# Solubility enhancement of an antifungal agent by association with dendrimers

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**ABSTRACT**

**Background**: Amphotericin B is a polyene antifungal agent which is not freely soluble in water. The clinical use of amphotericin B is limited by nephrotoxicity and poor water solubility, which leads in some cases to permanent renal impairment, especially in the presence of other nephrotoxic drugs. PAMAM dendrimer provides a uniform platform for drug attachment that has the ability to bind and release drugs through several mechanisms. **Objectives& Methods:** In the present study we investigated the potential of polyamidoamine (PAMAM) dendrimers to increase the solubility of amphotericin B. **Results:** The experimental results suggested that the solubility of amphotericin B was greatly enhanced in the presence of PAMAM dendrimer solutions. Results showed that the solubility of amphotericin B increased with increase in dendrimer concentration as well as generations. *In vitro* release behavior of amphotericin B in presence of G3 PAMAM dendrimers was performed by dialysis method. **Conclusion:** Our work demonstrated that encapsulation of amphotericin B into dendrimers led to sustained release of the drug *in vitro.*

**Key words:** PAMAM Dendrimer, amphotericin B, drug dendrimer conjugates, solubilization

## INTRODUCTION

Every year approximately $65 billion in drug revenues is accounted by active pharmaceutical ingredients (APIs) with suboptimal bioavailability. About forty percent of newly developed drugs are rejected by the pharmaceutical industry and will never benefit a patient because of poor bioavailability due to low water solubility and/or cell membrane permeability. In addition, about seventeen percent of launched APIs exhibit suboptimal performance for the same reasons. New delivery technologies could help to overcome this challenge. Over the past few years, nanotechnology has emerged as a new and exciting research field that deals with the design, synthesis and fabrication of structures at the molecular scale. As polymer science has evolved over the past two centuries, the number of compositions and architectures of macromolecules synthetically accessible has also grown (C.L. Cameron, 2003). The highly branched and symmetrical molecules known as dendrimers are the most recently recognized members of the polymer family. Dendrimers have often been referred to as the "Polymers of the 21st century". Over the past three decades, dendrimers have evolved from a concept to become a new class of polymers with a unique architecture and versatile chemical structures (D.A.Tomalia, 1985) the high loading capacity of dendrimers renders them highly attractive as carriers for delivery of drugs. It has been proposed that dendrimers may have potential alications in enhancing the solubility of low aqueous solubility drugs and as delivery systems for bioactive materials (U. Gupta, 2006). In particular polyamidoamine (PAMAM) dendrimer is one of the most popular species and is extensively investigated as an enhancer to improve dissolution of drugs, such as ketoprofen (Y.Y. Cheng, 2005), ibuprofen (O.M. Milhem, 2000), aceclofenac (J. Patel, 2011), and riboflavin (A.Filipowicz, 2011).

Amphotericin is a polyene antifungal agent, often used intravenously for systemic fungal infections. The clinical use of amphotericin B (AmB) is limited by nephrotoxicity and poor water solubility, which leads in some cases to permanent renal impairment, especially in the presence of other nephrotoxic drugs**.** Over the past few years, various efforts have been made to improve the solubility and *in vitro* dissolution property of amphotericin B. Easily controllable features of dendrimers such as their size, shape, branching length, their surface functionality allow to modify the dendrimers as per the requirements, makes these compounds ideal carrier in many of the alications and enhancing the solubility of poorly water soluble drugs (M. Liu, 1999). Here we made an attempt to incorporate amphotericin B in different generations of PAMAM dendrimers (G1-G3) to investigate the potential of PAMAM dendrimers to increase the solubility of amphotericin B. The solubilization mechanism and *in vitro* release behavior of amphotericin B- PAMAM complex were investigated. To improve the tolerability profile and enhance efficacy, extensive efforts were made to reformulate AmB in to a suitable drug delivery system. Thus, the search for an optimal formulations is still of great importance.

## MATERIALS AND METHODS

**Materials:** Amphotericn B was purchased from Cipla Ltd. (Mumbai, India). PAMAM dendrimer of different generations were received from Sigma Aldrich (USA). All other chemicals used were of analytical grade and procured from S.D. Fine Chemicals (Mumbai, India)

**Synthesis of PAMAM-AmB conjugates:** Results of dendrimer – mediated solublility studies suggested that the solubility of amphotericin B was increased with increase in dendrimer concentration as well as generation. The optimized G3 dendrimer- based formulations was selected for further studies. The amphotericin B was dissolved in dendrimer solutions and diluted by distilled water to a final concentration of 2 mg/ml. These were vortexed for 24 h at room temperature. The vortexed samples were spun at 14000 rpm for 10 min. These were then filtered through 0.22 µm membrane filter. These conjugates were used for *in vitro* release studies (M. Minglu, 2007).

**UV- Vis Spectroscopy:** Amphotericin B in phosphate buffer (pH 7.4) gives maximum absorbance in UV region at its characteristic wavelength (407nm). A calibration curve of AmB was prepared at different concentrations. Jasco V-630 Spectrophotometer was used to estimate the amount of drug incorporated in the dendrimer. The drug dendrimer solution was diluted by same distilled water. Since dendrimer in the diluted solutions give no absorbance at 407nm, the absorbance obtained from AmB- dendrimer solution would be solely from amphotericin B. This absorbance was correlated with the calibration curve and amount of amphotericin B was determined.

### Solubility Studies

**Determination of effect of dendrimer generartion and concentration on the solubility of amphotericn B:** The solubility of amphotericin B was determined using equilibrium solubility technique (T. Higuchi and A. Connors, 1965). Diluted solutions of PAMAM dendrimers (G1-G3) in concentration from 0.05 to 0.2 % w/v were prepared. The excess amount of drug was then added to each of the test solutions. These were vortexed for 24 h at room temperature. The vortexed samples were spun at 10000 rpm for 10 min. These were then filtered through 0.22 µm membrane filter followed by spectrophotometric measurements of absorbance using UV spectroscopy (D. Bharathi, 2005). Drug loaded dendrimer solutions were analyzed over the range between 200 to 450 nm in a UV visible spectrophotometer to analyze the effect of solubilization as well as drug loading at $\lambda$max. Three repeats were conducted. The dendrimer generation that showed the maximum drug loading/solubilization was selected for further characterization.

**Determination of pH –dependent solubility of amphotericin B:** This study was performed under three different pH conditions (4, 7.4 and 10) to interpret the effect of pH on dendrimer- mediated solubilization of amphotericin B (M. Minglu, 2007). These pH values were chosen to provide typical acidic/basic conditions because we believed that the ionization status of amphotericin B significantly affects its solubilization process. For the determination of the effect of pH on the solubility of amphotericin B, an excess amount of drug was added to glass vials containing concentrations of 0.05 to 0.2 % w/v of G3 PAMAM dendrimers in acetate buffer (pH 4.0). A similar procedure was followed at pH 7.4 and 10 also. The rest of the procedure was similar to the procedure followed to determine the effect of dendrimer concentration on the solubility of amphotericin B.

***In vitro* release studies:** *In vitro* release behavior of amphotericin B in presence of G3 PAMAM dendrimers was performed by dialysis method (N. Man, 2006). The AmB was dissolved in dendrimer solutions and diluted by distilled water to a final concentration of 2 mg/ml. Pure amphoteicin B was dissolved in small quantity of dimethyl sulphoxide, then diluted with distilled water and used as a control. Five milliliters of drug dendrimer solution was filled in dialysis bags (M.W. cut off =1000 Daltons, Himedia), which was pretreated with phosphate buffer of pH 7.4. The bags were suspended in 100 ml of dissolution medium (phosphate buffer of pH 7.4) under constant stirring using a magnetic stirrer maintained at 37$\pm$0.5ºC. After a scheduled interval of time for 12 h, 1ml of the sample was withdrawn from the outer phase, and the outer phase was again replenished with 1 ml of the same medium to maintain perfect sink conditions. The amount of drug released was determined spectrophotometrically at 407 nm.

## RESULTS AND DISCUSSIONS

**Solubility studies:** The effect of PAMAM dendrimer concentration and generation on solubility of amphotericin B (AmB) was measured at room temperature and the results are shown in Fig.1. It was observed that the extremely low water solubility of AmB has been significantly improved by PAMAM dendrimers compared with that in distilled water. The solubility of AmB in the dendrimer solutions increased in an aroximately linear manner with increase of dendrimer concentration. The increase of solubility of extremely low water solubility of AmB was contributed to the internal cavities that are available to encapsulate AmB molecules

and these properties make dendrimers suitable for drug delivery systems (D. Bharathi, 2005). From the Fig.1, it was clear that the solubility of AmB was affected by different generations of PAMAM dendrimers. The solubility of AmB in higher generation PAMAM solution was in fact higher than those in lower ones (Y. Hu, 2004). The solubility of amphotericin B in PAMAM solutions depend on the surface area and amino groups of PAMAM particles, thus a molecule of higher generation of PAMAM particle has a higher ability to absorb and interact with the amphotericin B molecule than that of lower one. Results suggested that solubility of amphotericin B increased with increase in dendrimer concentration as well as generation.

Dendrimers are considered as static unimolecular micelles and their micellar structure remains stable at even higher concentrations of solvents. Dendrimers enhance the solubility of drugs probably due to hydrophobic interactions, hydrogen bonding and electrostatic interaction between terminal functional groups of the dendrimers and drug molecules (C.J. Hawker,1993; G.R. Newkome, 1991; S. Stevelmens, 1996).

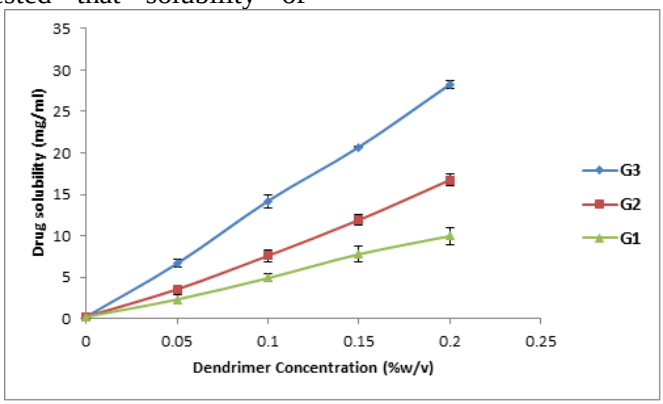


**Fig.1.Solubility of amphotericin B in the presence of increasing concentrations of PAMAM dendrimers**

To ascertain the effective pH condition on solubility of amphotericin B using PAMAM G3 denendrimer. The results are shown in Fig.2 and, as can be seen, the process was pH dependent. The solubility of AmB increased in the order of pH 7.4 > 10.0 > 4.0 in PAMAM G3 based dendrimer formulations. At low pH there is lower significant increase of solubility of amphotericin B in dendrimer solution compared to that at high pH. The solubility enhancement is due to the interaction between surface amine groups of dendrimer molecule and functional groups present in the drug molecule. However the mechanism involved could be different at different pH conditions. At pH 10.0 the hydrophobic encapsulation of drug and hydrogen bonding between OH and $NH_2$ groups of the drug and dendrimer, respectively were thought to be responsible for solubility enhancement. At pH 7.4 solubility enhancements was attributed to electrostatic interaction between COOH groups of drug and primary amines of dendrimer. At pH 4.0 however, a relatively lesser solubility enhancement was observed and it is due to the ionization status of the interaction species, which reduces the interaction between them as compared to two other pH conditions. The differences in solubility in different buffer solutions can be correlated with a combination effect of the ionization state of the drug and the PAMAM dendrimers.

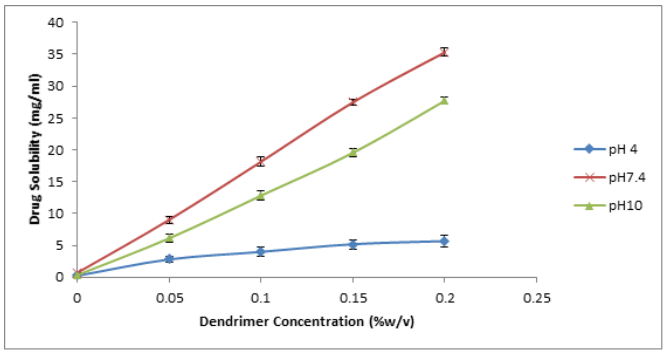


**Fig.2.Solubility profile of amphotericin B at different pH with increasing concentration of PAMAM G3 Dendrimers**

***In vitro* release studies:** The strength and stability of the drug – dendrimer complex were investigated using *in vitro* release studies. *In vitro* release of Amphotericin B - PAMAM G3 formulations was performed in phosphate buffer of pH 7.4. Pure amphotericin B was released (63.36 %) in 5 h whereas drug–dendrimer formulations displayed the delayed release of the drug (Fig. 3). After 10h, 75.76 % release was obtained for the pure drug while 42.72 % was obtained for AmB-G3 PAMAM conjugates. The release of AmB from the drug dendrimer conjugates was significantly slower compared to pure amphotericin B. These results strongly suggested that electrostatic interaction might play an important role in release of drugs from dendritic mixtures.

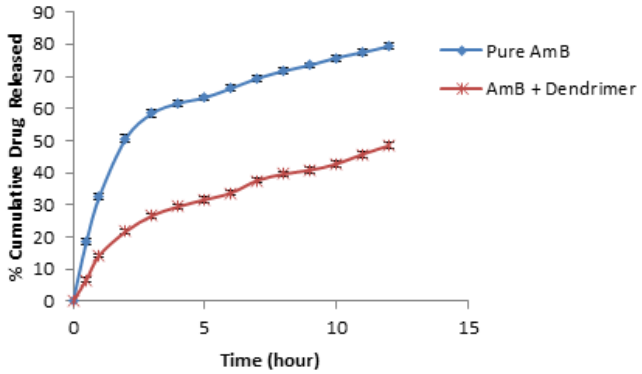


**Fig.3.*In vitro* release of AmB in G3 PAMAM dendrimer solution compared with the pure AmB(amphotericin B) release behavior**

## CONCLUSION

The amine terminated PAMAM dendrimers have the potential to increase significantly the aqueous solubility of poorly water soluble drugs, such as amphotericin B. The solubility of amphotericin B depends on the generation of the dendrimer, concentration of the dendrimer and pH value of the solution. They also offer the advantage of controlled release of the drug from the drug-dendrimer complexes. Among the different generations of PAMAM dendrimers, G3-PAMAM dendrimers show better *in vitro* performance. Our work demonstrated that the conjugation of amphotericin B with PAMAM dendrimers led to sustained release of drug *in vitro*. Although dendrimer drug-delivery is in its infancy, it offers several attractive features in drug delivery alications. Although toxicity problem may exist, modification of the structure of dendrimers should resolve these issues.

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