Indian Journal of Chemical Technology
Vol. 29, November 2022, pp. 647-657
DOI: 10.56042/ijct.v29i6.67405

Biocompatible dendrimer for the solubility enhancement and sustained release of piroxicam

E Murugan*, V Yogaraj & D P Geetharani

Department of Physical Chemistry, School of Chemical Sciences, University of Madras,Guindy Campus, Chennai 600 025, Tamil Nadu, India

E-mail: dr.e.murugan@gmail.com

Received: 19 August 2022; accepted: 21 October 2022

Piroxicam (PRM) a nonsteroidal anti-inflammatory medication is an oxicam-class used orally to treat gout, arthritis, and other inflammatory diseases. However, its poor aqueous solubility and bioavailability has hampered further clinical applications in health industries. This work emphasize on development of four types of functionalised biocompatible dendrimer with generation 0 and 1and examine its solubility, drug release and antibacterial activity. The maximum solubility enhancement of PRM up to 48 folds has been achieved by PAMAM (G1)-CH_3 at a concentration of 9.9×10^{-4} M. The *in vitro* release gets sustained up to 450 mins for releasing 90% of drug from PAMAM (G1)-$COCH_3$ compared to its parent dendrimer which is 120 min. The anti-bacterial studies reflected that when dendrimers are used as drug carriers, the inherent property of drug is not disturbed, instead the activity has been increased. The activity interms of zone of inhibition results in 1.5-2.0 folds increase and it is more pronounced in the case of *B. subtilis* rather than *E.coli*. This observation indicates evidence that dendrimer and their derivatives are promising candidates for drug solubility enhancer and effective delivery of drugs with drastic reduction in side effect and improved efficiency.

Keywords: Antibacterial activity, PAMAM dendrimer, Piroxicam (PRM), Solubility enhancement, Sustained release

Piroxicam is non- steroidal anti-inflammatory, anti-rheumatoid arthritis[1] and analgesic[2] agent. The side effects on the gastrointestinal system and the poor aqueous solubility have limited its use in therapeutic treatments[3,4]. In order to overcome the obstacles in poor aqueous solubility is to develop a drug carrier using dendrimer. About 50% of new drugs limited their application in water solubility which results in poor bioavailability during oral administration[5,6]. Drug delivery methods are influenced by drugs physicochemical properties. Two parameters stability and solubility should be considered for effective drug formulation[7].cyclodextrin inclusion complex, micronation, Solid dispersions, permeation enhancers, surfactants, salt formulation, and nano-particles are the strategies used in formulations to overcome these issues[8-10]. Therefore, solubility enhancement of drugs is an important task in pharmaceutical technology as it leads to improved bioavailability[11]. Poorly soluble drugs lead to the development of drug delivery technologies through chemical or mechanical modification of the drug environment or physically altering the macromolecule[12]. Encapsulation of these pharmaceuticals can be done via emulsions/emulsion pre-concentrates and lipid solutions[13]. The digestive motility of the gastrointestinal tract provides the agitation required to generate nanoemulsions in the body. SNEDDS focus to increase the absorption of drugs by enhancing colloidal dispersibility in oral-systemic with low aqueous solubility which recollecting the drug dispersed in the gastrointestinal tract's transit[14,15].

In this background, several researchers have used three-dimensional polymeric architecture dendrimers with tailor making properties with its nano size used as a carrier for solubility increment, stability, cytotoxicity waiver and bioavailability enhancement of drugs in administration for therapeutic applications. Additionally, the surface functional groups of dendrimers can be modified/functionalized with biologically active components such as drugs and targeting moieties[16]. Drugs and targeting moieties can conjugated to the surface group of dendrimers via covalent methods or can be encapsulated within the dendrimer cavities via non-covalent approaches such as ionic, hydrophobic, and hydrogen bond interactions[17]. Poly (amidoamine) (PAMAM) and poly (propylene imine) (PPI) dendrimers possess cationic primary amine groups on their surface. Most of the dendrimer drug carrier formulations limits in clinical applications due to their cytotoxic or haemolytic

activity leads to cell death[18-20]. To overwhelmed this issue, the haemolytic activity of cationic dendrimer surface and cytotoxicity can be reduced by PEGylation, hydroxylation, glycosylation or acetylation[21-25]. An attempt was made earlier on development of PPI dendrimer drug carriers[26-28] for solubility enhancement of drugs such as Norfloxacin, Nimesulide and Piroxicam. In this study, a biocompatible poly (amidoamine) dendrimer drug carrier with lower generation that results in enhanced aqueous solubility and sustained release in vitro. Further, anti-bacterial activity proved that the inherent property of drug is not disturbed, instead the activity was increased.

Experimental Section

Materials

Methyl acrylate, Methyl iodide andamberlite anion exchange resin IRA 402 from Sigma Aldrich, Ethylenediammine from SRL, Acetyl chloride from Sd fine, Dialysis Membrane [M.W.Cut off 500-1000] from Spectra pore, Piroxicam as a gift sample from Mesha Pharma Ltd, Mumbai, India. Phosphate buffer solution was used for both solubility and *in vitro* release studies whereas Glass Distilled Water was used for antibacterial activity studies.

Synthesis of PAMAM (G0)/(G1) dendrimers with methyl as the surface group

PAMAM (G0)/(G1) (0.1 g) dendrimer was dissolved in 5 mL DMF and 0.9 mL of methyl iodide diluted in DMF was added and stirred for 24 h at room temperature in N_2 atmosphere. The product was precipitated in diethyl ether and the residue was vacuum- dried for overnight. The dried residue was dissolved in water and this was passed through amberlite anion resin to exchange I^- into Cl^- ions. The eluted solution from the resin was lyophilized to get PAMAM-(G0)/(G1)-CH_3 (Scheme 1).

Synthesis of PAMAM (G0)/(G1) dendrimers with -COCH3 as the surface group

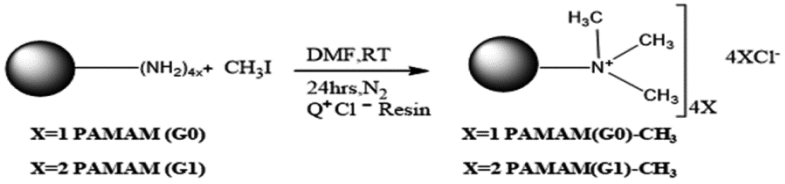
The synthesis was performed in two steps. In first step, 0.1 g of PAMAM (G0)/(G1) dendrimers dissolved in 2 mL of triethylamine and 10 mL of chloroform at 0°C. 2 mL of acetyl chloride was added at RT and stirred for 36 h at N_2atmosphere. The extensive dialysis with the frequent replacement of outer medium to remove unreacted substances and the final solution was lyophilized to get a semi solid product viz., Ac-PAMAM-(G0)/(G1) (Scheme 2, Step 1). In the second step, excess methyl iodide diluted in DMF was added slowly to 0.1 g of Ac-PAMAM (G0)/(G1) dissolved in DMF and stirred for 48h in N_2 atmosphere at RT. Diethyl ether was used to precipitate the resulting solution and vacuum oven dried for overnight. The ions were exchanged by passing the dried residue through amberlite anion resin and the eluted solution was lyophilized to get PAMAM-(G0)/(G1)-$COCH_3$ (Scheme 2, Step 2). The resulting4 types of dendrimer drug carriers that is (i) PAMAM (G0)-CH_3, (ii) PAMAM (G1)-CH_3, (iii) PAMAM (G0)-$COCH_3$ and (iv) PAMAM (G1)-$COCH_3$ were characterized by FTIR, ^{1}H NMR, ESI MASS & MALDI-TOFF Spectral techniques and employed as a drug carrier for Piroxicam in terms of drug solubilizing ability, sustained release (*in vitro* release) and their effect on inherent activity of the drug were also studied extensively.

Standard calibration curve for Piroxicam using UV-vis spectroscopy

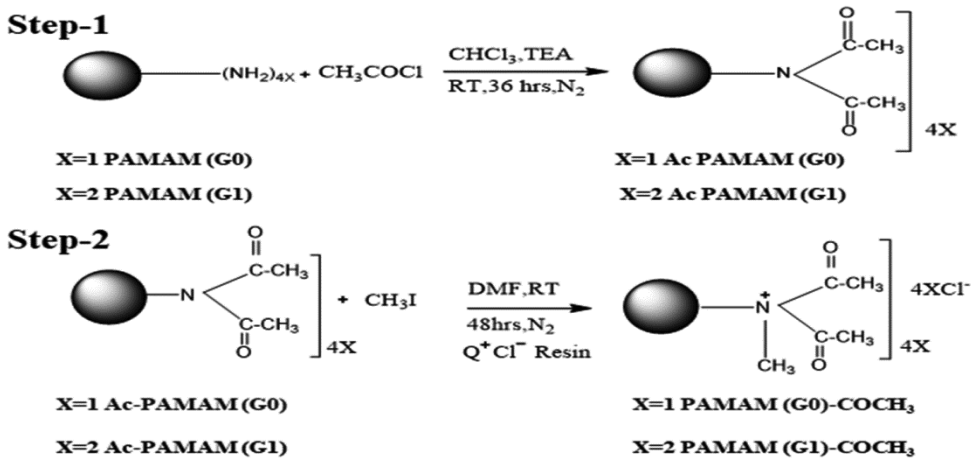
To prepare standard calibration curve, 2.3 mg of PRM was accurately weighed and dissolved into a 50 mL volumetric flask and filled to volume using phosphate buffer (*p*H 7.4), and the solution is made up to the mark. From this stock solution, serial dilutions were made with the same buffer to obtain standard solutions of PRM in the concentration ranging from 3 to 75 µM. The absorbance of the solutions was measured using UV-vis spectrophotometer. The standard curve was constructed by plotting the absorbance versus respective concentration. The calibration curve was used to calculate the amount of PRM using Beer-Lambert's law.

Phase solubility experiments for solubility estimation of piroxicam

Higuchi and Connors method was used for Phase solubility analysis[29]. Here, we have employed the



Scheme 1 — Synthesis of PAMAM (G0)/(G1) dendrimers with methyl as the surface group



Scheme 2 — Synthesis of PAMAM (G0)/(G1) dendrimers with acetyl as the surface group

above dendrimer drug carriers as the accelerators of solubility for the drug PRM. The degree of solubility of the drug was quantified by monitoring the characteristic absorbance of the drug at 356 nm using UV spectrophotometer. The concentrations of carriers were fixed in the range of 0.5×10^{-4} to 9.9×10^{-4} M. The experiments were conducted repeatedly three times. The experimental procedure involves adding excess of PRM in 20 mL vials containing 5mL of each test solution. These vials were then stirred for 24 h at 35ºC. Centrifugation at 2000 rpm for 20 min, and filtered using cellulose acetate filter of pore size 0.45µm. The samples were analysed in UV Visible spectrophotometer at 356 nm correlated with calibration curve to quantify the amount of PRM dissolved in the respective solution and also blanks were performed on the same concentrations of carriers. Phase solubility diagrams were constructed by plotting solubility of PRM versus molar concentration of carriers.

***In vitro* drug release studies**

Equilibrium dialysis method was used to study the release behaviour of drug from all the carriers i.e., PAMAM (G0) and (G1) and modified 4 types of developed dendrimers under *in vitro* condition[30]. In general, PRM/PAMAM (G0)-CH_3, PRM/PAMAM (G1)-CH3, PRM/PAMAM (G0)-$COCH_3$ and PRM/PAMAM (G1)-$COCH_3$ were prepared with different concentrations. The concentrations 6×10^{-4} M for the drug and 4.9×10^{-4} M for all the carriers were maintained as a constant. Pure PRM was dissolved in methanol to get a concentration of 6×10^{-4} M and used as control. The complex formation was accelerated by excess sonication before release studies and transferred to dialysis bag having molecular weight cut off (MWCO) 1000Da. The whole setup was immediately immersed in a buffer solution container. The outer phase of dialysis bag stirred continuously till 95 % of the drug gets released. Equal amount of sample was taken and replenished in the outer phase at regular time intervals. The PRM concentrations released in the outer phase was determined by sample absorbances using UV-vis spectroscopy**.**

Anti-bacterial activity studies

Activity of all the complexes were tested by the well diffusion method using nutrient agar against bacteria such as *Bacillus subtillis* (ATCC 6633) and *Escherichia coli* (ATCC 35218). Both the microorganisms were incubated at 37ºC for 24h in nutrient agar broth. The culture suspensions were prepared and adjusted by comparing against 0.4-0.5 McFarland turbidity standard tubes. Nutrient agar (20mL) was poured into each sterilized Petri dish (10mm×100mm diameter) and allowed to solidify. After the solidification, the bacterial cultures were swabbed in the nutrient agar plates for antibacterial activity. The drug formulations in the presence/absence of carriers were prepared as follows, PRM-PAMAM (G0)/(G1), PRM-PAMAM (G0)/(G1)-CH_3 and PRM-PAMAM (G0)/(G1)-$COCH_3$ solutions were prepared by fixing the concentration as 6.04×10^{-4}M and 4.9×10^{-4}M irrespective of carriers. Plain drug was dissolved in DMSO at the same concentration of 6.04×10^{-4} M. Each sample (50 µL) was filled directly with the serial dilutions of Neat in to the wells of agar plates, 1:2, 1:4 and 1:8. During the 24 h incubation period, radial growth of the colony was noted and inhibition

zones were evaluated at the end of the incubation period and compared with that of the plain drug. The test for DMSO inhibitory activity also carried out.

Results and Discussion

It is well known that the therapeutic effectiveness of any drug, generally depends on the degree of therapeutic effect without creating any side effect/toxicity. Further, the degree of therapeutic effect has been influenced on the extent of solubility of drugs with higher order of biocompatibility. As most of the drugs are hydrophobic in nature, solubility of them in aqueous medium is normally minimum thereby therapeutic effect is not upto the mark. Therefore, many pharma based industries have paid much attention to find suitable means to accelerate the solubility of drugs and act as drug carriers for the effective delivery of the same. It is learned from the studies that PRM belongs to class of acidic non-steroidal anti-inflammatory drugs. It is quite efficient in the treatment of rheumatoid arthritis, osteoarthritis and other painful inflammatory disorders. But there are a number of aspects that limit its pharmaceutical applications. Among the limitations the chief being its water insolubility that immensely creates troubles in formulation.

Even though amine terminated PAMAM dendrimers can solubilize different families of hydrophobic drugs, but the cationic charges on dendrimer surface may disturb the cell membrane which results in cytotoxicity[31]. Therefore, neutralization of dendrimer surface charge has been proposed as a necessary step to improve dendrimer biocompatibility. To date, there is no reports regarding the modifications of lower generation PAMAM dendrimers with different surface groups and exploring their ability as drug carriers. In view of this background, we have taken steps to utilize the tailor make properties of dendrimers to modify the terminal functional groups by different strategies which includes direct methylation and acetylation. Initially we have synthesized the lower generation (G0) and (G1) PAMAM by the reported method[32]. Taking them, we have synthesized four types of modified dendrimers with surface functional groups such as $–CH_3$ and $–COCH_3$. Further, we have assessed their ability as drug carriers in terms of drug solubility, sustained release and anti-bacterial activity.

Synthesis of PAMAM (G0) and (G1) dendrimers

PAMAM **(**G0) and (G1) dendrimers were synthesized by employing ethylene diamine as initiator core and methyl acrylate in the appropriate molar ratio. Completion of each reaction step in the synthesis was confirmed with copper sulphate colour reaction[33] where in full generation dendrimers gave a purple colour, while half generation gave a deep blue colour due to copper chelation at the $–NH_2$ terminal groups of the dendrimers as reported earlier[34]. The synthesised dendrimers were confirmed by FTIR, ^{1}HNMR and Mass spectroscopy and these data agree with the published reports[35]. Added to this, conductivity measurements were made to estimate the inherent conductivity of (G0) and (G1) PAMAM dendrimers (Table 1).

Synthesis and characterization of PAMAM G0 and G1 dendrimers with $–CH_3$as the surface group

Methylation was carried out in the appropriate condition (Scheme 1). The introduction of $–CH_3$ were characterized by FTIR, ^{1}H NMR and mass spectral techniques. In FTIR spectrum of PAMAM (G0)-CH_3, (Fig.1a) prominent –C-H stretching peaks at 2969 cm^{-1} and 2780 $cm^{-1}$and 1472 cm^{-1} for –CH bending, –NH-CO stretching at 1644 cm^{-1}, and –C-N stretching at 1023 $cm^{-1}$and –NH- stretching at 3371 cm^{-1}. ^{1}H NMR spectrum of the same shows δ values at 2.51 ppm (CH_2CO) strong singlet at 2.724 ppm (N-CH_3), 3.2-3.4 ppm –N CH_2CH_3, 3.71 ppm (CONH CH_2), 3.64 (CH_2-N^+) gives proof for the terminal functionalization of the PAMAM G0. MALDI-TOFF

Table 1 — Conductivity of dendrimer drug carriers at different concentrations

S. No	[Dendrimer drug carriers] $\times10^4$	Conductivity in Mhos $\times10^2$					
		PAMAM (G0)	PAMAM (G1)	PAMAM (G0)-CH_3	PAMAM (G1)-CH_3	PAMAM (G0)-$COCH_3$	PAMAM (G1)-$COCH_3$
1.	0.825	2.6	0.52	0.32	0.78	0.313	0.58
2.	1.65	3.13	0.59	0.56	0.92	0.68	1.93
3.	3.3	3.2	0.84	0.79	1.05	1.10	2.70
4.	4.9	3.72	0.97	0.95	1.22	1.64	3.51
5.	6.6	4.18	1.18	1.13	1.41	2.18	4.27
6.	8.25	4.67	1.37	1.29	1.58	2.59	4.85
7.	9.9	5.31	1.59	1.54	1.73	3.23	5.95

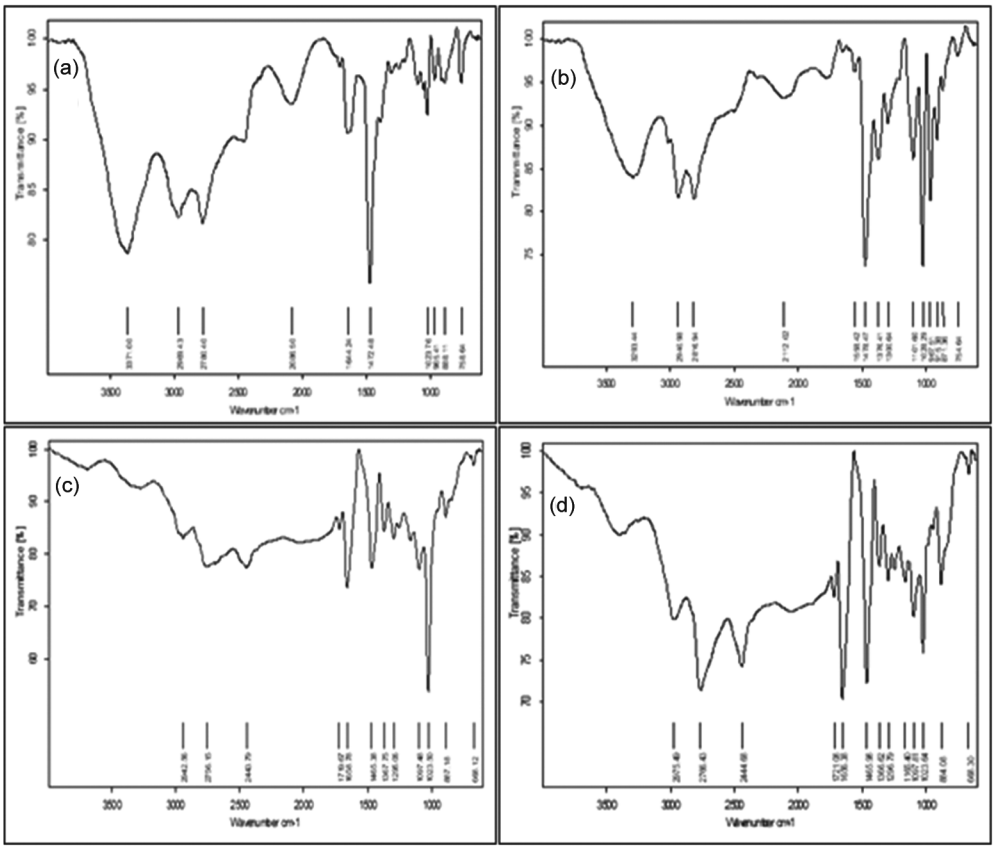


Fig. 1 — FTIR spectrum of (a) PAMAM (G0)-CH_3; (b)PAMAM (G1)-CH_3; (c)PAMAM (G0)-$COCH_3$ and (d) PAMAM (G1)-$COCH_3$

gave m/z value at 821 (816 calculated) with considerable intensity, gave convincing evidence for the successful synthesis of PAMAM G0-CH_3 [Fig. 2(a)]. Similarly, FTIR spectrum of PAMAM (G1)-CH_3 [Fig. 1(b)] gives strong –C-H stretching peak at 2940 and 21816 cm^{-1} along with peak at 1478 cm^{-1} assigned to –C-H bending. ^{1}H NMR spectrum shows δ values at 2.6 ppm (CH_2CO) strong singlet at 2.724 ppm (N-CH_3),3.2-3.4 ppm –N CH_2CH_3, 3.71ppm (CONH CH_2), 3.64 (CH_2-N^+) gives proof for the terminal functionalization of the PAMAM (G1). The m/z value obtained from MALDI-TOFF gave peak at 2059 (2056 calculated) confirms the synthesis of PAMAM (G1)-CH_3 [Fig. 2(b)]. Further, the conductivity measurements were made for PAMAM (G0)-CH_3 and PAMAM(G1)-CH_3 dendrimers which shows considerable increased values (Table 2).

Synthesis and characterization of PAMAM G0 and G1 dendrimers with –$COCH_3$ as the surface group

In the first step acetylation was carried out using acetyl chloride (Scheme 2, step1), the acetylated products were methylated to get the quaternized dendrimers (Scheme 2, step-2). The acetylation and methylation were confirmed with FTIR [Fig.1(c) & 1(d)], ^{1}HNMR and ESI Mass and MALDI-TOFF [Fig. 2(c) and 2(d)] spectral techniques. Further, the conductivity measurements were made for PAMAM (G0)-$COCH_3$ and PAMAM (G1)-$COCH_3$ dendrimers which shows significant increased values (Table 2). FTIR, ^{1}HNMR and MALDI mass spectral results along with the conductivity measurements proves the successful synthesis of all the four modified dendrimers i.e., PAMAM (G0)/(G1)-$CH_3$and PAMAM (G0)/(G1)-$COCH_3$. Where, the neutralization of terminal functional groups was generated with maintaining the internal cationic character.

Solubilization of PRM using four types of modified dendrimers and two parent dendrimers(control)

Standard calibration curve was drawn for PRM using standard solutions of it keeping the concentration range from 3 to 75 µM. A straight line, y=17345x passing through the origin was

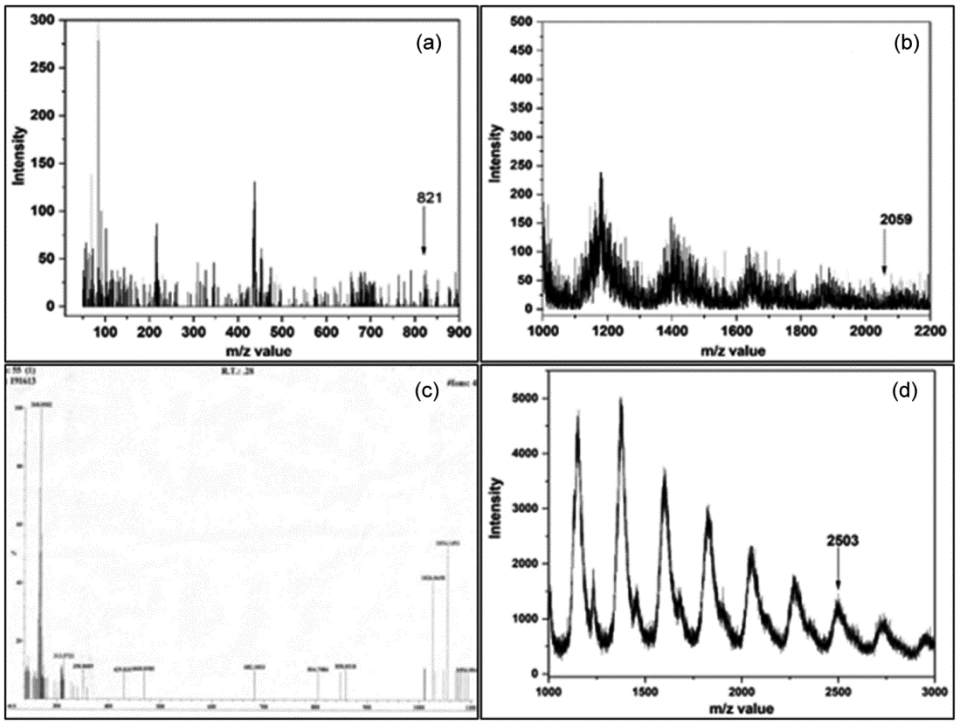


Fig. 2 — MALDI-TOFF spectrum of (a) PAMAM (G0)-CH_3; (b)PAMAM (G1)-CH_3; (c) PAMAM (G0)-$COCH_3$ and (d) PAMAM (G1)-$COCH_3$

Table 2 — Influence of dendrimer drug carriers on PRM solubility

S. No	[Dendrimer drug carriers] $\times10^{-4}$	Sol in mg/mL (St)						Solubility increment (St/S0)					
		PAMAM (G0)	PAMAM (G1)	PAMAM (G0)-CH3	PAMAM (G1)-CH3	PAMAM (G0)-COCH3	PAMAM (G1)-COCH3	PAMAM (G0)	PAMAM (G1)	PAMAM (G0)-CH3	PAMAM (G1)-CH3	PAMAM (G0)-COCH3	PAMAM (G1)-COCH3
1.	0.825	0.2384	0.2696	0.310	0.3560	0.2912	0.3406	10.36	11.72	13.48	15.48	12.66	14.81
2.	1.65	0.3294	0.5898	0.397	0.4475	0.3499	0.4072	14.32	25.65	17.29	19.46	15.22	17.71
3.	3.3	0.3856	0.722	0.442	0.5807	0.4293	0.4877	16.77	31.39	19.23	25.25	18.66	21.21
4.	4.9	0.4810	0.752	0.525	0.7284	0.5036	0.5700	20.92	32.72	22.84	31.67	21.89	24.78
5.	6.6	0.576	0.812	0.593	0.8224	0.5816	0.649	25.03	35.34	25.78	35.76	25.28	28.25
6.	8.25	0.626	0.8356	0.667	0.9629	0.6707	0.747	27.24	36.33	29.00	41.86	29.16	32.49
7.	9.9	0.656	0.886	0.755	1.112	0.7686	0.851	28.54	38.52	32.83	48.31	33.42	37.00

Intrinsic Solubility of Piroxicam S_0 is 0.023mg/mL

obtained and thus proving the validity of Beer-Lambert's law in the specified concentration and giving the molar absorptivity ε at 354nm in aqueous medium at room temperature as 1.7345×10^4. The developed dendrimer drug carriers along with control PAMAM (G0)/(G1) dendrimers were employed as drug solubilizers and thereby examined their potential as drug carriers. Solubility studies were performed keeping the [dendrimer carriers] from 0.83×10^{-4}M to 9.9×10^{-4}M and the solubility of PRM has been increased significantly in the presence of modified dendrimer and their control. The absorbance of the characteristic peak noticed at 354 nm in UV spectrophotometer has been monitored at different dendrimer concentration. The so obtained absorbance was correlated with the standard calibration curve for PRM and the solubility of the drug was calculated in the presence of individual carriers such as PAMAM (G0)&(G1), PAMAM (G0)/(G1)-CH_3 and PAMAM (G0)/ (G1)-$COCH_3$ and given in Table 2. Similarly using these values plots were drawn and found to be linear which are evident from the Fig. 3. Further, the UV-vis spectra show the increase in absorbance of PRM with increase in concentrations of all the carriers.

Inherent/intrinsic solubility (S_0), of PRM without any drug carrier is taken from the literature[36], which is 0.023 mg/mL. Similarly, the solubility of PRM in different [dendrimers] is labelled as S_t and the relative solubility is labelled as S_t/S_0. Now, using the relative

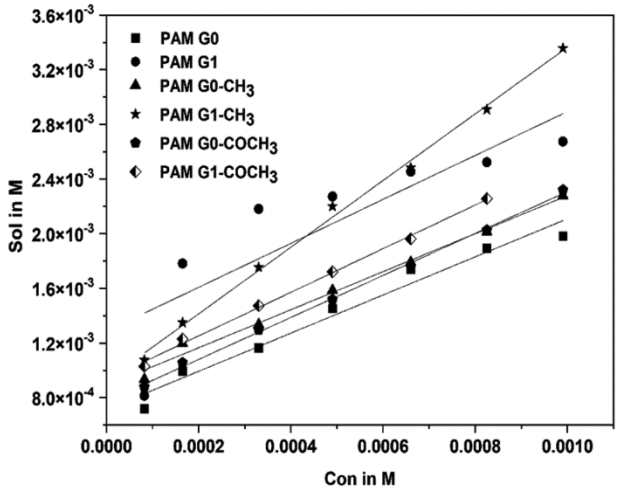


Fig. 3 — Plot showing the variation in solubility with respect to different carriers

solubility (S_t/S_0) of the [PRM] was calculated given in the Table 2. The various results observed from the solubility measurements have strongly supported that the unmodified and modified dendrimers were found to effectively accelerate the solubilities of PRM. The possible reasons for solubility enhancement are the electrostatic interaction between anionic sites of the drug and the cationic sites of the respective carriers and this agrees well with earlier studies[37,38]. The degree of electrostatic interaction between the drug and carriers were found to increase on par with the generation number and nature of surface group of the respective dendrimer carriers. That is, higher the generation number more is the surface group that in turn reflected higher interaction of drugs and thus improved the solubility of PRM.

Sustained release behaviour of developed dendrimer drug carrier and unmodified dendrimers

The very purpose of drug carrier is to carry the drugs and deliver them at the site of action. One of the prerequisites of a good carrier is, it should deliver the drug in a controlled/sustained manner. If the delivery is in a controlled/sustained manner, the side effect of drug can be minimized by preventing the fluctuation of the therapeutic concentration of the drug in the body. It also eliminates the chance of over or under dosing. One of the methods to achieve this sustained/controlled release is normally possible via diffusion through a matrix[39]. In view of this background, we have monitored the *in vitro* release of the drug from the carrier through the well-established equilibrium dialysis method. First, the release of drug alone was noted. subsequently, the release of drug

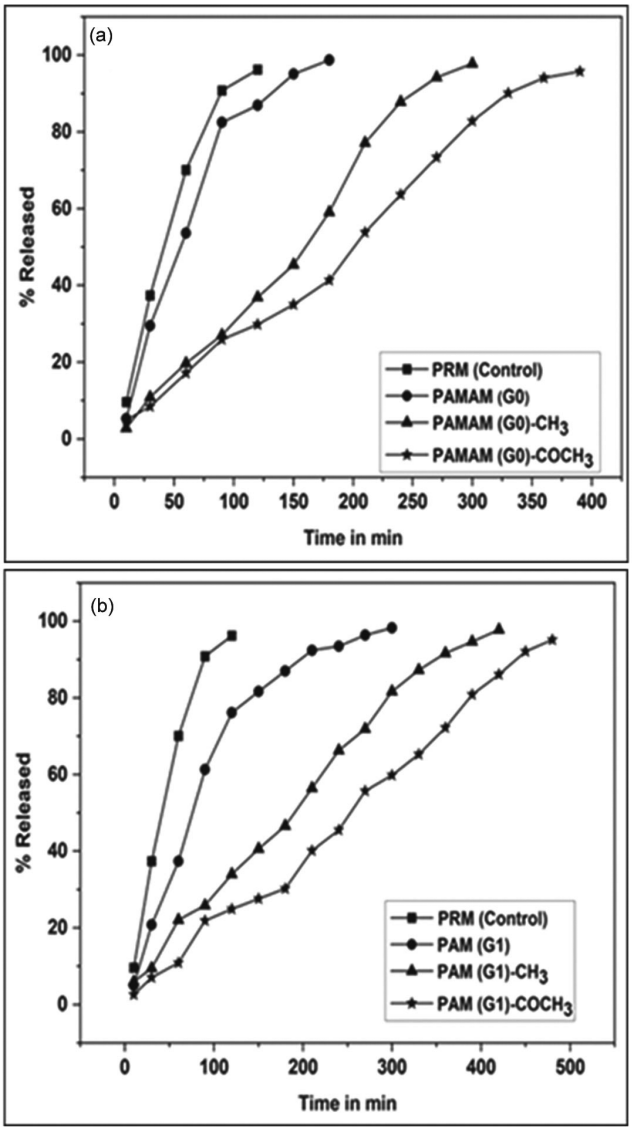


Fig. 4 — *In vitro* release of PRM from (a) G0 dendrimer drug carriers and (b) G1 dendrimer drug carrier.

from PAMAM (G0), PAMAM (G1), PAMAM-G0-CH_3, PAMAM-G1-CH_3, PAMAM-G0-$COCH_3$ and PAMAM-G1-$COCH_3$ were monitored by keeping the [drug] and [carrier] as constant throughout. *In vitro* release test was carried out using appropriate dialysis bags against double distilled water. We have observed significant sustained release pattern from all the carriers. The percentage release of the drug from the each of the carriers were monitored at different time intervals and the release pattern was plotted as Percentage released versus Time [Fig. 4(a) and 4(b)].

After 40 min, 50% of the drug was found to release from the dialysis bag, whereas, it takes more than 75 min to get release of the same amount from

PAMAM (G0) and (G1) respectively. Interestingly, 150 min required from PAMAM (G0)-CH_3 and more than 180 min from PAMAM (G1)-CH_3 for the release of same 50% of drug. Within 120 min, 96.22% of the plain drug gets released, whereas it takes 180 min to release 98.71% from PAMAM (G0) and 270 min to release 98.28% for PAMAM (G1). Further, it takes 300 min to release 97.78% from PAMAM G0-CH_3, 420 min to release 97.8% from PAMAM G1-CH_3, 390 min to release 95.79% from PAMAM (G0)-$COCH_3$ and 480 min to release 95.18% from PAMAM (G1)-$COCH_3$. The consolidated values for the percentage of drug release determined irrespective of the surface functional groups of (G0) and (G1) based hydrophilic PAMAM dendrimers was given in Table 3. The plots for [PRM] release versus time were drawn and shown in Fig. 4(a) for (G0) dendrimers and Fig. 4(b) for (G1) dendrimers. The sharp reduction in the rate of release of a drug irrespective of dendrimer carriers were due to the existence of ionic and hydrophobic interaction between the dendrimer matrices and the drug molecules. The delay in drug release is due to the interaction between the carrier and drug molecule[40]. In other words, in the absence of any carrier, stronger the interaction slower is the release. In the absence of any carrier, drug comes out faster. Further the release of drug gets extended reasonably from unmodified dendrimers. The release time can be quantified to the amount of interaction between the carrier and the drug molecule which is well reflected in the release behaviour of drug from modified PAMAM dendrimers. With the above findings, it proves that all the carriers offer the possibility of sustained release of the drug during delivery. Among the carriers under study, modified PAMAM dendrimers gave better sustained release on comparison with the unmodified dendrimers.

Table 3 — Comparison of solubilizing potential and *in vitro* release of PRM from dendrimer drug carriers under study

S. No.	Dendrimer drug carriers	Solubility increment (S_t/S_0) [9.9×10^{-4} M]	Time required to release 90% of drug(mins)
1	PRM (Control)	0.023	90
2	PAMAM (G0)	28.54	135
3	PAMAM (G1)	38.52	210
4	PAMAM (G0)-CH_3	32.83	270
5	PAMAM (G1)-CH_3	48.30	360
6	PAMAM (G0)-$COCH_3$	33.42	330
7	PAMAM G1-$COCH_3$	37.00	450

Comparative study of PRM solubility and *in vitro* release with different dendrimer carriers

From the Table 3 values, it is observed that the solubility of PRM in PAMAM (G0) is enhanced up to 29 folds, whereas, in PAMAM (G1), the enhancement is up to 39 folds. These values prove that the generation number has largely influenced the solubility. The same kind influences were also noticed in the modified dendrimers also. The increase in solubility is up to 33 folds for PAMAM (G0)-CH_3, a highest of 48 folds for PAMAM (G1)-CH_3, 33 folds for PAMAM G0-$COCH_3$ and up to 37 folds for PAMAM (G1)-$COCH_3$. The solubility of PRM were observed in the order of PAMAM (G0) < PAMAM (G0)-CH_3 < PAMAM (G0)-$COCH_3$ for (G0) generation. Whereas, the order for (G1) based dendrimers were determined as PAMAM (G1)-$COCH_3$ < PAMAM G1 < PAMAM (G1)-CH_3. The reason for this trend can be attributed to the increased binding capacity of the modified dendrimers with the drug molecules compared with the unmodified dendrimers with the drug molecules. Modified dendrimers have definite cationic character which leads to stronger interaction with the drug molecules facilitating more solubility. On the other hand, in PAMAM (G1)-$COCH_3$, a little lesser solubility is observed which can be interpreted in this way. As the terminal functional groups increases with increase in generation steric hindrance of the modified functionalities plays a substantial role, while the drug molecules approaching the carrier molecule/solubilizer.

The comparison of sustained release from Table 3, 90% of plain drug gets released as early as 120 min of its administration. Whereas, the release substantially gets slower upto 450 min for the same amount of drug to gets released from G1-$COCH_3$. The order of release with respect to different carriers is as follows; Plain drug < PAMAM G0 < PAMAM G0-CH_3< PAMAM G0-$COCH_3$.Plain drug < PAMAM G1 < PAMAM G1-CH_3< PAMAM G1-$COCH_3$. With the above results, it is evident that the modified dendrimers are promising candidates to solubilise and to sustain the release of PRM drug. Further, due to surface modification, the cytotoxicity has strongly reduced and biocompatibility is improved.

Antibacterial activity of drug in presence of developed dendrimer drug carrier and unmodified dendrimers

When the drug carriers are used for the purpose of effective delivery, the inherent activity of

the drug should not be disturbed. Rather the presence of carrier molecule should increase the activity of drug to qualify itself as better carrier. In this perspective, we have analysed the antibacterial activity of drug alone and drug-dendrimer complexes i.e., PRM-PAMAM (G0), PRM-PAMAM (G1), PRM-PAMAM (G0)-CH_3, PRM-PAMAM (G1)-CH_3, PRM-PAMAM (G0)-$COCH_3$ and PRM-PAMAM (G1)-$COCH_3$ at proper concentrations using *E. coli* and *B. subtilis* of bacterial strains. When equal amounts of free PRM and PRM-dendrimer are considered (the actual amount of PRM in carrier solution was equal to the free drug used). PRM- dendrimer complexes are shown reasonably more potent than free PRM dissolved in DMSO. Zone of inhibition was used to quantifythe activity with respect to drug and dendrimers complexes and the obtained results are given in the Table 4. The corresponding photographs are shown in Fig. 5(a-n). From the tables and photographs, it is understood that the increase in activity of the drug in presence of carriers was due to its increase in water soluble nature and because of which they diffuse to a greater extent in the aqueous agar medium and hence the zone of inhibition was found to be greater for complexes than the pure drug. Further, the carriers favour the interaction of the drug with its target or help it to penetrate through the bacterial membrane which in turn causes the inhibition in bacterial growth[41]. Such a development is promising feature to use dendrimers as drug carriers.

Table 4 — Anti-bacterial activity of drug-dendrimer complexes against *Bacillus subtillis* and *Escherichia coli*

S. No.	Formulation	*Bacillus subtillis* Inhibition zone diameter(mm)				*Escherichia coli* Inhibition zone diameter(mm)			
		S	1:2	1:4	1:8	S	1:2	1:4	1:8
1	DMSO- (Blank)	--	--	--	--	--	--	--	--
2	PXM (Control)	12	--	--	--	12	--	--	--
3	PXM+PAMAM (G0)	15	12	9	7	13	11	8	7
4	PXM+PAMAM (G1)	18	15	11	9	17	15	12	9
5	PXM+PAMAM (G0)-CH_3	16	14	12	10	16	13	11	10
6	PXM+PAMAM (G1)-CH_3	20	18	15	10	18	16	14	11
7	PXM+PAMAM (G0)-$COCH_3$	17	14	11	9	14	12	10	8
8	PXM+PAMAM (G1)-$COCH_3$	18	16	13	10	16	14	11	8

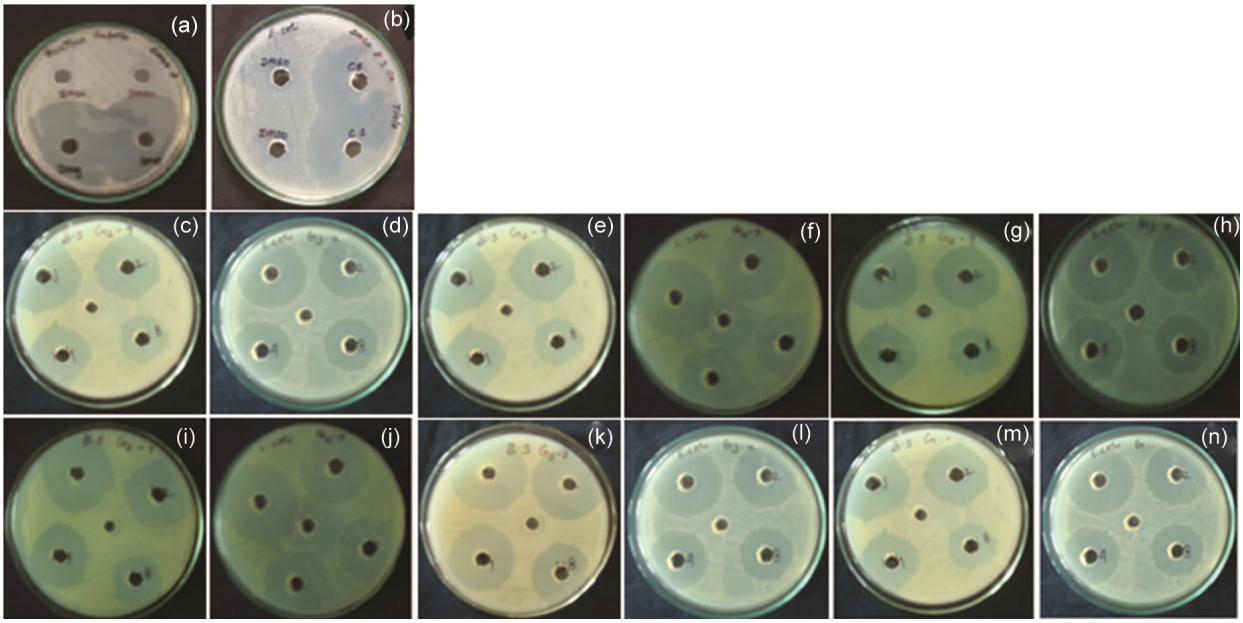


Fig. 5 — Activity of drug in presence of dendrimer drug carriers against *B.subtillis* (a) PRM drug control; (b) DMSO (blank); (c) PAMAM (G0); (d) PAMAM (G1); (e) PAMAM (G0)-CH_3; (f)PAMAM (G1)-CH_3; (g)PAMAM (G0)-$COCH_3$; and (h) PAMAM (G1)-$COCH_3$ and against *E.coli* (i) PAMAM (G0); (j) PAMAM (G1); (k) PAMAM (G0)-CH_3; (l) PAMAM (G1)-CH_3; (m) PAMAM (G0)-$COCH_3$ and (n) PAMAM (G1)-$COCH_3$.

Conclusion

In a nutshell, this study has been intended to modify the terminal functional groups of lower generation (G0) and (G1) PAMAM dendrimers and hence led to the new window of exploring their ability as drug carriers. The change of surface groups of dendrimers has neutralized the toxic primary amine groups and replaces with neutral moieties which are more hydrophilic and more biocompatible. The maximum solubility enhancement of piroxicam up to 48 folds was achieved by using PAMAM (G1)-CH_3 as solubilizer at a concentration of 9.9×10^{-4} M. The 90% plain drug gets released as early as 120 min of its administration, whereas, the release substantially gets slower upto 450 min from G1-$COCH_3$. This observation indicates evidence that the dendrimers is prove to be an effective delivery of drugs with drastic reduction in side effect and improved efficiency. The activity in terms of zone of inhibition reveals that the inhibition value was increased to 1.5-2.0 folds in the presence of carriers and it reflects that the inherent property of drug is not disturbed, instead the activity was increased. The increase in zone of inhibition was to be more pronounced in the case of *B. subtilis* rather than *E.coli.* This work demonstrated that the dendrimer drug carriers provide a platform for drug attachment that can bind and release drugs and leads to solubility enhancement, sustained release of the drug in vitro and increased anti-bacterial activity. Therefore, the dendrimer carrier with -CH_3 and -$COCH_3$ surface groups has proved to be a prominent candidate for effective delivery of Piroxicam drug.

Acknowledgement

Authors would like to acknowledge MHRD-RUSA 2.0 RI & QI Theme-2 for the financial support.

Conflicts of Interest

The author declares no conflict of interest.

References

1 Hedner T, Samulesson O, Wahrborg P, Wadenvik H, Ung K A, Ekbom A, *Drugs*, 64 (2004) 2315.
2 Izdes S, Orhun S, Turanli S, Erkilic E & Kanbak O, *Anesth Analg*, 97 (2003) 1016.
3 Castellsague J, Riera-Guardia N, Calingaert B, Varas-Lorenzo C, Fourrier-Reglat A & Nicotra F, *Int J Med Toxicol Drug Exp*, 35 (2012) 1127.
4 Jinno J, Oh D, Crison J R & Amidon G L, *J Pharm Sci*, 89 (2000) 268.
5 Pushkar S, Philip A, Pathak K & Pathak D, *Indian J Pharm Educ Res*,40(2006) 153.
6 Sakthivel T & Florence A T, *Int J Pharm*, 254 (2003) 23.
7 Gupta U, Agashe H B, Asthana A & Jain N K, *Biomacromolecules*, 7 (2006) 649.
8 Mahapatra A K, Murthy P N, Swadeep B & Swain R P, *Int J Pharm Tech Res*, 6 (2014) 546.
9 Tang J, Sun J & He Z G, *Curr Drug Ther*, 2 (2008) 85.
10 Mishra V, Nayak P, Yadav N, Singh M & Tambuwala M M, *Expert Opin Drug Deliv*, 18 (2021) 315.
11 Yiyun C, Zhenhua X, Minglu M & Tonguen X, *J Pharma Sci*, 97 (2008) 123.
12 Hawker C & Fréchet J M J, *J Chem Soc Chem Commun*, 15 (1990) 1010.
13 AL-Timimi Z, *Heliyon*, 7 (2021) 06863.
14 Al-Timimi Z, *Int J Low Extrem Wounds*, 21 (2022) 640.
15 Barakat D A, Al-Abbas G & Al-Timimi Z, *Iraqi Laser Sci J*, 1 (2019) 21.
16 Grainger D W & Okano T, *Adv Drug Delivery Rev*, 55 (2003) 311.
17 Emanuele AD & Attwood D, *Adv Drug Deliv Rev*, 57 (2005) 2147.
18 Jain K, Kesharwani P, Gupta U & Jain N K, *Int J Pharm*, 394 (2010)122.
19 Duncan R & Izzo L, *Adv Drug Deliv Rev*, 57 (2005) 2215.
20 Smith P E, Brender J R, Durr U H, Xu J, Mullen D G & Banaszak Holl M M, *J Am Chem Soc*, 132(2010) 8087.
21 Zhang Y, Sun Y, Xu X, Zhang X, Zhu H & Huang L, *J Med Chem*, 53(2010) 3262.
22 MajorosI J, Thomas T P, Mehta C B & Baker J R, *J Med Chem*, 48 (2005) 5892.
23 Roy R & Baek M G, *J Biotechnol*, 90(2002) 291.
24 Kolhatkar R, Kitchens K, Swaan P & Ghandehari H, *Bioconjug Chem*, 18 (2007) 2054.
25 Zhang Y, Sun Y, Xu X, Zhu H, Huang L & Zhang X, *Bioorg Med Chem Lett*, 20(2010) 927.
26 Murugan E, Geetha Rani D P, Srinivasan K & Muthumary J, *Expert Opin Drug Delivery*, 10 (2013) 1319.
27 Murugan E, Geetha Rani D P & Yogaraj V, *Colloids Surf B Biointerfaces*, 114 (2014) 21.
28 Murugan E, Yogaraj V, Geetha Rani D P & Alok Kumar S, *RSC Adv*, 5 (2015) 106461.
29 Yogaraj V, Gowtham G, Akshata C R, Manikandan R, Murugan E & Arumugam M, *J Drug Deliv Sci Technol*, 58 (2020) 101785.
30 Martin A, Bustamante P, Chun AHC.,*Physical Pharmacy,* 4th Edition, B.I. Waverly Pvt. Ltd. New Delhi 274 (1999) 53.
31 Ventura C A, Giannone I, Paolino D, Pistora V, Corasaro A & Puglisi G, *Eur J Med Chem*, 40 (2005) 624.
32 Shi X Y, Wang S H, Van Antwerp M E, Chen X & Baker J R, *Analyst*, 134 (2009) 1373.
33 Shi X Y, Wang S H, Meshinchi S, Van Antwerp M E, Bi X D, Lee I H & Baker J R, *Small*, 3 (2007) 1245.
34 Cheng Y Y, Li Y W, Wu Q L, Zhang J H & Xu T W, *Eur J Med Chem*, 44 (2009) 2219.
35 Komiyana M & Bender M L J, *Am Chem Soc*, 100 (1977) 2259.

36 Yang W J, Li Y W, Cheng Y Y, Wu Q L, Wen L P, Xu T W J, *Pharm Sci*, 98 (2009) 1075.

37 Liu J Y, Pang Y, Huang W, Zhu X Y, Zhou Y F & Yan, D Y, *Biomaterials,* 31 (2010) 1334.

38 Brand T, Cabrita E J & Berger S, *Prog Nucl Magn Reson Spectrosc*, 46 (2005) 159.

39 Chai M H, Holley A K & Kruskamp M, *Chem Commun*, (2007) 168.

40 Hao H X, Wang J K & Wang Y L J, *Chem Eng Data*, 49 (2004) 1697.

41 Nirschl R P, Rodin D M, Ochiai D H & Maartmann-Moe C, *Am J Sports Med*, 31 (2003) 189.