

“TRIAZINE BASED DENDRIMER AS SOLUBILITY ENHANCERS OF KETOPROFEN: EFFECT OF CONCENTRATION, PH AND GENERATION”

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ABSTRACT

Objective: To synthesize novel dendritic architecture from *N,N'*-bis(4,6-dichloro-1,3,5-triazin-2-yl)propane-1,3-diamine as core. To apply of different generations of synthesized triazine based dendrimer (G1-G3) to improve aqueous solubility of hydrophobic drug Ketoprofen and to study the effect of concentration, pH and generation on aqueous solubility of Ketoprofen.

Methods: Triazine based dendrimer was synthesized up to generation 3 using divergent method. Synthesized dendrimer generations were characterized by FT-IR, ¹H-NMR and ¹³C-NMR. Potential of full generation triazine based dendrimers (G1-G3) as solubility enhancers of Ketoprofen were investigated by Higuchi and Connors method at different dendrimer concentrations, pH and generations.

Results: Triazine based dendrimer significantly enhances solubility of Ketoprofen by either hydrophobic interaction or hydrogen bonding or both.

Conclusion: Synthesized triazine based dendrimers (G1-G3) enhances solubility of Ketoprofen in water. Solubility of Ketoprofen increased with increase in concentration of the dendrimer, pH and dendrimer generation.

Keywords: Triazine Trichloride, Dendrimer, Ketoprofen, Solubility enhancers

INTRODUCTION

Poor aqueous solubility of an active pharmaceutical ingredient (API) is one of the most demanding issues in pharmaceutical research because most of the new API candidates under development have poor water solubility [1]. Since a large portion of the body is made up of water, drugs must have sufficient water solubility to have an acceptable bioavailability. Poorly water soluble drugs tend to be eliminated from the gastrointestinal tract before they get an opportunity to dissolve and absorbed in blood circulation which results in poor bioavailability, and limits their use [2, 3].

Ketoprofen (2-(3-benzoylphenyl) propionic acid) is an aryl propionic acid derivative, is a Nonsteroidal anti-inflammatory drug (NSAID) with well-known anti-inflammatory, antipyretic as well as analgesic properties [4]. It is also an inhibitor of prostaglandin synthetase [5]. However Ketoprofen is not freely soluble in water, causes local or systematic disturbances in the gastrointestinal tract and reduces its bioavailability [6] which restricts its use in topical and parenteral applications [7]. 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 [8-10].

Dendrimers are a new class of macromolecules known for their large no of reactive end groups, tree like structure with branches and monodisperse molecular weight distribution [11]. Dendrimers with hydrophilic exterior and hydrophobic interiors have shown to exhibit Missile like behavior and have a “container” like properties in solution [12]. Dendrimers enhances solubility of drugs probably by hydrophobic interactions, hydrogen bonding and electrostatic interaction between dendrimers and drugs [13, 14]. Dendrimers have enhanced solubility of many drugs ranging from 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. Mainly Polyamidoamine (PAMAM) type dendrimers have been exploited as solubility enhancers of drugs. A few reports are available on solubility enhancement of drugs using other types of dendrimer such as by Polypropylene-imine type dendrimer

[21], Polyglycerol dendrimer [20], and triazine based dendrimer with triazine trichloride as core [22].

We have synthesized novel dendritic architecture with *N,N'*-bis(4,6-dichloro-1,3,5-triazin-2-yl)propane-1,3-diamine as core and terminated by diethanolamine linkages [23] using divergent method. Dendrimer was synthesized up to generation 3 and was characterized by Infrared spectroscopy (FT-IR), ¹H-NMR and ¹³C-NMR. The Candidature of dendrimer generations (G1-G3) as solubility enhancers of Ketoprofen were investigated. Effect of pH, concentration and generation of dendrimer on solubility of Ketoprofen were studied.

MATERIALS AND METHODS

Ketoprofen was generously provided by A.R. College of Pharmacy, Vallabh Vidhyanagar as gift sample. Triazine trichloride (cyanuric chloride), propane-1,3-diamine, 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. ¹H-NMR and ¹³C-NMR spectra were recorded at 400 MHz in Bruker Avance 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)propane-1,3-diamine (core)

Cyanuric Chloride (0.02mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of propane 1,3-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 : 3291(NH), 2872, 2780(Aliphatic C-H), 1722, 1625(C=N of triazine), 841, 792 (C-Cl); $^1\text{H-NMR}$ (400MHz, $\text{DMSO-}d_6$) δ ppm: 1.8031-1.8649 (m, 2H, N-CH₂-CH₂-CH₂-N), 3.4304-3.4660 (m, 4H, N-CH₂-CH₂-CH₂-N); $^{13}\text{C-NMR}$ (75MHz, $\text{DMSO-}d_6$) δ ppm: 28.70 (N-CH₂-CH₂-CH₂-N), 38.18 (N-CH₂-CH₂-CH₂-N), 166.01, 171.66 (Triazine part).

Synthesis of generation 1 dendrimer (G1)

N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl)propane-1,3-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 : 3373(O-H), 2949, 2881(aliphatic C-H), 1664(C=N of triazine), 1056 (C-O); $^1\text{H-NMR}$ (400MHz, D_2O) δ ppm: 1.9091-1.9403(m, 2H, N-CH₂-CH₂-CH₂-N), 3.3211-3.3460 (m, 2H, N-CH₂-CH₂-CH₂-N), 3.5980-3.6485 (m, 16H, N-CH₂-CH₂-OH), 3.9480-3.9856 (m, 16H, N-CH₂-CH₂-OH); $^{13}\text{C-NMR}$ (75MHz, D_2O) δ ppm: 28.72 (CH₂), 38.22 (N-CH₂), 50.66 (N-CH₂-CH₂-OH), 60.18 (N-CH₂-CH₂-OH), 166.61, 171.16 (triazine part).

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 : 3217(N-H), 2833,2780(Aliphatic C-H), 1777, 1752, 1721 (C=N of triazine), 1053(C-O), 775(C-Cl); $^1\text{H-NMR}$ (400MHz, $\text{DMSO-}d_6$) δ ppm: 1.9201-1.9549 (m, 2H, N-CH₂-CH₂-CH₂-N), 3.3425-3.3759 (m, 4H, CH₂-NH), 4.0175-4.0759 (m, 16H, N-CH₂-CH₂-O-Tri), 4.1641-4.2015 (m, 16H, N-CH₂-CH₂-O-Tri); $^{13}\text{C-NMR}$ (75MHz, $\text{DMSO-}d_6$) δ ppm: 28.70 (CH₂), 38.10 (N-CH₂), 51.01 (N-CH₂-CH₂-O-tria), 60.58 (N-CH₂-CH₂-O-tri), 163.01, 168.66, 170.18, 172.28 (Triazine part).

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 : 3356(O-H), 2942, 2881, 2840 (aliphatic C-H), 1676, 1619(C=N of triazine), 1065(C-O); $^1\text{H-NMR}$ (400MHz, D_2O)

δ ppm: 1.9331-1.9694 (m, 2H, N-CH₂-CH₂-CH₂-N), 3.3225-3.3519 (m, 4H, CH₂-NH), 3.6813-3.7303 (m, 64H, N-CH₂-CH₂-OH), 3.8631-3.9182 (m, 64H, N-CH₂-CH₂-OH), 4.0175-4.0759 (m, 16H, N-CH₂-CH₂-O-tri), 4.1641-4.2015 (m, 16H, N-CH₂-CH₂-O-tri); $^{13}\text{C-NMR}$ (75MHz, D_2O) δ ppm: 28.50 (CH₂), 38.08 (N-CH₂), 60.81(N-CH₂-CH₂-O-tri), 61.28 (N-CH₂-CH₂-OH), 63.88(N-CH₂-CH₂-O-tri), 65.70 (N-CH₂-CH₂-OH), 163.01, 168.66, 170.78, 172.28 (Triazine portion).

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 : 3215(NH), 2881, 2833(aliphatic C-H), 1778, 1752, 1722(C=N of triazine), 1054 (C-O), 776 (C-Cl); $^1\text{H-NMR}$ (400MHz, $\text{DMSO-}d_6$) δ ppm: 3.3382-3.3728(t, 4H, CH₂-N), 3.9662-4.0226(m, 80H, N-CH₂-CH₂-O-Tri), 4.0777-4.1440(m, 80H, N-CH₂-CH₂-O-Tri); $^{13}\text{C-NMR}$ (75MHz, $\text{DMSO-}d_6$) δ ppm: 28.8 (N-CH₂-CH₂-N), 38.2(N-CH₂-CH₂-N), 60.0(N-CH₂-CH₂-OH), 61.2(N-CH₂-CH₂-O-tri), 63.3(N-CH₂-CH₂-OH), 65.5(N-CH₂-CH₂-O-Tri), 164.1, 166.4, 168.8, 171.7, 172.5, 174.0 (Triazine Portion).

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 : 3371(OH), 2948, 2839(Aliphatic C-H), 1733(C=N of triazine), 1063(C-O); $^1\text{H-NMR}$ (400MHz, D_2O) δ ppm: 3.3211-3.3440 (t, 4H, CH₂-N), 3.6438-3.7243(m, 264H, N-CH₂-CH₂-OH), 3.7929-3.8586(m, 264H, N-CH₂-CH₂-OH), 3.9107-3.9520 (m, 80H, N-CH₂-CH₂-O-Tri), 4.0197-4.0315(m,80H,N-CH₂-CH₂-O-Tri); $^{13}\text{C-NMR}$ (75MHz, D_2O): 28.00(N-CH₂-CH₂-CH₂-N), 38.14(N-CH₂-CH₂-CH₂-N), 59.90(N-CH₂-CH₂-OH), 60.62 (N-CH₂-CH₂-O-Tri), 63.31(N-CH₂-CH₂-OH), 66.69(N-CH₂-CH₂-O-tri), 168.16, 169.91, 171.51, 175.57, 177.20, 179.11(Triazine portion).

Solubility Studies

Solubility study was carried out according to the method described by Higuchi and Connors[24]. 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.

Table 1: Physical description of dendrimer generations

Compound	Molecular formula	Appearance	Solubility in water	Surface groups (number)
Core	C ₉ H ₈ C ₁₄ N ₈	White solid	Insoluble	Cl (4)
G1	C ₂₅ H ₄₈ N ₁₂ O ₈	Brown liquid	Soluble	OH (8)
G1.5	C ₄₉ H ₄₀ Cl ₁₆ N ₃₆ O ₈	White solid	Insoluble	Cl(16)
G2	C ₁₁₃ H ₂₀₀ N ₅₂ O ₄₀	Brown liquid	Soluble	OH(32)
G2.5	C ₂₀₉ H ₁₆₈ Cl ₆₄ N ₁₄₈ O ₄₀	White solid	Insoluble	Cl(64)
G3	C ₄₆₅ H ₈₀₈ N ₂₁₂ O ₁₆₈	Brown liquid	Soluble	OH (128)

RESULTS AND DISCUSSION

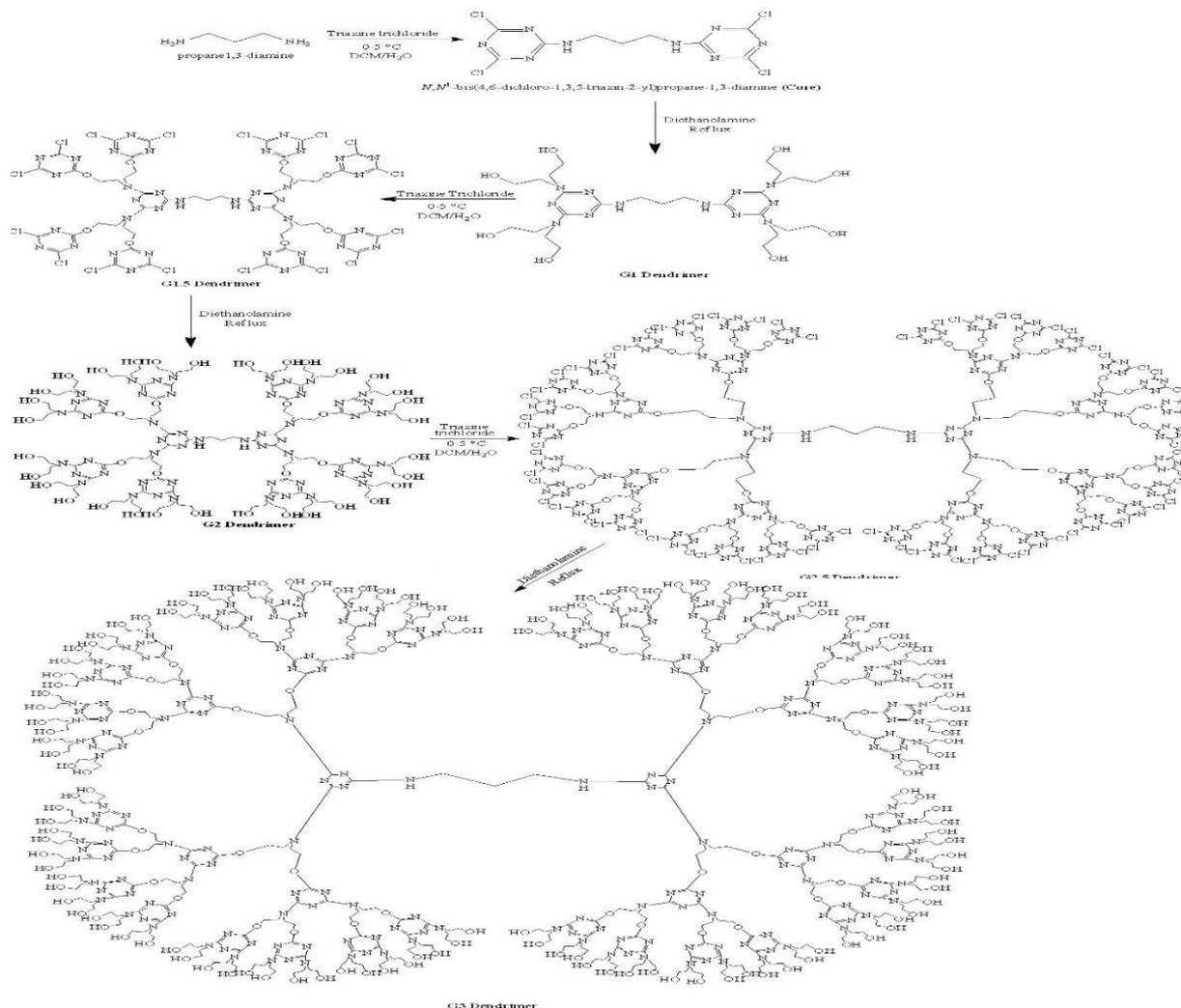
Synthesis and Characterization of dendrimers

Dendrimer was synthesized up to generation 3 using divergent method reported in literature [23]. As shown in scheme 1, propane-1,3-diamine was reacted with two moles of cyanuric chloride at 0-5°C in the first step to give *N,N'*-bis(4,6-dichloro-1,3,5-triazin-2-yl)propane-1,3-diamine as a core for dendrimer synthesis. Core was

then refluxed with diethanolamine which was used as both solvent and reactant in second step to give hydroxyl terminated G1 dendrimer. G1 dendrimer was again reacted with cyanuric chloride to give chlorine terminated G1.5 dendrimer. G1.5 dendrimer was again reacted with diethanolamine to give hydroxyl terminated G2 dendrimer. The above two steps were repeated to give G2.5 and G3 dendrimer. Purity of synthesized dendrimer was investigated by techniques such as FTIR, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ [23]. Physical

description of dendrimer generations is given in Table 1. It is evident that core, G1.5 and G2.5 dendrimers were chlorine terminated and water insoluble so, they were not used for solubility studies, whereas G1, G2 and G3 were hydroxyl terminated and water

soluble, hence used as solubility enhancers of Ketoprofen. It was also worth noting that with every increase in dendrimer generations there was a significant increase in terminal hydroxyl groups, molecular weight and surface area.



Scheme 1: Shows Synthetic Route to Dendrimer

Solubility studies

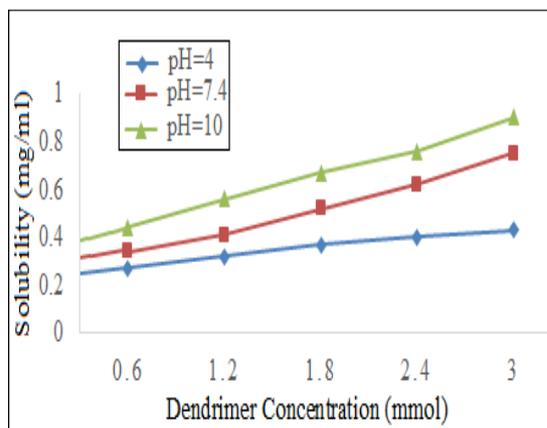


Fig. 1: shows solubility of Ketoprofen with increasing concentration of Dendrimer (G1)

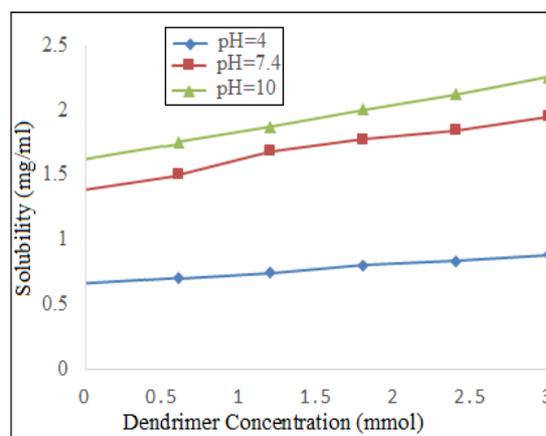


Fig. 2: Solubility of Ketoprofen with increasing concentration of Dendrimer (G2)

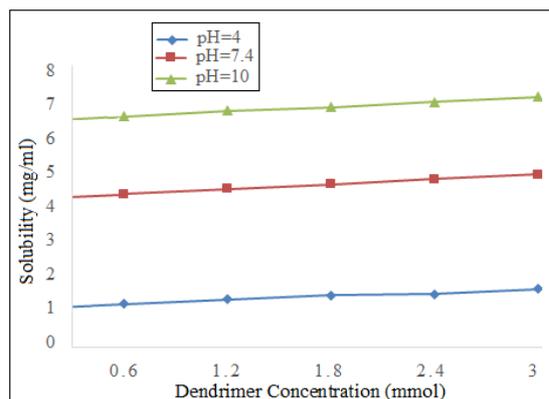


Fig. 3: Solubility of Ketoprofen with increasing concentration of Dendrimer (G3)

A series of solubility experiments were carried out using different concentrations (0.6 mmol to 3 mmol) of dendrimer generations (G1-G3) at pH 4.0, 7.4 and 10.0. It was observed (Figure 1-3) that for every generation (G1-G3), the solubility of Ketoprofen was increased with increase in dendrimer concentration in almost linear manner. 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 Ketoprofen by dendrimer could be either hydrophilic interaction or hydrogen bonding or both [22]. For all dendrimer generations, increased aqueous solubility of Ketoprofen was observed to increase in pH from 4.0 to 10.0. Solubility of Ketoprofen was lowest at pH 4.0 and highest at pH 10 for all dendrimer generations. It was proposed that weakly acidic Ketoprofen is not fully ionized at lower pH, therefore it cannot freely interact with dendrimer results in lower solubility of Ketoprofen at low pH [16]. It was evident from the results (Figure 1-3), the solubility of Ketoprofen was significantly affected by generation of the dendrimer. Solubility of Ketoprofen was highest in generation 3 dendrimer, low in generation 2 and lowest for generation 1 dendrimer. 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 Ketoprofen molecule was significantly increased. Hence solubility of Ketoprofen was significantly increased with increase in dendrimer generation [16-17].

CONCLUSION

Triazine based dendrimers were synthesized and characterized by techniques such as FTIR, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$. It was concluded that Triazine based dendrimers (G1-G3) significantly enhances solubility of hydrophobic drug Ketoprofen in water. It was proposed that dendrimer enhances solubility of Ketoprofen by either hydrophobic interaction or hydrogen bonding or both. Aqueous solubility of Ketoprofen was dependent upon factors such as pH, concentration and the generation of dendrimer and solubility was increased with increase in the above factors.

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