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FOLATE ANCHORED CONJUGATES OF POLY (AMIDOAMINE) (PAMAM) DENDRIMER FOR CONTROLLED SITE SPECIFIC DELIVERY OF PIROXICAM IN ARTHRITIC RATS

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G4.0-PAMAM dendrimer, folate-dendrimer conjugates, Arthritis, Targeted drug delivery, Pharmacodynamic

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ABSTRACT: The purpose of the present study was to synthesize and evaluate the potential of folate-G.4.0-PAMAM dendrimer conjugates as acceptable vehicle for site specific delivery of piroxicam (PXM) to inflammatory regions and to determine its targeting efficiency in carreganan induced arthritic rats. PXM was selected as a model acidic anti inflammatory drug. Folic acid was linked to the surface amino groups of G4.0-PAMAM dendrimer through a carbodiimide reaction and loaded with PXM. The coupled conjugates were characterized by ^{1}H-NMR, IR spectroscopy and polyacrylamide gel electrophoresis (PAGE). The drug content and % encapsulation efficiency increased with increasing folate content for the dendrimer conjugates. The *in- vitro* release rate was decreased for the PXM-F1, PXM-F2 and PXM-F3 conjugates when compared with PXM-D1. Drug dendrimer and conjugates were further evaluated for haemolytic toxicity and stability study. There was no change in turbidity, colour, consistency in stability study. Efficacy of plain PXM and dendrimer conjugates was tested by carrageenan induced paw edema model. Pharmacodynamic study revealed 74%, 78%, 85%, 90% inhibition at 4th hour by PXM-F1, PXM-F2 and PXM-F3 conjugates that was maintained above 50% till 12th hour. This study revealed the supremacy of active targeting over dendrimer mediated passive targeting of an anti-arthritic drug to the inflammatory tissues. The folate-PAMAM dendrimer conjugates are the ideal choice for targeted delivery of antiarthritic drugs to inflammatory regions with reduced side-effects. However, comparison of overall data suggested folate-G-4.0- dendrimer conjugate based formulations to be superior to PXM-D1 as well as pure PXM.

INTRODUCTION: Polyamidoamine dendrimers (PAMAM) are hyperbranched, ordered, mono-disperse, and the most investigated polymers in drug delivery [1]. These molecular boxes have utilized as drug delivery vehicles because of their ability to form complexes [2]. The controlled multivalency of PAMAM dendrimers can be used to attach several drug molecules, targeting groups and other agents (solublizing/sensing) to the periphery of the PAMAM dendrimers in a well-defined manner.

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The development of PAMAM dendrimer based efficient drug delivery systems has attracted a great deal of attention over the last few years. Folic acid (FA), an essential ingredient in DNA replication, is required in extreme amounts to support the rapid cellular division characteristic of cancer. Many types of cancer, including lung, ovary, colon, and epithelial cancers, over express the high affinity folic acid receptor (FAR).

The folate-PAMAM conjugate remains the ideal candidate for site-specific drug delivery with high efficiency to target the inflammatory tissues accompanied by reduced side-effects. In rheumatoid arthritis (RA), for example, FR-β has been shown to be specifically expressed on activated macrophages in inflamed joints, thus providing a target for the delivery of therapeutics [3, 4]. Piroxicam (PXM) belongs to the class of acidic, nonsteroidal

anti-inflammatory drugs (NSAIDs). It is quite efficient in the short or long-term treatment of rheumatoid arthritis, osteoarthritis and other painful inflammatory disorders [4, 5]. Apart from this, oral administration shows low absorption [6], considerably high food interaction, GI tract associated adverse effects, which displays poor bioavailability, limiting its oral usage. Moreover, PXM shows extensive plasma protein binding and hence unavailability at desired site in therapeutic dose [7]. Site-specific delivery of PXM with folate-dendrimer conjugate to the target site remains the best choice to overcome the side effects and to increase its efficacy. This strategy of targeting via the FR may be utilized for the site-specific delivery for drugs and prevent unwanted side effects.

In the present study, our objective was to synthesize folate-PAMAM dendrimer conjugates, as suitable drug delivery system for carrying PXM **Fig. 1** and to investigate its site specific targeting efficiency to inflammatory region in carrageenan induced arthritic rats.

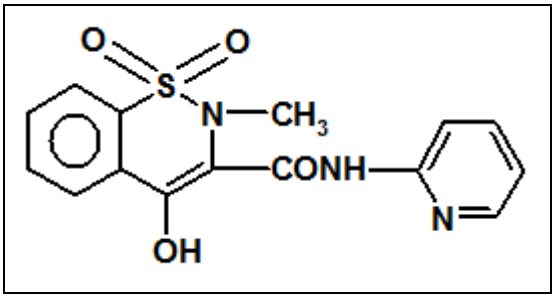

FIG. 1: CHEMICAL STRUCTURE OF PIROXICAM

MATERIAL AND METHODS:

Materials: Methanolic solution of Starburst Polyamidoamine dendrimer (G4.0-PAMAM) was received from Aldrich (USA). Piroxicam was received as a generous gift sample from Sun Pharma, Vadodara, India. Folic acid, hydrochloride salt of 1-(3-dimethylaminopropyl)-3-ethylcarbo-diimide (EDC) and benzolylated dialysis tubing (Mw cut off 12,000) was obtained from Sigma Chemicals Company (USA). All remaining chemicals were of analytical reagent (AR) grade.

Synthesis of Folate-Dendrimer Conjugates: Folate was coupled to the surface amino groups of G4.0-PAMAM dendrimer via a carbodiimide reaction as described by Wiener et al. (1997). Folic acid (8, 16 or 32 M times of G4.0-PAMAM) was dissolved in 4 mL of Dimethylformamide (DMF) and reacted with hydrochloride salt of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) (14M times of folic acid) for 1 h. The activated folic acid was added drop wise to 2.86 mM of G4.0-PAMAM dendrimer in 20 mL of deionized water at a pH of 9.3. The reaction was stirred for 3 days under nitrogen atmosphere. The solution was filtered and dialyzed against deionized water for 12 h to remove excess of unreacted folic acid. The content was lyophilized and stored in dark.

Drug Loading into Folate-Dendrimer Conjugates: Drug loading was done by adding an excess of PXM (15 mg) to 0.3% w/v aqueous solution of G4.0-PAMAM dendrimer and PBS (pH 7.4) solution of folate-dendrimer conjugates in screw-capped vials (10 ml). The solution was kept in a water bath sonicator (Bandelin Sonorex, Germany) at 37 ºC for 1 h followed by at 300 rpm in a metabolic shaker (Indian Equipment Corporation, Mumbai, India) for 24 h and allowed to stand for 12 h to attain equilibrium at ambient temperature.

The unloaded drug was removed by filtration through a 0.45 µm membrane filter (Sigma, Germany) and washed thrice with double distilled water. It was solubilised in methanol, suitably diluted using mixture of methanol and water (1:1) and determine indirectly by spectrophotometer (UV-vis 1601, Shimadzu, Japan) at 334 nm for drug content. The clear solution mixture was lyophilized (Heto Lyophilizer, Germany) and characterized by FTIR spectrophotometer (Shimadzu 8201 PC, Japan). The free G 4.0 PAMAM dendrimer-piroxicam complex was coded PXM-D1 and drug loaded folate-dendrimer conjugates were coded PXM-F1 for 8M, PXM-F2 for 16M and PXM-F3 for 32M, respectively.

The drug encapsulation was calculated by following formula:

$$\% \text{ drug encapsulated} = \frac{(\text{Amount of drug initial dispersed} - \text{Amount of drug in membrane filter})}{\text{Amount of drug initial dispersed}} \times 100$$

Characterization of Folate-Dendrimer Conjugates: FT-IR spectrum of the folate conjugates **Fig. 2** supported the conjugate formation. The number of folate molecules conjugated per mole of G4.0-PAMAM dendrimer was also estimated using ^{1}H-NMR spectroscopy (Varian Gemini 300).

The G4.0-PAMAM dendrimer and its folate conjugates were also characterized by polyacrylamide gel electrophoresis (PAGE) **Fig. 3** as previously reported by Brothers II *et al*. [9]

***In-vitro* Drug Release Study:** *In-vitro* release studies were performed under sink conditions in PBS (pH 7.4). The *in-vitro* release study was initiated by dispersing weighed amount of the lyophilized in water and placed in a hermetically tied dialysis sac 7.4 and placed in a hermitically tied dialysis sac. The dialysis sac was placed into 100 mL of PBS of pH 7.4 and maintained at 35 ± 2ºC on a magnetic stirrer. The samples were withdrawn at every 1 h interval upto 12 h and finally at 24th h and replenished with the fresh PBS solution to maintain perfect sink conditions. The amount of drug released was analyzed by UV spectrophotometer (UV-Vis 1601 Shimadzu, Japan) at 334 nm against blank, indirectly **Fig. 4**.

Hemolytic Toxicity Study: Hemolytic studies were performed as per previously reported studies [10, 11, 12]. Whole human blood was collected using anticlot blood collection vials (Himedia Laboratories, Mumbai, India). This was centrifuged at 3000 rpm for 10 minute and RBCs were separated at the bottom of tube. The RBCs were washed by normal saline (0.9% w/v), made in double distilled water until a clear, colourless 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 (Biopharm Bulletin, 1999).

To 1 ml of RBC suspension, in a centrifuge tube, distilled water (5 ml) was added, which was considered 100% haemolytic. Similarly 5 ml of normal saline was added to 1 ml of RBC suspension in another tube assumed to produce no haemolysis, acting as negative control (0% haemolytic). 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:

$$\text{Hemolysis (\%)} = (\text{Abs} - \text{Abs0}/\text{Ab100-} \text{Abs0}) \times 100$$

where Abs, Abs0 and Abs100 are the absorbances of formulation treated sample, a solution of 0% haemolysis (absorbance of negative control) and a solution of 100% haemolysis (absorbance of control), respectively. The calculated values were graphically presented in **Fig. 5**.

Microscopic Study: Transmission electron microscopy (TEM) of folic acid-4.0 G PAMAM dendrimer conjugate (PXM-F3) was done to characterize the folic acid-dendrimers in terms of their size. TEM was performed after drying on 3 mm forman (0.5% plastic powder in amyl acetate) coated copper grid (300 mesh) at 60 KV and 80 KV (Philips Morgani, 268 D; Transmission Electron Microscope, Holland) after staining negatively using uranyl acetate (4%) and photographs were taken at suitable magnification from Electron Microscopy division of All India Institute of Medical Sciences (AIMMS), New Delhi **Fig. 6**.

Stability Study of Formulation: Based on the loading efficiency, controlled *in-vitro* release of drug, and haemolytic properties of the deigned formulations, the stability of dendritic formulation (PXM-F3) 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 ºC, room temperature (25 ± 2 ºC) and accelerated temperature (60 ± 2 ºC) in controlled oven for a period of 6 weeks. The samples were analyzed initially and periodically after every week for up to six weeks for any precipitation, turbidity, crystallization, color change, consistency and drug leakage. The data obtained was used for the analysis of any physical and chemical degradation at the specified conditions and for determining the precaution required for storage. The observations are recorded in **Table 1** and graphically presented in **Fig. 7**.

***In-vivo* Anti-Inflammatory Activity:** 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/16/13).

The present study was performed using non immunological carrageenan induced hind paw edema method, which had been our previously reproduced [11]. In the screening of anti-inflammatory activity 0.1 ml of 1% carrageenan was taken as phlogistic agent. Acute inflammatory activity was determined by measuring change in the volume of inflamed paw produced by injection of carrageenan. The paw volume was measured using 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. A constant temperature was maintained in the animal house, and stresses of any kind were avoided. Every time, the study was carried out at daytime to avoid any variation due to circadian rhythms. Animals were divided into six groups group, each group comprising four animals. Test formulations PXM-D1, PXM-F1, PXM-F2, and PXM-F3 were solublized in PBS (pH 7.4; 0.3% w/v). The plain drug (PXM) was solubilized in PBS pH 7.4 employing a minimum quantity of DMSO as cosolvent.

The dose of 2 mg/kg (equivalent to PXM) body weight was administered through an intravenous route in albino rats of the respective group, precluding the control. 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, 10 min post administration of the test formulation. The paw volume was measured every hour until the eighth hour, the last two observations were recorded at the 12^{th} and 24^{th} h, and a graph was plotted between percentage inhibitions of edema *vs* 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.

RESULTS AND DISCUSSION:

Characterization of Folate-Dendrimer Conjugates: The γ-carboxylic acid group of folic acid was covalently conjugated to the free surface amino groups of G4.0-PAMAM dendrimer through a carbodiimide mediated amide linkage. In order to find the optimum number of folate moieties that should be attached to the dendrimer for maximum delivery of piroxicam at the target site, three different molar ratio of folate (8, 16 and 32 times the molar ratio of G4.0-PAMAM dendrimer) were synthesized. The conjugates were confirmed by using ^{1}H-NMR and FTIR spectroscopy.

The drug-dendrimer complexes were subjected to FTIR studies to understand the nature of complexation. FTIR spectra of pure PXM displayed N-H or O-H stretching vibration at 3391 cm^{-1}. As given in **Fig. 2**, the shift in the –NH– peak of PXM-folic acid and PXM-dendrimer to higher wave numbers (3468–3415 cm^{-1}) and shift in carbonyl absorbance of –OH group of PXM confirm the involvement of both these groups in the complex formation. FTIR spectra of pure PXM displayed N-H or O-H stretching vibration at 3391 cm^{-1}. The results agreed with that reported in the literature [8] **Fig. 3**.

The electron microscopic analysis of 4.0 G PAMAM-Folic acid (PXM-F3) conjugate proves them to be as nanometric sized vesicles as evident by TEM photographs where in circular nanometric aggregates were observed **Fig. 6**, The size of folate dendrimer conjugate was found between 60-110 nm. This data was in agreement with published reports [13, 14].

Drug Loading into Folate-Dendrimer Conjugates: PXM is a weak acid (pKa 4.6), and its solubility increases with an increase in pH. The loading of PXM was done in PBS pH 7.4 where the -OH group of PXM and the free primary amines of G4.0-PAMAM (pKa 10.0) remain 99 % ionized leading to maximum interaction and increased drug loading. Therefore, pH 7.4 is an ideal condition at which maximum potential for electrostatic bonding between the drug and dendrimer is expected [11].

The drug content and % encapsulation efficiency increased with increasing folate content in the dendrimer conjugates. The conjugation of folate moiety resulted in 1.74, 2.72, and 3.51 times increased PXM encapsulation when compared the PXM-F1, PXM-F2 and PXM-F3 formulation with

PXM-D1, respectively. The drug encapsulation efficiency of the folate-dendrimer conjugates increased with increasing folate content which can be attributed to the hydrophobic aromatic groups of the folate molecule. When more number of heavy folate moieties was attached to the surface amino groups of G4.0-PAMAM, they create more space that can accommodate large number of drug molecules.

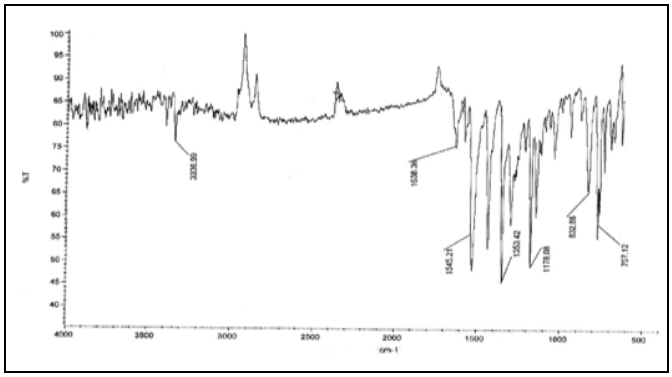

FIG. 2a: IR SPECTRA OF PXM

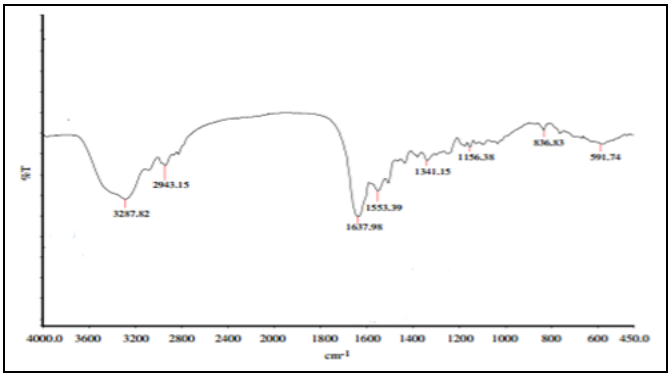

FIG. 2b: IR SPECTRA OF PXM-F3 FORMULATION

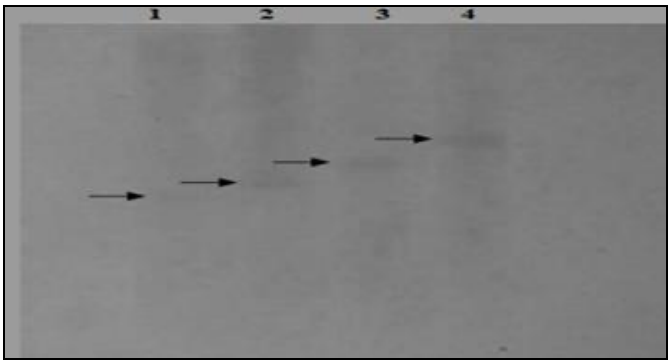

FIG. 3: ELECTROPHEROGRAM OF G4.0-PAMAM AND ITS FOLATE CONJUGATES
Lane 1 is G4.0-PAMAM dendrimer, Lane 2 is PXM-F1, Lane 3 is PXM-F2and Lane 4 is PXM-F3

***In-vitro* Drug Release Study:** The *in-vitro* release of PXM from PXM-D1, PXM-F1, PXM-F2, and PXM-F3 was explored in PBS pH 7.4. The percent release of PXM by PXM-F1 and PXM-F2 were significantly ($p<0.05$) higher than PXM-D1 at only 0.5 and 1 hr, but were similar to that of PXM-D1 upto 3 h followed by slower release. The release rate of drug from PXM-D1 was faster with 59.80 % drug released at 4 h.

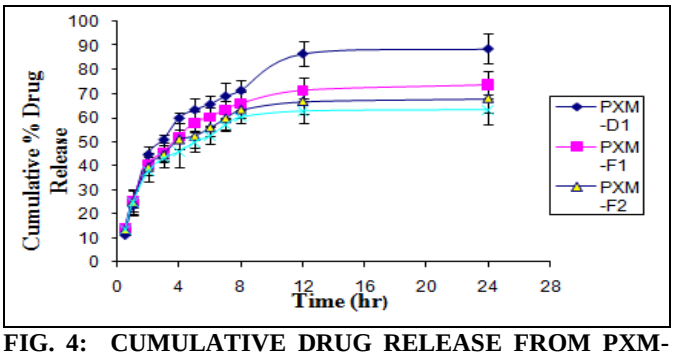

FIG. 4: CUMULATIVE DRUG RELEASE FROM PXM-D1, PXM-F1, PXM-F2, PXM-F3 FORMULATIONS IN PHOSPHATE BUFFER SALINE (pH 7.4 at 37 ºC)
Each value represents mean ±SD (n=3)

This untimely higher release was owing to the surface adsorbed PXM. Later on slower release rate might be because of the shielding effect of folates at the surface. Though, the release of PXM from PXM-F3 was significantly ($p<0.05$) lesser than the PXM-D1 at all studied time points except at 0.5 h where similar amounts were released (11.20% by PXM-D1 and 13.15% by PXM-F3). More controlled release was found with PXM-F3 where only 63.39% of drug was released upto 24 h. Extended release of PXM was observed with all three conjugates upto 24 h, which can be expected to the supplementary shielding effect of folate-moieties at the surface [8].

The increased folate coverage by PXM-F3 provided more shielding effect to the entrapped drug from external aqueous environment leading to the slower release rate. The overall release rate displayed in the order of PXM-D1>PXM-F1>PXM-F2>PXM-F3. The decrease in release rate correlated well with the increase in folate

content of the conjugates. Thus the folate-conjugation to the PAMAM dendrimer enhanced the drug loading efficiency and also served as a controlled delivery system. It is also quite evident that the release profile was more sustained and controlled with PXM-F3 formulation.

Haemolytic Toxicity Study: The haemolytic toxicity of the dendrimers was enough to impose a constraint in its use as a drug delivery system. The toxicity is due to the polycationic nature of the G.4-PAMAM dendrimers. However, conjugation of dendrimers with folic acid was found to decrease the haemolysis of the RBC considerably at 0.3% due to shielding of the charged quaternary ammonium ion that generally formed on the amine-terminated full generation of PAMAM dendrimers. The D1 (plain dendrimers), PXM-D1, PXM-F1, PXM-F2 and PXM-F3 formulations showed haemolytic toxicities of 13.0 ± 1.8, 9.8 ± 1.2, 7.4 ± 2.0, 6.2 ± 1.5, and 3.8 ± 1.4 at 0.3% w/v concentration, respectively **Fig. 5**.

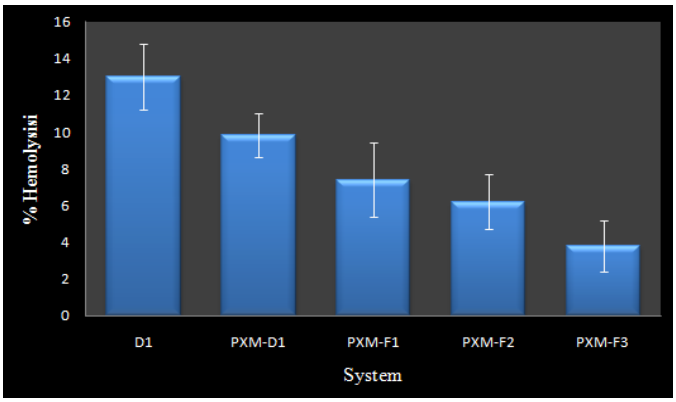

FIG. 5: HAEMOLYTIC TOXICITY PROFILE OF DIFFEREN FORMULATIONS
Each value represents mean ±SD (n=3**)**

The overall haemolytic toxicity showed in the order of D1>PXM-D1>PXM-F1>PXM-F2>PXM-F3. The decrease in haemolytic toxicity correlated well with the increase in folate content of the conjugates (PXM-F1, PXM-F2, PXM-F3), hence folic acid conjugated dendrimer decreases the haemolytic toxicity. This result was in agreement with previous findings [10, 12]. However, it is also envisaged here with that this toxicity can be further brought down by modifying the surface of PAMAM dendrimers by PEGylation.

Microscopic Study: The electron microscopic analysis of 4.0 G PAMAM-Folic acid (PXM-F3) conjugate proves them to be as nanometric sized vesicles as evident by TEM photographs where in circular nanometric aggregates were observed **Fig. 6**. The size of folate dendrimer conjugate was found between 60-110 nm. This data was in agreement with published reports [13, 14].

Stability Study of Formulation: Based on the loading efficiency, controlled *in vitro* release of drug, and haemolytic properties of the deigned formulations, the stability of dendritic formulation (PXM-F3) was performed under different conditions of temperature (0 °C; room temperature, 25 ± 2 °C; and 60 ± 2 °C.

The designed dendrimer-based system was found to be sufficiently stable even at elevated temperatures up to 60 ± 2 °C, dark (amber-colored vials). However, a slight change in colour as well as a sign of precipitation was noted after six weeks when kept at 60 ± 2 °C, in the presence of light (transparent vials). Considerable drug loss was observed under higher temperature stipulations in the presence of light **Fig. 7, Table 1**.

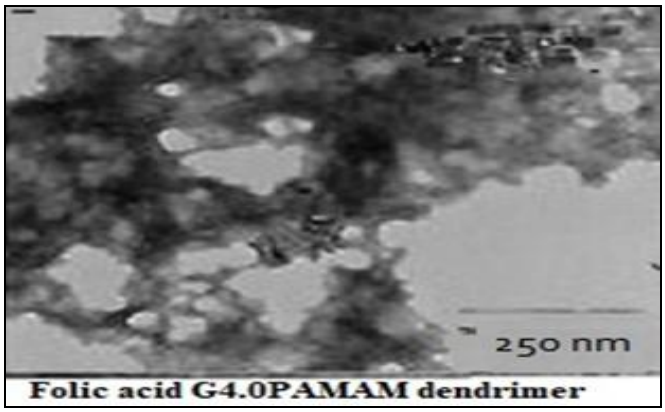

FIG. 6: TEM STRUCTURE OF PXM-F3 CONJUGATE

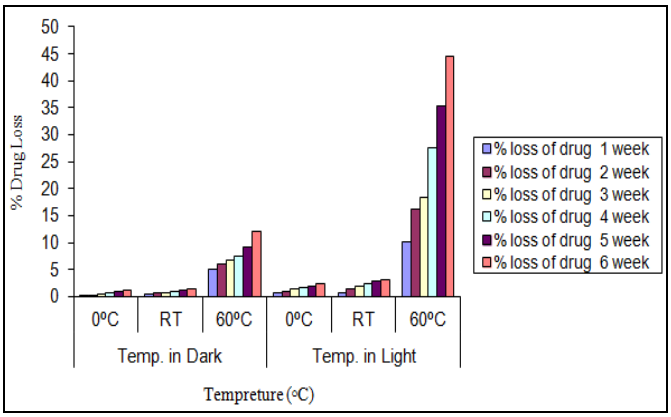

FIG 7: %DRUG LOSS FROM PXM-F3 FORMULATION UNDER DIFFERENT STORAGE CONDITIONS

TABLE 1: STABILITY DATA OF PXM-F3 FORMULATION

Parameter	Time Period	Temp. in Dark			Temp. in Light		
		0 ºC	RT	60 ºC	0 ºC	RT	60 ºC
Turbidity	After 6 weeks	-	-	+	-	+	++
Precipitation	After 6 weeks	-	-	-	-	+	+
Colour change	After 6 weeks	-	-	++	-	+	++
Change in consistency	After 6 weeks	-	-	++	-	+	+++
% loss of drug	1 week	0.15	0.25	4.00	0.60	0.70	10.05
	2 week	0.25	0.50	5.00	0.75	1.20	15.70
	3 week	0.50	0.70	5.80	1.40	1.70	17.35
	4 week	0.75	0.95	6.50	1.50	1.90	27.40
	5 week	0.88	1.1	7.10	1.80	2.70	34.65
	6 week	1.05	1.3	11.00	2.10	3.00	43.40

'-' indicates no change, '+' indicate smaller change, '++' indicate considerable change, '+++' indicate more change. Each value represents mean ±SD (n=3)

There was change in color and precipitation noted after six weeks when kept at 60 ºC in presence of light. At higher temperature loss of drug was observed greater in the presence of light. The dendritic structure is supposed to be more open at higher temperature and this change in surface characteristics might cause the conformational changes in the structure and release of drug. This may be due to higher temperature reaction kinetics in the presence of light at higher temperature (60 ºC) [11, 15]. No change in turbidity, colour, consistency was noticed at low temperature and room temperature. Therefore it is concluded that the formulation can be stored at cool and dark place.

***In-vivo* Anti-Inflammatory Activity:** Folate receptor is overexpressed on the activated macrophages in equally animal models and human patients with actually taking place rheumatoid arthritis. The pharmacodynamic assessment revealed that, in the case of plain drug, relatively lower level inhibition was observed 26.90 ± 0.9%, 12 h and 20.79 ± 0.76%, 24 h, as compared to folate conjugates PXM-F1, PXM-F2, PXM-F3, that displayed maximum inhibition after 4 h and a significant inhibition level was observed even after 12 and 24 h that was found to 38.42 ± 0.28%, 43.42 ± 0.61%, 48.42 ± 0.35%, 52.42 ± 0.48%, respectively **Fig. 8**.

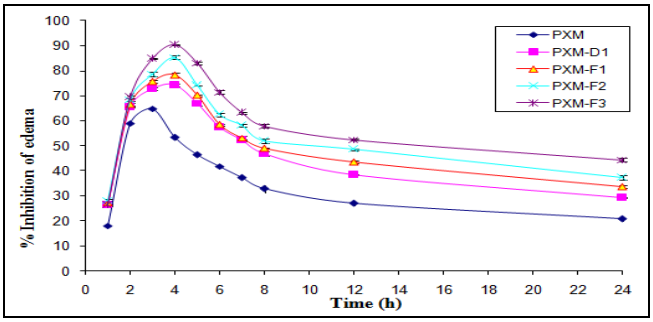

FIG. 8: ANTI-INFLAMMATORY ACTIVITY OF VARIOUS FORMULATIONS (n=5)

From the pharmacokinetic study it was inferred that the drug levels observed in 'edema induced' paw tissues with folate dendritic conjugates were much higher than the free drug **Fig. 8** at all the time intervals of the study.

Though, the enhanced permeation of the folate dentric complex is due to the leaky vasculature in the inflamed paw, redistribution of dendrimer from inflamed area may occur via lymphatic system, unlike tumors where the lymphatic drainage is poor. One such possibility of the localization/ retention of the folate dentric complex at the inflammatory site may be due to the affinity of dendrimer forwards the glycosaminoglycan abundantly present in arthritic joints or inflamed areas [11, 16]. The increased accumulation of the PXM-DI 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) [16]. In the present study, the active targeting strategy was utilized where folate (ligand for over expressed active FR) conjugated dendrimer carriers were targeted to inflammatory region with more efficiency.

CONCLUSION: The present investigation was focused on the anti-inflammatory activity of G4.0-PAMAM-PXM complex and Folic acid-G4.0-PAMAM-PXM conjugate to the inflammatory region using an arthritic rat model. Folate-PAMAM dendrimer conjugates with different degree of folate substitutions were synthesized and characterized. PXM encapsulation/complexation efficiency enhanced with enhancing folate content. The *in-vitro* release profile displayed a more controlled release of the drug with increasing folate content.

Pharmacodynamic study in arthritic rats had revealed preferential higher accumulation of piroxicam at the inflamed paw by the folate conjugates when compared with dendrimer at equivalent dose. Among the conjugates, the formulation PXM-F3 was established to possess superior encapsulation efficiency, controlled release nature and highest locating at the inflammation site to the inflammatory region.

The folate-PAMAM conjugates are the ultimate choice for site-specific drug delivery with high efficiency to target the inflammatory tissues accompanied by reduced side-effects. 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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CONFLICT OF INTEREST: Nil

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