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Original Article

A NANOSCALE DENDRITIC MACROMOLECULES BASED ON ETHANE 1, 2-DIAMINE AS POTENTIAL DRUG CARRIERS FOR NSAIDS: SYNTHESIS, CHARACTERIZATION AND APPLICATIONS

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ABSTRACT

Objective: The present work deals with the objective of development and characterization of novel dendritic macromolecules as solubility enhancer and carrier for sustained release of Ketoprofen. Cytotoxicity and hemolytic assay of dendritic macromolecules were also estimated as an objective to evaluate its toxicity and biocompatibility.

Methods: Dendritic macromolecules were synthesized using divergent method. Synthesized macromolecules were characterized by spectral techniques such as FTIR, ^{1}H-NMR, ^{13}C-NMR electro-spray ionization mass spectrometry and elemental analysis. Enhanced aqueous solubility of ketoprofen was evaluated with respect to pH, generation number and concentration of dendrimer using Higuchi and Connors method. Sustained release of ketoprofen from ketoprofen loaded dendrimers was measured and compared with that of free ketoprofen. Hemolytic assay and cytotoxicity of G3 dendrimer on A-549 cell lines were studied to evaluate toxicity and biocompatibility of dendrimer. All dendritic macromolecules were fully characterized by spectral techniques.

Results: Solubility study revealed that aqueous solubility of ketoprofen by dendrimer increased with increase in pH, concentration and generation of dendrimer. Ketoprofen was released slowly from ketoprofen loaded dendrimer compared to that of free ketoprofen. Dendritic macromolecules were less cytotoxic and showed less hemolytic potential.

Conclusion: It can be concluded that dendrimer have high potential as carriers and solubility enhancers of hydrophobic drug.

Keywords: Triazine based dendrimer, Ketoprofen, Drug Carrier, Cytotoxicity, Hemolysis.

INTRODUCTION

As products of repetitive reactions dendrimers have attracted attention of many fields of applications due to several unique properties such as nanoscale monodispersity, scaffolding properties, amplifiable and functionable surface groups and dimensions that mimics biomolecules such as protein [1]. Currently dendrimers have fascinated attention in many applications in the field of applications such as drug solubilisation [2], drug delivery [3-5], dendrimer encapsulated nanoparticles [6], gene therapy [7], membrane separation technology [8], cancer therapy [9] and catalysis [10].

Mainly divergent and convergent routes are most prominent for dendrimer synthesis[11, 12] which requires protection or manipulation of functional group thus synthesis of dendrimer is often tedious or time consuming. It has been reported earlier that by using triazine trichloride is active monomer, dendrimer can be constructed by facile route obviating protection/deprotection or manipulation of functional group [16, 17]. We have already reported synthesis and application of triazine based dendrimers [13-17].

Delivery of hydrophobic drugs is one of the challenging issues in pharmaceutical research and development [18]. Most part of the body is made up of water. Therefore low water solubility of these drugs causes early elimination from the gastrointestinal tract which results in poor bioavailability of drugs [19, 20]. As drug development is time consuming and costly process low solubility of these drugs is an issue for both drug discovery and pharmaceutical development process which should be addressed early on during compound development [21].

In this paper, we have described triazine based dendrimers with N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl)ethane-1,2-diamine used as core for dendrimer synthesis. N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl)ethane-1,2-diamine was synthesized from triazine trichloride and ethane-1,2 diamine. Diethanolamine and triazine trichloride were utilized as linkers for growth of dendrimer generation. Dendrimer was synthesized up to generation three [17]. Synthesized dendrimers were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, ESI-Mass spectrometry and elemental analysis. Full generation dendrimer G1, G2 and G3 were used as solubility enhancers of ketoprofen. Effect of pH, generation and concentration of dendrimer on ketoprofen solubilisation was studied. Ketoprofen was loaded by dendrimers using inclusion complex technique. Sustained release study of ketoprofen loaded dendrimer was carried out and compared with free ketoprofen. Cytotoxicity and hemolytic assay were carried out to evaluate toxicity of dendrimers.

MATERIALS AND METHODS

Triazine trichloride (cyanuric chloride), ethane-1,2 diamine, acetone, dichloromethane and methanol were purchased from Sigma-Aldrich (India) Ltd. Ketoprofen was generously provided by A. R. College of Pharmacy, Vallabh Vidhyanagar. All the reagents and solvents for the synthesis and analysis were used as received. Absorbance was measured on Shimadzu UV-1800 spectrophotometer. Double distilled water was used for solubility studies. 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). FT-IR was carried out in the range of 250–4000 cm^{-1} using Perkin Elmer-Spectrum RX-FTIR spectrometer instrument through KBr disc and pellet method for solid samples or nujol mull method for liquid samples.^{1}H-NMR and ^{13}C-NMR spectra were recorded at 400 MHz in Brucker Avance II 400 (Germany) using TMS as internal standard using either D_2O or DMSO-d_6 as solvents. Mass spectra were recorded on Waters Micromass Q-ToF Micro (USA) instrument equipped with electro spray ionization. Shimadzu UV-1800 spectrophotometer was used to measure absorbance of ketoprofen at its characteristic wavelength of 260 nm.

Synthesis of N, N'-biz(4,6-dichloro-1,3,5-triazin-2-yl) ethane-1,2-diamine (Core)

Cyanuric Chloride (0.02 mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of ethane-1,2 diamine (0.01 mmol) 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: 75%. FT-IR (KBr, cm^{-1}) ν: 3203, 2992(Aliphatic C-H stretching), 1780, 1754(aromatic C=N), 845, 786 (C-Cl stretching). ^{1}H-NMR (400MHz, DMSO) δ ppm: 3.5549 (s, 2H, N-CH_2-CH_2-N-), 4.0288 (NH). ^{13}C-NMR (75 MHZ, DMSO) δ ppm: 49.55 (N-CH_2-CH_2-N), 167.76 (triazine C-N), 169.10 (triazine C-Cl). ESI-Mass: Calculated Molecular Weight: 356; Obtained: 357 (M+1). Anal Calcd. for $C_8H_6Cl_4N_8$: C, 26.99; H, 1.70; N, 31.48; found C, 27.01; H, 1.75; N, 31.55.

Synthesis of generation 1 dendrimer (G1)

N, N'-biz(4,6-dichloro-1,3,5-triazin-2-yl) ethane-1,2-diamine (0.01 mmol) was dissolved in an excess of diethanolamine (0.04 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 with honey like consistency.

Yield: 80 %. FT-IR (Nujol, cm^{-1}) ν: 3364 (O-H stretching), 2942 (Aliphatic C-H streching), 1672, 1620 (Aromatic C=N stretching), 1068 (C-O stretching). ^{1}H-NMR (400MHz, D2O) δ ppm: 3.5549 (s, 2H, N-CH_2-CH_2-N), 3.6650-3.6968 (t, 16H,-CH_2-CH_2-OH), 3.7642-3.7904 (t, 16H,-CH_2-CH2-OH). ^{13}C-NMR (75 MHZ, DMSO) δ ppm: 49.55 (N-CH_2-CH_2-N), 59.10 (N-CH_2-CH_2-OH), 61.70 (N-CH_2-CH_2-OH) 167.76 (triazine C-N), 169.10 (Triazine C-O). ESI-Mass: Calculated Molecular weight: 630; Obtained: 631 [M+1], 632 [M+2].

Synthesis of generation 1.5 dendrimer (G1.5)

Cyanuric chloride (0.08 mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of G1 dendrimer (0.01 mmol) 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: 75 %. FT-IR (KBr, cm^{-1}) ν: 3220(Secondary N-H stretching), 2888, 2850 (Aliphatic C-H stretching), 1710, 1605 (Aromatic C=N stretching), 1052 (C-O stretching), 786 (C-Cl stretching). ^{1}H-NMR (400MHz, DMSO) δ ppm: 3.5455 (s, 4H, N-CH_2-CH_2-N-), 3.9642-3.9902 (t, 16 H, N-CH_2-CH_2-O-tri), 4.0410-4.0890(t, 16H, N-CH_2-CH_2-O-tri). ^{13}C-NMR (75 MHZ, DMSO) δ ppm: 49.70 (N-CH_2-CH_2-N), 58.20 (N-CH_2-CH_2-O-tria), 64.10 (N-CH_2-CH_2-O-tria), 167.76 (triazine C-N), 176.70 (Triazine C-O), 179.10 (Triazine C-Cl). ESI-Mass: Calculated Molecular Weight 1812; obtained: 1813 (M+1).

Synthesis of generation 2 dendrimer (G2)

G1.5 dendrimer (0.01 mmol) 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 with honey like consistency.

Yield: 75 %. FT-IR (Nujol, cm^{-1}) ν: 3366 (O-H stretching), 2942 (Aliphatic C-H stretching), 1645, 1733 (Aromatic C=N stretching), 1031 (C-O stretching). ^{1}H-NMR (400MHz, D_2O) δ ppm: 3.5455 (s, 4H, N-CH_2-CH_2-N), 3.6566-3.6950 (m, 64H, N-CH_2-CH_2-OH), 3.7901-3.8250 (m, 64H, N-CH_2-CH_2-OH), 3.9480-3.9685 (m, 16H, N-CH_2-CH_2-O-tri), 4.0351-4.0581(m, 16H, N-CH_2-CH_2-O-tri).^{13}C-NMR(400MHz, D_2O) δ ppm: 49.51 (N-CH_2-CH_2-N), 59.22 (outer N-CH_2-CH_2-O-triazine), 61.44(outer N-CH_2-CH_2-O-triazine), 64.64(outer N-CH_2-CH_2-O-tria), 66.11 (inner N-CH_2-CH_2-O-tria), 167.57(Triazine C-N), 169.97 (Triazine C-N-(CH_2-CH_2-O-)$_2$), 177.66 (Triazine C-O), 179.67 (Triazine C-N(CH_2-CH_2-OH)$_2$). ESI-Mass: Calculated Molecular weight: 2912; Obtained: 2913 (M+1).

Synthesis of generation 2.5 dendrimer (G2.5)

Cyanuric Chloride (0.32 mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of G2 dendrimer (0.01 mmol) containing sodium hydroxide (0.32 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: 80%. FT-IR (KBr, cm^{-1}) ν: 3220 (Secondary N-H stretching), 2888, 2850, 2814(aliphatic C-H), 1773, 1753, 1713 (Aromatic C=N stretching), 1054 (C-O stretching), 776 (C-Cl stretching).^{1}H-NMR (400MHz, D_2O) δ ppm: 3.4833 (s, 4H, N-CH_2-CH_2-N), 3.9688-3.9849 (m, 80H, N-CH_2-CH_2-Otri), 4.0411-4.0888 (m, 80H, N-CH_2-CH_2-O-tri).^{13}C-NMR (75 MHZ, D_2O) δ ppm: 40.51 (N-CH_2-CH_2-N), 61.44 (outer-N-CH_2-CH_2-O-tri), 59.22(inner-N-CH_2-CH_2-O-tri), 64.64 (outer-N-CH_2-CH_2-O-tri), 66.11 (inner-N-CH_2-CH_2-O-tri), 167.57 (Triazine C-N), 169.97 (inner C-N-(CH_2-CH_2-O-)$_2$), 177.66 (outer C-N-(CH_2-CH_2-O-)$_2$, 179.67(outer C-Cl), 180.78 (inner triazine C-O). ESI-Mass: Calculated Molecular Weight: 7646: Obtained: 7646 (M+), 7647 (M+1).

Synthesis of generation 3 dendrimer (G3)

Generation 2.5 dendrimer (0.01 mmol) 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 3 dendrimer which was light brown colored with honey like consistency.

Yield: 80%. FT-IR (Nujol, cm^{-1}) ν: 3389 (O-H stretching), 2950, 2889 (Aliphatic C-H stretching), 1671, 1619 (Aromatic C=N stretching), 1068 (C-O stretching).^{1}H-NMR (400 MHz, D_2O) δ ppm: 3.5425 (4H, N-CH_2-), 3.7245-3.7668 (m, 264H, outer N-CH_2-CH_2-OH), 3.9442-3.9642 (m, 264H, outer N-CH_2-CH_2-OH), 4.0388-4.0616 (m, 80H, inner N-CH_2-CH_2-O-Tri), 4.1494-4.1977 (m, 80H, inner N-CH_2-CH_2-O-Tri).^{13}C-NMR (75 MHz, D_2O) δ ppm: 40.59 (N-CH_2), 59.95 (outer N-CH_2-CH_2-OH), 60.66 (inner CH_2-CH_2-O-Tri), 63.41 (outer N-CH_2-CH_2-OH), 66.70 (inner CH_2-CH_2-O-Tri), 168.18 (Triazine C-N), 169.97 (inner C-N-(CH_2-CH_2-O-)$_2$), 171.59 (Outer triazine C-O), 175.71(outer C-N-(CH_2-CH_2-O-)$_2$), 177.27 (outer C-N-(CH_2-CH_2-O-)$_2$), 179.18 (Outer Triazine C-O). ESI-Mass: Calculated Mole. Wt.; 12028; Obtained: 12028.4 [M+].

Solubility study

Solubility study was carried out according to the method described by Higuchi and Connors [22]. Excess of ketoprofen 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 ketoprofen and absorbance of ketoprofen were measured at its characteristic wavelength 260 nm using Shimadzu UV-1800 spectrophotometer.

Drug loading

Generally there are two approached for drug loading in dendrimer either by inclusion complex or by conjugation. In the present approach, we have utilized inclusion complex technique. Drug loading was performed by reported methods with little modifications [16, 18]. A known amount of ketoprofen was added to generation 3 dendrimer G3(OH)$_{128}$ (3 mmol in 10 ml of distilled water) solution. The mixture was stirred for 72 h at room temperature. The mixture was then filtered and 5 ml of methanol was passed through five times through the filter to remove excess of ketoprofen. Excess Ketoprofen from filter and each fraction of methanol was analyzed by UV spectrophotometer to determine amount of encapsulated drug indirectly.

***In vitro* drug release**

Pure ketoprofen was dissolved in methanol (2 mg/ml) and used as control. The prepared ketoprofen loaded dendrimer was dissolved in distilled water at a concentration of 2 mg/ml (the same concentration of ketoprofen as 2 mg/ml pure drug solution). This solution (2 ml in volume) was transferred to a dialysis bag (size cut off = 2.5 nm) immediately. The dialysis bag was placed in a 50 ml-beaker containing 40 ml distilled water. The outer phase was stirred continuously. After a scheduled interval of time for 0.5 h, 100 µl of sample was withdrawn from the outer phase, and the outer phase was again replenished with 100 µl distilled water. The absorbance of the outer phase was monitored at 260 nm using a spectrophotometer in order to characterize the concentration of ketoprofen.

Hemolysis study

About 5 ml of the human blood from healthy individual was collected in a tube containing heparin. The blood was centrifuged at 1500 RPM for 3 minutes. The supernatant (Erythrocyte) was collected and plasma was discarded. The pellet was washed for 3 times using 0.75% NaCl and centrifuged at 1500 RPM for 5 mins. The cells were re suspended in normal saline to 0.5%. Washed erythrocytes were stored at 4 °C and used within 6 h for the hemolysis assay. To 0.5 ml of cell suspension, 0.5 ml of different concentration of the test sample (40, 60, 80 and 100 µg/ml in phosphate buffer saline (pH 7.2)) was added and incubated for 1 hr. After centrifugation, the supernatants were taken and diluted with an equal volume of normal saline and absorbance was measured at 540 nm. The phosphate buffer saline and distilled water were used as minimal and maximum hemolytic control.

Cytotoxicity study

The monolayer cell culture was trypsinized and the cell count was adjusted to 3 Lac cells/ml using medium containing 10% fetal bovine serum. Pre incubate cells at a concentration of 1× 106 cells/ml in culture medium for 3 h at 37 °C and 5% CO_2. The cells were seeded at a concentration of 5× 104 cells/well in 100 µl culture medium and incubated at 37 °C in 5 % CO_2 incubator for 24 hrs. After 24 h, when the monolayer formed, the supernatant was flicked off and added previously diluted with media of 100 µl of different concentrations of test extract in microtitre plates and kept for incubation at 37 °C in 5 % CO_2 incubator for 48 h and cells were periodically checked for granularity, shrinkage, swelling. After 48 h, the sample solution in the wells was flicked off and 10 µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 h at 37 °C in 5% CO_2 incubator. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at 570 nm.

Statistical analysis

Data are expressed as the mean, standard deviation (SD) of obtaining results. The statistical analysis of data was performed using analysis of variance (ANOVA) (Graphpad, Version 2.01, San Diego, CA). A value of $p<0.05$ was considered as statistically significant.

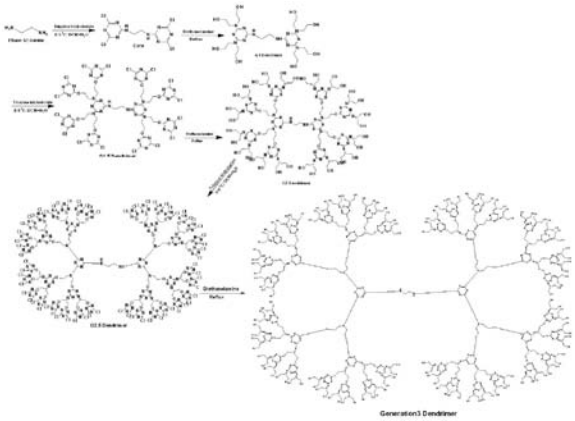

Fig. 1: Structures of dendritic generations: a) G1$(OH)_8$ b) G2$(OH)_{32}$ and c) G3$(OH)_{128}$ dendrimer

RESULTS AND DISCUSSION

Synthesis of triazine based dendrimers is depicted in [fig. 1] N,N'-biz(4,6-dichloro-1,3,5 triazin-2-yl) ethane-1,2-diamine was synthesized as core by the reaction of two moles of triazine trichloride with a mole of ethylene diamine at low temperature. Product was isolated and purified by washing with acetone and methanol. In the second step, core compound was refluxed with diethanolamine to obtain G1 dendrimer. G1 dendrimer was isolated and purified by washing and dispersing in dichloromethane. Similar to first step G1 dendrimer was reacted with cyanuric chloride to obtain G1.5 dendrimer, which was subsequently reacted with diethanolamine to obtain G2 dendrimer. The above two steps was repeated to give G2.5 and G3 dendrimer [17, 18].

Table 1: Physical description of dendrimer generations

Compound	Molecular formula	Appearance	Solubility in water	Surface groups
G0.5	$C_8H_6Cl_4N_8$	White solid	Insoluble	Cl (4)
G1	$C_{24}H_{46}N_{12}O_8$	Brown liquid	Soluble	OH (8)
G1.5	$C_{49}H_{39}Cl_{16}N_{35}O_8$	White solid	Insoluble	Cl(16)
G2	$C_{112}H_{198}N_{52}O_{40}$	Brown liquid	Soluble	OH(32)
G2.5	$C_{208}H_{166}Cl_{64}N_{148}O_{40}$	White solid	Insoluble	Cl(64)
G3	$C_{464}H_{806}N_{212}O_{168}$	Brown liquid	Soluble	OH (128)

Physical description of dendrimer generations is given in table. 1 It was observed that hydroxyl terminated dendrimers were freely soluble in water and were light brown liquids. Whereas chlorine terminated half generation dendrimers were insoluble in water and were white solids. Half generation dendrimers were terminated by hydrophobic triazine rings which may have accounted for their poor water solubility. Full generation dendrimers were terminated by hydroxyl groups on the periphery which might have contributed to its good water solubility [17].

Table 2: Prominent peaks in infrared spectrums of dendrimer generations

Compound	IR absorption band(cm^{-1}) for functional group		
	O-H	C-Cl	C-O
Core	-----	786	---
G1	3364	----	1068
G1.5	---	786	1052
G2	3366	----	1031
G2.5	----	776	1054
G3	3389	-----	1068

Infrared spectrum of the core showed absorption bands at 3291 cm^{-1} for secondary NH-stretching, 2860, 2780 cm^{-1} aliphatic C-H stretching, 1721, 1620 cm^{-1}aromatic C=N stretching of triazine ring and 786 cm^{-1} for stretching of terminal C-Cl groups. As shown in table. 2, Infrared spectrums of full generation dendrimer G1, G2 and G3 dendrimers exhibited absorption bands in the range of 3350-3400 cm^{-1}, along with bands in the range of 1500-1700 cm^{-1} for C=N stretching of triazine ring, 3000-2800 cm^{-1} for C-H stretching and 1040-1060 cm^{-1} for C-O stretching which confirmed presence of hydroxyl group. It was noted that an absorption band for C-Cl stretching were absent in FT-IR spectrums of full generation dendrimers. Infrared spectrums of chlorine terminated dendrimers

showed absorption bands for C-Cl stretching in the range of 700-800 cm^{-1}, 1500-1700 cm^{-1} for C=N stretching of triazine ring, 3000-2800 cm^{-1} for C-H stretching and 1040-1060 cm^{-1} for C-O stretching. Infrared spectrums of half generation dendrimers showed absence of bands for O-H stretching. It was also noted that IR spectrums of both full and half generation dendrimers showed absorption bands in the range of 1030-1070 cm^{-1} for C-O stretching and absorption band for C-O stretching was absent in infrared spectrums of core.

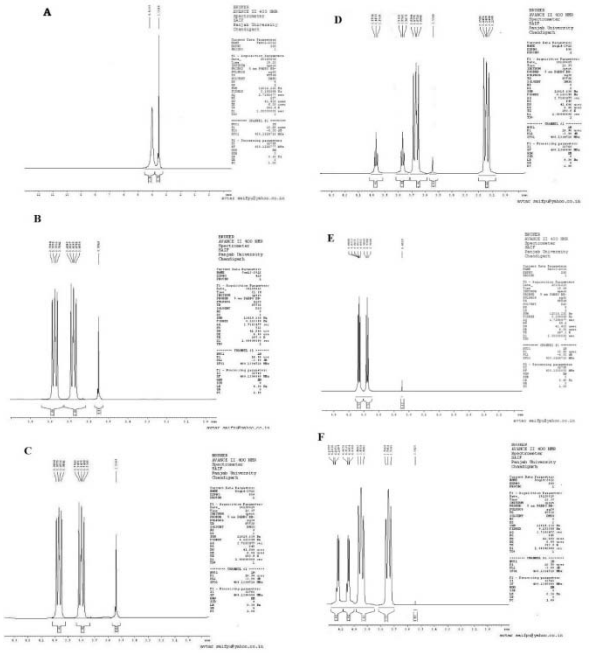

Fig. 2: ^{1}H-NMR spectrum of A) Core, B) G1 dendrimer, C) G1.5 dendrimer, D) G2 dendrimer, E) G2.5 dendrimer and F) G3 dendrimer

^{1}H-NMR spectrum for core [fig. 2A.] showed singlet at δ 3.5549 ppm for ethyl group. ^{1}H-NMR spectrum [fig. 2B] of G1 dendrimer demonstrated the above peaks for core moiety and two additional triplets at δ 3.6650-3.6968 and 3.7642-3.7904 ppm for methylene protons of diethanolamine moiety. In ^{1}H-NMR spectrum of G1.5 dendrimer [fig. 2C], the above two triplets were appeared in the downfield region at δ 3.9642-3.9902 and 4.0410-4.0890 ppm as G1.5 dendrimer was terminated by triazine rings. ^{1}H-NMR spectrum of G2 dendrimer [fig. 2D] displayed triplets at δ 3.6566-3.6950, 3.7901-3.8250 for outer diethanolamine moiety, triplets at δ 3.9480-3.9685, 4.0351-4.0581 ppm for inner diethanolamine moiety and peaks at δ 3.5455 ppm for core moiety. ^{1}H-NMR spectrum of G2.5 dendrimer [fig. 2E] showed two triplets at δ 3.9688-3.9849 and 4.0411-4.0888 ppm as both inner and outer diethanolamine groups as they were terminated by triazine rings. ^{1}H-NMR spectrum of G3 dendrimer [fig. 2F] showed triplets at δ 3.7245-3.7668, 3.9442-3.9642 ppm for outer diethanolamine moiety, triplets at δ 4.0388-4.0616, 4.1494-4.1977 ppm for inner diethanolamine moiety and peaks at δ 3.5425 ppm for ethylene proton.

^{13}C-NMR spectrum of core [fig. 3A] showed peaks δ 49.55 ppm for aromatic carbons and δ 167.76 169.10 ppm for triazine carbons. ^{13}C-NMR of G1 dendrimer [fig. 3B] showed peaks at 49.55 for aliphatic carbon, δ 59.10, 61.70 ppm for diethanolamine moiety, and peaks at δ 167.76, 169.10 ppm for triazine carbons. ^{13}C-NMR of G1.5 dendrimer [fig. 3C] showed peaks at δ 58.20, 64.10 ppm for diethanolamine moiety, peaks at δ 49.70 ppm for aliphatic carbon and δ 167.76, 176.70, 179.10 ppm for triazine carbon. ^{13}C-NMR spectrum of G2 [fig. 3D] dendrimer showed peaks at δ 59.22, 61.44 ppm for outer diethanolamine moiety, peaks at δ 66.11 ppm for inner diethanolamine moiety, peaks at δ 167.57, 169.97, 177.66, 179.67 ppm for triazine carbons. ^{13}C-NMR spectrum [fig. 3E] of G2.5 dendrimer showed peaks at 40.51 ppm for aliphatic carbon, peaks at δ 61.44, 64.64 ppm for outer diethanolamine moiety, peaks at δ 59.22, 66.11 ppm for inner diethanolamine moiety, and peaks at δ 167.57, 169.97, 177.66, 179.67, 180.78 for triazine carbons. ^{13}C-NMR spectrum of G3 dendrimer [fig. 3F] showed peaks at δ 40.59 ppm for aliphatic carbons, peaks at δ 60.66, 66.70 ppm for inner diethanolamine moiety, peaks at δ 59.95, 63.41 for outer diethanolamine moiety and peaks at δ 168.18, 169.97, 171.59, 175.71, 177.27, 179.18 ppm to triazine carbons.

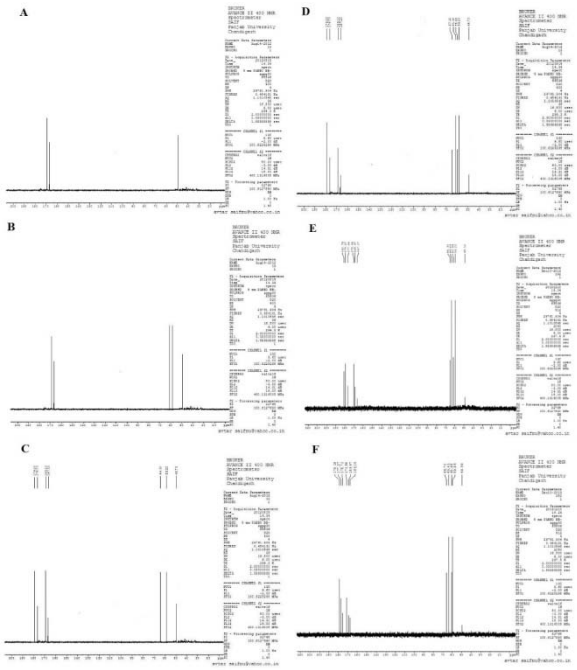

Fig. 3: ^{13}C-NMR spectrum of A) Core, B) G1 dendrimer, C) G1.5 dendrimer, D) G2 dendrimer, E) G2.5 dendrimer and F) G3 dendrimer

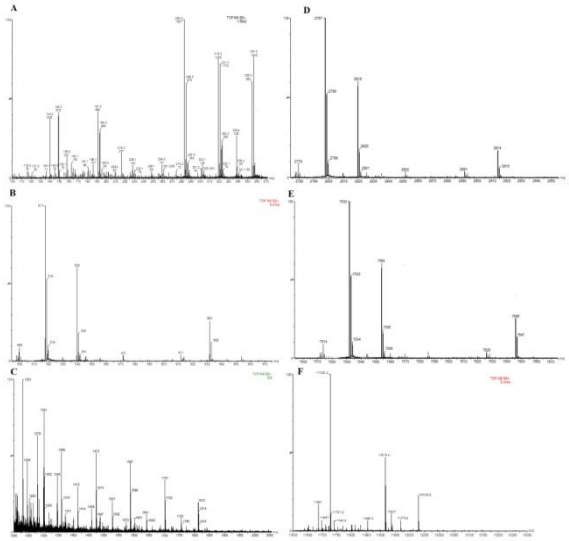

Fig. 4: ESI-Mass spectrums of A) Core, B) G1 dendrimer, C) G1.5 dendrimer, D) G2 dendrimer, E) G2.5 dendrimer and F) G3 dendrimer

All the dendrimer generations were characterized by ESI-Mass spectrometry. It was revealed that all molecular ion peaks of dendrimer generations correspond to their calculated molecular weight.

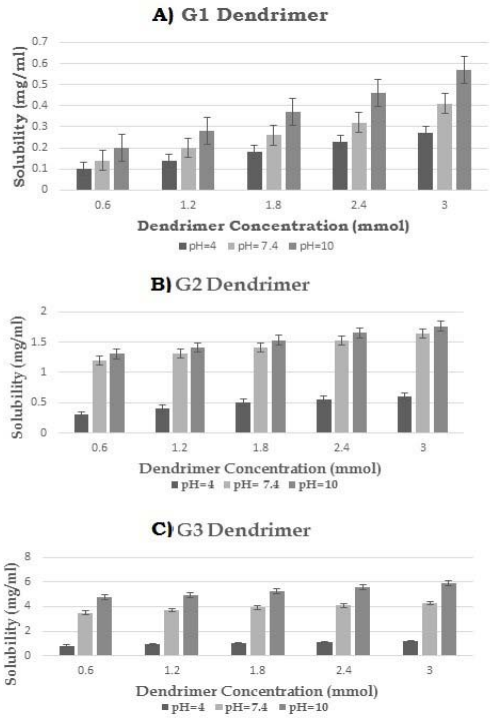

Fig. 5: Effect of the generations of triazine dendrimers and pH on aqueous solubilisation of Ketoprofen (n = 3)

Solubility study

A series of solubility studies for ketoprofen by dendrimer generations were carried out using different concentrations (0.6 mmol to 3 mmol) of dendrimer generations at pH 4.0, 7.4 and 10.0 [Figure. 5]. Solubility of practically water insoluble drug ketoprofen was improved approximately up to 4 mg/ml by generation 3 dendrimer at pH 7.4. It was also witnessed that with increase in concentration of dendrimer generations, solubility of ketoprofen was increased in a linear manner. It was proposed that as a dendrimer contains a 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 ketoprofen by dendrimer could be either hydrophilic interaction or hydrogen bonding or both[2]. It was revealed that with increased in pH generation number of dendrimer.

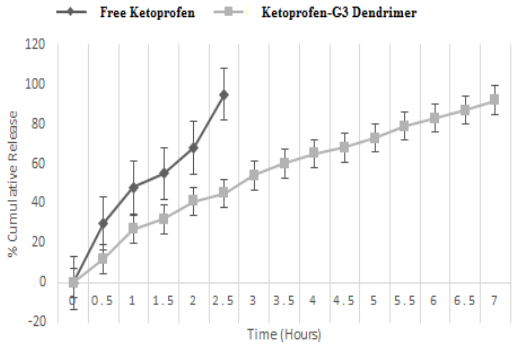

Fig. 6: % Cumulative release profile ketoprofen from free ketoprofen and ketoprofen containing G3 dendrimer (n=3)

Drug loading and *in vitro* release

Ketoprofen loaded dendrimer was prepared as per reported method [16, 18]. Drug loaded dendrimer further characterized by UV spectrophotometer which revealed that about 24.60% of ketoprofen was loaded by dendrimer. About 95% Ketoprofen was released in 2.5 h from free ketoprofen whereas same amount of drug was released after 7 h from ketoprofen dendrimer complex. This revealed that ketoprofen was released slowly from ketoprofen dendrimer complex compared to free drug [23-25].

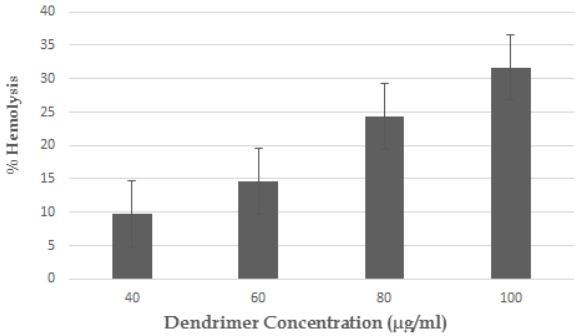

Fig. 7: % Hemolysis of Red blood cells by G3 dendrimer after 1 hour of incubation (n=3)

Hemolysis

It was observed that G3 dendrimer showed concentration dependent hemolysis. However, triazine based G3 dendrimer were less hemolysis compared to commercialized PAMAM dendrimer[26] [fig. 7]. Positively charged amine terminated PAMAM dendrimer interacts with negatively charged surfaces of red blood cells and caused hemolysis[27]. In comparison, G3 dendrimers have less toxicity due to hydroxyl end groups at periphery.

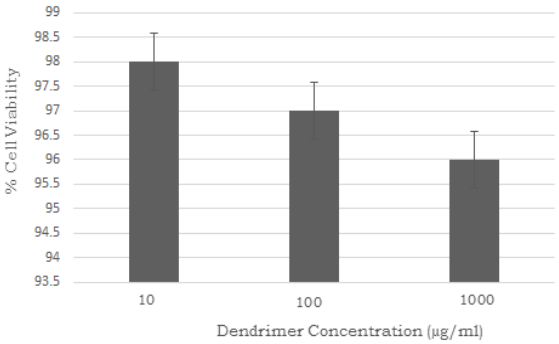

Fig. 8: Cytotoxicity of AG3, BG3 and CG3 dendrimers on A-549 cell lines after 48 h of incubation

Cytotoxicity

Cellular toxicity of G3 dendrimers on A-549 cell lines were investigated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide) assay technique. It is water soluble, yellow colored dye. Living cells are able to converted MTT into water insoluble, blue colored formazan crystals by reductive cleavage of tetrazoluim ring. Formazan crystals extracted by organic solvents and measured at 550 nm, and the result is correlated with living cells to measure cell viability. Our results [fig. 8] displayed that dendrimers showed more than 90% cell viability at concentration levels ranging from 10 µg/ml to 1000 µg/ml. So, synthesized dendrimers having less cytotoxic compare to PAMAM dendrimers.

CONCLUSION

Synthesized dendrimer generations have significantly enhanced solubility of hydrophobic drug ketoprofen. Ketoprofen solubility was affected by pH, generation, and concentration of dendrimer. Sustained

release of ketoprofen was comparatively slower from loaded ketoprofen dendrimer. Cytotoxicity and hemolytic potential displayed that dendrimers were less toxic and can be explored as drug carriers.

CONFLICT OF INTERESTS

Declared None

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