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Research Article

ENHANCED SOLUBILITY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS BY HYDROXYL TERMINATED S-TRIAZINE BASED DENDRIMERS

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ABSTRACT

Objective: To synthesize and characterized s-triazine based dendrimer generations from*N,N'*-bis(4,6-dichloro-1,3,5-triazin-2-yl)hexane-1,6-diamine as core. To use dendrimer generation (G1-G3) as solubility enhancers of Ketoprofen, Ibuprofen, and Diflunisal and to study effects of factors such as pH, concentration of dendrimer and generation number on solubility of NSAIDS.

Methods: s-triazine based dendrimer was synthesized up to generation 3 by divergent method. Synthesized dendrimer generations were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, ESI-Mass spectrometryand elemental analysis. Candidature of full generation triazine based dendrimers (G1-G3) as solubility enhancers of NSAIDS were investigated by Higuchi and Connors method at different dendrimer concentrations, pH and generations.

Results: Dendrimer was synthesized up to generation 3 and structures of dendrimer generations were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR and ESI-Mass spectrometry. Dendrimer significantly enhances solubility of NSAIDS by either hydrophobic interaction or hydrogen bonding or both.

Conclusion: Synthesized triazine based dendrimers (G1-G3) enhances solubility of NSAIDs in water. Solubility of NSAIDS increased with increase in concentration of the dendrimer, pH and dendrimer generation. The order of solubility of NSAIDS was found to be Ketoprofen> Ibuprofen >Diflunisal by dendrimer.

Keywords:triazine trichloride, dendrimer, Nonsteroidal Anti-Inflammatory Drugs, solubility enhancers

INTRODUCTION

One of the major hurdles to develop a highly potent drug is the poor water solubility of many drugs. It is identified that about 40% of newly developed drugs are poorly soluble in water, therefore, it never benefit patient and thus rejected [1, 2]. Poor water solubility also leads to poor bioavailability and absorption of drugs [3].

Non-steroidal anti-inflammatory drugs (NSAIDS) are one of the most frequently used drugs all over the world for the symptoms associated with osteoarthritis and other chronic musculoskeletal conditions [4]. NSAIDs reduce the risk of and mortality from colon cancer by about half and constitute the ideal colon cancer chemo preventive agents [5]. However the use of NSAIDS have been limited due to problems related to gastrointestinal side effects, renal side effects and additional side effects [6]. It was reported that most NSAIDs can damage the esophagus, stomach, duodenum, small and large intestines, and can impair platelet function systemically, with a consequent increase in bleeding from a variety of GI lesions [7]. It was also reported that use of NSAIDS in parenteral could control these side effects, however poor bioavailability related to low water solubility of the NSAIDS hinders success of this formulation [8]. Several techniques have been used to improve solubility of drugs in water, such as the addition of surface active agents, formation of water soluble salts, polymers to enhance solubility and bioavailability of drug [9-11].

Dendrimers represent a novel type of polymeric material that has generated much interest in many diverse areas due to their unique structure and properties. Dendrimer-mediated solubility enhancement of drugs has been reportedand depends on factors such as generation size and terminal functionality [12].Dendrimers have hydrophilic exteriors and hydrophilic interiors, which are responsible for its unimolecular micelle nature. They form covalent as well as non-covalent complexes with drug molecules and hydrophobes, which are responsible for its solubilisation behavior [13].Thus, dendrimer have the potential to enhance the bioavailability of drugs that are poorly soluble[14]. A few reports are available on solubility enhancement of non-steroidal anti-inflammatory drugs (NSAIDS) like Ibuprofen [15], Ketoprofen [16], Naproxen, Diflunisal [17], Indomethacin [18] to anti-cancer drugs such as Methotrexate [19], Paclitaxel [20] etc. by using dendrimer.

In this paper, we have synthesized novel dendritic architecture with *N,N'*-bis(4,6-dichloro-1,3,5-triazin-2-yl)hexane-1,6-diamine as a core and terminated by diethanolamine linkages [21, 22] using divergent method. Dendrimer was synthesized up to generation 3 and was characterized by Infrared spectroscopy (FT-IR), ^{1}H-NMR,^{13}C-NMR, Electrospray Ionization Mass Spectrometry and Elemental Analysis. Dendrimer generations (G1-G3) as solubility enhancer non-steroidal anti-inflammatory drugs (NSAIDS) of Ketoprofen, Ibuprofen and Difusinal were investigated. Effect of pH, concentration and generation of dendrimer on solubility of said drugs were studied.

MATERIALS AND METHODS

Ketoprofen, Ibuprofen, and Difusinal weregenerously provided by A.R. College of Pharmacy, Vallabh Vidhyanagar as gift samples. Triazine trichloride (cyanuric chloride), hexane1,6diamine, acetone, dichloromethane and methanol were purchased from Sigma-Aldrich (India) Ltd.Acid phthalate buffer (pH 4.0), Borate alkaline buffer (pH 10.0) and Phosphate buffer saline (pH 7.4) were prepared according to Indian Pharmacopoeia (1996).All the reagents and solvents for the synthesis and analysis were used as received. FTIR studies were carried out in the range of 250–4000 cm^{-1} using Perkin Elmer-Spectrum RX-FTIR spectrometer instrument through KBr disc and pellet method and nujol mull method. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 400 MHz in BruckerAvance II 400 (Germany) using TMS as internal standard.Mass spectra were recorded on Waters Micromass Q-ToF Micro (USA) instrument equipped with electrospray ionization. Absorbance was measured on Shimadzu UV-1800 spectrophotometer. Double distilled water was used for solubility studies.

Synthesis of *N,N'*-bis(4,6-dichloro-1,3,5-triazin-2-yl)hexane-1,6-diamine (Core)

Cyanuric Chloride (0.02mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of hexane 1,6-diamine (0.01mmol) containing sodium hydroxide (0.02 mmol) in water was added drop wise in the solution of cyanuric chloride at 0-5 °C with stirring. The solution was stirred at 0-5 °C for 2 hrs. Then the solution was filtered, washed with methanol and acetone and dried under vacuum: A white colored solid was formed.

Yield, 83%; FT-IR (KBr, cm^{-1}) *ν*: 3281(N-H), 2884, 2779(aliphatic C-H), 1722, 1624(C=N of triazine), 844, 796(C-Cl); ^{1}H-NMR (400MHz, DMSO-*d6*) δ ppm: 1.2898- 1.3558 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 1.4941-1.5383 (m,4H,N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N) 3.2445- 3.2881(m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N);^{13}C-NMR (75MHz, DMSO-*d6*) δ ppm: 25.20, 25.29 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 30.52 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 40.52, 41.47(N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N)168.34, 169.40 (Triazine part); Anal. Calcd. for $C_{12}H_{14}Cl_4N_8$: C, 34.97; H, 3.42; N, 27.19 found C, 35.02; H, 3.49; N, 27.26.

Synthesis of generation 1 dendrimer (G1)

N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl)hexane-1,6-diamine(0.01mmol) was dissolved in an excess of diethanolamine (0.04mmol) which was used as both solvent and reactant. The resulting mixture was refluxed for 2 hrs. After cooling, it was dispersed and washed by acetone repeatedly to give generation 1 dendrimer which was light brown colored.

Yield.75%; FTIR (KBr, cm^{-1}) *ν*: 3364 (O-H), 2941 (aliphatic C-H), 1668 (C=N of triazine), 1063 (C-O); ^{1}H-NMR (400MHz, D_2O) δ ppm: 1.3138-1.3358 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 1.4945-1.5145 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 3.4191- 3.4381 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N) 3.5785-3.6151 (m, 16H, N-CH_2-CH_2-OH), 3.7801-3.8145 (m, 16H, N-CH_2-CH_2-OH); ^{13}C-NMR (75MHz, D_2O) δ ppm: 25.20,25.29 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 30.52 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 58.04 (N-CH_2-CH_2-OH), 61.04 (N-CH_2-CH_2-OH), 168.04, 169.90 (triazine part); Anal. Calcd. for $C_{28}H_{54}N_{12}O_8$: C, 48.97; H, 7.92; N, 24.47; Found C, 49.02; H, 7.98; N, 24.50.

Synthesis of generation 1.5 dendrimer (G1.5)

Cyanuric Chloride (0.08mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of G1 dendrimer (0.01mmol) containing sodium hydroxide (0.08 mmol) in water was added drop wise in the solution of cyanuric chloride at 0-5 °C with stirring. The solution was stirred at 0-5 °C for 2 hrs and refluxed for 6 hrs. Then the solution was filtered, washed with methanol and acetone and dried under vacuum: A white colored solid was formed.

Yield 84%; FT-IR (KBr, cm^{-1}) *ν*: 3214 (N-H), 2834, 2832, 2780 (aliphatic C-H), 1779, 1753, 1717(C=N of triazine), 1061(C-O), 786 (C-Cl); ^{1}H-NMR (400MHz, D_2O) δ ppm: 1.3138-1.3358 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 1.4945- 1.5145 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 3.4191-3.4381 (m, 4H, CH_2-NH); 3.8585-3.9350 (m, 16H, N-CH_2-CH_2-O-Tri), 4.0301-4.0832 (m, 16H, N-CH_2-CH_2-O-Tri);^{13}C-NMR (75MHz, D_2O) δ ppm: 25.20, 25.29 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 30.52 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 41.47 (N-CH_2-CH_2-O-tria), 61.04 (N-CH_2-CH_2-O-tria), 163.01, 166.66, 170.18, 172.28 (Triazine part); Anal. Calcd. for $C_{52}H_{46}Cl_{16}N_{36}O_8$: C, 33.39; H, 2.48; N, 26.96; Found: C, 34.02; H, 2.50; N, 27.01.

Synthesis of generation 2 dendrimer (G2)

Generation 1.5 dendrimer (0.01mmol) was dissolved in an excess of diethanolamine (0.16 mmol) which was used as both solvent and reactant. The resulting mixture was refluxed for 2 hrs. After cooling, it was dispersed and washed by acetone repeatedly to give generation 2 dendrimer which was light brown colored.

Yield 75%; FTIR (KBr) *ν*: 3389(O-H), 2939, 2950(aliphatic C-H), 1671, 1619(C=N of triazine), 1068 (C-O).; ^{1}H-NMR (400MHz, D_2O) δ ppm: 1.3158-1.3355 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 1.4984-1.5138 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 3.4191-3.4381 (m, 4H, CH_2-NH), 3.6743-3.7837 (m, 16H, N-CH_2-CH_2-OH), 3.8126-3.8970 (m, 16H, N-CH_2-CH_2-OH), 3.9803-4.0433 (m, 64H, N-CH_2-CH_2-O-tri), 4.0917-4.1672 (m, 64H, N-CH_2-CH_2-O-tri);^{13}C-NMR (75MHz, D_2O) δ ppm: 25.70 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 30.31 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 40.81 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 60.81 (N-CH_2-CH_2-O-tria), 61.28 (N-CH_2-CH_2-OH), 63.78 (N-CH_2-CH_2-O-tria), 65.80 (N-CH_2-CH_2-OH), 163.66, 168.66, 170.18, 172.21 (Triazine part); Anal. Calcd. for $C_{116}H_{206}N_{52}O_{40}$: C, 46.92; H, 6.99; N, 24.53; Found C, 47.01; H, 7.02; N, 24.59.

Synthesis of generation 2.5 dendrimer (G2.5)

Cyanuric Chloride (0.32mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of G2 dendrimer (0.01mmol) containing sodium hydroxide (0.32 mmol) in water was added dropwise in the solution of cyanuric chloride at 0-5 °C with stirring. The solution was stirred at 0-5 °C for 2 hrs and refluxed for 6 hrs. Then the solution was filtered, washed with methanol and acetone and dried under vacuum: A white colored solid was formed.

Yield: 70%; FT-IR (KBr, cm^{-1}) *ν*: 3280 (N-H), 2852, 2819 (aliphatic C-H), 1750, (C=N of triazine), 1045(C-O), 787 (C-Cl);^{1}H-NMR (400MHz, DMSO-*d6*) δ ppm: 1.3118-1.3351 (m, 4H, m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-N), 1.4984-1.5150 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 3.4151-3.4389 (m, 4H, CH_2-NH), 4.0153- 4.1380 (m, 16H, N-CH_2-CH_2-O-tri), 4.2168-4.2929 (m, 16H, N-CH_2-CH_2-O-tri); ^{13}C-NMR (75MHz, DMSO-*d6*) δ ppm: 25.20 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 30.05 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 40.45 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 60.06 (N-CH_2-CH_2-O-tria), 61.23 (N-CH_2-CH_2-OH), 63.30 (N-CH_2-CH_2-O-tria), 65.53 (N-CH_2-CH_2-OH), 164.11, 166.41, 168.81, 171.74, 172.57, 174.94 (Triazine part); Anal Calcd. for $C_{212}H_{174}Cl_{64}N_{148}O_{40}$: C, 33.05; H, 2.28; N, 26.91; Found C, 33.12; H, 2.30; N, 26.99.

Synthesis of generation 3 dendrimer (G3)

Generation 2.5 dendrimer (0.01mmol) was dissolved in an excess of diethanolamine (0.64 mmol) which was used as both solvent and reactant. The resulting mixture was refluxed for 2 hrs. After cooling, it was dispersed and washed by acetone repeatedly to give generation 1 dendrimer which was light brown colored.

Yield: 71 %; FT-IR (KBr, cm^{-1}) *ν*: 3368 (O-H), 2947, 2872 (aliphatic C-H), 1639 (C=N of triazine), 1033 (C-O); ^{1}H-NMR (400MHz, D_2O) δ ppm: 1.3138-1.3353 (m, 4H, m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 1.4954-1.5181 (m, 4H, N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 3.4144-3.4333 (m, 4H, CH_2-NH), 3.6833-3.7574 (m, 264H, N-CH_2-CH_2-OH), 3.8829-3.9243 (m, 264H, N-CH_2-CH_2-OH) 4.0953- 4.1880 (m, 80H, N-CH_2-CH_2-O-tri), 4.2432-4.3168 (m, 80H, N-CH_2-CH_2-O-tri); ^{13}C-NMR (75MHz, D_2O):25.50 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 30.14 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 40.44 (N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N), 59.92 (N-CH_2-CH_2-O-tria), 60.60 (N-CH_2-CH_2-OH), 63.39 (N-CH_2-CH_2-O-tria), 66.69 (N-CH_2-CH_2-OH), 168.11, 169.99, 171.55, 175.81, 177.28, 179.11 (Triazine part); Anal Calcd. for $C_{468}H_{814}N_{212}O_{168}$: C, 46.46; H, 6.78; N, 24.54; Found C, 46.51; H, 6.80; N, 24.59; ESI-Mass: Molecular Wt. 12086 found (M+1): 12087.

Solubility Studies

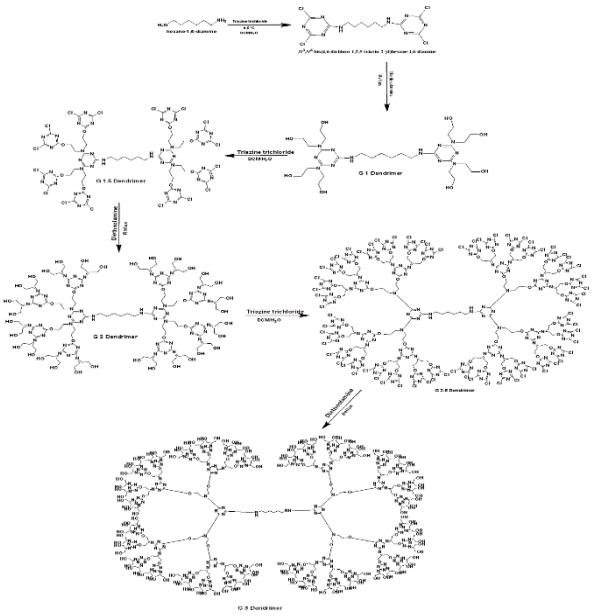
Solubility study was carried out accordingto the method described by Higuchi and Connors[23]. Excess of drug was added to screw-capped vials containing different concentrations (0.6 mmol to 3 mmol) of dendrimer generations in buffers of 4.0, 7.4 and 10 pH. Vials were shaken for 48 h at 37 °C in shaking water bath. The vials were centrifuged to remove undissolved drug and absorbance of drug were measured at its characteristic wavelength 260 nm, 258 nm and 250 nmfor Ketoprofen, Ibuprofen and Diflunisal respectively using Shimadzu UV-1800 spectrophotometer.

RESULT AND DISCUSSION

Synthesis

Synthesis of G3 s-triazine based dendrimer is outline in scheme 1. Temperature controlled nucleophillic substitution of triazine trichloride and selectivity of triazine trichloride toward aliphatic amino to hydramine was utilized [21, 22]. Hexane-1,6-diamine was reacted with triazine trichloride at low temperature to give bis(4,6-dichloro-1,3,5-triazin-2-yl)hexane-1,6-diamine as core for dendrimer synthesis. Core compound was reacted with diethanolamine in second step, later was utilized as both solvent and

reactant to give generation 1 (G1) dendrimer. G1 dendrimer was again reacted with triazine trichloride to give 1.5 (G1.5) dendrimer. Last two steps were repeated until generation 3 dendrimer was synthesized.



Scheme 1: Shows synthetic route of dendrimer

Table 1: Physical description of dendrimer generations

COMPOUND	MOLECULAR FORMULA	APPEARANCE	SOLUBILITY IN WATER	SURFACE GROUPS (NUMBER)
Core	$C_{12}H_{14}Cl_4N_8$	White solid	Insoluble	Cl (4)
G1	$C_{28}H_{54}N_{12}O_8$	Brown liquid	Soluble	OH (8)
G1.5	$C_{52}H_{46}Cl_{16}N_{36}O_8$	White solid	Insoluble	Cl(16)
G 2	$C_{116}H_{206}N_{52}O_{40}$	Brown liquid	Soluble	OH(32)
G2.5	$C_{212}H_{174}Cl_{64}N_{148}O_{40}$	White solid	Insoluble	Cl(64)
G3	$C_{468}H_{814}N_{212}O_{168}$	Brown liquid	Soluble	OH (128)

Physical appearance and solubility in water of both chlorine terminated half generation dendrimers and hydroxyl terminated full generation dendrimers were different. As shown in Table 1, Chlorine terminated core compound, G1.5 dendrimer and G2.5 dendrimer were white solids and insoluble in water. Hydroxyl terminated full generation dendrimer G1, G2 and G3 were brown colored and soluble in water.

Characterization of dendrimer

Infrared Spectroscopy

Table 2: Infra-Red spectroscopy data of dendrimer generations

Compound	IR absorption band(cm^{-1}) for functional group			
	O-H	C=N	C-O	C-Cl
Core	---	1722	---	796
G1	3364	1668	1063	---
G1.5	---	1779	1061	786
G2	3389	1671	1068	---
G2.5	---	1680	1045	787
G3	3368	1630	1033	---

Dendrimer generations were characterized by Infrared spectroscopy, the results are furnished in Table 2. In Infra-red spectrum of chlorine terminated compounds core, G1.5 and G2.5 dendrimers, characteristic absorption bands appeared at in range of 1680-1780 cm^{-1} for C=N stretching, 1055-1065 cm^{-1} of C-O stretching and 750-780 $cm^{-1}$for C-Cl stretching. In infrared spectrum of hydroxyl terminated compounds G1, G2 and G3 dendrimer, characteristic absorption bands appeared at in range of 1680-1780 cm^{-1} for C=N stretching, 1055-1065 cm^{-1} of C-O stretching and 3350-3380 $cm^{-1}$for O-H stretching.

^{1}H-NMR Spectroscopy

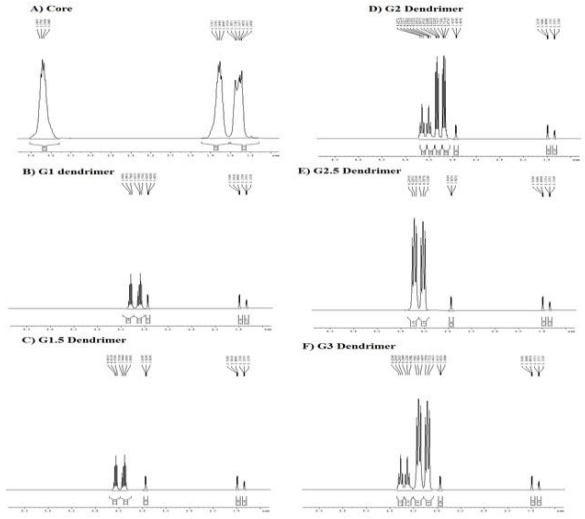


Figure 2: Shows ^{1}H-NMR spectrums of A) core, B) G1 Dendrimer, C) G1.5 Dendrimer, D) G2 dendrimer, E) G2.5 Dendrimer and F) G3 Dendrimer

All dendrimer generations were characterized by ^{1}H-NMR spectroscopy to investigate their purity ^{1}H-NMR spectrum of core [Fig 2 A] compound showed multiplets at δ 1.2898- 1.3558, 1.4941-1.5383 and 3.2445- 3.2881 ppm for 1,6-hexanediamine moiety at core. Half generation dendrimers [Fig 2 C, E] showed two multiplets in the region of δ 4-4.25 ppm values for methylene groups of diethanolamine branching unit as both inner and outer diethanolamine linkages were terminated by triazine trichloride and under same environment. ^{1}H-NMR spectra [Fig 2 B] of full generation dendrimer G1 showed these multiplets in the region of δ3.5-3.9ppm as diethanolamine moiety was terminated by hydroxyl groups shifted these peaks in the up field region. Full generation dendrimer G2 and G3 [Fig 2 D, F] showed four triplets, two in the regions of δ3.5-3.8 ppm for methylene protons terminated by hydroxyl groups and other in the region of δ4-4.25 ppm for methylene groups terminated by triazine groups.

^{13}C-NMR Spectroscopy

^{13}C-NMR spectrum of core compound [Fig 3 A] showed peaks at δ 25.20, 25.29, 30.52, 40.52, 41.47 ppm for aliphatic carbons of hexane group at core and peaks δ 168.34, 169.40 for triazine part of structure. ^{13}C-NMR spectrum [Fig 3 B] of G1 dendrimer showed additional peaks at δ 58.04 and δ 61.04 for diethanolamine part of the structure. ^{13}C-NMR spectrum [Fig 3 C] of G1.5 dendrimer showed peaks at δ 25.20, 25.29, 30.52 for core carbons, δ 41.47, 61.04 for diethanolamine part and δ 163.01, 166.66, 170.18, 172.28 triazine part of structure. ^{13}C-NMR spectrum [Fig 3 D] of G2 dendrimer showed δ 25.70, 30.31, 40.81 for core, δ 60.81, 63.78 for inner diethanolamine part, δ 61.28, 65.80 for outer diethanolamine part and δ 163.66, 168.66, 170.18, 172.21 for triazine part. ^{13}C-NMR spectrum [Fig 3 E] showed δ 25.20, 30.05, 40.45 for core part, 60.06, 63.30 for inner diethanolamine part δ 61.23, 65.53 for outer diethanolamine part and δ 164.11, 166.41, 168.81, 171.74, 172.57, 174.94 triazine part of structure. ^{13}C-NMR spectrum [Fig 3 F] of G3

dendrimer, 25.50, 30.14, 40.44 for core, 59.92, 60.60 for outer diethanolamine part, 63.39, 66.69 for inner diethanolamine part and 168.11, 169.99, 171.55, 175.81, 177.28, 179.11 triazine part of structure.

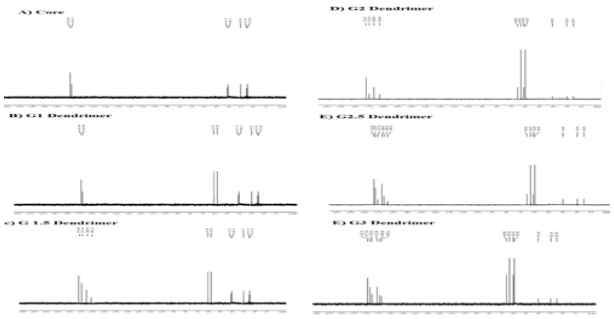


Fig.3: Shows ^{13}C-NMR spectrums of A) core, B) G1 Dendrimer, C) G1.5 Dendrimer, D) G2 dendrimer, E) G2.5 Dendrimer and F) G3 Dendrimer

ESI-MS and Elemental Analysis

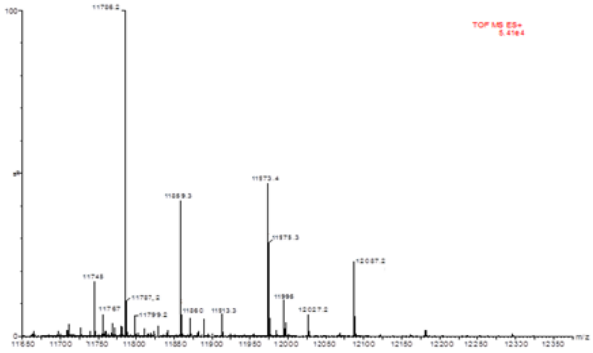


Fig.3: ESI-Mass Spectrum of Generation 3 Dendrimer

Figure 3 showed ESI mass spectrum of generation 3 dendrimer with M+1 ion peak at 12087 m/z which corresponded to calculated molecular weight of 12086 Daltons.

Table 3: Elemental Data of dendrimers

Compound	Percentage of Elements					
	Theoretical			Practical		
	C	H	N	C	H	N
Core	34.97	3.42	27.19	35.02	3.49	27.26
G1	48.97	7.92	24.47	49.02	7.98	24.50
G1.5	33.39	2.48	26.96	34.02	2.50	27.01
G 2	46.92	6.99	24.53	47.01	7.02	24.59
G2.5	33.05	2.28	26.91	33.12	2.30	26.99
G3	46.46	6.78	24.54	46.51	6.80	24.59

Elemental data of dendrimers are furnished in Table 3. All the elemental percentage of elements for dendrimer generations matched the theoretical percentages.

Only full generation dendrimers G1, G2 and G3 were water soluble, so, solubility studies were carried out by only using full generation dendrimers as per Higuchi and Connors method [23]. Solubilisation behavior of drugs were studied in relation to pH, concentration and generationnumber. Ketoprofen,Ibuprofen and Diflunisal were selected as model drugs to study solubilisation behaviour. The characteristic data of the drugs are given in Table 4.

Dendrimer generation (G1-G3) significantly enhances solubility of Ketoprofen, Ibuprofen and Diflunisal and the results are displayed in Fig. 3-5(A-C). G1, G2 and G3 dendrimers enhances aqueous solubility of the practically insoluble Ketoprofen up to 0.83 mg/ml, 2.01 mg/ml and 4.95 mg/ml respectively at pH 7.4. Similarly, G1, G2 and G3 dendrimer increased aqueous solubility of practically insoluble Ibuprofen up to 0.7mg/ml, 1.87mg/ml and 4.67 mg/ml and Diflunisal up to 0.47mg/ml, 1.70mg/ml and 4.36 mg/ml at 7.4 pH. It was evident that solubility of NSAIDs was increased with increase in

Solubilisation behaviour of drugs

Table 4: Characteristic Data of Drugs

Chemical Name	Ketoprofen	Ibuprofen	Diflunisal
Chemical Structure			
Mole. Formula	$C_{16}H_{14}O_3$	$C_{13}H_{18}O_2$	$C_{13}H_8F_2O_3$
Mole. Weight (gm/mole)	254.28	206.28	250.20
Solubility	Practically insoluble in water	Practically insoluble in water	Practically insoluble in water
Characteristic Wavelength	260	258	250
pKa	4.5	5.3	3.3

dendrimer concentration in almost linearmanner. It was proposed that as dendrimer contains hydrophobic triazine ring in interior regions which may impart hydrophobic interaction and the hydroxyl groups in the exterior, which may impart hydrogen bonding so, thus mechanism for enhanced solubility of NSAIDs by dendrimer could be either hydrophilic interaction or hydrogen bonding or both [24].

It was also evident from solubility resultsFig. 3-5(A-C) that solubility of NSAIDS increased with increase in pH from 4.0 to 10.0. For all dendrimer generations, at 4.0 pH lowest solubility of NSAIDS was observed and at pH 10.0 maximum solubility of NSAIDS was observed. It was proposed that weakly acidic NSAIDS were not fully ionized at lower pH, therefore it cannot freely interact with dendrimer which results in lower solubility of NSAIDS at low pH [16].

Solubilisation of NSAIDS was also significantly affected by dendrimer generation. With increase in dendrimer generation,

solubilisation of NSAIDS were also increased. With every increase in generation of dendrimer there was significant increase in surface area, terminal hydroxyl groups and size of dendrimer so, the ability of dendrimer to interact with Drug molecule was significantly increased. Hence solubility of Drug was significantly increased with increase in dendrimer generation [16-17].

It was also observed that order of solubility of NSAIDS at constant dendrimer generations was found to be Ketoprofen> Ibuprofen>Diflunisal. It was observed that solubilisation of Ketoprofen by dendrimer was maximum and minimum for Diflunisal.

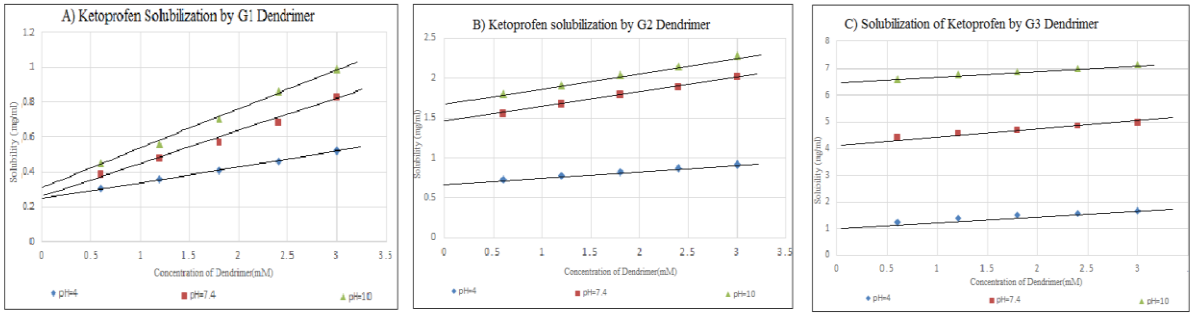


Fig.4: Shows Solubilisation of Ketoprofen at pH 4.0, 7.4 and pH=10 by A) G1 dendrimer, B) G2 Dendrimer and C) G3 Dendrimer

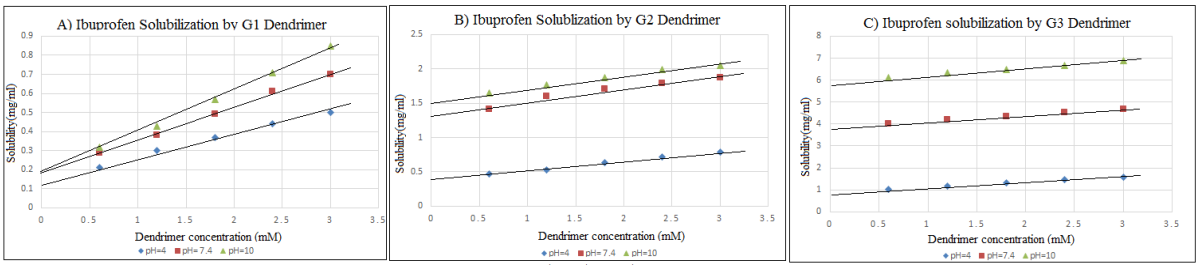


Fig.5: shows Solubilisation of Ibuprofen at pH 4.0, 7.4 and pH=10 by A) G1 dendrimer, B) G2 Dendrimer and C) G3 Dendrimer

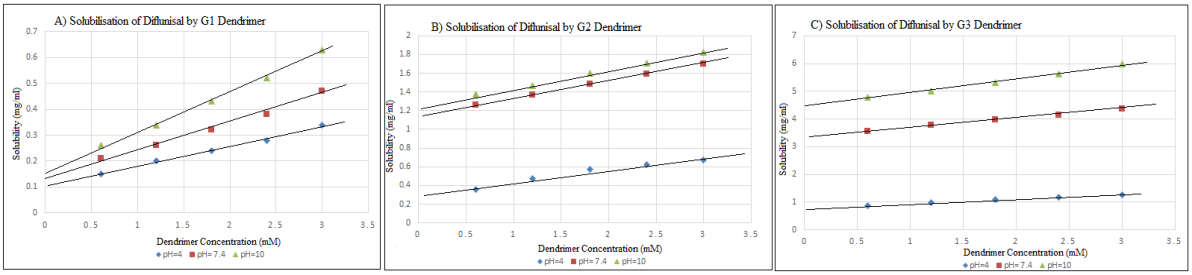


Fig.6: shows Solubilisation of Ibuprofen at pH 4.0, 7.4 and pH=10 by A) G1 dendrimer, B) G2 Dendrimer and C) G3 Dendrimer

CONCLUSION

s-Triazine based dendrimer generations were synthesized up to generation 3 and characterized by spectral analysis. Full generation dendrimer (G1-G3) significantly enhances solubility of NSAIDS Ketoprofen, Ibuprofen and Diflunisal. It was evident that solubilisation of NSAIDS was increased with increase in pH, generation number and concentration of dendrimer. It was proposed that dendrimer enhances solubility of NSAIDS by either hydrophobic interaction or hydrogen bonding or both. The order of solubility of NSAIDS was found to be Ketoprofen> Ibuprofen> Diflunisal.

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