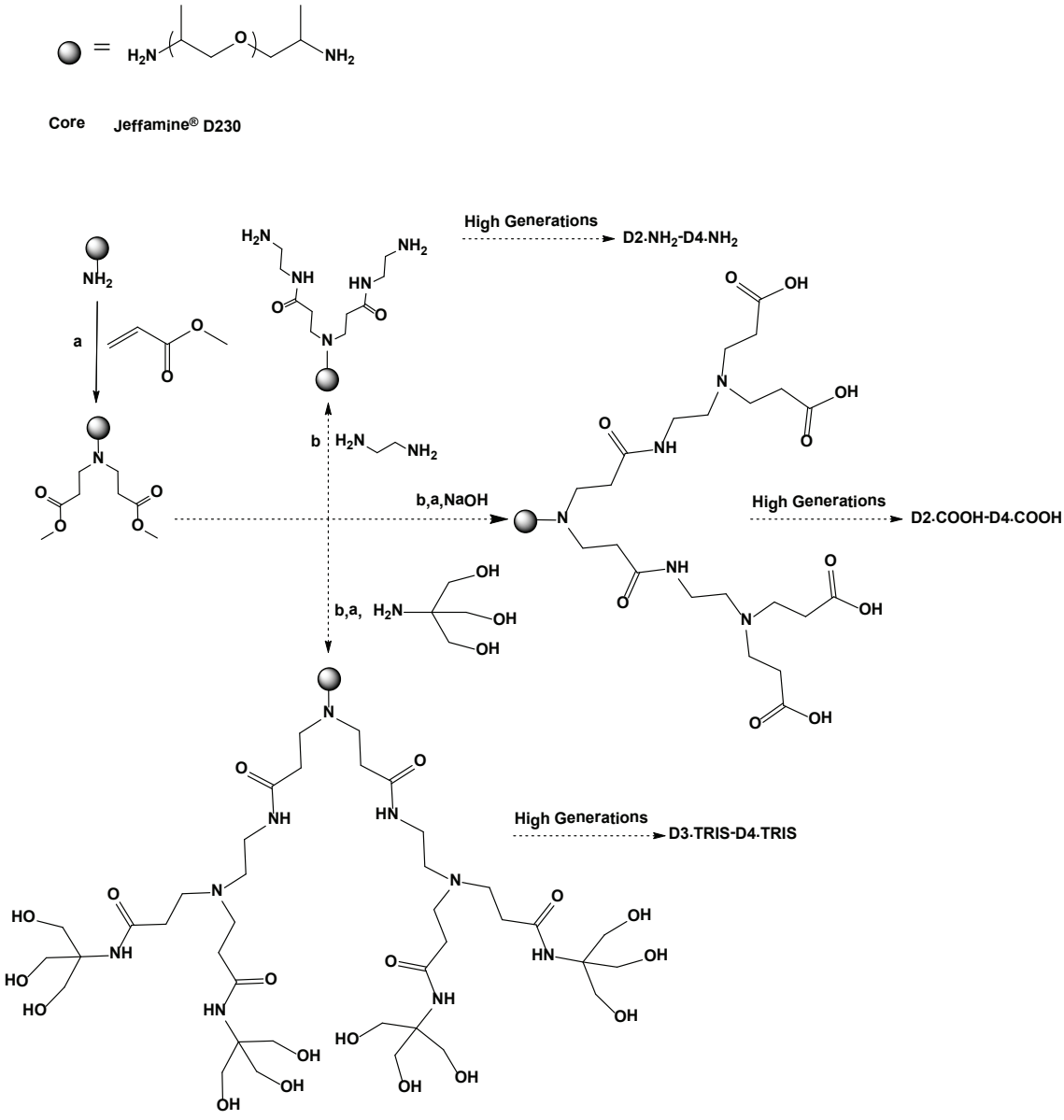
2.2. Synthesis of PAMAMs

Microwave-assisted synthesis of PAMAM-NH_2 (D2.NH_2-D4.NH_2) and PAMAM-TRIS (D2.TRIS-D4.TRIS) dendrimers with (**D**) core, from half generation PAMAM-OCH_3

dendrimers were performed according to our previous studies (19) and (18), respectively. On the other hand, half generation ester terminated PAMAMs (PAMAM-OCH_3) (D1.5 OCH_3-D3.5.OCH_3) were converted to PAMAM-COOH dendrimers (D2.COOH-D4.COOH) by using the slight modification of the literature procedure (20) and our recent study (13) (Scheme 1). Purity of the synthesized new type of PAMAMs was characterized by ATR-FTIR, ^{1}H NMR, ^{13}C NMR and, supported by spectroscopic titrations. Some physico-chemical properties of the synthesized PAMAMs were presented in Table 1.

2.2.1. General procedure for the microwave-assisted synthesis PAMAM-NH_2 dendrimers

Jeffamine® D230 (**D**) cored PAMAM-NH_2 dendrimers were synthesized according to our previous study (19), and briefly summarized hereafter. This method involves the alkylation (a) and amidation (b) steps (Scheme 1). In the alkylation steps, a methanolic solution of methyl acrylate (2.5 M eq. per terminal amine) was added over to a stirring methanolic solution of dendrimer core (***D***) Jeffamine® D230 (**D**) or full generation PAMAM-NH_2 dendrimers (***Dn.NH***$_2$). After



Scheme 1. Synthetic routes for higher generation PAMAM-NH_2, PAMAM-TRIS and PAMAM-COOH dendrimer synthesis.

stirring the resulting reaction mixture for 24 h at room temperature, excess reagents and solvents were removed under vacuum at 65 °C and purified by means of LPR. The resulting PAMAM-OCH_3 dendrimers (***Dn.5.OCH***$_3$) D0.5.OCH_3-D3.5.OCH_3 were colorless oils and product yields were between 95-98% (Table 2).

D0.5. OCH_3

Yellowish gel (12.22 g, 96%). ATR-IR ν_{max}/cm^{-1} 1739 (C=O). ^{1}H-NMR δH(500 MHz; $CDCl_3$) 2.38 (8H, t, $CH_2CH_2COOCH_3$), 2.75 (8H, m, $CH_2CH_2COOCH_3$), 3.25 (4H, m, $OCH_2CH_2NR_2$), 3.62 (12H, s, $COOCH_3$). ^{13}C-NMR δC(125 MHz; $CDCl_3$) 173.05 ($COOCH_3$), 77.36, 77.10, 76.85 (OCH_2CH_2O), 51.36 ($COOCH_3$), 46.34 ($CH_2CH_2COOCH_3$), 34.46 ($CH_2CH_2COOCH_3$).

D1.5. OCH_3

Yellowish gel (16.55 g, 95%). ATR-IR ν_{max}/cm^{-1} 3331 (NH), 1731 (C=O), 1647 (HNC=O), 1545 (HNC=O). ^{1}H-NMR δH(500 MHz; $CDCl_3$) 2.34 (16H, m, $CH_2CH_2COOCH_3$), 2.43 (16H, m, $CH_2CH_2COOCH_3$), 2.67 (8H, m, $CONHCH_2CH_2NR_2$), 3.57 (24H, s, $COOCH_3$). ^{13}C-NMR δC(125 MHz; $CDCl_3$) 172.90 ($COOCH_3$), 172.35 (NCH_2CH_2CONH), 51.52 ($COOCH_3$), 49.17 ($CH_2CH_2COOCH_3$), 32.59 ($CH_2CH_2COOCH_3$).

D2.5. OCH_3

Yellowish gel (17.91 g, 96%). ATR-IR ν_{max}/cm^{-1} 3305 (NH), 1732 (C=O), 1645 (HNC=O), 1538 (HNC=O). ^{1}H-NMR δH(500 MHz; $CDCl_3$) 2.28 (32H, m, $CH_2CH_2COOCH_3$), 2.46 (32H, m, $CH_2CH_2COOCH_3$), 2.71 (16H, m, $CONHCH_2CH_2NR_2$), 3.57 (48H, s, $COOCH_3$). ^{13}C-NMR δC(125 MHz; $CDCl_3$) 172.95 ($COOCH_3$), 172.06 (NCH_2CH_2CONH), 51.58 ($COOCH_3$), 49.19 ($CH_2CH_2COOCH_3$), 32.62 ($CH_2CH_2COOCH_3$).

D3.5.OCH_3

Yellowish gel (10.72 g, 98%). ATR-FTIR ν_{max}/cm^{-1} 3294 (NH), 1731 (C=O), 1644 (HNC=O), 1537 (HNC=O). ^{1}H-NMR δH(500 MHz; $CDCl_3$) 2.29 (64H, m, $CH_2CH_2COOCH_3$), 2.48 (64H, m, $CH_2CH_2COOCH_3$), 2.70 (32H, m, $CONHCH_2CH_2NR_2$), 3.61 (96H, s, $COOCH_3$).

Table 1. Some physico-chemical properties of the synthesized PAMAMs (See also Scheme 1).

Dendrimer	Mw[a] (g/mol)	Number of tertiary amines (NR_3)	Number of terminal amines (NH_2)	Number of terminal hydroxyls (OH)	Number of terminal carboxyl (COOH)
NH_2-terminated					
D2.NH_2	1600	6	8	-	-
D3.NH_2	3427	14	16	-	-
D4.NH_2	7081	30	32	-	-
TRIS-terminated					
D2.TRIS	2088	6	-	24	-
D3.TRIS	4403	14	-	48	-
D4.TRIS	9034	30	-	96	-
Carboxyl-terminated					
D2.COOH	1263	6	-	-	8
D3.COOH	2753	14	-	-	16
D4.COOH	5734	30	-	-	32

[a]Molecular weight

Table 2. Preparition of Jeffamine® D230 core ester-terminated PAMAM dendrimers (**Dn.5**).

PAMAM-OCH_3 dendrimers	R-NH_2 g (mmol)	MA g, (mmol)	MeOH (mL)	Time (h)	Yield (%)
D0.5.OCH_3	5.1 (22.17)	9.54 (110)	30	24	96
D1.5.OCH_3	8.7 (12.66)	10.90 (126)	30	24	95
D2.5.OCH_3	10.03 (6.26)	10.79 (125)	30	24	96
D3.5.OCH_3	6.07 (1.77)	6.09 (70)	30	24	98

^{13}C-NMR δC(125 MHz; $CDCl_3$) 173.02 ($COOCH_3$), 172.05 (NCH_2CH_2CONH), 51.59 ($COOCH_3$), 49.19 ($CH_2CH_2COOCH_3$), 32.62 ($CH_2CH_2COOCH_3$).

In the amidation steps, a 1-5 mL methanolic solution of ethylenediamine (EDA) involving 10 M eq. of excess EDA per ester branched PAMAM-OCH_3 dendrimers were added to a vigorously stirring methanolic solution of half generation PAMAM-OCH_3 dendrimers (Cn.5). The final mixture was irradiated with microwave (MW) at 250 W for 80 min. Final traces of EDA were removed under vacuum below a bath temperature of 65 °C by the azeotrapic mixture of n-butanol three times. The resulting product was purified by using LPR. The final products were full generation PAMAM-NH_2 dendrimers (***Dn.NH***$_2$) D1.NH_2-D4.NH_2. Product yields were in the range of 90-97% (Table 3).

D1.NH$_2$

Yellowish gel (11.81 g, 97%). ATR-IR ν_{max}/cm^{-1} 3286 (NH), 1642 (HNC=O), 1553 (HNC=O). ^{1}H-NMR δH(500 MHz; DMSO-d_6) 2.20 (8H, m, CH_2CH_2CONH), 2.56 (16H, m, CH_2CH_2CONH), 2.80 (8H, s, $CONHCH_2CH_2NH_2$), 3.04 (8H, t, $CONHCH_2CH_2NH_2$), 8.09 (4H, s, NCH_2CH_2CONH). ^{13}C-NMR δC(125 MHz; DMSO-d_6) 171.51 (NCH_2CH_2CONH), 74.38, 74.16, 74.05 (OCH_2CH_2O), 45.43 (NCH_2CH_2CONH), 43.05 ($CONHCH_2CH_2NH_2$), 41.83 ($CONHCH_2CH_2NH_2$), 35.91 (NCH_2CH_2CONH).

D2.NH$_2$

Yellowish gel (13.23 g, 90%). ATR-IR ν_{max}/cm^{-1} 3281 (NH), 1644 (HNC=O), 1550 (HNC=O). ^{1}H-NMR δH(500 MHz; DMSO-d_6) 2.21 (16H, m, CH_2CH_2CONH), 2.57 (16H, m, CH_2CH_2CONH), 2.68 (16H, m, $CONHCH_2CH_2NH_2$), 3.03 (16H, m, $CONHCH_2CH_2NH_2$), 8.02 (8H, s, NCH_2CH_2CONH). ^{13}C-NMR δC(125 MHz; DMSO-d_6) 171.49 (NCH_2CH_2CONH), 45.48 (NCH_2CH_2CONH), 43.02 ($CONHCH_2CH_2NH_2$), 41.89 ($CONHCH_2CH_2NH_2$), 35.96 (NCH_2CH_2CONH).

D3.NH$_2$

Yellowish gel (13.42 g, 90%). ATR-IR ν_{max}/cm^{-1} 3271 (NH), 1654 (HNC=O), 1566 (HNC=O). ^{1}H-NMR δH(500 MHz; D_2O) 2.25 (32H, m, CH_2CH_2CONH), 2.58 (32H, m, CH_2CH_2CONH), 2.64 (32H, m, $CONHCH_2CH_2NH_2$), 3.13 (32H, m, $CONHCH_2CH_2NH_2$). ^{13}C-NMR δC(125 MHz; D_2O) 171.42 (NCH_2CH_2CONH), 45.54 (NCH_2CH_2CONH), 43.06 ($CONHCH_2CH_2NH_2$), 41.83 ($CONHCH_2CH_2NH_2$), 35.91 (NCH_2CH_2CONH).

D4.NH$_2$

Yellowish gel (4.81 g, 93%). ATR-IR ν_{max}/cm^{-1} 3275 (NH), 1634 (HNC=O), 1548 (HNC=O). ^{1}H-NMR δH(500 MHz; D_2O) 2.25 (64H, m, CH_2CH_2CONH), 2.57 (64H, m, CH_2CH_2CONH), 2.64 (64H, m, $CONHCH_2CH_2NH_2$), 3.12 (64H, m, $CONHCH_2CH_2NH_2$). ^{13}C-NMR δC(125 MHz; D_2O) 174.90 (NCH_2CH_2CONH), 47.04 (NCH_2CH_2CONH), 44.38 ($CONHCH_2CH_2NH_2$), 41.22 ($CONHCH_2CH_2NH_2$), 35.87 (NCH_2CH_2CONH).

2.2.2. General procedure for the microwave-assisted synthesis of PAMAM-TRIS dendrimers

PAMAM-TRIS dendrimers were prepared according to procedure in our recent study (18), and briefly summarized hereafter. A methanolic solution of PAMAM–OCH_3 dendrimers (**D1.5.OCH$_3$-D3.5.OCH$_3$**) were added to a stirred suspension of TRIS (1.2 M equiv. per terminal ester) and anhydrous K_2CO_3 (1.5 M equiv. per terminal ester) in 10–15 mL of MeOH. The following mixture was irradiated at 200 W for 120–130 min by refluxing at a bulk temperature of 70–90 °C. The final reaction mixture was filtered to remove excess solid reagents, and the filtrate collected. Then product

Table 3. Preparition of Jeffamine® D230 core amine-terminated PAMAM dendrimers (**Dn.NH$_2$**).

PAMAM-NH_2 dendrimers	R-OCH_3 g (mmol)	EDA g (mol)	MeOH[a] (mL)	MW (watt)	Time (min)	Yield (%)
D1.NH$_2$	10.19 (17.74)	42.65 (0.79)	5.0	200	80	97
D2.NH$_2$	12.64 (9.19)	44.18 (0.73)	5.0	200	80	90
D3.NH$_2$	12.68 (4.25)	40.94 (0.68)	5.0	200	80	90
D4.NH$_2$	4.53 (0.73)	14.09 (0.23)	5.0	200	80	93

was purified with the LPR method. The final methanolic solution of retained product (***Dn.TRIS***) was concentrated under vacuum below a bath temperature of 65 °C, The resulting products were D2.TRIS-D4.TRIS, and yields were in the range of 92–95% (Table 4).

D2.TRIS

Opaque oil (1.27 g, 94%). ATR-IR ν_{max}/cm^{-1} 3272 (COH), 1645 (HNC=O), 1567 (HNC=O), 1395 (O-H). ^{1}H NMR δH(500 MHz; D_2O) 2.57 (16H, m, $CH_2CH_2CONHCR_3$), 2.72 (16H, m, $CH_2CH_2CONHCR_3$), 3.67 (48H, s, CH_2OH). ^{13}C NMR δC(125 MHz; D_2O) 181.12, 174.62, 174.46 (NCH_2CH_2CONH), 62.06 ($CONHCR_2CH_2OH$), 56.43 ($CONHCR_2CH_2OH$), 50.32 ($CH_2CH_2CONHCR_3$), 33.21 ($CH_2CH_2CONHCR_3$).

D3.TRIS

Opaque oil (1.29 g, 95%). ATR-IR ν_{max}/cm^{-1} 3288 (COH), 1647 (HNC=O), 1569 (HNC=O), 1396 (O-H). ^{1}H NMR δH(500 MHz; D_2O) 2.58 (32H, m, $CH_2CH_2CONHCR_3$), 2.79 (32H, m, $CH_2CH_2CONHCR_3$), 3.73 (96H, s, CH_2OH). ^{13}C NMR δC(125 MHz; D_2O) 181.01, 174.82, 174.66 (NCH_2CH_2CONH), 62.26 ($CONHCR_2CH_2OH$), 56.51 ($CONHCR_2CH_2OH$), 50.70 ($CH_2CH_2CONHCR_3$), 33.34 ($CH_2CH_2CONHCR_3$).

D4.TRIS

Opaque oil (1.24 g, 92%). ATR-FTIR ν_{max}/cm^{-1} 3292 (COH), 1649 (HNC=O), 1571 (HNC=O), 1396 (O-H). ^{1}H NMR δH(500 MHz; D_2O) 2.55 (64H, m, $CH_2CH_2CONHCR_3$), 2.76 (64H, m, $CH_2CH_2CONHCR_3$), 3.70 (192H, s, CH_2OH). ^{13}C NMR δC(125 MHz; D_2O) 181.92, 174.49, 174.23 (NCH_2CH_2CONH), 62.53 ($CONHCR_2CH_2OH$), 56.94 ($CONHCR_2CH_2OH$), 50.41 ($CH_2CH_2CONHCR_3$), 33.15 ($CH_2CH_2CONHCR_3$).

2.3.3. General procedure for the synthesis of PAMAM-COOH dendrimers

PAMAM-COOH dendrimers were prepared by slight modification of literature procedure (20) and our previous recent study (13). A methanolic solution of ester-terminated half generation PAMAM-OCH_3 (***Dn.OCH$_3$***) 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. The resulting products were D2.COOH-D4.COOH, and yields were 100% (Table 5.)

D2.COOH

White solid (0,54 g, 100%). ATR-IR ν_{max}/cm^{-1} 3299 (COOH), 1647 (HNC=O), 1569 (HNC=O), 1402 (O-H). ^{1}H NMR

Table 4. Preparition of Jeffamine® D230 core TRIS-terminated PAMAM dendrimers (**Dn.TRIS**).

PAMAM-TRIS dendrimers	R-OCH_3 g (mmol)	TRIS g (mmol)	K_2CO_3 g (mmol)	MeOH (mL)	MW (watt)	Time (min)	Yield (%)
D2.TRIS	0.9 (0.65)	0.75 (6.2)	1.1 (7.97)	10.0	200	130	94
D3.TRIS	0.94 (0.31)	0.72 5.95)	1.02 (7.39)	10.0	200	150	95
D4.TRIS	0.98 (0.15)	0.69 (5.7)	0.99 (7.17)	10.0	200	140	92

Table 5. Preparition of Jeffamine® D230 core carboxyl-terminated PAMAM dendrimers (**Dn.COOH**).

PAMAM COOH Dendrimers	R-OCH_3 g (mmol)	NaOH g (mmol)	MeOH (mL)	Time (h)	Yield (%)
D2.COOH	0.64 (0.43)	0.2 (5.15)	5.0	24	100
D3.COOH	0.44 (0.15)	0.14 (3.6)	4.0	24	100
D4.COOH	0.88 (0.14)	0.27 (6.87)	4.0	24	100

δH(500 MHz; D_2O) 2.29 (16H, bm, CH_2CH_2COOH), 2.56 (16H, bm, CH_2CH_2COOH). ^{13}C NMR δC(125 MHz; D_2O) 181.21, 181.06 (*C*OOH), 174.38 (NCH_2CH_2CONH), 48.94 (CH_2CH_2COOH), 34.03 (CH_2CH_2COOH).

D3.COOH

White solid (0,41 g, 100%). ATR-IR ν_{max}/cm^{-1} 3309 (COOH), 1650 (HNC=O), 1573 (HNC=O), 1405 (O-H). ^1H NMR δH(500 MHz; D_2O) 2.23 (32H, bm, CH_2CH_2COOH), 2.59 (32H, m, CH_2CH_2COOH). ^{13}C NMR δC(125 MHz; D_2O) 181.23, 181.17 (*C*OOH), 174.64 (NCH_2CH_2CONH), 48.79 (CH_2CH_2COOH), 34.12 (CH_2CH_2COOH).

D4.COOH

White powder (0.8 g, 100%). ATR-FTIR ν_{max}/cm^{-1} 3305 (COOH), 1641 (HNC=O), 1566 (HNC=O), 1398 (O-H). ^1H NMR δH(500 MHz; D_2O) 2.28 (64H, bm, CH_2CH_2COOH), 2.68 (64H, bm, CH_2CH_2COOH). ^{13}C NMR δC(125 MHz; D_2O) 181.01, 180.68 (*C*OOH), 174.68 (NCH_2CH_2CONH), 48.86 (CH_2CH_2COOH), 33.86 (CH_2CH_2COOH).

2.3. The spectroscopic titrations of PAMAM-COOH and PAMAM-TRIS dendrimers with Cu^{2+} ions

Spectrocopic 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 (18). In summary, Table 6 presents the concentrations of dendrimer and $CuSO_4$ solutions used in the spectroscopic titration.

2.4. Preparation and characterization of drug-PAMAM dendrimer complexes

2.4.1. Preparation of drug-PAMAM dendrimer complexes

Preparation of IBU-PAMAM dendrimer complexes were performed according to literature procedures (21, 22) and mentioned hereafter briefly. The initial molar rations of IBU to dendrimer were 1:1. The reaction mixtures were stirred at 250 rpm and 37 °C for 24 h. Then, a rotary evaporator was used to remove methanol. The precipitates were dried under vacuum to remove the methanol completely, followed by the addition of deionized water and stirred for another 2 h to extract out drug-dendrimer complex. This solution then filtered through the 0.45 μm and lyophilized to remove the water completely. Resulting drug-dendrimer complex was subjected to ATR-FTIR (4000-600 cm^{-1}) and UV-Vis (230-400 nm) analysis.

2.4.2.. UV-Vis characterization

IBU-PAMAM dendrimer complexes were diluted properly with methanol to the final concentration of 10 μg/mL. The UV spectra of these complexes were collected between the wavelength ranges of 210-400 nm. λ_{max} = 221 nm was observed to be the maximum absorption band. Any shift

Table 6. Concentrations used to titrate aqueous solutions of TRIS and COOH terminated PAMAMs with Cu^{2+} ions.

Type of Dendrimer	Dendrimer conc. (mM)	$CuSO_4$ conc. (mM)	Increment of $CuSO_4$ (μL)
D4.TRIS	0.301	66.25	630.00
D4.COOH	0.320	66.25	697.00

Table 7. Number of tertiary amine groups on PAMAM-TRIS and PAMAM-COOH dendrimers available for binding with Cu^{2+} ions[a]

Type of Dendrimer	^3N theoretical value	^3N practical value	% Correlation
D4.TRIS	30	28.55 ± 0.78	95.16
D4.COOH	30	28.92 ± 0.69	96.40

[a]Results were calculated from potentiometric titrations for five repeated experiments, and presented as mean ± confidence intervals.

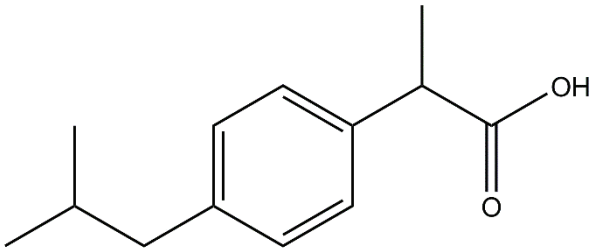


Figure 1. Molecular structure and some physicochemical properties of IBU: λmax = 221 nm, pKa = 4.85 Mw = 206.3 g/mol

or suppression in this strong band was assigned to complex formation (21-24).

2.4.3. ATR-FTIR spectral studies

PAMAM-COOH dendrimer, IBU, and IBU-PAMAM drug-dendrimer complexes were subjected to ATR-FTIR. The FTIR spectra were recorded after 20 scans at 4 cm^{-1} in a wavenumber range of 4000-600 cm^{-1}.

2.5. Solubility tests of drugs

The solubility of drugs was determined by using the rotating bottle technique of Higuchi and Connors (25). 5 mL aqueous solutions of PAMAMs with different generation size (D2-D4), surface functional groups (NH_2, COOH, TRIS), and molar concentration (0-2 mM) were prepared. Then, excess amounts of drugs were added to each PAMAM dendrimer solution. The resulting suspensions were shaken at 250 rpm and 37 °C for 24 h. Then, the solutions were centrifuged at 6000 rpm for five minutes, and insoluble excess drugs was removed by filtering through 0.45 μm cellulose acetate filter. The absorbances of the test solutions and PAMAMs were measured by PG T-70 UV-Vis spectrophotometer in the characteristic wavelength (λ_{max} = 221 nm for IBU). Absorbances of PAMAMs were subtracted from drug-dendrimer test solution absorbances. The absorbances were

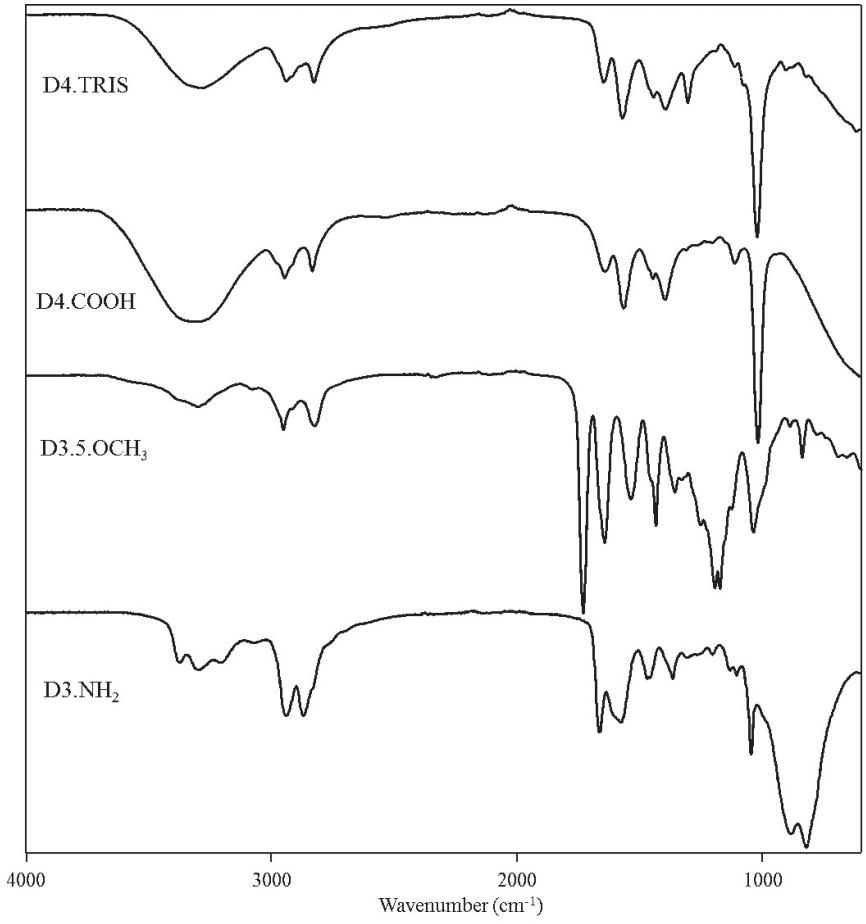


Figure 2. Sample representative ATR-FTIR spectra of D3.NH_2, D3.5.OCH_3, D4.COOH D4.TRIS PAMAM dendrimers.

correlated with the calibration curve prepared for drugs and the amount of drugs were determined. Three repeated measurements were conducted.

3. Results and discussion

3.1. Characterization of PAMAMs

Conversion of PAMAM-OCH_3 dendrimers to PAMAM-NH_2, PAMAM-COOH and PAMAM-TRIS dendrimers were followed by ATR-FTIR, ^{1}H NMR, and ^{13}C NMR spectra. In the ATR-FTIR characterization, replacement of amide I (~1640 cm^{-1}) and amide II (~1540 cm^{-1}) peaks, and disappearance of ~1730 cm^{-1} peak originating from the ester functional groups of PAMAM-OCH_3 dendrimers evidenced to the fully conversion of PAMAM-OCH_3 dendrimers to PAMAM-NH_2 dendrimers (Fig.2) . Similar observation were obtained for PAMAM-OCH_3 to PAMAM-COOH and PAMAM-TRIS dendrimer conversions. Moreover formation of new band at 3500-3100 cm^{-1} was assigned as OH part of carboxylic acid and TRIS, which could readily make H-Bond resulting broadening the spectrum (Fig. 2).

Fig. 3 shows a representative sample NMR monitoring spectra of the conversion of D3.5 to D4. COOH and D4.TRIS dendrimers as an example to PAMAM-OCH_3 to PAMAM-COOH and PAMAM-TRIS dendrimers. Investigation of the Fig. 3 reveals that methyl ester peak of D3.5.OCH_3 at 3.61 ppm cannot be observed in the spectra of D4.COOH and D4.TRIS, and confirms the complete conversion of ester groups to acids (D4.COOH, middle in Fig. 3). In addition, formation of a new singlet peak of D4.TRIS at 3.70 ppm affirms the full modification of the ester functional groups to TRIS (D4.TRIS, top in Fig. 3). On the other hand, the fully disappearance of the methy ester peak of D3.5 at 51.59 ppm during the synthesis of D4.COOH and D4.TRIS was observed. Instead, a new peak at 56.94 ppm ($NHCR_3$) and 62.53 pmm (-CH_2OH) were appeared at TRIS conversions. This was also an indication of good purity. In addition, the signals at 181.92 from exterior amides (C=O), and 174.23, 174.49 ppm from interior amides (C=O) correspond to TRIS, and the interior amides prove the complete conversion of D3.5 to D4.TRIS. Moreover, formation of the 181.01, 180.68, 174.68 ppm corresponding to acids and interior amides proves the fully conversion of D3.5.OCH_3 to D4.COOH. Thus, ^{1}H NMR and ^{13}C NMR spectroscopy evaluations prove good purity. Similar results were observed in the NMR monitoring of the all conversions from PAMAM-OCH_3 dendrimers to synthesized PAMAM-COOH and PAMAM-TRIS dendrimers.

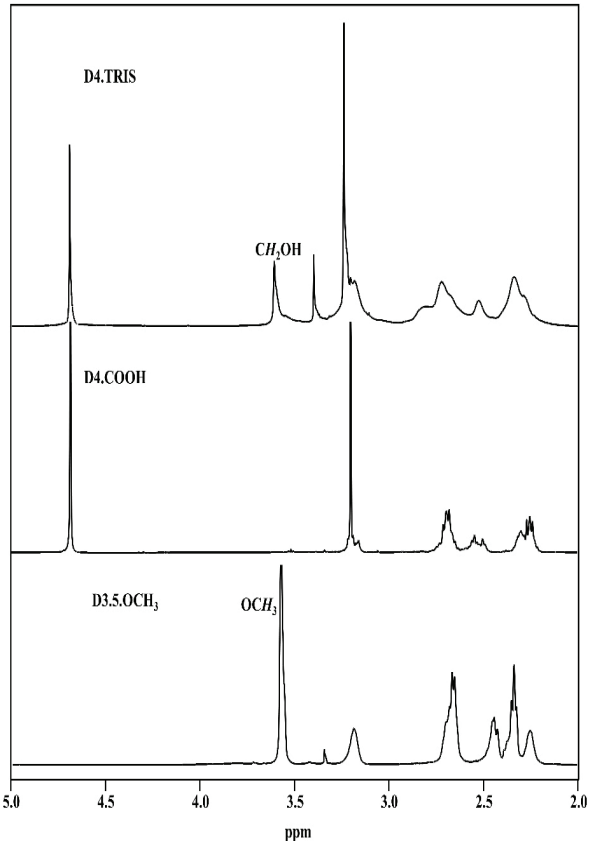


Figure 3. ^{1}H NMR spectrum monitoring of the conversion of Jeffamine core PAMAMs (D3.5.OCH_3 in $CDCI_3$, D4.COOH and D4.TRIS in MeOD).

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

3.3. Spectroscopic characterization of IBU-PAMAM dendrimer complexes

Spectroscopic titration experiments were conducted according to our recent study (18) by simply adapting from Crooks et. al former studies (26, 27). The results of these studies showed that Cu^{2+} ions coordinate with a maximum number of four tertiary amines. In similar, our aim in this study was to show the ideal growth of the synthesized PAMAM dendrimers and monodispersity by calculating the exact number of tertiary amines present at D4.TRIS and D4.COOH with the aid of spectroscopic titrations. After addition of Cu^{2+} ions at the specific increments (Table 6) to the dendrimer solutions, a sudden change in color to deep blue was observed. The intensity of this color was dependent on Cu(II)-PAMAM-COOH and Cu(II)-PAMAM-TRIS dendrimers complexes of D4.COOH and D4.TRIS characterized with a broad absorption

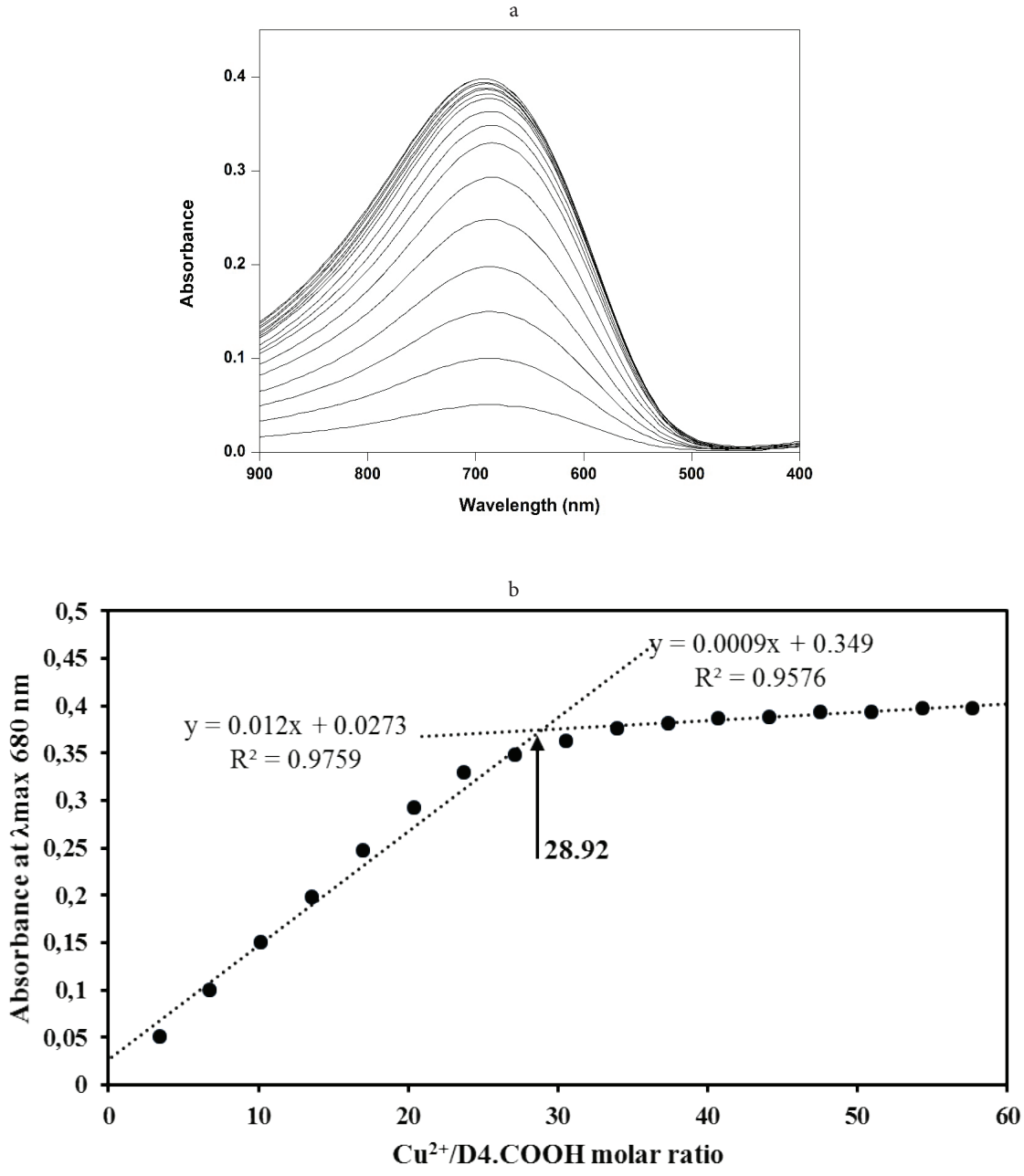


Figure 4. Absorption spectra of PAMAM-COOH dendrimers at 680 nm (a) D4.COOH (0.320 mM) solution titrated with Cu^{2+} (66.25 mM), and (b) spectroscopic titration curve of D4.COOH.

band at λ_{max} 680 nm in UV-Vis spectrophotometer (Fig. 4 and Fig. 5). This band (copper *d-d* transition) is the indication of complexation between Cu(II), and the tertiary amine groups of dendrimers at 1:4 molar ratio and coordination (28). The maximum molar excess of Cu^{2+} that can be loaded onto PAMAM–TRIS and PAMAM-COOH dendrimers were determined from absorbance at λ_{max} 680 nm versus Cu^{2+}/PAMAM–TRIS and Cu^{2+}/PAMAM-COOH dendrimers molar ratio plots (Fig. 4 and Fig. 5).

Table 7 presents the theoretical number of tertiary amines (^3N) present in PAMAM-TRIS and PAMAM-COOH dendrimers (D4.TRIS and D4.COOH) and the experimental found numbers. The results showed that there exist a good correlation between theoretical and practical number of tertiary amine numbers in PAMAM-TRIS and PAMAM-COOH dendrimers. These results are also evidence for the purity, monodispersity therefore ideal growth of synthesized

PAMAM dendrimers with no such kind of impurity like trailing generations.

3.3.1. UV-Vis characterization

Fig. 6 shows the UV-Vis spectra of IBU, PAMAM dendrimer, IBU-PAMAM dendrimer complexes in methanol. The λ_{max} of IBU is 221 nm and PAMAMs is ~280 nm. The suppression of the 221 nm band in the spectrum of alone and IBU-PAMAM dendrimer complexes indicates that the bonding between among dendrimer-drug molecules could be attributed to non-covalent interactions or entrapping of drug molecules to the cavity of dendrimers (29).

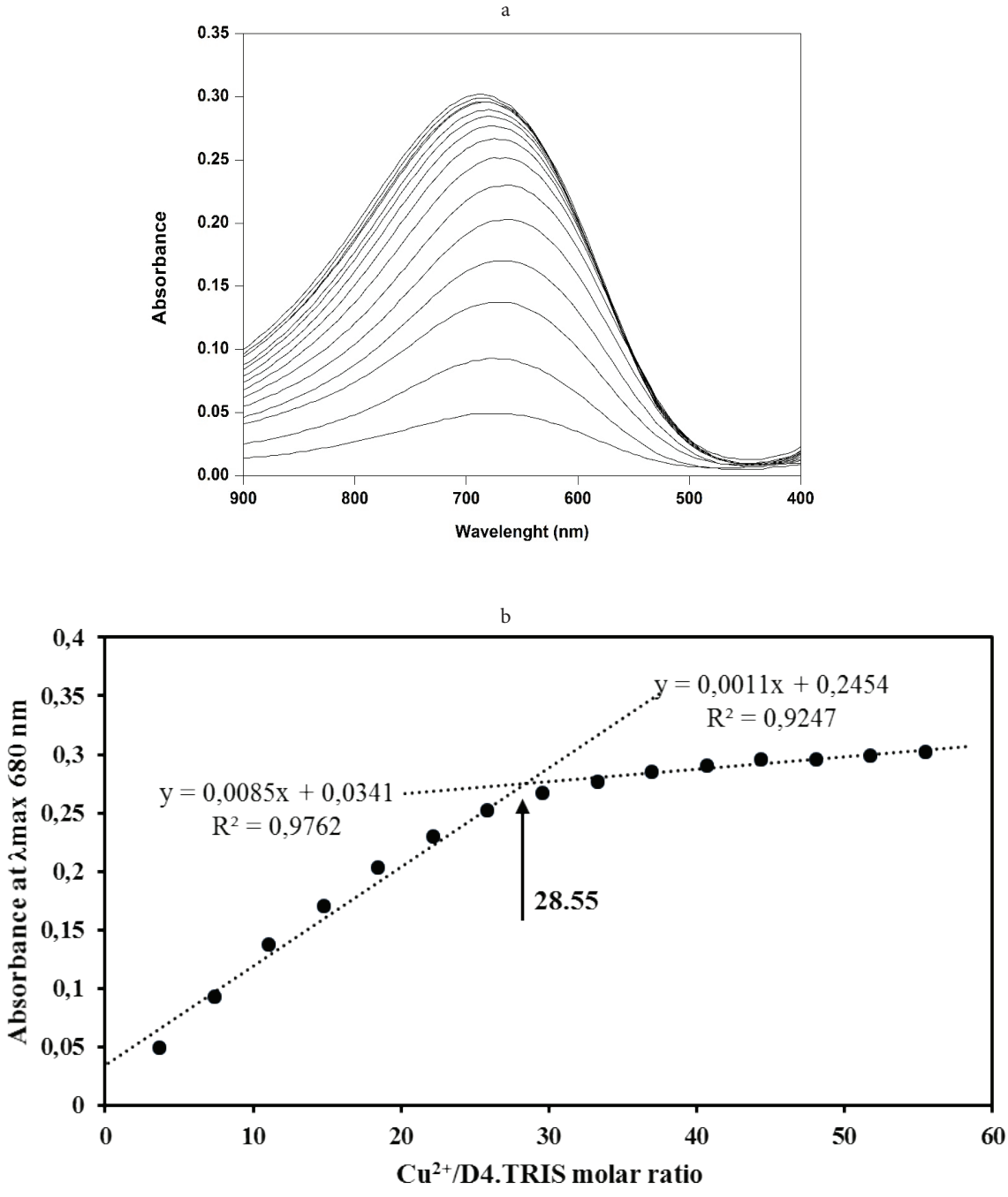


Figure 5. Absorption spectra of PAMAM-TRIS dendrimers at 680 nm (a) D4.TRIS (0.301 mM) solution titrated with Cu^{2+} (66.25 mM), and (b) spectroscopic titration curve of D4.TRIS.

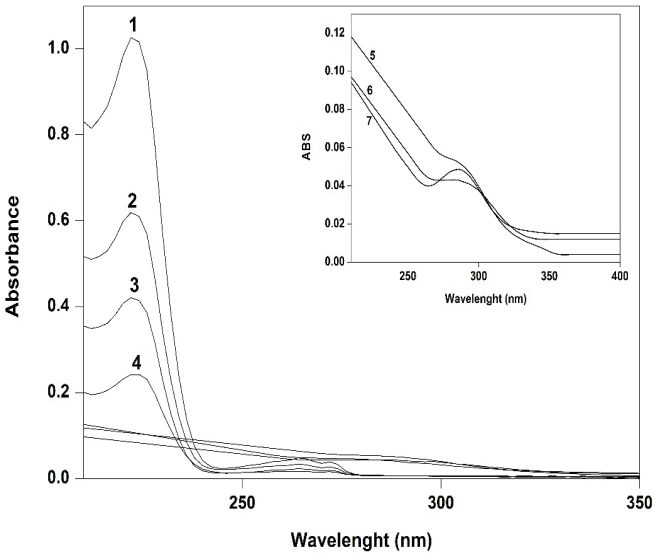


Figure 6. UV-spectra of (1) IBU (10 µg/mL); (2) IBU-D4.COOH complex (10 µg/mL); (3) IBU-D4.TRIS complex (10 µg/mL); (4) IBU-D4.NH_2 complex (10 µg/mL); (5) D4.TRIS (25 µM); (6) 25 µM D4.NH_2 (25 µM); (7) D4.COOH (25 µM).

3.3.2. ATR-FTIR characterization

ATR-FTIR spectra of the IBU, D4.COOH, and IBU-D4.COOH complex were recorded between 4000-600 cm^{-1}, and presented in Fig. 7. Characteristic bands of IBU were found at 2954 and 2867 cm^{-1} (C-H strecthing); 1704 cm^{-1} (C=O stretching); 1429 (C-H strecthing) and 1230 cm^{-1} (C-O stretching). The ATR-FTIR spectrum of pure D4.COOH showed about at 3270 cm^{-1}, 3000-2800 cm^{-1}, 1645 cm^{-1}, 1560 cm^{-1}, 1402 cm^{-1}, and at 1031 cm^{-1} corresponding to the vibrations of O-H stretching, C-H stretching, C=O stretching, N-H bending, C-O stretching, and C-O stretch groups, respectively. After complexation of PAMAMs with IBU, spectral shifts were observed for the dendrimer characteristic bands at between 0-15 cm^{-1} because of the hydrophilic interactions with dendrimer polar groups

3.4. Effect of dendrimer concentration and generation on the solubility of IBU

The effect of dendrimer concentration and generation size on the aqueous solubility of IBU was studied by using (**D**)

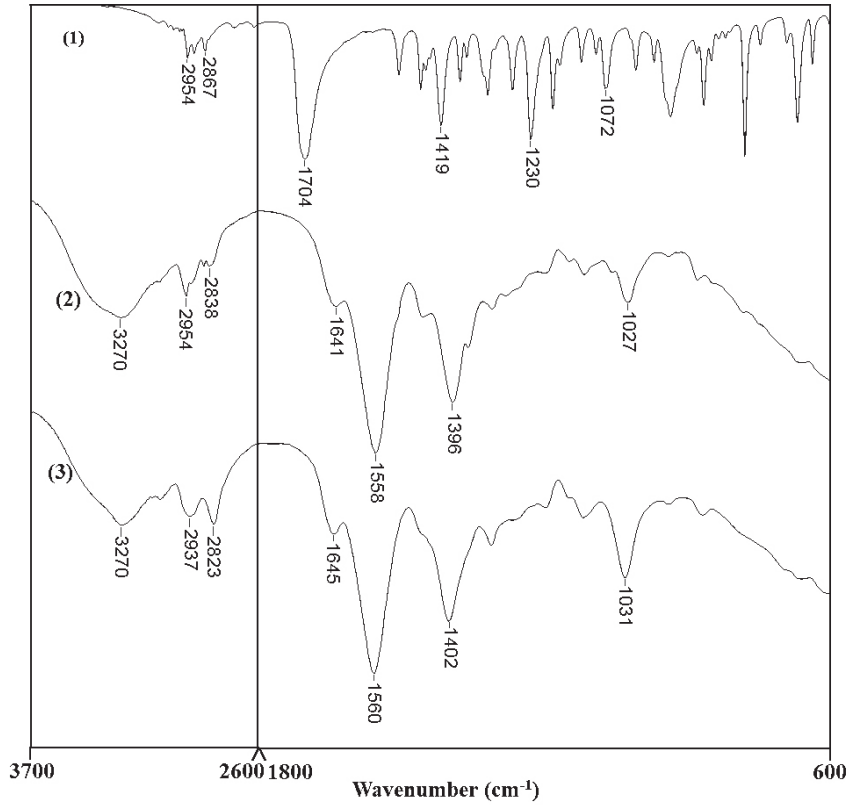


Figure 7. ATR-FTIR spectra of (1) IBU; (2) IBU-D4.COOH complex, and (3) D4.COOH

core and NH_2, TRIS and COOH-terminated PAMAMs, and the results were shown in Fig. 8. Results showed that the solubility of IBU enhanced significantly in the presence of PAMAMs with an increasing generation size (D2-D4) and dendrimer concentration (0-2 mM). Form these results, it could be concluded that the encapsulation efficiency of PAMAMs increases especially by the increasing generation size and varying functional groups, which could be attributed to dendrimer trapping the drugs by electrostatic interaction, and encapsulation of the inner cavities of dendrimers (30).

3.5. Effect of surface functional group on solubility of IBU

Presence of high density of surface functional groups in the structure of PAMAMs make them attractive for solubility enhancement applications of poor soluble drugs in aqueous media. Inner cavities and surface functionality of PAMAMs are responsible for increased solubility and, therefore, they can be acceptable as suitable drug-delivery systems (13). Up to know, the best reported solubility enhancement for IBU was obtained when EDA cored generation 4 amine-terminated PAMAM dendrimer (7.45 (mg/mL) (31) and β CD (~ 0.96 mg/mL) (32) ecepients used. IBU is practically water insoluble (0.08 mg/ml). As shown in Fig. 8, the solubility of IBU was increased significantly in comparison with the before mentioned literature in the presence of D4.COOH (18.21 mg/mL), D3.COOH (13.21 mg/mL), D4.TRIS (10.30 mg/mL), D2.COOH (8.55 mg/mL). The observed solubility enhancement was in the decreasing order of D4.COOH (18.21 mg/mL)> D3.COOH (13.21 mg/mL)> D4.TRIS (10.30 mg/mL)> D2.COOH (8.55 mg/mL)> D3.TRIS (6.04 mg/mL)> D4.NH_2 (4.56 mg/mL)> D3.NH_2 (3.36 mg/mL)> D2.TRIS (2.42 mg/mL)> D2.NH_2 (1.86 mg/mL), and in the ranges of 30 to 247-fold. Overall, results indicated that that the solubility of IBU increased with the increasing generation number of all NH_2, TRIS, and COOH-terminated PAMAM dendrimers. Water-soluble TRIS and COOH-terminated PAMAM dendrimers synthesized in this study could be potential drug carriers for NSAIDs for the future clinal applications and biological studies by eliminating the disadvantages of amine-terminated PAMAMs without causing any lysis of the living cells.

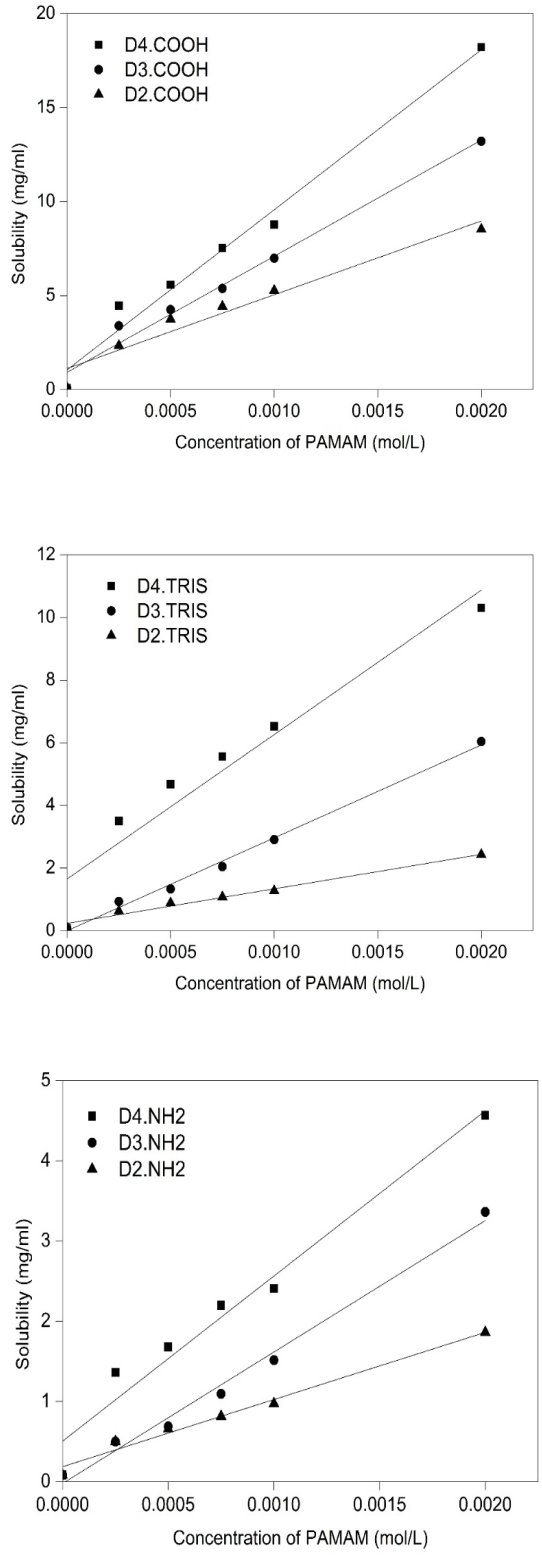


Figure 8. Solubility profiles of IBU in the presence of increasing generation and concentrations of COOH, TRIS, and NH_2-terminated PAMAMs.

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ÖZ

Birçok terapötik olarak aktif olan ilaçların sudaki çözünürlüğü zayıf ve bundan dolayı canlı hücrelerde biyoyararlanımları düşük ve büyük bir problem oluşturmaktadır. Bu çalışmada, yeni nesil Jeffamine® D230 çekirdekli amin (NH_2), Tris(hidroksimetil)aminometan (TRIS) ve karboksil (COOH) sonlu poli(amidoamine) PAMAM dendrimerler (PAMAMs) sentezlendi. Sentezlenen yeni tip PAMAMs'lar ^{1}H NMR, ^{13}C NMR, ATR-FTIR kullanılarak karakterize edildi ve örnek bir non-steroidal anti-inflamatuar ilaç (NSAID) olan Ibuprofen (IBU) için çözünürlük arttırıcı olarak araştırıldı. Sentezlenen yeni nesil PAMAMs'ların, jenerasyon büyüklüğünün (D2-D4), konsantrasyonun (0-2.0 mM) ve yüzey fonksiyonel gruplarının (NH_2, COOH, TRIS), IBU'nun çözünürlüğüne olan etkisi ayrıca araştırıldı. IBU'nun gözlemlenen çözünürlük artışı, D4.COOH (18.21 mg/mL)> D3.COOH (13.21 mg/mL)> D4.TRIS (10.30 mg/mL)> D2.COOH (8.55 mg/mL)> D3.TRIS (6.04 mg/mL)> D4.NH_2 (4.56 mg/mL)> D3.NH_2 (3.36 mg/mL)> D2.TRIS (2.42 mg/mL)> D2.NH_2 (1.86 mg/mL) sırasındadır. Sonuçlar gösteriyor ki, sentezlenen PAMAMs'ların jenerasyon büyüklüğü ve konsantrasyonun artmasıyla birlikte IBU'nu çözünürlüğü önemli ölçüde artmıştır (30 ile 247 kat arası).

Anahtar kelimeler: Dendrimer, poly(amidoamin) PAMAM, Jeffamin, ilaç taşıyıcı, NSAID, ibuprofen

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