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Original Research Paper

Dendritic macromolecules as nano-scale drug carriers: Phase solubility, *in vitro* drug release, hemolysis and cytotoxicity study

Pravinkumar M. Patel [a,*], *Rinkesh Patel* [a], *Devang Wadia* [b], *Rajni M. Patel* [c]

[a] *Industrial Chemistry Department, V.P. & R.P.T.P. Science College, Affiliated to Sardar Patel University, Vallabh Vidhyanagar, Gujarat, India*

[b] *Nirlife Healthcare Division, Nirma Limited, Ahmedabad, Gujarat, India*

[c] *P. D. Patel Institute of Pure and Applied Sciences, Charusat University, Changa, Gujarat, India*

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ABSTRACT

Potential of nanoscale triazine based dendritic macromolecules G1, G2 and G3 as solubility enhancers of drug was investigated. Effect of pH, concentration and generation of synthesized dendritic macromolecules on solubility of ketoprofen was studied. G3 dendrimer was further exploited as carrier for sustained release. Ketoprofen was encapsulated by inclusion complex method and also characterized by Flourier Transform Infrared spectroscopy. Sustained release study of ketoprofen from ketoprofen loaded dendrimer was carried out and compared with free ketoprofen. Hemolytic potential and Cytotoxicity assay using A-549 lung cancer cell lines revealed that synthesized triazine based dendritic macromolecules having more potential that commercially available PAMAM dendrimer.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely prescribed medications in the world. Their main benefit originates from their anti-inflammatory and analgesic properties [1]. These drugs are commonly employed for the conditions associated with osteoarthritis and other chronic musculoskeletal conditions [2,3]. Ketoprofen is a member of nonsteroidal anti-inflammatory drug, also used as an inhibitor of prostaglandin synthetase [4], administered orally, three or four times per day [5]. Several side effects are related with ketoprofen such as gastrointestinal side effects, renal side effects and additional side effects such as

* *Corresponding author*. Industrial Chemistry Department, V.P. & R.P.T.P. Science College, Affiliated to Sardar Patel University, Vallabh Vidhyanagar 388120, Anand, Gujarat, India.
E-mail addresses: drpravinpatel@rediffmail.com, zhhundds@163.com (P.M. Patel).
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hypersensitivity limits its application [6,7]. It was advised that the use of ketoprofen in the parenteral application could control these critical side effects. However, low water solubility of ketoprofen leads to poor bioavailability pose a great challenge for the formulation of drug [7] for use in topical and parenteral applications. Many methodologies have been applied to improve solubility and reduce side effects of ketoprofen for example use of liposomes, synthetic polymer as drug delivery agents [8,9]. Ketoprofen has often employed as model drug for such studies [10].

Dendrimers are three dimensional, nanosized macromolecules having monodisperse molecular weight distribution obtained by repetitive sequence of reactions [11]. Dendrimer have several unique properties such as nano-scale monodispersity, scaffolding properties, amplifiable and functionable surface groups and dimensions that mimics biomolecules such as protein [12]. Therefore dendrimers are often used in biomedical applications such as drug solubilization [13], drug delivery [14], MRI contrast agents [15] etc. Application of dendrimer as a vehicle for drug delivery has been of great interest [16].

Dendrimers based on triazine are well known. Synthesis of triazine dendrimer is facile since it does not require functional group manipulations compared to other classes of dendrimers such as PAMAM dendrimer [17]. Recently, dendrimers based on triazine have been used in a wide range of applications such as in optics [18,19], for the delivery of anti-tumor agents [20], in molecular recognition [21], catalytic supports [22] etc.

Previously, synthesis and characterization and application of s-triazine based dendrimer for water remediation has been reported [23–25]. Synthesized dendrimer has hydroxyl groups on periphery and previously it has been reported that hydroxyl terminated dendrimers are less cytotoxic than amine terminated PAMAM dendrimers [26] and recent biomedical applications on triazine based dendrimers [26–28] motivating us to investigate triazine based dendrimer as a carrier of sustained release using ketoprofen as a model drug.

In the present investigation, potential of triazine based dendrimer as potential drug carrier of ketoprofen was evaluated. Ketoprofen was loaded to G3 dendrimer by inclusion complex method. Ketoprofen loaded dendrimer was further investigated by Infrared spectroscopy. Release of Ketoprofen from Ketoprofen-dendrimer complex in dialysis bag was measured and compared with that of free Ketoprofen. Cytotoxicity and hemolysis was carried out to evaluate toxicity and biocompatibility of the dendrimer.

2. Materials and methods

2.1. Materials

Ketoprofen was generously provided by A.R. College of Pharmacy, Vallabh Vidhyanagar as gift sample. Triazine trichloride (cyanuric chloride), 1,4-butanediamine, acetone, dichloromethane and methanol were purchased from Sigma-Aldrich (India) Ltd. 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. FTIR was carried out in the range of 250–4000 cm^{-1} using Perkin Elmer-Spectrum RX-FTIR spectrometer instrument. Carl Ziess-Primovert inverted microscope was used for microscopic images of A-549 cell lines.

2.2. Synthesis and characterization of triazine based dendrimer

Triazine based dendrimer was synthesized by following method. Triazine trichloride (0.02 mmol) was reacted with 1,4-butanediamine (0.01 mmol) at 0–5 °C to give N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl)butane-1,4-diamine as core for dendrimer synthesis. N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl) butane-1,4-diamine was purified by washing with Acetone and Methanol. N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl)butane-1,4-diamine (0.01 mmol) was reacted with diethanolamine (0.04 mmol) to give hydroxyl terminated generation 1 (G1) dendrimer. G1 dendrimer was purified by washing and dispersing in dichloromethane. Similar to first step, G1 dendrimer (0.01 mmol) was reacted with triazine trichloride (0.08 mmol) at 0–5 °C to give chlorine terminated half generation G1.5 dendrimer (G1.5). Similar to second step, chlorine terminated half generation dendrimer (G1.5) (0.01 mmol) was reacted with diethanolamine (0.16 mmol) to give full generation hydroxyl terminated dendrimer (G2) [23]. The above two steps were repeated to give half generation G2.5 and full generation G3 dendrimers respectively. Synthesized core and all dendrimer generations were fully characterized by spectral analysis such as FT-IR, ^{1}H-NMR, ^{13}C-NMR and ESI-Mass Spectrometry [23].

2.3. Solubility study

Solubility study was carried out according to the method described by Higuchi and Connors (1965). Excess of ketoprofen was added to screw-capped vials containing different concentrations (0.6 mmol–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.

2.4. Drug encapsulation

Drug loading was performed by reported methods with little modifications [26,29]. Known amount of ketoprofen was added to a solution containing G3 dendrimer (3 mmol in 10 ml of distilled water). 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.

2.5. In vitro drug release

Pure ketoprofen was dissolved in methanol (2 mg/ml) and used as control. The prepared ketoprofen loaded dendrimer