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 enhancer of Ketoprofen, Ibuprofen, and Diflunisal and to study effect 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, 1H-NMR, 13C-NMR, ESI-Mass spectrometry and elemental analysis. Candidature of full generation triazine based dendrimers (G1-G3) as solubility enhancer 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, 1H-NMR, 13C-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 hurdle 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 chemopreventive 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 reported and depends on factors such as generation size and terminal functionality [12]. Dendrimers have hydrophilic exteriors and hydrophobic 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 their solubilization 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 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), 1H-NMR, 13C-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 Diflunisal were investigated. Effect of pH, concentration and generation of dendrimer on solubility of said drugs were studied.

MATERIALS AND METHODS

Ketoprofen, Ibuprofen, and Diflunisal were generously provided by A.R. College of Pharmacy, Vallabh Vidhyanagar as gift samples. Triazine trichloride (cyanuric chloride), hexane, 1,6-diamine, acetone, dichloromethane and methanol were purchased from Sigma-Aldrich (India) Ltd. A.C. 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-FTR spectrometer instrument through KBr disc and pellet method and nujol null method. 1H-NMR and 13C-NMR spectra were recorded at 400 MHz in Brucker Advance H-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)

Cyanoauric 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 dropwise in the solution of cyanoauric 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 color solid was formed.

Yield, 83%: FT-IR (KBr, cm⁻¹): 3281 (N-H), 2894, 2779 (aliphatic C-H), 1722, 1624 (C=N of triazine), 844, 796 (C-Cl). 1H-NMR (400 MHz, DMSO-d6) δ ppm: 3.138-3.1355 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 1.4941-1.5154 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 3.4191-3.4381 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 3.7891 (m, 16H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 5.804 (m, N-CH₂N(CH₂CH₂CH₂OH). 16.01 (CH₃). 16.59 (CH₂). 20.25.25.29 (N-CH₂N(CH₂CH₂CH₂N-). 30.52 (N-CH₂N(CH₂CH₂CH₂N-). 58.04 (N-CH₂N(CH₂CH₂OH). 64.01 (N-CH₂N(CH₂CH₂OH). 168.04, 169.90 (Trizine part); Anal. Calcd. for C₃₁H₂₆N₉O₄C₪: C, 43.97; H, 3.42; N, 37.21, Found: C, 43.02; H, 3.46; 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): 3364 (O-H), 2941 (aliphatic C-H), 1668 (C=N of triazine), 1063 (C-O). 1H-NMR (400 MHz, DMSO-d6) δ ppm: 1.3138-3.1355 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 1.4945-1.5154 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 3.4191-3.4381 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 3.7891 (m, 16H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 5.804 (m, N-CH₂N(CH₂CH₂CH₂OH). 16.01 (CH₃). 16.59 (CH₂). 20.25.25.29 (N-CH₂N(CH₂CH₂CH₂N-). 30.52 (N-CH₂N(CH₂CH₂CH₂N-). 58.04 (N-CH₂N(CH₂CH₂OH). 61.04 (N-CH₂N(CH₂CH₂OH). 168.04, 169.90 (Trizine part); Anal. Calcd. for C₁₁H₂₆N₉O₄C₄: C, 43.97; H, 7.92; N, 24.47; Found: C, 49.02; H, 7.98; N, 24.50.

Synthesis of generation 1.5 dendrimer (G1.5)

Cyanoauric Chloride (0.08mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of G1 dendrimer (0.01mmol) containing sodium hydroxide (0.008 mmol) in water was added dropwise in the solution of cyanoauric 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 color solid was formed.

Yield 84%; FTIR (KBr, cm⁻¹): 3214 (N-H), 2833, 2832, 2780 (aliphatic C-H). 1779, 1753, 1717 (C=N of triazine), 1061 (C-O). 786 (C-O). 1H-NMR (400 MHz, DMSO-d6) δ ppm: 1.3138-3.1355 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 1.4945-1.5154 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 3.4191-3.4381 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 3.7891 (m, 16H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 5.804 (m, N-CH₂N(CH₂CH₂CH₂OH). 16.01 (CH₃). 16.59 (CH₂). 20.25.25.29 (N-CH₂N(CH₂CH₂CH₂N-). 30.52 (N-CH₂N(CH₂CH₂CH₂N-). 58.04 (N-CH₂N(CH₂CH₂OH). 168.04, 169.90 (Trizine part); Anal. Calcd. for C₁₄H₂₈N₁₂O₆C₄: C, 43.97; H, 7.92; N, 24.47; Found: C, 49.02; H, 7.98; N, 24.50.

Synthesis of generation 2 dendrimer (G2)

Generation 1.5 dendrimer (0.01mmol) was dissolved in an excess of diethanolamine (0.064 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): 3368 (O-H), 2947, 2872 (aliphatic C-H). 1629 (C=N of triazine), 1033 (C-O). 1H-NMR (400 MHz, DMSO-d6) δ ppm: 1.3138-3.1355 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 1.4954-1.5181 (m, 4H, N-CH₂N(CH₂CH₂CH₂CH₂N-). 3.4144-3.4333 (m, 4H, CH₃). 3.6833-3.7574 (m, 264, N-CH₂N(CH₂CH₂OH). 3.8832-3.9243 (m, 264, N-CH₂N(CH₂OH). 4.0953-4.1880 (m, 80H, N-CH₂N(CH₂O-tri). 4.2342-4.3168 (m, 80H, N-CH₂N(CH₂O-tri). ¹³C-NMR (75MHz, DMSO-d6) δ ppm: 25.20, 25.29 (N-CH₂N(CH₂CH₂CH₂CH₂N-). 44.44 (N-CH₂N(CH₂CH₂CH₂CH₂N-). 59.92 (N-CH₂N(CH₂O-tri). 60.60 (N-CH₂N(CH₂OH). 63.39 (N-CH₂N(CH₂O-tri). 66.69 (N-CH₂N(CH₂OH). 168.11, 169.99, 171.55, 175.81, 177.28, 179.11 (Trizine part); Anal. Calcd. for C₁₂H₂₅N₁₄O₇C₁₆: C, 46.46; H, 6.78; N, 24.54; Found: C, 46.51; H, 6.80; N, 24.39; ESI-Mass: Molecular Wt: 12066 found (M+): 12087.

Solubility Studies

Solubility study was carried out according to 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 nm for Ketoprofen, Ibufrofen 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 nucleophilic substitution of triazine trichloride and selectivity of triazine trichloride toward aliphatic amino to hydrazine 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

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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.

Dendrimer generations were characterized by Infrared spectroscopy, the results are furnished in Table 2. In Infrared 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

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

Dendrimer generations were characterized by \( ^1\)H-NMR spectroscopy to investigate their purity. All dendrimer generations were characterized by \( ^1\)H-NMR spectroscopy to investigate their purity. 

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Table 1: Physical description of dendrimer generations

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>MOLECULAR FORMULA</th>
<th>APPEARANCE</th>
<th>SOLUBILITY IN WATER</th>
<th>SURFACES GROUPS (NUMBER)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>C(_2)H(_4)C(_6)N(_8)</td>
<td>White solid</td>
<td>Insoluble</td>
<td>Cl (4)</td>
</tr>
<tr>
<td>G1</td>
<td>C(_6)H(_8)N(_5)O(_2)</td>
<td>Brown liquid</td>
<td>Soluble</td>
<td>OH (8)</td>
</tr>
<tr>
<td>G1.5</td>
<td>C(_6)H(_6)C(_6)O(_2)N(_8)</td>
<td>White solid</td>
<td>Insoluble</td>
<td>Cl (16)</td>
</tr>
<tr>
<td>G2</td>
<td>C(_6)H(_8)N(_5)O(_2)</td>
<td>Brown liquid</td>
<td>Soluble</td>
<td>OH (32)</td>
</tr>
<tr>
<td>G2.5</td>
<td>C(_6)H(_12)C(_6)O(_2)N(_4)</td>
<td>White solid</td>
<td>Insoluble</td>
<td>Cl (64)</td>
</tr>
<tr>
<td>G3</td>
<td>C(_6)H(_12)C(_6)O(_2)N(_4)</td>
<td>Brown liquid</td>
<td>Soluble</td>
<td>OH (128)</td>
</tr>
</tbody>
</table>

Physical appearance and solubility in water of both chlorine terminated half generation dendrimers and hydroxyl terminated full generation dendrimers were different.

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Table 2: Infra-Red spectroscopy data of dendrimer generations

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>IR absorption band(cm(^{-1})) for functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O-H</td>
</tr>
<tr>
<td>Core</td>
<td>--</td>
</tr>
<tr>
<td>G1</td>
<td>3364</td>
</tr>
<tr>
<td>G1.5</td>
<td>--</td>
</tr>
<tr>
<td>G2</td>
<td>3389</td>
</tr>
<tr>
<td>G2.5</td>
<td>--</td>
</tr>
<tr>
<td>G3</td>
<td>3368</td>
</tr>
</tbody>
</table>

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**Scheme 1:** Shows synthetic route of dendrimer

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**Table 2:** Shows \( ^1\)H-NMR spectra of A) core, B) G1 Dendrimer, C) G1.5 Dendrimer, D) G2 dendrimer, E) G2.5 Dendrimer and F) G3 Dendrimer

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**Figure 2:** Shows \( ^1\)H-NMR spectra of A) core, B) G1 Dendrimer, C) G1.5 Dendrimer, D) G2 dendrimer, E) G2.5 Dendrimer and F) G3 Dendrimer
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 13C-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**

![ESI-MS and Elemental Analysis](image)

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

Table 3: Elemental Data of dendrimers

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

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 generation number. 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 0.7 mg/ml, 1.87 mg/ml and 4.67 mg/ml and Diflunisal up to 0.47 mg/ml, 1.70 mg/ml and 4.36 mg/ml at 7.4 pH. It was evident that solubility of NSAIDs 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 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](image1.png) 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](image2.png) 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](image3.png) 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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