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Performance evaluation of PAMAM dendrimer based simvastatin formulations

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ABSTRACT

The purpose of this investigation was to evaluate the performance of poly (amidoamine) (PAMAM) dendrimers, with three different surface groups, to be used as drug carriers. Drug–dendrimers complexes were investigated for solubility studies, dissolution studies, in vitro drug release studies, and for stability studies. The solubility enhancement was found maximum with PEGylated dendrimers (33 times) followed by amine (23 times) and hydroxyl (17.5 times) dendrimers. The solubility profile of simvastatin–dendrimer complex showed a linear correlation (Higuchi A_L-type diagram) between solubility and dendrimers concentration. The formation of the complexes between drug molecules and dendrimers were characterized by the FTIR spectra of these complexes, showing the appearance of the bond formed between the functional groups of the drug (OH and COOH) and dendrimers (NH_2 and OH). The drug–dendrimer complexes displayed the controlled release action during in vitro release studies. Pure simvastatin (SMV) was released in 5 h whereas the PEGylated dendrimers–SMV complexes released the drug up to 5 days. The non-PEGylated formulations released the drug up to 24 h. Formulations with amine and PEGylated dendrimers were subjected to accelerated stability studies. Formulations with amine dendrimers were found to be most stable in dark, low temperature (0 °C) whereas the dark, RT was most suitable storage conditions for formulation with PEGylated dendrimers.

1. Introduction

Currently available all novel drug delivery systems basically involve the use of lipids or polymers. Each type of system has its limitations: lipid based drug delivery systems (i.e., liposomes, solid lipid nanoparticles, nanostructured nanocarriers) have poor physical stability, drug leakage, difficulty in drug targeting (Arulsudar et al., 2004; Tabatt et al., 2004) and low drug loading capacity due to the formation of a perfect lipid crystal matrix (Mehnert and Mader, 2001) whereas polymer based systems use linear polymers which are polydisperse in nature, in addition to regulatory issues and scaling up problems.

Dendrimers are a unique class of synthetic macromolecules having a highly branched, three dimensional, nanoscale architecture with very low polydispersity (1.00002–1.005) and high functionality. The unique structure makes dendrimers an excellent building block to create an ideal polymeric drug-delivery system with multiple functionalities, which is otherwise difficult to achieve with linear polymers. The basic advantage of dendrimers is to deliver drugs efficiently and effectively, at the same time they also improve the biopharmaceutical and pharmacokinetic properties of drugs (Svenson and Tomalia, 2005). Various studies have been carried out to use dendrimers as drug delivery via various routes of administration: oral (Tripathi et al., 2002; D'Emanuele et al., 2004; Man et al., 2006), intravenous (Malik et al., 1999; Padilla et al., 2002; Kukowska-Latallo et al., 2005; Bhadra et al., 2003, 2005; Chauhan et al., 2004; Asthana et al., 2005), transdermal (Chauhan et al., 2003; Wang et al., 2003a,b; Cheng et al., 2006), and ocular (Shaunak et al., 2004; Vandamme and Brobeck, 2005).

Among the three basic family of dendrimers: poly (amidoamine) (PAMAM), diaminobutane (DAB) and polypropyleneimine (PPI), PAMAM dendrimers have been extensively used in drug delivery because they allow the precise control of size, shape and placement of functional group (dimensional stability), controlled method of synthesis, minimum toxicity and wide availability.

In this investigation, performance of PAMAM dendrimers with different surface groups was evaluated for their application as drug delivery system using simvastatin as model drug. Simvastatin is a hypolipidemic drug and is used to control hyper-cholesterolemia and to prevent cardiovascular diseases. Simvastatin inhibits the rate determining step in cholesterol biosynthesis; catalyzed by 3-hydroxy-3-methylgluteryl coenzyme A (HMG-Co-A) reductase (Alberts et al., 1980; Goodman et al., 2001). This inhibition leads to up-regulation of low-density lipoprotein (LDL) receptors and increase in catabolism of LDL cholesterol (Brown and Goldstein, 1986). Simvastatin is practically insoluble in water and hence poorly absorbed from the gastro-intestinal tract; oral bioavailability is less (<5%) (Martindale, 2005).

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Attempts have been made to deliver simvastatin by the use of lipids (as self-emulsifying drug delivery system) (Patil et al., 2007; Kang et al., 2004) and cyclodextrins (Patel and Patel, 2007; Yoshinari et al., 2007). But all the formulations suffer with some and other disadvantages. The major problem associated with SEDDS is thermodynamically instability. A SEDDS is either diluted just prior to administration or else in the body, the required droplet stability is less than 6 h (i.e., the transit time of materials down the small intestine) (Lawrence and Warisnoicharoen, 2006). Secondly, as per lipid formulation classification system, proposed by Pouton (2000, 2006), self emulsifying drug delivery systems are type-II lipid formulation, which requires 20–60% water-insoluble surfactants (HLB < 12) to make the stable formulations. The use of such a large quantity of surfactants can induce GI irritation (Tang et al., 2008).

Patel et al. increased the solubility of SMV by 7.35 fold and 13.3 fold at 14 mM/L concentration of β-CD and HP-β-CD, respectively. But the use of cyclodextrins in more than 6 mM/L concentration induces very rapid hemolysis (due to extraction of lipids from the erythrocytic membrane) (Arikan, 2003).

So the basic objective of this project was to evaluate the performance of three different G4 poly (amidoamine) (PAMAM) dendrimers to be used as drug carriers and to simultaneously develop the controlled release formulation of lipid lowering drug simvastatin. The reason behind to develop this formulation is that the patient suffered with hypercholesterolemia requires a long-term treatment and secondly statins take 2–3 days to display their effects.

2. Materials and methods

2.1. Materials

G4-PAMAM–NH_2, G4-PAMAM–OH and G4-PAMAM–PEG were purchased from Dendritic Nanotechnologies, USA. Simvastatin was obtained as gift sample from Ranbaxy Laboratory (Gurgaon, India). Cellulose dialysis tubing (Mw ~1000) and membrane filter of pore size 0.2 μm were purchased from Himedia Lab. (Mumbai, India). Rest all chemicals were of analytical grade and were purchased from CDH (India).

2.2. Phase solubility studies

Solubility studies of SMV with G4-PAMAM–NH_2, G4-PAMAM–OH and G4-PAMAM–PEG were carried out by the method described by Higuchi and Connors with minor modifications (Higuchi and Connors, 1965). The studies were performed in amber colored bottles to avoid any degradation of dendrimer (Chauhan et al., 2004). The aqueous solubility of SMV was determined in the presence of increasing concentration of dendrimers to evaluate the effect of dendrimers concentration. Three different pH values (pH 5.0, pH 7.0 and pH 10.2) were selected to determine the pH-dependent solubilization of SMV. The similar study was also performed with all three types of dendrimers to evaluate the effect of dendrimer surface groups on solubility of SMV.

2.2.1. Determination of effects of dendrimer concentration on SMV solubility

An excess (10 mg) of SMV was added into screw capped amber colored vials containing varying concentration (0.05–0.4%, w/v) of G4-PAMAM–NH_2 dendrimers in double distilled water. Separately excess drug (10 mg) was added in a vial containing only double distilled water and used as a control. These suspensions were sonicated briefly, and incubated overnight at 32 °C and 100 rpm in a shaking bath. The vials were allowed to stand for 24 h to attain equilibrium. After equilibration these suspensions were filtered through 0.2 μm membrane filter. Aliquots (0.5 ml) of filtrates were withdrawn from each vial and diluted with appropriate quantity of double distilled water. These samples were analysed for drug content by UV-spectrophotometer at 238 nm and are expressed as the molar ratio (drug/dendrimer) and also as drug concentration vs dendrimer concentration.

2.2.2. Determination of pH-dependent solubility of SMV

For the determination of the effect of pH on the solubility of simvastatin, an excess amount of drug (10 mg) was added in aqueous dendrimer solution (concentration 0.1%, w/v); with a preadjusted pH to pH 5.0, pH 7.0 and pH 10.2 using 0.1 M HCl and 1 M NaOH with the help of pH meter. The rest procedure was similar to the procedure followed to determine the effect of dendrimer concentration on solubility of SMV.

2.2.3. Determination of effect of surface groups on SMV solubility

For the determination of effect of dendrimer surface groups on the solubility of simvastatin, an excess amount of drug (10 mg) was added into three screw capped amber colored vials containing 0.05–0.4% (w/v) aqueous dendrimer solution, preadjusted at pH 10.2, of G4-PAMAM–NH_2, G4-PAMAM–OH and G4-PAMAM–PEG dendrimers separately. The rest procedure was similar to the procedure followed to determine the effect of dendrimer concentration and pH on solubility of SMV.

2.3. Characterization of drug–dendrimer complexes

FTIR spectrums of simvastatin and drug–dendrimer complexes were obtained by means of a FTIR spectrophotometer (FTIR-8400s Shimadzu, Japan). The samples were prepared by the potassium bromide disk method and measurements were attempted with the accumulation of 20 scans and a resolution of 4 cm^{-1} over the range of 400–4000 cm^{-1}. After running the spectra, significant peaks relating to major functional groups were identified; spectra of the subsequent sample of the same compound were compared with the original.

2.4. Dissolution studies

The dissolution of pure simvastatin and drug dendrimer formulations was determined in simulated gastric fluid (SGF) and USP dissolution medium pH 7.0. Three ml of drug–dendrimer formulations (DN_2, DO_2 and DP_2, containing 0.1% (w/v) of amine, hydroxyl and PEGylated PAMAM dendrimers, respectively) were taken in round bottom flask and lyophilized. These lyophilized formulations and pure simvastatin were exposed to the 200 ml of dissolution medium, at 37 ± 0.5 °C and 75 rpm on magnetic stirrer. At scheduled intervals, 2 ml of samples were withdrawn and replenished the dissolution medium with the same volume. After appropriate dilutions, the amount of drug dissolved as a function of time was determined by UV-spectrophotometer at 238 nm.

2.5. In vitro drug release studies

In vitro drug release from the different drug–dendrimer complexes was determined using the dialysis tube diffusion technique. Three ml solution of drug–dendrimer complexes (DN_2, DO_2, and DP_2) were placed in the hermetically tied dialysis sacs, separately. These dialysis sacs were placed into 80 ml of 0.1 M phosphate buffer saline (PBS) pH 7.4, kept at 37 ± 0.5 °C with continuous magnetic stirring to maintain sink conditions. At scheduled intervals, 1 ml of sample was withdrawn from the external medium and replaced with the same volume of fresh PBS to maintain the sink conditions. After appropriate dilutions, the amount of drug released as

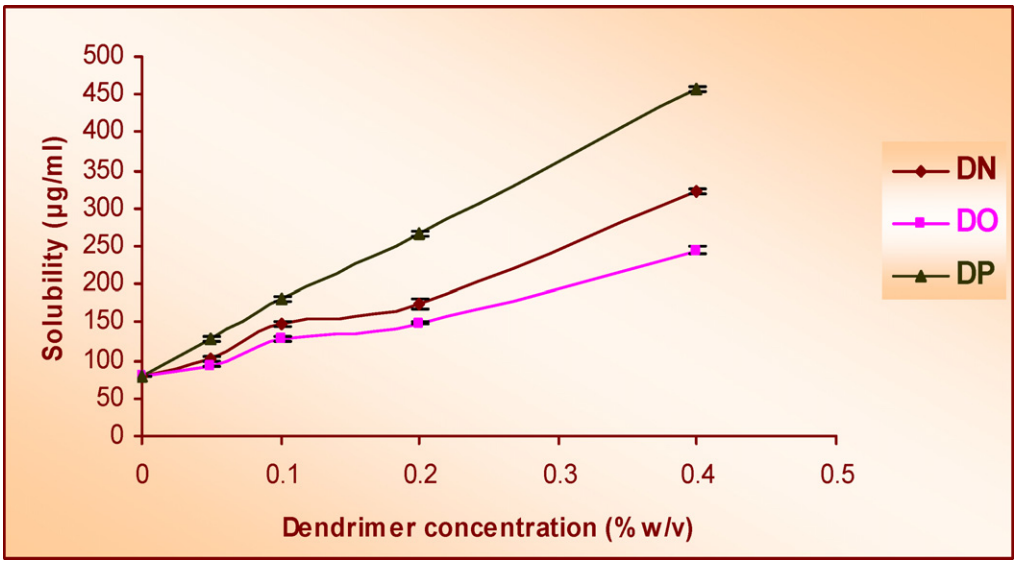

Fig. 1. Effect of dendrimers concentration and surface groups (NH_2, OH and PEG) on simvastatin solubility (μg/ml).

a function of time was determined by UV-spectrophotometer at 238 nm.

2.6. Stability studies

The dendrimer–drug formulations were kept in amber colored and colorless vials at 0 °C, room temperature and 60 °C for a period of 5 weeks. The samples were analysed initially and periodically for up to 5 weeks for any precipitation, turbidity, crystallization, change in color, change in consistency and increase in drug loss. The drug leakage was indirectly determined by checking the increase in release rate of drug from the formulations after storage. The obtained data were used for the analysis of any physical or chemical degradation at specified storage conditions.

3. Results and discussion

3.1. Solubility studies

The solubility enhancement was found maximum with PEGylated dendrimers (33 times) followed by NH_2 (23 times) and OH (17.5 times) dendrimers. The solubility profile of PEG dendrimers–SMV complex showed a linear correlation (Higuchi A_L-type diagram) between solubility and dendrimers concentration (Fig. 1). The solubility was increased from 33.4 to 1093.25 μM/L with 109.04 M (0.4%, w/v) PEGylated dendrimer solution. The solubility enhancement with PEGylated dendrimers may contribute to interaction between SMV and tertiary amino groups, availability of large space for molecular encapsulation and H-bonding. The linearity of curve may due to more spherical and symmetric shape with PEG chain on dendrimer surface.

The solubility profile with NH_2 and OH dendrimers showed a non-linear (Higuchi A_N-type diagram) between solubility and dendrimers concentration (Fig. 1). The curve showed a linear relationship up to a certain concentration (up to 0.1%) after that the increase was no longer linear, presumably due to self association of dendrimers at higher concentrations leading to the nano-scale separation (Chauhan et al., 2004). The solubility enhancement with amine dendrimers can be explained on the basis of electrostatic interaction between amino groups of dendrimers and –COOH group of simvastatin, molecular encapsulation and weak H-bonding. Because of the presence of similar hydroxyl groups

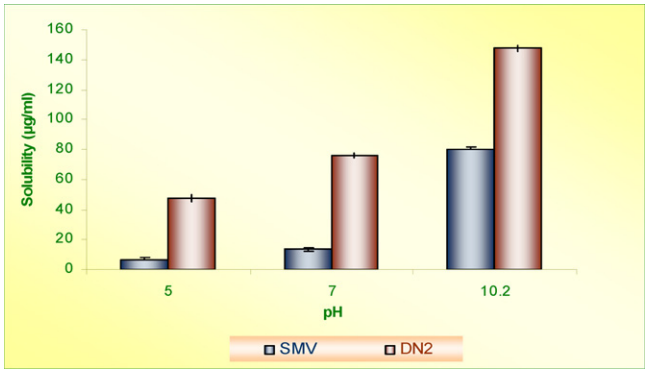

Fig. 2. Comparative effect of pH on SMV solubility in water and 0.1% (w/v) G4 NH_2 PAMAM dendrimer solution.

(having a net similar charge) on both SMV and dendrimer surface, there is weak interaction between hydroxyl dendrimers and SMV. Thus the increase in solubility could be result of weak hydrogen bonding along with molecular encapsulation.

The effect of pH on the solubility of SMV in the presence of amine dendrimers was also studied (Fig. 2). The enhancement in solubility was highest at pH 10.2, less at pH 7 and least at pH 5. The solubility was found to have enhanced nearly 10.6 times at pH 10.2, 5.4 times at pH 7 and 3.4 times at pH 5. At low pH (pH 5), the drug was remain unionized (because simvastatin is weakly acidic drug) and amines groups of dendrimers (primary amine groups at surface and tertiary groups in interior) were protonated, resulting into the weak interaction between drug and dendrimers.

At higher pH (pH 10), the increase in solubility of SMV may attributed to strong electrostatic interaction between deprotonated dendrimers and completely ionized drug molecules. Another factor may be the pH dependent solubility enhancement of weakly acidic drug at higher pH. Overall it was found that the increase in SMV solubility in three different types of PAMAM dendrimer solutions depended on concentration of dendrimers, the pH of solution and the type of functional group present on dendrimer surface.

3.2. Characterization of drug–dendrimer complexes

The use of the dendrimers as drug delivery vehicle depends on their ability to form a complex with the drug and the forma-

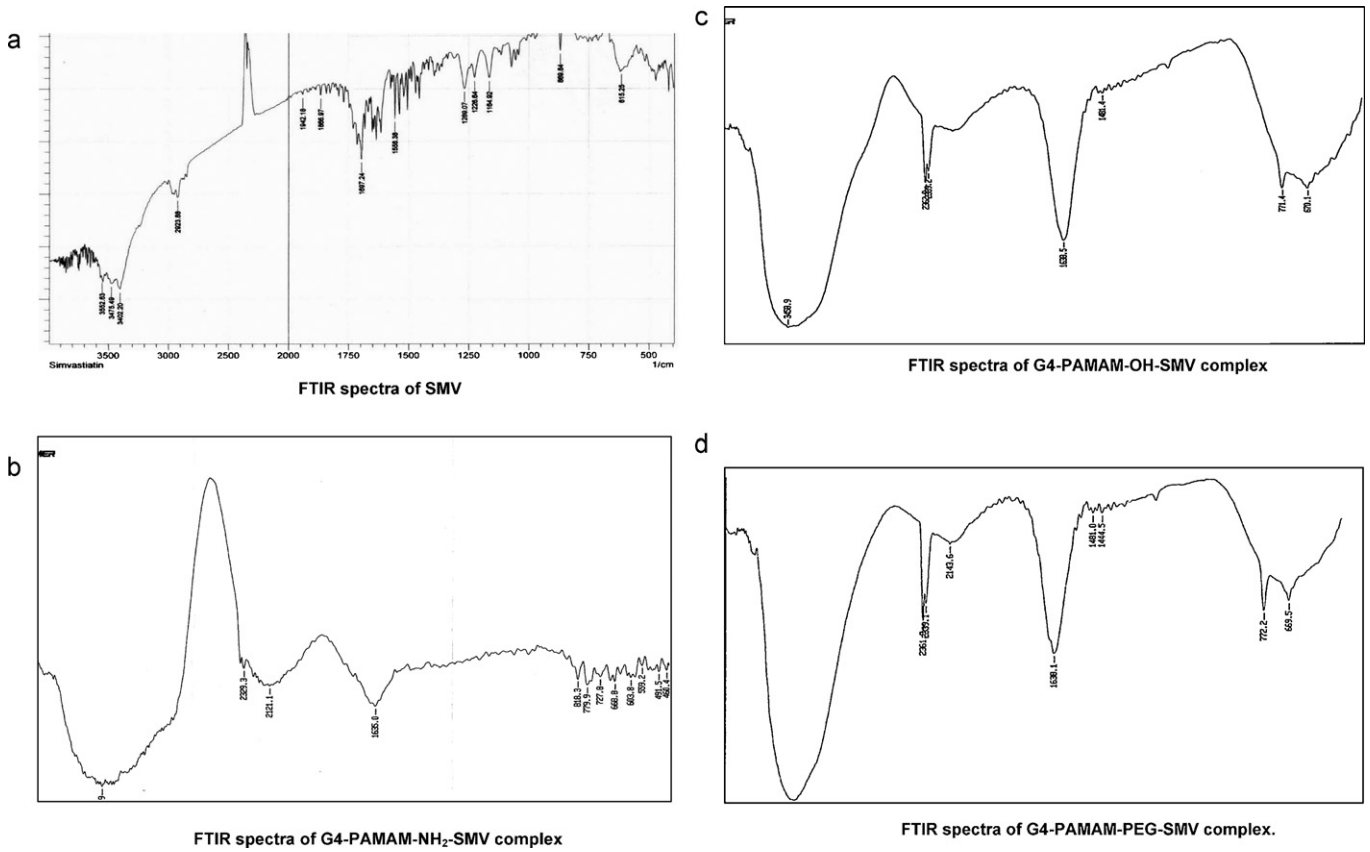

Fig. 3. (a) FTIR spectra of SMV, (b) FTIR spectra of G4-PAMAM–NH_2–SMV complex, (c) FTIR spectra of G4-PAMAM–OH–SMV complex and (d) FTIR spectra of G4-PAMAM–PEG–SMV complex.

tion of drug–dendrimer complex further depends on the nature of the core–surface groups of the dendrimer, electrostatic interaction between the dendrimer and the drug and the ability of the drug to form a conjugate with the dendrimers through chemical bonding. So the drug–dendrimer complex formation between the SMV and dendrimers (G4-PAMAM–NH_2, G4-PAMAM–OH and G4-PAMAM–PEG) used in this project were characterized by their FTIR spectra.

The FTIR spectrum of pure simvastatin (Fig. 3a) has four characteristic peaks at 3552 cm^{-1}, 1697, 1267 and at 870 cm^{-1} for O–H stretching vibration, strong carbonyl (C=O) ester stretching vibration, lactone C–O–C stretching vibration and for trisubstituted olefinic C–H wagging vibrations, respectively (Florey, 2001).

The FTIR spectrum of pure G4-PAMAM–NH_2 has three characteristic peaks at 3292 cm^{-1}, 1644 cm^{-1} and at 1035 cm^{-1} for N–H stretching vibration of primary amine groups present on the periphery, C=O stretching of amide group and for C–N stretching of primary amine groups, respectively. The FTIR spectrum of G4-PAMAM–NH_2–SMV complex (Fig. 3b) shows the disappearance of two characteristic peaks: one peak of N–H stretching vibration at 3292 cm^{-1} and another peak of C–N stretching vibration of primary amine groups. Further the interaction was confirmed by the strong C=O stretching peak of amide at 1635 cm^{-1}.

The FTIR spectrum of pure G4-PAMAM–OH has three characteristic peaks at 3287 cm^{-1}, 1639 cm^{-1} and at 1060 cm^{-1} for O–H stretching vibration of primary amine groups present on the periphery, C=O stretching of amide group and for C–O stretching of primary hydroxyl groups, respectively. The FTIR spectrum of G4-PAMAM–OH–SMV complex (Fig. 3c) shows the appearance of the characteristic peak at 1638 cm^{-1} for the formation of β-hydroxy ester bond between the –OH group of dendrimer and the –COOH group of the simvastatin. A broad band appeared at 3300–3550 cm^{-1} may be due to intermolecular hydrogen bonding of the molecules.

The FTIR spectrum of pure G4-PAMAM–PEG and pure G4-PAMAM–OH were almost similar because of the same functional groups. So the FTIR spectrum of G4-PAMAM–PEG–simvastatin complex (Fig. 3d) and G4-PAMAM–OH–SMV complex were also similar.

3.3. Dissolution studies

The dissolution of pure drug and drug–dendrimer complexes was determined in USP dissolution medium pH 7.0 and in SGF pH 1.2. The results are shown in Figs. 4 and 5. At the end of 3 h, the dissolution of the simvastatin from the drug–dendrimer complexes was significantly greater than that for pure drug.

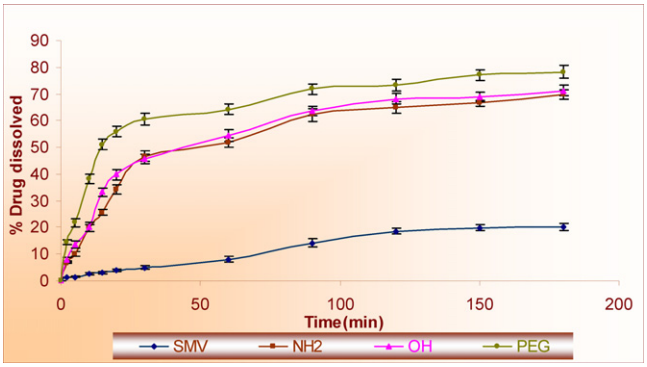

Fig. 4. Dissolution profile of SMV and different drug–dendrimer formulations in USP dissolution medium (pH 7.0).

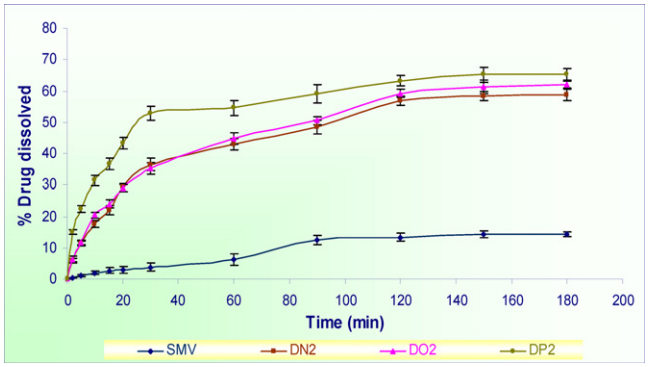

Fig. 5. Dissolution profile of SMV and different drug–dendrimer formulations in simulated gastric fluid (pH 1.2).

This may be the result of interaction between drug and dendrimer molecules which leads to the enhancement of solubility of the drug in dissolution medium. The increase in dissolution of simvastatin was also found in simulated gastric fluid (SGF pH 1.2) but was less in compared to USP dissolution medium pH 7.0.

3.4. In vitro drug release studies

In vitro release of uncomplexed SMV and drug–dendrimer formulations was performed in phosphate buffer saline pH 7.4. Pure SMV was released (89%) in 5 h whereas drug–dendrimer formulations displayed initial rapid release followed by the delayed release of the drug in later half (Fig. 6). Formulations with amine and hydroxyl dendrimers (DN_2 and DO_2) displayed about 50% release in first 3 h and 84.39% and 86.72% in 24 h, respectively, indicating the delayed release of the drug in the later half.

This is possibly due to initial release of the drug encapsulated in dendrimer cavities and drug attached to surface groups (primary amines and hydroxyl groups). The internal tertiary nitrogens are basic and are therefore involved in deprotonating the acidic drug molecules. These quaternized nitrogens bind the counter ions such as carboxylate and hydroxyl ions and control their dissociation.

Another possible reason behind delayed release (Asthana et al., 2005) 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 surface of the dendritic units.

The average release rate for DN_2, DO_2 and DP_2 formulations was found to be 3.52%, 3.61% and 0.69%, respectively. The slow drug release from PEGylated dendrimers, nearly 1/5, that of non-

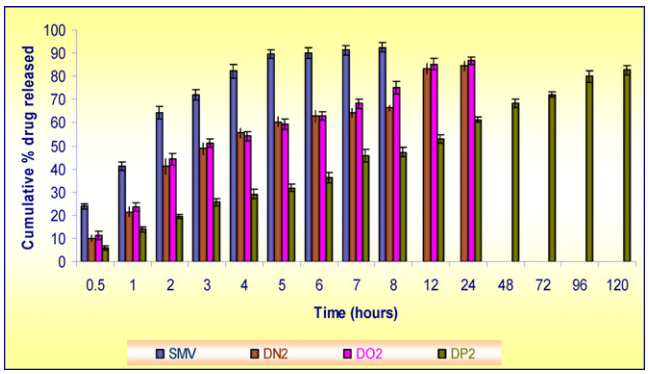

Fig. 6. In vitro drug release profile of SMV and different formulations.

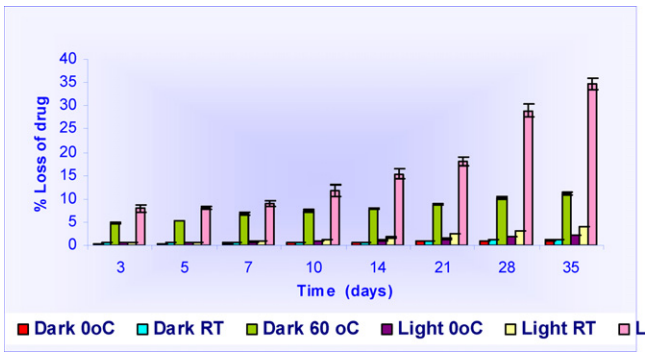

Fig. 7. Stability curves for G4 NH_2 PAMAM dendrimer–drug formulation (DN_2).

PEGylated may be due to dense chains of PEG closing and covering the periphery.

3.5. Stability studies

The stability of G4-PAMAM dendrimers with different functionalities (NH_2 and PEG) were evaluated at various conditions of temperature (0 °C, RT and 60 °C) after keeping in dark (in amber colored vials) and under light in colorless vials. The observed results suggested that the dendrimer-based systems are stable even at elevated temperature up to 60 °C if kept in dark. Formulations with amine and hydroxyl surface groups were found to be most stable in dark, low temperature (0 °C) whereas the dark, RT was most suitable storage conditions for formulation with PEGylated dendrimers (Table 1). The drug release was the minimum with PEGylated dendrimers as compared to NH_2 dendrimers. This may be attributed to greater ring closure at periphery by PEG chains.

At higher temperature, loss of drug was observed greater in the presence of light (Figs. 7 and 8). This can be explained by destabilization of the uniform ring like structure of dendrimers by increase in free energy level of molecules and higher chemical kinetics at elevated temperature. Secondly, the PEG linkage may also break which could make the structures wide open resulting into further release of drug. It was also found that drug leakage was more in formulation stored in light than stored in dark. The drug leakage was found minimum at room temperature as compared to that at 0 °C, which may be due to hypothetical shrinking of the dendritic structure because of reduction in energy levels that lead to decrease in energy for efficient interaction with drug molecules (Bhadra et al., 2005). But this shrinking is found less in amine and hydroxyl dendrimers that is why they are more stable at 0 °C than RT.

After seven weeks, there was decrease in consistency and change in color in the formulations kept at 60 °C in the presence

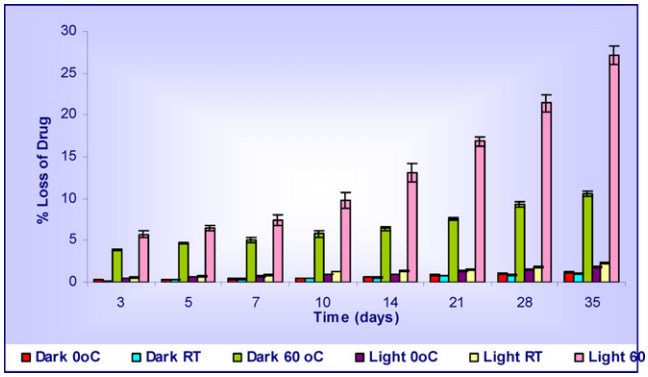

Fig. 8. Stability curves for G4 PEGylated PAMAM dendrimer–drug formulation (DP_2).

Table 1
Stability studies of G4 NH_2 PAMAM dendrimer–drug formulation (DN_2) and G4 PEGylated PAMAM dendrimer–drug formulation (DP_2).

Parameters	Time period	DN_2						DP_2					
		Dark			Light			Dark			Light		
		0 °C	RT	60 °C	0 °C	RT	60 °C	0 °C	RT	60 °C	0 °C	RT	60 °C
Turbidity	After 7 weeks	–	–	+	–	+	++	+	–	++	+	+	+++
Precipitation	Days after which first ppt. appeared	–	–	29	–	26	14	32	–	23	30	23	16
Color change	After 7 weeks	–	–	+	–	+	++	–	–	+	–	+	++
Change in consistency	After 7 weeks	–	–	≪	–	<	⋘	+	–	<	+	≪	⋘

of light. A small change in these parameters was observed at room temperature with DP_2 formulation. The increase in consistency was also found at dark, low temperature with PEGylated dendrimers. Turbidity was observed at both low and high temperatures with DP_2 formulation. At low temperature, it may attributed to desolubilization whereas at high temperature due to polymerization tendency of free groups by degeneration of structure. At higher temperature polymerization was accelerated by light. Another observed physical change was precipitation. No precipitation was observed at dark, room temperature with DP_2 formulation whereas DN_2 formulation showed physical stability from low to room temperatures.

4. Conclusion

PAMAM dendrimers could be exploited to develop the formulation of a weakly acidic and practically water insoluble drug simvastatin. The dendrimers improve the solubility and dissolution of simvastatin, however, the enhancement depends on the concentration of dendrimer, pH of the solution and the surface functional group of the dendrimer. In addition, they also offer the advantage of controlled release of the drug from the drug–dendrimer complexes. Among the various G4-PAMAM dendrimers, PEGylated dendrimers show better in vitro performance. PEGylation of the dendrimer results in to the more solubility enhancement, better dissolution, slower release of the drug, more biocompatibility and more stability of the drug compared to the non-PEGylated dendrimers. However, the in vivo potential of the formulations is under investigation.

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