

Poly(amidoamine) (PAMAM) Dendritic Nanostructures for Controlled Site-specific Delivery of Acidic Anti-inflammatory Active Ingredient

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Abhay Asthana,¹ Abhay Singh Chauhan,² Prakash Vamanrao Diwan,² and Narendra Kumar Jain¹

¹Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar 470 003, India

²Pharmacology Division, Indian Institute of Chemical Technology, Habsiguda, Tarnaka, Hyderabad 500 007, India

ABSTRACT

The purpose of the investigation was to evaluate the potential of polyamidoamine (PAMAM) dendrimer as nano-scale drug delivery units for controlled release of water insoluble and acidic anti-inflammatory drug. Flurbiprofen (FB) was selected as a model acidic anti-inflammatory drug. The aqueous solutions of 4.0 generation (G) PAMAM dendrimer in different concentrations were prepared and used further for solubilizing FB. Formation of dendrimer complex was characterized by Fourier transform infrared spectroscopy. The effect of pH on the solubility of FB in dendrimer was evaluated. Dendrimer formulations were further evaluated for *in vitro* release study and hemolytic toxicity. Pharmacokinetic and biodistribution were studied in male albino rats. Efficacy of dendrimer formulation was tested by carrageenan induced paw edema model. It was observed that the loaded drug displayed initial rapid release (more than 40% till 3rd hour) followed by rather slow release. Pharmacodynamic study revealed 75% inhibition at 4th hour that was maintained above 50% till 8th hour. The mean residence time (MRT) and terminal half-life (THF) of the dendritic formulation increased by 2-fold and 3-fold, respectively, compared with free drug. Hence, with dendritic system the drug is retained for longer duration in the biosystem with 5-fold greater distribution. It may be concluded that the drug-loaded dendrimers not only enhanced the solubility but also controlled the delivery of the bioactive with localized action at the site of inflammation.

KEYWORDS: dendrimer, flurbiprofen, *in vitro* studies, pharmacokinetics, biodistribution.

INTRODUCTION

Pain associated with inflammation is a state that could augment disorders such as mental depression, variations in blood pressure, and personality disorganization, if not taken

under control early and satisfactorily. Thus, the active moiety must be readily available to display its action. But most nonsteroidal anti-inflammatory drugs (NSAIDs) used for the purpose are hydrophobic and thus not easy to formulate in a more suitable bioavailable dosage form. Unfortunately many side effects are associated with these drugs at higher doses. Various proposed techniques to aid solubilization of these hydrophobic bioactives are available. The most common approach is to either design water-soluble derivatives of the drug moiety or encapsulate the drug in the hydrophobic interior of a surfactant-based system as in the case of emulsions or micelles. However, many of these approaches are associated with some or other limitations. Surfactant-encapsulated systems have drawbacks concerning premature and incomplete release of drug owing to their sensitivity to the biological environment, where they display unstable dynamics. Also bioavailability of the system in different regions while distributed to the body may be a restrictive factor for the purpose of drug localization.

Dendrimers are one of the emerging delivery systems with the capability to present such hydrophobic agents in a formulation with better prospective. These dendritic macromolecules with a large number of surface terminal groups and interior cavities offer a better opportunity for delivery by becoming charged and acting as static covalent micelles.¹ These are biocompatible, nonimmunogenic, and water-soluble and possess terminal functional groups for binding various targeted or guest molecules.² The host-guest properties of dendrimers based on hydrophobic and ionic interactions apart from physical entrapment have been thoroughly studied.³ The involvement of dendrimer in enhancing solubility of acidic anti-inflammatory drug through ionic interaction has been explored.⁴ Dendrimers have also been explored for intracellular delivery of the anti-inflammatory drug ibuprofen.⁵ As the dendrimer has many end functional groups, these would determine their solubility, and physical and chemical interaction in the immediate surrounding environment.⁶ Dendritic architecture and uniformly positioned functionality have been recently reported to carry the anti-inflammatory drug indomethacin for its transdermal delivery as well as anticancer drugs (eg, 6-mercaptopurine).^{7,8} Recent studies reported investigation on the effect of 4.0 generation (G)

Corresponding Author: Narendra Kumar Jain,
Department of Pharmaceutical Sciences, Dr. H. S. Gour
University, Sagar 470 003, India. Tel: +917582-264712;
Fax: +917582-264712. E-mail: jnarendr@yahoo.co.in

PAMAM dendrimer present in an anionic phospholipid composition, consisting of hydrogenated soyaphosphatidylcholine, cholesterol, dicetylphosphate, and poly(ethylene glycol)-derivatized phosphatidylethanolamine, on the hydration and liquid crystalline structure formation.⁹

Flurbiprofen (FB), a phenylpropionic acid derivative with analgesic and anti-inflammatory action, is widely used in the treatment of rheumatoid arthritis and other rheumatic disorders. Parenteral dosage form of FB is not available in the market because of its very low aqueous solubility. Microemulsion technology was used by some researchers to formulate a parenteral formulation of FB.^{10,11} But this technique was not so successful owing to long-term stability and toxicity problems. Also, the presence of alcohol in these formulations for parenteral administration is questionable.

In the present study, the PAMAM dendrimers were exhaustively studied as controlled-release systems for parenteral administration of a model drug FB and analyzed using various release kinetic studies. This study gives us an insight about the biodistribution pattern of acidic anti-inflammatory drug and its localization at the site of inflammation with PAMAM dendrimers.

MATERIALS AND METHODS

Materials

PAMAM dendrimers were purchased from Sigma-Aldrich (Dorset, UK) and the gift sample of FB was provided by M/S Knoll Pharma Ltd (Mumbai, India). The benzoylated dialysis cellulose membrane with molecular weight cut off in the range of 12 000 to 14 000 Da was purchased from Sigma. High-performance liquid chromatography (HPLC)-grade acetonitrile, acetic acid, and water were used as solvents in HPLC analysis. All other solvents and buffers were of analytical grade.

Drug-payload and Phase Solubility Studies

PAMAM dendrimer of generation 4 amine-terminated (4.0 G-NH₂) was used in concentration of 0.1%, 0.2%, 0.3%, and 0.4% wt/vol with drug FB in various formulations DF₁, DF₂, DF₃, and DF₄ (DF represents drug-dendrimer complex), respectively, for in vitro characterization. The drug loading was performed by dispersing excess of drug in dendrimer solution contained in amber-colored vials. The vials were kept in refrigerated incubator shaker chamber (Innova/4320, New Brunswick Scientific, Edison, NJ) at 25°C ± 2°C for 24 hours. The similar studies were further performed at different pH conditions to evaluate solubility of the drug in dendrimer solutions. The pH was adjusted in different vials using 0.1M HCl and 1M NaOH with the

help of pH meter (Systronic, New Delhi, India). After equilibration these solutions were filtered through membrane filter (Millipore, Billerica, MA) of pore size 0.2 µm. The amount of FB in filtrate was determined by HPLC analysis following the method given below.

For estimation and analysis of drug in every study an HPLC system (Shimadzu, LC-10, Ai, Kyoto, Japan) was used. The mixture of acetonitrile, acetic acid, and water was used as mobile phase in proportions of 60:35:5, respectively, at a flow rate of 1 mL/min with operating pressure of 102/101 bars.¹² The run time was adjusted to 15 minutes. The peak area and its retention time were noted for the peaks observed at 247 nm. The drug-to-dendrimer ratio was determined to estimate the drug payload of the dendrimers in each of the above cases.

Characterization of the Formulation

The association of drug to dendrimer was confirmed by Fourier transform infrared spectroscopy (FTIR, Spectrum One, PerkinElmer, Inc, Boston, MA). Here the FTIR spectrum of 4.0 G PAMAM dendrimer, plain drug (FB), and drug dendrimer complex (DF) were taken and studied.

In Vitro Release Kinetic Studies

In vitro drug release from the formulation was determined using the dialysis tube diffusion technique. The benzoylated dialysis cellulose membrane (MW cut-off [MWCO] 12 000-14 000, Sigma) was selected following screening of various membranes. It was found that no FB adsorption occurred, and the membrane was freely permeable to the active ingredient. Also, it restricted dendritic system from passing through it. Five-milliliter solution of dendritic formulation or FB standard was placed in the dialysis sac, hermetically tied, and suspended into 50 mL of aqueous receptor medium. The entire system was kept at 37°C with continuous magnetic stirring at 200 rpm of the sink solution. The in vitro release experiments were performed in phosphate buffer saline (PBS) 0.1M pH 7.4 alone (medium 1) or PBS 0.1M pH 7.4 containing 0.1% of albumin (medium 2), PBS 0.1M pH 6.2 (medium 3) or double-distilled deionized water alone under strict sink conditions in the receptor compartment. The volume of the receptor compartment was maintained constant by replenishing 1 mL of sink solution at 37°C immediately after withdrawal of 1-mL sample from receptor solution.

Hemolytic Toxicity of Drug-loaded Dendrimer

The reported procedure was followed to perform the hemolytic toxicity studies.¹³ Briefly, the red blood cell (RBC) suspension was dispersed in distilled water and

normal saline. Distilled water was considered as 100% hemolytic, and normal saline as nonhemolytic, hence as control. One milliliter of adequately diluted drug-loaded dendrimer as well as plain dendrimer solution was added to either 5.0 mL of normal saline or 5.0 mL distilled water and interacted with RBC suspension. The amount of dendrimers in both plain and drug-loaded state was kept equivalent to allow the comparison of drug-loaded dendrimer and plain dendrimer on hemolysis data. However, the study was performed with increasing concentration of dendrimer in both blank dendrimers and drug-loaded dendrimers. Further, the hemolysis from the plain drug (FB) was also observed separately on RBC suspension. The UV-spectrophotometric assay was performed on suitably diluted supernatant in normal saline obtained after centrifugation of the above mixtures. The absorbance was taken at 540 nm against blank (in normal saline) and percentage hemolysis was calculated using absorbance factor of 100% hemolytic sample (in distilled water).

In Vivo Studies

All the studies conducted on animals were in accordance with standard institutional guidelines and norms as specified by the ethical committee. Detailed in vivo kinetic studies were performed, which were later correlated using various pharmacokinetic parameters. The inferences from pharmacodynamic data were examined for the formulation with pharmacokinetic and biodistribution studies. The male albino rats of Sprague Dawley strain (120 ± 5 g) were used for this experimental study.

Pharmacodynamic study was performed using carrageenan induced paw edema method.¹⁴ The DF₂ formulation was selected for the in vivo studies as it displayed better in vitro characteristics. Albino rats were weighed and numbered. Marks were made on the right hind paw just behind the tibia-tarsal junction on each animal. A constant temperature was maintained in the laboratory, stress on rats was avoided, and rats were fasted for 12 hours before dose administration. The daytime was chosen for the study to avoid any significant changes in circadian rhythm. Animals were divided into 4 groups including one control group; each group comprised 5 animals. Test formulation of DF₂, blank dendrimer solution (0.2% wt/vol), and free drug solution (0.1M NaOH) in the dose equivalent to 3.8 mg/kg body weight were administered intravenously, respectively in the animals of 3 groups. While the control group was injected with saline (0.9% NaCl). All the formulations were prepared in water for injection. A dose of 0.1-mL solution of carrageenan (1% wt/vol normal saline) was given by intraplantar injection in right hind paw of the test animals 10 minutes after administration of the test dose. The paw volume was measured using a plethysmometer (UGO

Basile, Comerio VA, Italy) after every hour till 8th hour and then at 24th hour. The percentage inhibition of edema induced by carrageenan was calculated for each group.

Pharmacokinetic and biodistribution studies were further performed in rats with carrageenan-induced inflammation. Here again 0.1 mL solution of carrageenan (1% wt/vol in normal saline) was given by intraplantar injection in rats 10 minutes after administration of test dose in each group. Drug levels in blood and various tissues up to 24 hours were estimated by present method of analysis including both the pure drug as well as drug associated with the dendrimers.

Statistical Analysis

The results were expressed as mean \pm SD and the statistical analysis was done by analysis of variance (ANOVA). A probability level of $P < .05$ was considered to be significant.

RESULTS AND DISCUSSION

Drug-payload and Phase Solubility Studies

The drug carrier capacity of the dendrimers with respect to FB was determined through the study of drug-to-dendrimer ratio. It was noticed that the drug loading with DF₂ and DF₄ formulations was 960 μ g/mL and 1177 μ g/mL, respectively, at pH 7.0 (Table 1). The drug-loading capacity increased linearly with the increase in concentration of dendrimer. It is expected that amino groups of dendrimers interact electrostatically with the carboxyl group of FB as reported in case of ibuprofen.⁴

The solubility enhancement factor was calculated by taking the ratio of the drug solubilized with the aid of dendrimer to that of distilled water at a particular pH. From the solubility enhancement ratio it was found that the contribution of dendrimer in drug loading was much higher at pH 7.0 (Figure 1) in all cases. This might be owing to the fully ionized state achieved by the dendrimers at pH 7.0 as

Table 1. Effect of pH on Loading Efficiency of Flurbiprofen With Different Concentrations of 4.0 G-NH₂ PAMAM Dendrimer Solutions at 25°C \pm 2°C (RSD < 5%)*

S No.	System	FB (μ g/mL) Loading at Different pH		
		pH 2.0	pH 7.0	pH 10.0
1	Water	0.23	3.57	50.4
2	D ₁	0.74	6.72×10^2	7.12×10^2
3	D ₂	1.02	9.60×10^2	1.10×10^3
4	D ₃	1.22	1.10×10^3	1.25×10^3
5	D ₄	1.34	1.18×10^3	1.31×10^3

*S indicates serial; RSD, relative standard deviation; and FB, flurbiprofen. D₁, D₂, D₃, and D₄ are 0.1%, 0.2%, 0.3%, and 0.4% (wt/vol) aqueous solutions of 4.0 G PAMAM dendrimer.

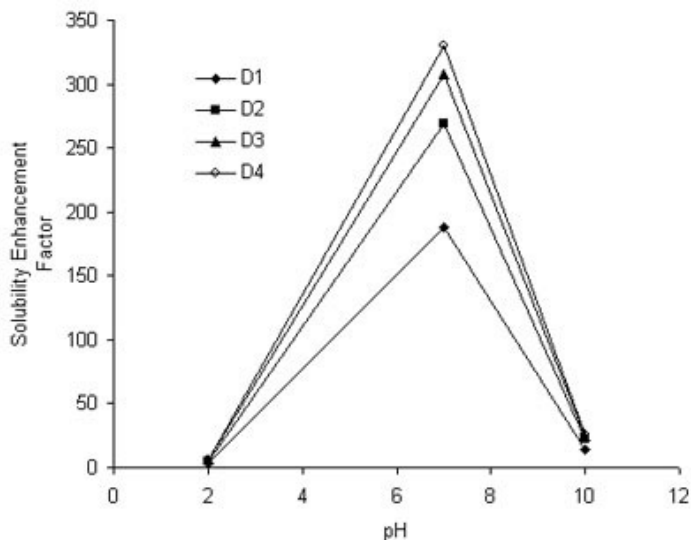


Figure 1. Solubility enhancement ratio (factor) of FB dissolved in dendrimer solutions (D₁, D₂, D₃, and D₄) at various pH at 25°C ± 2°C.

compared with acidic or basic states and hence higher degree of association of FB with dendrimer, which further enhanced its solubility in the aqueous phase.

Characterization of the Formulation

The peaks from the FTIR spectrum of blank PAMAM 4.0 G dendrimers at 3250 cm⁻¹ and 3060 cm⁻¹ showed the

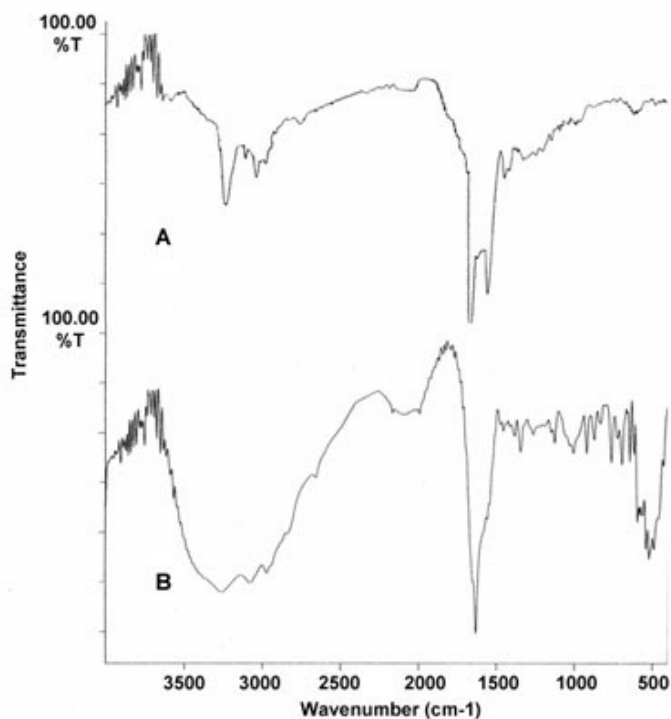


Figure 2. (A) FTIR spectra of 4.0 G PAMAM dendrimer; (B) FTIR spectra of drug-dendrimer complex.

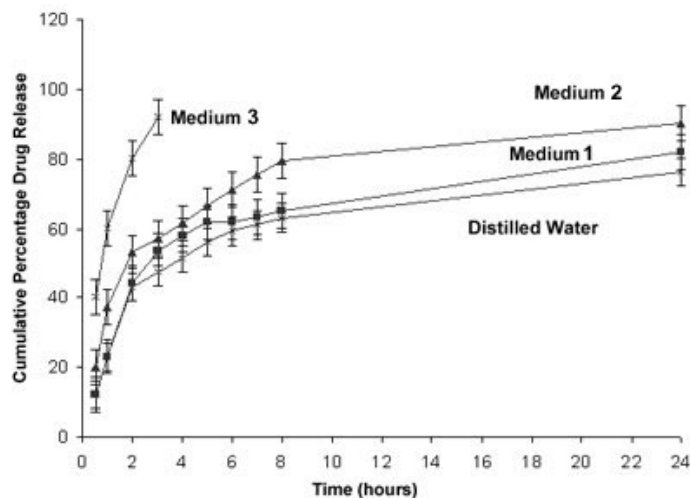


Figure 3. Cumulative percentage drug release from dendrimer formulation DF₂ in different media.

presence of terminal primary amino groups (Figure 2A). The peak obtained from plain drug, FB, at 1710 cm⁻¹ represents C=O stretch of carbonyl group. In case of drug-dendrimer complex, the broad peak at 3200cm⁻¹ is the strong evidence of the presence of NH₃⁺ showing the electrostatic association of drug with dendrimer (Figure 2B). Also, an appreciable shift in COO⁻ stretch from 1700 cm⁻¹ to 1610 cm⁻¹ as observed with free drug and drug-dendrimer complex justifies the association of drug with 4.0 G-NH₂ PAMAM dendrimers.

In Vitro Release Kinetic Studies

The in vitro release study of the sample batches displayed initial rapid release followed by the delayed release of the drug in the later half in all formulations (Figures 3 and 4).

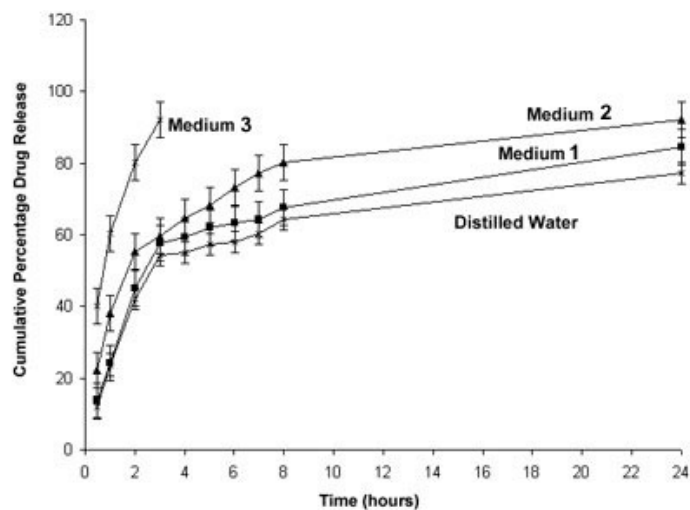


Figure 4. Cumulative percentage drug release from dendrimer formulation DF₄ in different media.

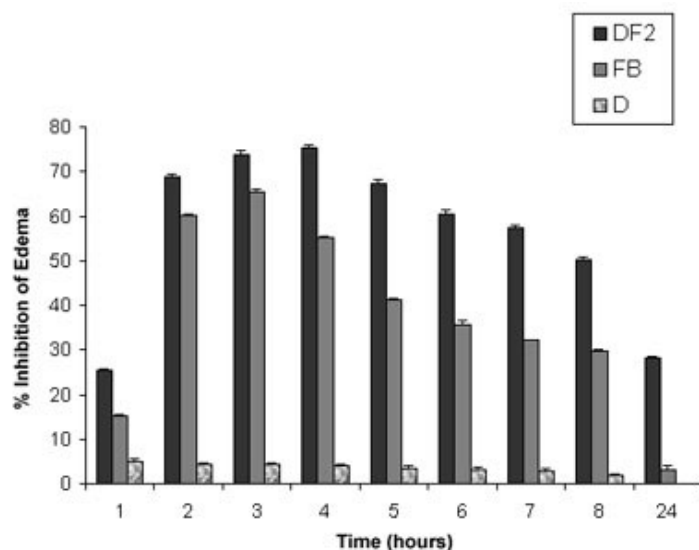


Figure 5. Effect of free drug (FB) and dendrimer formulation (DF₂) on carrageenan-induced paw edema after intravenous administration in male Sprague-Dawley rats (n = 6).

The formulations DF₂ and DF₄ displayed initial rapid release phase and above 40% of the drug was released until 3rd hour. The later half observed a rather slow release. This result might be due to the drug attachment to the primary amines, which release the drug earlier and the tertiary nitrogens within the dendritic cavities, which delayed the drug release. The internal tertiary nitrogens are strongly basic (pK_a = 9.5) and are therefore involved in deprotonating the acidic guest molecules. These quaternized nitrogens bind the counter ions, such as carboxylate ions, and control their dissociation. Another possible reason behind delayed release might be the hydrophobicity of these acidic drug molecules, which allow them to stay a little longer in the relatively more hydrophobic interior cavities that act as sink to retain the drug molecules for extended duration than the surface of the dendritic unit cells. The higher release rate was observed in medium 2 than in medium 1. The higher affinity of FB for albumin and modified physiological conditions might be responsible for the variation in the release profile. Also, higher release rate was observed under low pH states (less than 7) in medium 3 and bound drug starts precipitating below pH 6.0. The possibility of nitrogen getting protonated at low pH and loss of its ability to keep intact the acidic drug molecules might be the reason behind such characteristic release profile under low

pH conditions. The slight variation in the release rate observed for the 2 formulations was possibly due to changed surface characteristics of the dendrimer with concentration. The distilled deionized water was used to study any effect of miscellaneous ions on release profile of the drug from the formulation and relatively no distinguishable variations were observed as compared with release profile in PBS pH 7.4.

Hemolytic Toxicity of Drug-loaded Dendrimer

Full generation dendrimer displayed hemolytic toxicity up to 18%, a value close to as reported elsewhere.¹⁵ The study revealed that the dendrimer display hemolysis as a function of their increasing concentration. Also, the drug-dendrimer complex showed lesser toxicity at an equivalent concentration of dendrimer than the plain dendrimers. Thus, the presences of free amino groups on the surface of plain dendrimers, which are occupied by the drug molecules in case of dendritic drug complex, are responsible for the toxicity. As these blank dendritic units with free terminal amino groups interact with RBCs, they directly expose their polycationic nature that causes hemolysis. The DF₂ formulation, found less toxic than DF₄ formulation, was considered the optimum drug:dendrimer ratio among all other formulations and therefore selected for further in vivo studies.

In Vivo Studies

The in vivo investigations showed that there was a marked difference in the percentage drug distribution from the bound drug through dendrimer when compared with the free drug ($P < .05$). The pharmacodynamic study of dendritic formulation gave an idea about the percentage inhibition of the edema with time. It was observed that the formulation under study not only decreased the inflammation to a larger magnitude but also sustained this magnitude (Figure 5). In case of dendrimer-drug formulation (DF₂), the maximum inhibition was observed at the 4th hour with higher value (75%) and until the 8th hour inhibition was maintained above 50%, and even after 24 hours, 25% inhibition was observed. However, in case of plain drug (FB) maximum inhibition was displayed at the 3rd hour with magnitude of 65% and just after 4th hour it scored below 50%. The possible reason for this result may be the drug concentration in the body that was maintained for a

Table 2. Pharmacokinetic Parameters of FB and DF₂ in Serum of Male Albino rats (n = 6)*

S No.	System	AUC (0→t) (µg/mL/h)	Vol _{SS} (mL/Kg)	MRT (hours)	CL (mL/h/kg)	THF (hours)
1.	DF ₂	96.80 ± 0.90	209.54 ± 2.31	5.99 ± 0.25	38.14 ± 0.26	4.08 ± 0.19
2.	FB	88.88 ± 0.76	142.22 ± 0.98	3.18 ± 0.29	43.97 ± 0.14	1.33 ± 0.11

FB indicates fluribiprofen; S, serial; AUC, area under the curve; Vol_{SS}, volume of distribution at steady-state; MRT, mean residence time; CL, systemic clearance; and THF, terminal half life.

Table 3. Pharmacokinetic Data Displaying Elimination and Distribution Patterns of FB and DF₂*

System	$K_{et\frac{1}{2}}$	α	β	$\alpha_{\frac{1}{2}}$	$\beta_{\frac{1}{2}}$	K_{12}	K_{21}
DF ₂	1.60	8.65	0.23	0.08	2.96	5.12	3.11
FB	1.07	2.07	0.21	0.33	3.22	0.95	0.69

$K_{et\frac{1}{2}}$ is the elimination half-life constant; α and β are constants representing distribution and elimination phases; $\alpha_{\frac{1}{2}}$ is distribution half-life constant; and $\beta_{\frac{1}{2}}$ is elimination half-life constant; and K_{12} and K_{21} are distribution rate constants from central to peripheral compartment and peripheral to central compartment, respectively.

longer duration in case of DF as compared with that of FB. Also, “greater magnitude” of inhibition signifies the localized action of DF₂ in inflamed paw.

From the pharmacokinetic study it was found that both FB and DF₂ were cleared from the plasma in a biphasic manner. The data were analyzed using pharmacokinetic (PK) analyst software to calculate various PK parameters (Tables 2 and 3). Significant difference was observed in terminal half-life, distribution volume at steady-state, and mean residence time (MRT). Almost 4-fold greater distribution was observed for DF₂ compared with FB ($P < .05$). Also, there was greater distribution of the drug from central to peripheral compartment (K_{12}) with DF₂, but the in-flow from peripheral to central compartment (K_{21}) was less. On the other hand, FB also showed the same pattern but the magnitude was approximately 5-fold less than DF₂ ($P < .05$). At the same time, the MRT of DF₂ was found to be nearly 2-fold ($P < .05$) and “terminal half-life” was almost 3-fold that of FB ($P < .05$). This finding may be the reason for drug concentration being retained longer in blood.

From the biodistribution data it is evident that the preferred sites of drug associated with dendrimer are the liver and inflamed paw (Table 4). Hydrophobic particles are immediately recognized as “foreign” and are generally covered by plasma proteins known to function as opsonins, which facilitate phagocytosis, thus in the present strategy, surface characteristics increase hydrophilicity and hence decrease macrophage phagocyte systems (MPS) clearance. Because

of this the drug levels observed in edema-induced paw tissues with dendritic formulation were much higher than the free drug at all time intervals of study. A host of mechanical, chemical, and other insults release prostaglandins and leukotrienes, and they contribute the genesis of the signs and symptoms of inflammation. Although prostaglandins do not appear to have direct effects on vascular permeability, both PGE₂ and PGI₂ markedly enhance edema formation and leukocyte infiltration by promoting blood flow in the inflamed region.¹⁶ The MRT was higher and clearance was lower in the case of DF₂ than FB ($P < .05$). This finding represents the localized action of DF₂. As the dendritic formulation exhibits prolonged retention in blood, it may be proposed that the greater perfusion of blood at the inflamed site could bring a larger amount of DF₂ in paw tissues. Also, as there is considerable change in vasculature at the site of inflammation, the chances of DF₂ localization increase further owing to the enhanced permeability and retention (EPR) effect at such sites as reported for macromolecular polymeric structures like PAMAM.^{17,18} The other more plausible reason could be the interaction of PAMAM dendrimer with albumin protein as reported with bovine serum albumin.¹⁹ This was further evidenced by the enhanced in vitro release of FB from the dendrimer formulation in the presence of albumin. Further, the lower drug concentration observed in stomach with dendritic formulation is beneficial, as it would reduce the chances of gastric irritation and ulcerative conditions that are associated with these acidic hydrophobic NSAIDs.

Table 4. Various Parameters Displaying Biodistribution of FB and DF₂ in Different Organs (n = 6)*

S No.	Organs		AUC (0→∞) (μg/mL/h)	THF (hours)	CL (mL/h/kg)	MRT (hours)
1.	Liver	DF ₂	31.1 ± 0.24	2.8	118.7 ± 1.02	3.8
		FB	30.3 ± 0.32	3.8	126.0 ± 0.62	5.5
2.	Kidney	DF ₂	4.9 ± 0.10	2.2	751.6 ± 3.32	2.7
		FB	8.1 ± 0.23	3.8	474.6 ± 2.36	5.5
3.	Stomach	DF ₂	3.6 ± 0.08	3.8	1006.0 ± 7.34	5.6
		FB	6.3 ± 0.11	5.3	603.4 ± 4.37	7.7
4.	Paw	DF ₂	19.1 ± 0.30	9.6	193.2 ± 3.29	16.1
		FB	10.5 ± 0.16	6.7	363.7 ± 1.98	11.9
5.	Spleen	DF ₂	2.1 ± 0.07	2.8	1736.8 ± 5.52	3.9
		FB	3.3 ± 0.05	3.2	1145.1 ± 4.35	4.6
6.	Lung	DF ₂	9.8 ± 0.32	4.5	374.1 ± 2.38	6.6
		FB	20.5 ± 0.42	3.3	187.4 ± 4.87	4.7

*S indicates serial; AUC, area under the curve; THF, terminal half life; CL, organ clearance; and MRT, mean residence time.

CONCLUSION

The present study reveals that the dendrimers interact with hydrophobic FB molecules to bring it in its ionized state and hence enhance its aqueous solubility. At the same time dendrimers can localize the drug at the site of inflammation and the drug can provide effective pharmacological action by selectively inhibiting cyclooxygenase (COX) located at the inflammation site. This would finally optimize its therapeutic efficacy by reducing its adverse effects. However, the potential role of the proposed system in various other categories of the drugs for targeted drug delivery is still under investigation.

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REFERENCES

1. Beezer AE, King ASH, Martin IK, Mitchel JC, Twyman LJ, Wain CF. Dendrimers as potential drug carriers; encapsulation of acidic hydrophobes within water soluble PAMAM derivatives. *Tetrahedron*. 2003;59:3873–3880.
2. Patri AK Jr, Majoros IJ Jr, Baker JR Jr. Dendritic polymer macromolecular carriers for drug delivery. *Curr Opin Chem Biol*. 2002;6:466–471.
3. Bosman AW, Janssen HM, Meijer EW. About dendrimers: structure, physical properties, and applications. *Chem Rev*. 1999;99:1665–1688.
4. Milhem OM, Myles C, McKeown NB, Attwood D, Emanuele AD. Polyamidoamine starburst dendrimers as solubility enhancers. *Int J Pharm*. 2000;197:239–241.
5. Kolhe P, Misra E, Kannan RM, Kannan S, Lieh-Lai M. Drug complexation, in vitro release and cellular entry of dendrimers and hyperbranched polymers. *Int J Pharm*. 2003;259:143–160.
6. Zeng F, Zimmerman SC. Dendrimers in supramolecular chemistry: from molecular recognition to self-assembly. *Chem Rev*. 1997;97:1681–1712.
7. Chauhan AS, Sridevi S, Chalasani KB, et al. Dendrimer-mediated transdermal delivery: enhanced bioavailability of indomethacin. *J Control Release*. 2003;90:335–343.
8. Khopade AJ, Caruso F, Tripathi P, Nagaich S, Jain NK. Effect of dendrimer on entrapment and release of bioactive from liposomes. *Int J Pharm*. 2002;232:157–162.
9. Khopade AJ, Shenoy DB, Khopade SA, Jain NK. Phase structures of a hydrogenated anionic phospholipid composition containing cationic dendrimers and pegylated lipids. *Langmuir*. 2004;20:7368–7373.
10. Park KM, Kim CK. Preparation and evaluation of flurbiprofen-loaded microemulsion for parenteral delivery. *Int J Pharm*. 1999;181:173–179.
11. Park KM, Lee MK, Hwang KJ, Kim CK. Phospholipid-based microemulsions of flurbiprofen by the spontaneous emulsification process. *Int J Pharm*. 1999;183:145–154.
12. Government of India. *Indian Pharmacopoeia*. New Delhi, India: Ministry of Health and Family Welfare, Controller of Publications; 1996:328.
13. Singhai AK, Jain S, Jain NK. Evaluation of an aqueous injection of ketoprofen. *Pharmazie*. 1997;52:149–151.
14. Chauhan AS, Jain NK, Diwan PV, Khopade AJ. Solubility enhancement of indomethacin with poly(amidoamine) dendrimers and targeting to inflammatory regions of arthritic rats. *J Drug Target*. 2004;12:575–583.
15. Bhadra D, Bhadra S, Jain S, Jain NKA. PEGylated dendritic nanoparticulate carrier of fluorouracil. *Int J Pharm*. 2003;257:111–124.
16. Hardman JG, Limbird LE, Molinoff PB, Ruddeon RW, Gilman AG. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*, Vol 9. New York, NY: McGraw-Hill; 1996:640–647.
17. Kissel M, Peschke P, Subr V, et al. Synthetic macromolecular drug carriers: biodistribution of poly[(N-2-hydroxypropyl)methacrylamide] copolymers and their accumulation in solid rat tumors. *PDA J Pharm Sci Technol*. 2001;55:191–201.
18. Crommelin DJA, Hennik WE, Strom G. Drug targeting systems: fundamentals and applications to parenteral drug delivery. In: Hillery AM, Lloyd AW, Swarbrick J, eds. *Drug Delivery and Targeting*. New York, NY: Taylor and Francis Inc; 2001:119–125.
19. Klajnert B, Stanislawska L, Bryszewska M, Palecz B. Interactions between PAMAM dendrimers and bovine serum albumin. *Biochim Biophys Acta*. 2003;1648:115–126.