RESEARCH ARTICLE

# Polyamidoamine Dendrimer-mediated Formulation Development and *In Vitro-In Vivo* Evaluation of Ketorolac

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## Abstract

**Aim:** The aim of study was to explore dendrimer-mediated solubilization of separated ketorolac (KTC) from ketorolac trometamol (KTM) followed by formulation development and *in vitro* as well as *in vivo* evaluation. **Material and Methods:** Amine-terminated polyamidoamine (PAMAM) dendrimers of 3.0G and 4.0G were synthesized and characterized by infrared,$^1$H-NMR spectroscopy. Solubility studies of KTC using G3.0-$NH_2$ and G4.0-$NH_2$ dendrimer were carried out in three different pH values to evaluate the effect of pH on the solubility of KTC. Hemolytic studies and *in vitro* release rate behavior of optimized formulation $DKTC_2$ were performed. Optimized formulation was further assessed by pharmacodynamic, pharmacokinetics studies along with accelerated stability study. **Results and Discussion:** The optimized formulation $DKTC_2$ resulted in significant improvements of KTC solubility. The *in vitro* release rate behavior of KTC from the formulation and stability studies were also favorable. Pharmacodynamic assessment by paw edema model of $KTC_2$ formulation exhibited significant inhibition level (36.82 ± 0.65%) which are higher than those of plain KTC (19.14 ± 0.52 %) after 12 h. Pharmacokinetic study displayed higher $AUC_{0\rightarrow t}$; µg/ml/h of 26.514 ± 1.95 with $DKTC_2$, as opposed to 21.945 ± 1.18 with plain KTC. Furthermore, the elimination rate constant of KTC observed with $DKTC_2$ formulation was lower as compared to free KTC, whereas the half-life of KTC experienced with $DKTC_2$ formulation was significantly higher compared to free drug. The designed dendrimer-based system was found to be adequately stable even at elevated temperatures. **Conclusion:** This study reveals a sustained, high plasma concentration and a slow elimination of the drug that can improve bioavailability, along with helps in the localization of drugs at the inflammatory site and thus provide better therapeutic efficacy at a lower dose.

**Key words:** Formulation development, ketorolac, polyamidoamine dendrimers, solubility

## INTRODUCTION

Ketorolac (KTC) [Figure 1], a pyrrolizine carboxylic acid derivative structurally related to indomethacin, is a non-steroidal agent with potent analgesic and moderate anti-inflammatory activity. KTC is frequently used in post-operative, acute musculoskeletal pain, and osteoarthritis. It may also be used for renal colic, migraine, and pain due to metastatis.[1] In clinical use, it is administered as the trometamol salt (KTM), which is freely soluble in water, while KTC and free acid have limited solubility in water.[2] The majority of drawbacks associated with KTM involve gastrointestinal ulceration, gastric mucosal irritation or hemorrhage, and acute renal failure in long-term use.[3,4] It is identified that about 40% of newly developed drugs are poorly soluble in water, therefore, it never benefit patient and thus rejected.[5] It has been a constant ambition of formulation scientists to optimize drug delivery systems of hydrophobic drugs, which improve solubility, bioavailability, and minimize toxicity of drug.

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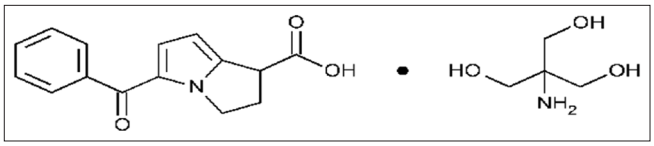


**Figure 1:** Structure of ketorolac

Polyamidoamine dendrimers (PAMAM) are hyperbranched, ordered, monodisperse, and the most investigated polymers in drug delivery.[6] These molecular boxes have utilized as drug delivery vehicles because of their ability to form complexes.[7] PAMAM dendrimers with hydrophobic core and hydrophilic periphery have been shown to exhibit static micelles-like behavior and have container properties in solution. This analogy highlighted the utility of PAMAM dendrimers as solubilizing agents and the interactions between drugs and dendrimers enhanced the solubility of poorly soluble drugs.[8-12] The controlled multivalency of PAMAM dendrimers can be used to attach a number of drug molecules, targeting groups and other agents (solubilizing/sensing) to the periphery of the PAMAM dendrimers in a well-defined manner. The development of PAMAM dendrimer-based efficient drug delivery systems has drawn a great deal of attention over the last few years.

In the present study, our objective was to synthesize PAMAM dendrimer, explore dendrimer-mediated solubilization of KTC followed by formulation development, and *in vitro* as well as *in vivo* evaluation in carrageenan-induced arthritic rats.

## MATERIALS AND METHODS

### Materials

Ethylenediamine (EDA) and methyl methacrylate were purchased from CDH, Mumbai, while methanol was procured from Qualigens Fine Chemicals, Mumbai, India. KTC was received as a generous gift sample from Dr. Reddy's Laboratories, Hyderabad, India. All remaining chemicals and solvents were of analytical reagent (AR) grade.

### Synthesis of PAMAM Dendrimers

Synthesis of PAMAM dendrimers up to the fourth generation was prepared on the basis of two steps process, involving

1. Michael addition of a suitable initiator core with methyl acrylate, and
2. Exhaustive amidation of the resulting esters with large excess of EDA, following reported methods.[10,13,14]

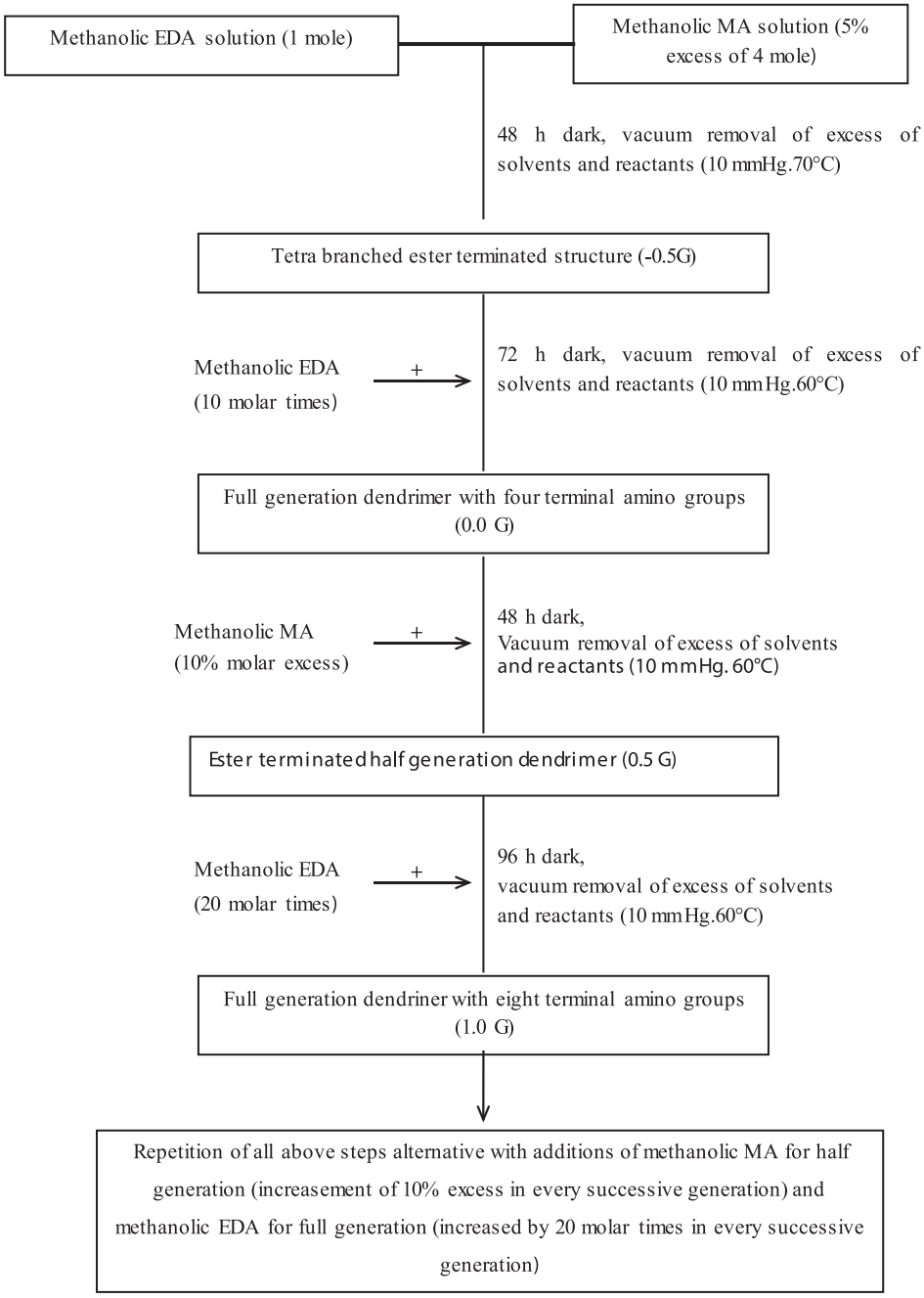
Michael addition reaction used EDA as an initiator core for starting the synthesis of dendrimers by attaching four acrylate moieties on each amino group of EDA. The resulting compound is referred to as "generation – 0.5 PAMAM ester," because initial EDA itself may be considered as the generation-1. The second step was used for amidation of the terminal carbomethoxy ($COCH_3$) of methyl acrylate with EDA. This tetraester with excess EDA resulted in "generation 0.0 PAMAM tetra amine." EDA was used in excess to about twenty to hundred molar times to avoid incomplete reactions and hence to improve the yield. The excess of EDA was removed under high vacuum (5 mmHg) to yield 0.0G PAMAM dendrimer. The reaction was carried out using methanol as medium. Redistilled EDA was used whenever required. The reaction was carried out in quick fit round bottom-flasks (RBF) as per the quantities given in Scheme 1 (Flowchart). To avoid direct light and moisture, the RBF was corked tightly and it was well covered with carbon black paper and silver foil. The whole reaction was carried in the dark at 25°C. The reactions were followed by removal of excess reagents by rotary vacuum evaporator (Superfit, Mumbai, India) under reduced pressure at 55°C–60°C, in every step. Addition reaction was allowed to complete in 2 days (48 h), whereas amidation reaction took 4 days for completion. Reiteration of this reaction sequence results in the synthesis of half and full-generation intermediates (i.e. ester and amine-terminated intermediates, respectively). Completion of every reaction step in the synthesis was confirmed with copper sulfate color reaction.[13,15] The synthesized G4.0-PAMAM dendrimers were characterized by Fourier-transform infrared (FT-IR) (Shimadzu 8201 PC, Japan) and $^1$H NMR (solvent, D2O using Bruker Advanced DRX, 300MHz).

### Separation of KTC from KTM

KTC acid is obtained by acidifying a solution of KTM following reported methods.[16,17] KTM was dispersed in 100 ml 0.5 N hydrochloric acid to acidify the solution mixture. The mixture was precipitated as a free acid at pH 4, which was washed free of tromethamine and the resultant KTC suspension was checked for the absence of trometamol by the TLC method given as an identification test for tromethamine in the USP (XXIV) monograph of KTM. The KTC was dried in a vacuum oven and used for the further studies.

### Solubilization and formulation development

Solubility studies were performed according to the phase solubility method reported earlier by Higuchi and Connors, with slight modifications.[18] Three different buffer conditions (4.0, 10.0, and 7.4 pH) were selected for the solubility study to evaluate the effect of pH on the solubility of KTC. Briefly, an excess of KTC was added into amber-colored screw-capped glass vials (10 ml) containing varying concentrations (0.02–0.2 w/v) of 3.0G and 4.0G PAMAM dendrimers in pH 4.0. Independently excess KTC was added in a vial containing only buffer pH 4.0. The vials were shaken for 24 h in a metabolic shaker (Indian Equipment Corporation, Mumbai, India) at room temperature and permitted to stand for 12 h to




**Scheme 1:** Flowchart for synthesis of polyamidoamine dendrimers dendrimers

achieve equilibrium. Solutions were filtered using 0.45 µm membrane filter (Sigma, Germany). The aliquots of the filtrate were diluted with appropriate amount of methanol and buffer. These solutions were analyzed spectrophotometrically at 322 nm using equal ratio of buffer pH 4.0 and methanol as blank. The amount of drug solubilized was calculated by following formula:

Amount of KTC solubilized = amount of KTC added − amount of KTC undissolved

An identical procedure was followed at pH 7.4 and 10.0, and the dendrimer formulation that proved the utmost drug loading/solubilization was chosen for further characterization.

## Characterization of formulation

Drug-loaded dendrimer solution (0.2 % w/v) was analyzed over the ultraviolet (UV) range between 200 and 500 **nm** in a UV visible spectrophotometer **(**UV-vis 1601 Shimadzu, Japan) to analyze the effect of solubilization as well as drug loading. The drug-dendrimer mixture was lyophilized (Heto Lyophilizer, Germany) and characterized by FTIR spectrophotometer (Shimadzu 8201 PC, Japan).

### Hemolytic study

Hemolytic studies were performed as per previously reported studies.[10,19] Whole human blood was collected using anticlot blood collection vials (Himedia Laboratories, Mumbai, India). This was centrifuged at 3000 rpm for 10 min and red blood cells (RBCs) were separated at the bottom of the tube. The RBCs were washed by normal saline (0.9% w/v), made in double-distilled water until a clear, colorless supernatant was obtained above the cell mass. The cells were resuspended in normal saline. The RBCs suspension, so obtained was used further for hemolytic study. To 1 ml of RBC suspension, in a centrifuge tube, distilled water (5 ml) was added, which was considered 100% hemolytic. Similarly, 5 ml of normal saline was added to 1 ml of RBC suspension in another tube assumed to produce no hemolysis, acting as negative control (0% hemolytic). 0.5 ml of drug dendrimer formulations was added to 4.5 ml of normal saline and 1 ml of RBC suspension. Similarly, 0.5 ml of drug solution and 0.5 ml of dendrimer solution were taken in separate tubes and mixed with 4.5 ml of normal saline and 1 ml of RBC. All the samples were incubated at 37 ± 2°C for 4 h and centrifuged at 3000 rpm for 15 min. The supernatant was analyzed spectrophotometrically at 550 nm (UV-vis 1601 Shimadzu, Japan) against blank. The degree of hemolysis was estimated by the following equation:

$$\text{Hemolysis (\%)} = (Ab_s - Ab_s0/Ab_s100 - Ab_s0) \times 100$$

where $Ab_s$, $Ab_s0$, and $Ab_s100$ are the absorbances of formulation-treated sample; a solution of 0% hemolysis (absorbance of negative control); and a solution of 100% hemolysis (absorbance of control), respectively.

### *In vitro* drug release study

*In vitro* release studies were performed under sink conditions in two media, one comprised phosphate-buffered saline (PBS) (PBS pH 7.4; medium-I) and the other comprising PBS with 1% of albumin (medium-II). Five ml of dialyzed drug-dendrimer solution (after complete removal of unentrapped drug) was filled in cellulose tubing (MWCO 2 KD, Anaspec, USA), which was earlier treated and washed well with 0.1 M phosphate buffer saline (pH 7.4). The tubing was dialyzed in 100 ml of both media (medium-I and medium-II) through constant stirring using magnetic stirrer (Remi Equipments, Vasai, India) at 37 ± 2°C. Two milliliter of samples were withdrawn from the external solution and replaced with the same volume of fresh PBS to maintain the sink condition. The samples were taken at every 1 h interval up to 8 h and finally at 24$^{th}$ h. The amount of drug released was determined following appropriate dilutions spectrophotometrically at 322 nm against blank, indirectly.

### *In vivo* anti-inflammatory activity

The pharmacodynamic study was performed using non-immunological carrageenan-induced paw edema method, which had been previously reproduced.[9,10,13] This edema assay was based on the subplantar injection of carrageenan. The advantage of using carrageenan is that non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the edema in a characteristic dose-responsive fashion and the inhibitory potency of NSAIDs in the system under evaluation roughly parallels their activity in human being.[20] All the animal studies were conducted in accordance with the protocol approved by the Institutional Animal Ethical Committee of Bundelkhand University University, Jhansi, India (Registration No BU/Pharma/IAEC/a/13/21).

In the screening of anti-inflammatory activity, 0.1 ml of 1% carrageenan was taken as phlogistic agent. Acute inflammatory activity was evaluated by measuring change in the volume of inflamed paw produced by injection of carrageenan. The paw volume was measured by means of a plethysmometer (UGO, Basile, Italy). Albino male rats (Sprague-Dawley strain) were weighed, numbered, and marked on the right hind paw, just behind the tibia–tarsal junction. Each time the paw was dipped in the plethysmometer up to the fixed mark to ensure constant paw volume. Every time, the study was carried out at daytime to avoid any variation as a result of circadian rhythms. Animals were divided into four groups group, each group comprising four animals. The plain drug (KTC) and the test formulation $DKTC_2$ were solubilized in PBS (pH 7.4; 0.3% w/v dendrimer). The dose of 3.2 mg/kg (equivalent to KTC) body weight was administered through an intravenous (IV) route in albino rats of the respective group, excluding the control. 10 min later, a dose of 0.1 ml solution of carrageenan (1% w/v in normal saline) was injected in the right hind paw of the test animals and the paw volume was measured every hour until the 8$^{th}$ h, the last two readings were recorded at scheduled intervals for 12 h. The average paw swelling in the formulation-treated animal group was compared with that of saline-treated animal group (control), and the mean percentage inhibition of edema was determined. The data were graphically presented between percentage inhibitions of edema versus time h. Percentage inhibition of edema was calculated for each group by following formula:

$$\text{\% Inhibition of edema} = \left(\frac{V_{control} - V_{treated}}{V_{control}}\right) \times 100$$

where, $V_{control}$ and $V_{treated}$ are the mean edema volume of rats in control group, and edema volume of each rat in test group, respectively.

### Pharmacokinetics and biodistribution studies

Pharmacokinetic and tissue distribution studies of free drug and dendrimer complexed drug were performed in albino rats with carrageenan-induced inflammation in the right hind

paw. Albino rats were divided in two groups, each group comprising ten animals, numbered and marked. Each one group received KTC and DKTC2 at a dose equivalent to 3.2 mg/kg body KTC by IV route, followed by the collection of blood samples from retroorbital plexus at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, and finally at 24 h. In each case, blood sample was allowed to stay for 15 min and then centrifuged at 3000 rpm for 15 min to separate RBCs and serum. The supernatant (serum) was collected with the help of micropipette (Microlit, 5-50 µL, Germany) and analyzed for the drug content employing HPLC (Shimadzu LC-10 ATVP, HPLC, Japan) for the determination of plasma drug concentration and various pharmacokinetic parameters.

For organ distribution studies, rats were classified into four groups with each group administered with same IV dose of 3.2 mg/kg (equivalent to KTC of DPXM2 and free drug in similar amount. The rats from each group were sacrificed at 2 h, 4 h, and 8 h immediately after sacrificing the rats the organs, namely, paw, kidney, liver, and spleen were carefully removed and weighed. These were stored in aluminum foil, which was kept in the refrigerator. Later, these organs were added with required amount of methanol and homogenized. Homogenized sample was centrifuged at 4000 rpm for 15 min. After centrifugation, supernatant was collected and assayed for KTC using HPLC procedure as reported earlier, with slight modifications employing a $C_{18}$ 150 mm× 4.5 mm internal diameter, 5 um particle size (Thermo Hypersil, ODS) analytical column protected by a compatible guard column.[21,22] The HPLC system (Shimadzu LC-10 ATVP, HPLC, Japan) consisted of a variable UV-visible detector, and the elution was carried out using 100 mM sodium dihydrogen phosphate pH 7.4:isopropyl alcohol (96:4) as mobile phase. The flow rate was kept at 1.0 ml/min throughout the process.

### Stability study of formulation

The stability was carried out at accelerated conditions of temperature and light. The samples (10 ml) were kept in amber-colored vials (dark) and in colorless vials (light) at 0°C, room temperature (25 ± 2°C) and accelerated temperature (60 ± 2°C) in controlled oven for 6 weeks. The samples were analyzed firstly and periodically after each week for up to 6 weeks for any precipitation, turbidity, crystallization, color change, uniformity, and drug leakage. Percent drug leakage was determined by estimating increase in drug release from the formulation during storage, under different conditions. The data were analyzed to conclude the specifications appropriate for the storage of the formulation.

## RESULTS AND DISCUSSION

The PAMAM dendrimers were synthesized using EDA as initiator core with methylacrylate in appropriate molar ratio. Two consecutive steps were involved: Michael addition of primary amine (EDA) to methyl acrylate followed by the amidation of the formed multiester with EDA. Every generation of dendrimers were confirmed by reacting with $CuSO_4$ solution.[13,15] Half generation developed deep blue color and full generation developed purple color because of copper chelation at the terminal groups of dendrimers, as reported earlier.[10] The FT-IR was used for confirmation of the synthesized 3.0G and 4.0G dendrimers. The synthesized G4.0-PAMAM dendrimers having $NH_2$ groups was confirmed by strong peak of N-H stretching at 3350.27 $cm^{-1}$. Quaternary ammonium ion peak at 3245.0 $cm^{-1}$ and N-H bending peak (1566 $cm^{-1}$, 1327 $cm^{-1}$) also showed the presence of amide [Figure 2]. Similarly in the FT-IR spectrum of 3.0G PAMAM dendrimer, confirmed the synthesis. Further, the synthesis was also confirmed by $^1$H NMR spectroscopy of 3.0G and 4.0G PAMAM dendrimer. The results supported with that reported in the literature.[10,13,15]

Two types of PAMAM dendrimers (G3.0-NH2 and G4.0-NH2) were thoroughly investigated for solubility enhancement profile of KTC. The solubilization data suggested that higher concentration of corresponding dendrimer generation was found to be solubilizing more KTC compared to lower concentrations. KTC solubility was enhanced 219 folds with G4.0-$NH_2$ PAMAM (0.2% w/v), while in case of G3.0-$NH_2$, it was 110. Enhanced solubility with higher generations might be due to increase in polymeric architecture in case of G4.0-NH2 compared to G3.0-NH2. These results were in accordance with previously reported studies. [13,23,24] Formulation development, as well as solubility studies, were also performed at three various pHs (4.0, 7.4, and 10.0). Results suggested that KTC solubility increased with an increase in pH. At the lower pH (pH 4.0), the solubility enhancement was only due to interaction, whereas at higher pH values (pH 7.4 and 10.0) the observed enhancement was a result of both dendrimeric interaction and pH-dependent solubility enhancement. Overall, it was found that the increase in drug solubility in aqueous PAMAM dendrimer solutions depended on the size of the dendrimer, the types of functional groups, and the pH of the medium [Figures 3 and 4].

The optimized 4.0G-based formulations were selected for further studies and were assigned DKTC2 in further discussion. The data showed that DKTC2 formulation enhanced maximum aqueous solubility of KTC by 216

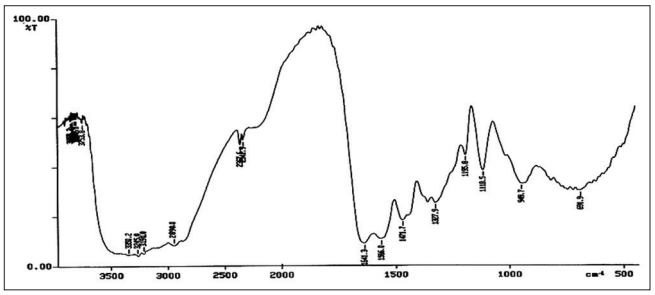



**Figure 2:** Fourier-transform infrared spectra of G 4.0- NH2 polyamidoamine dendrimers dendrimer

folds. The optimized formulation was characterized by FT-IR spectroscopy to confirm the drug-dendrimer complexation. IR spectrum of drug dendrimer complex, however, was almost similar to the IR spectrum of G4.0-$NH_2$ PAMAM with characteristic changes, might be due to encapsulation, electrostatic association of KTC. The broad peak at 3373.6 $cm^{-1}$ was the evidence of the presence of $NH_3^+$ showing the electrostatic interaction of drug with G4.0-$NH_2$ dendrimer. Furthermore, the peak shifts from 1638 $cm^{-1}$ to 1587.4 $cm^{-1}$ suggested the drug complex with dendrimer [Figure 5].

The study of percentage hemolytic toxicity revealed that the full-generation dendrimer displayed hemolysis as a function of their increasing concentration. Furthermore, the drug-dendrimer complex showed lesser toxicity at an equivalent concentration of dendrimer than the plain dendrimers. This may be due to the shielding of free amino groups present on the surface of the plain G 4.0-NH2 PAMAM dendrimer. DKTC2 formulation showed lesser hemolytic toxicity than DKTC4 formulation and therefore selected for further study [Table 1]. This result was in agreement with previous findings.[25,26]

The release study was conducted in the presence of a model protein (albumin), to qualitatively asses the fate of the G4.0-NH2 PAMAM/KTC complexes after injection in body fluids, which are rich in this protein content. After 2 h, the release was nearly 44.71 ± 3.50 and 51.67 ± 2.54 as opposed to 65.29 ± 2.10 and 73.80 ± 2.19, after 6 h from $DKTC_2$ formulation in medium-I and medium-II, respectively. In the medium-I and medium-II, after 24 h, the release of drug from $DKTC_2$ was nearly 86.56 ± 6.70 and 93.01 ± 4.99, respectively. The *in vitro* release study of the $DKTC_2$ formulation displayed initial rapid release followed by the delayed release of the drug in the later half. Delayed release of the drug in the medium-I was possibly due to the electrostatic interaction and entrapment of KTC in the internal channels of G4.0-NH2 PAMAM at pH 7.4. The internal tertiary nitrogens are strongly basic (pKa = 9.5) [10,13] and were therefore involved in deprotonating the KTC molecules within dendritic cavities. The higher release was experienced with medium-II (93.01 ± 4.99) as compared to that with medium I (86.56 ± 6.70) [Figure 6], and it may be attributed to interaction of drug-dendrimer complex and protein.[9] It is quite evident that the release profile was more sustained and controlled with $DKTC_2$ formulation.

**Table 1 :** Percentage hemolysis data of different formulation (*n*=3)

| System | % Hemolysis |
|---|---|
| $DKTC_1$ | 6.3±1.0 |
| $D_1$ | 7.5±1.3 |
| $DKTC_2$ | 8.4±1.0 |
| $D_2$ | 11.0±1.2 |
| $DKTC_4$ | 12.5±1.3 |
| $D_4$ | 14±1.4 |

$DKTC_1$, $DKTC_2$, and $DKTC_4$ represent drug dendrimers formulations (0.1%, 0.2%, and 0.4% concentration of plain dendrimers). Each value represents mean±SD (n=3)

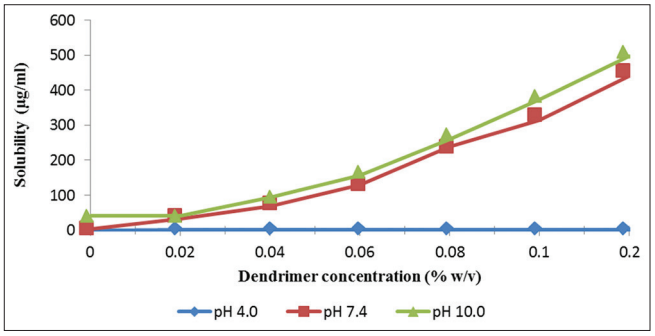



**Figure 3:** Solubility profile of ketorolac with G3.0-NH2 polyamidoamine dendrimers dendrimer at different pH states

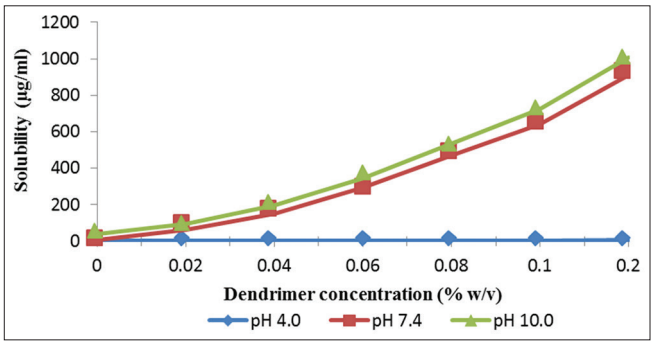



**Figure 4:** Solubility profile of ketorolac with G 4.0-$NH_2$ polyamidoamine dendrimers dendrimer at different pH states

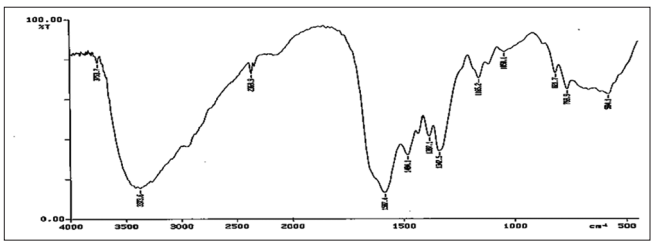


**Figure 5:** Infrared spectrum of drug dendrimer complex ($DKTC_2$)

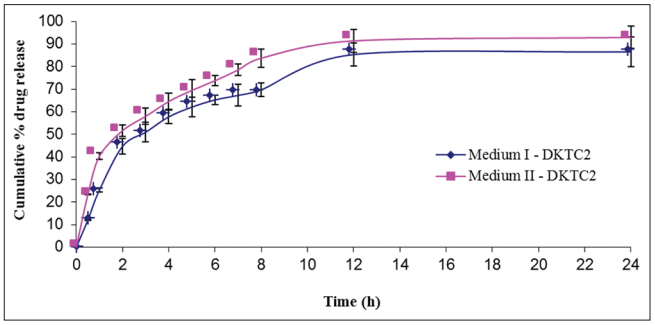



**Figure 6:** Cumulative drug release from $KTC_2$ formulation in medium-I (phosphate-buffered saline [PBS] pH 7.4) and medium-II (PBS pH 7.4, with 1% of albumin), each value represents mean ± standard deviation (*n*=3)

The pharmacodynamic assessment revealed that, in the case of $DKTC_2$, relatively higher level inhibition was observed as compared to that of plain drug KTC, under all the monitored time intervals ($P < 0.05$); [Figure 7]. In case of plain drug maximum inhibition was observed at 3rd h with magnitude of 62.59 ± 0.46% and just after 4th h, it scored below 50%, and the inhibition level was found to be 19.14 ± 0.52% and 13.11 ± 0.78%, after 12 and 24 h, respectively. The formulation under investigation ($DKTC_2$) displayed maximum inhibition at 4th h with higher value (74.96 ± 0.74%), and the inhibition was maintained at 36.82 ± 0.65% and 26.21 ± 2.1% even after 12 and 24 h, respectively. This could be due to drug concentration in the blood, which was maintained for longer duration in case of $DKTC_2$ compared to free, drug KTC. Furthermore, greater magnitude of inhibition signified the localized action of $DKTC_2$ in paw.

From the pharmacokinetic profile, it can be inferred that the blood levels of KTC in edema induced tissues were much higher with dendrimer complex ($DKTC_2$) as compared to the plain drug KTC, under all the monitored time intervals [$P < 0.05$; Figure 8]. This could be due to constant drug level in the blood, which was maintained for longer duration in case of DKTC2 formulation as compared to free drug KTC.

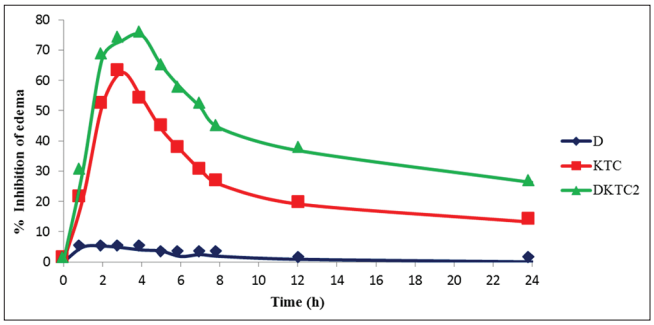



**Figure 7:** Anti-inflammatory activity of plain dendrimer(D), plain drug (ketorolac), and $DKTC_2$ formulation ($n$=5)

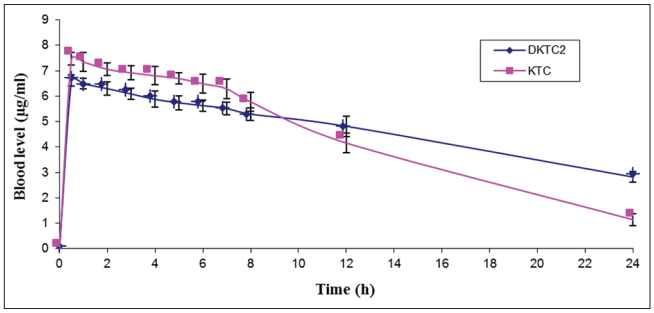



**Figure 8:** The blood plasma concentration of plain drug (ketorolac) and dendrimer complex

The significant changes in the $C_{max}$ values were observed for dendrimer complex and free drug [Table 2]. The plasma circulation half-life was significantly increased for the dendrimer complex with increased area under the plasma concentration-time profile ($AUC_{0\rightarrow t;}$ µg/mL/h) and decreased elimination rate constant (Kel). The $AUC_{0\rightarrow t;}$ µg/mL/h with $DKTC_2$ was 26.514 ± 1.95 µg/ml/h, which was 1.20 fold compared to free KTC. Furthermore, the elimination rate constant of KTC observed with $DKTC_2$ formulation was 0.2330 as against 0.2850 in the case of free KTC.

The half-life ($t_{1/2}$) of KTC experienced with $DKTC_2$ formulation was significantly higher compared to free drug (1.22 fold, $P < 0.05$). However, the increment in half-life does not seem to be significant practically. Along with this, the increased $AUC_{0\rightarrow t}$ *vis-a-vis* a diminished elimination rate as observed with $DKTC_2$ suggest extended plasma level of KTC.

The high percentage drug was recovered from liver and kidney with $DKTC_2$ complex indicates the preferential localization of the complexes in these organs. These results are in accordance with the previous reports regarding dendrimer's inherent characteristic to get localized in liver and kidney.[9,27] Accumulation of KTC in the spleen was statistically significantly ($p>0.05$) higher with $DKTC_2$ as compared to free drug (i.e. KTC). With free KTC formulation, the percentage of drug recovered in paw increased from 1.08 ± 0.35 % (2 h) to 2.41 ± 0.29 % (4 h), which was found to diminish significantly after 8 h ($P < 0.05$) with just 1.23 ± 0.37 % recovery. On the other hand, with $DKTC_2$ formulation a continuous rise in percentage of drug recovered from paw was observed, with 2.95 ± 0.38 %, 3.76 ± 0.39 % and 3.96 ± 0.32 % after 2, 4, and 8 h, respectively [Figure 9].

The enhanced permeation experience with $DKTC_2$ formulation may be due to the leaky vasculature in the inflamed paw, redistribution of dendrimer from inflamed area may occur through lymphatic system, unlike tumors where the lymphatic drainage is poor and is clearly evinced from the higher anti-inflammatory potential observed with $DKTC_2$ formulation [Figure 7]. One such possibility of the localization as well as retention of the $DKTC_2$ dentric complex at the inflammatory site may be due to the affinity

**Table 2:** Pharmacokinetic parameters of ketorolac after IV administration of free ketorolac and dendrimer complex in serum of male albino rats ($n$=4)

| System | $C_{max}$ (µg/ml) | $T_{max}$ (h) | Kel | $t_{1/2}$ (h) | AUC (µg/ml/h) |
|---|---|---|---|---|---|
| KTC | 7.469 | 0.5 | 0.2850 | 2.4308 | 21.945±1.18 |
| $DKTC_2$ | 6.615 | 0.5 | 0.2330 | 2.9730 | 26.514±1.95 |

$C_{max}$: Peak plasma concentration, $T_{max}$: Time of peak constant, Kel: Elimination rate constant, $t_{1/2}$: half life, AUC: Area under the curve ($P$<0.05). IV: Intravenous

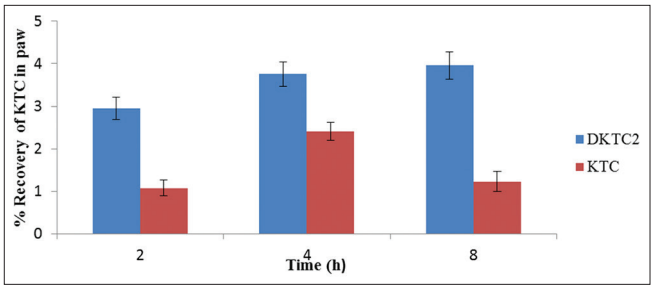



**Figure 9:** Percentage drug recovery in paw after intravenous. administration of plain drug (ketorolac) and dendrimer complex (*n*=4)

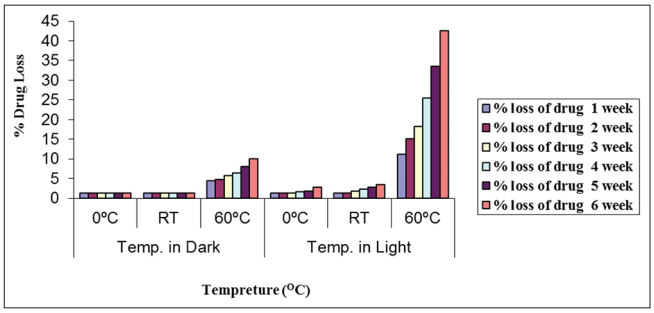



**Figure 10:** Drug loss from drug-dendrimer complex ($DKTC_2$) under different storage conditions

of dendrimer forward the glycosaminoglycan abundantly present in the arthritic joints or inflamed areas.[28,29] The increased accumulation of the $DKTC_2$ in inflamed paw can be attributed to the passive targeting nature of macromolecules, which leak out of the blood vessels into the interstitial spaces of highly permeable vasculature of inflamed tissues (EPR effect).[9] Consequently, higher concentration of drug was found in the inflamed paw in case of dendrimer formulation compared to free drug.

The designed dendrimer-based system was found to be adequately stable even at elevated temperatures up to 60 ± 2°C, dark (amber-colored vials). However, a minor vary in color as well as a sign of precipitation was found after 6 weeks when kept at 60 ± 2°C, in the presence of light (transparent vials). Significantly, drug loss was observed under higher temperature stipulations in the presence of light [Figure 10].

At higher temperature, loss of drug was observed greater in the presence of light. This may be due to higher temperature reaction kinetics in the presence of light at higher temperature (60°C).[30] No change in turbidity, color, and consistency was noticed at low temperature and room temperature. Therefore, it is concluded that the formulation can be stored at cool and dark place.

## CONCLUSION

It may be concluded that data demonstrate sustained, high plasma concentration and slow elimination of the drug that may improved the bioavailability. Dendrimer-based formulation helps in localization of drug at inflammation site and hence provide better therapeutic efficacy at a lower dose. A sustained release and slow elimination of the drug from the PAMAM dendrimer complex formulation, from the body with the parallel maintenance of high plasma concentration may be therapeutically promising. They also provide possibility to design pH-dependent controlled-release drug delivery systems containing the drug trapped inside the dendrimers. The study bears the employment of PAMAM dendrimer nanocarrier for development of safe, efficient, and biocompatible drug formulation desiring therapeutic efficiency with minimum dose.

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