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Performance Evaluation of Pamam Dendrimer Based Clotrimazole Formulations

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ABSTRACT

Clotrimazole (CLTZ) is a local imidazolic antifungal agent. A major problem associated with the successful formulation of effective dosage forms containing CLTZ is its poor aqueous solubility, which presents a hindrance for the local availability of CLTZ and limits the effective antifungal therapy. In the present study, the effects of various concentrations of poly(amidoamine) (PAMAM) dendrimers generation 3.5 (G3.5) and generation 4 (G4) with carboxylate (DCC), amine (DCN) and hydroxyl surface groups (DCO) on aqueous solubility, in vitro drug release studies, and for stability studies of CLTZ drug. The obtained results showed that all tested PAMAM dendrimers improved the solubility of CLTZ and the more potent were (DCC) dendrimers. The increase in solubility of CLTZ was highest at dendrimer concentration of 10 mg/ml. These observations indicate that PAMAM dendrimers enhance the solubility of CLTZ, The drug dendrimers complexes displayed the controlled release action during in vitro release studies. Formulation with amine and carboxylate were subjected to accelerated stability studies.

Key words: poly(amidoamine) dendrimers; clotrimazole; aqueous solubility; surface groups

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INTRODUCTION

Dendrimers are hyper branched and monodisperse three dimensional molecules, and have defined molecular weights and host- guest entrapment properties.[1]They possess central care with void spaces, radially extending repeat units, and a terminal functional group abundant surface. High surface density and empty inner spaces provide option for entrapment of bioactives and suggest suitability of dendrimers for gene and drug delivery applications.[2]

Lipid based drug delivery systems (ie., liposomes, solid lipid nanoparticles, nanostructured nanocarriers) have poor physical stability, drug leakage, difficulty in drug targeting[3] and low drug loading capacity due to the formation of a perfect lipid crystal matrix[4] whereas polymer based system use linear polymers which are polydisperse in nature, in addition to regulatory issues and scaling up problems. 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.[5]

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 Clotrimazole as model drug. Clotrimazole, an imidazole derivative with a broad spectrum of antimycotic activity, inhibits biosynthesis of the sterol ergostol, an important component of fungal cell membranes. Its action leads to increased membrane permeability and apparent disruption of enzyme systems bound to the membrane. Clotrimazole is practically insoluble in water and hence poor oral bioavailability.

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, In- vitro drug release studies, and for stability studies.

MATERIALS AND METHODS

Materials

G3.5-PAMAM-Co_2Na, G4-PAMAM–NH2 and G4-PAMAM–OH were purchased from Nanosynthons, USA. Clotrimazole was obtained as gift sample from Glenmark (Baddi, India) and membrane filter of pore size 0.2µm were purchased from Himedia Lab. (Mumbai, India).Rest all chemicals were of analytical grade and were purchased from CDH (India).

Methods

Phase solubility studies

Solubility studies of CLTZ with G3.5-DCC, G4-DCN and G4-DCO were carried out by the method described by Higuchi and Connors with minor modifications.[6] The studies were performed in amber colored bottles to avoid any degradation of dendrimers.[7] The aqueous solubility of CLTZ was determined in the presence of increasing concentration of dendrimers to evaluate the effect of dendrimers concentration. Three different pH values (pH 4.0, pH 7.4 and pH 10.0) were selected to determine the pH-dependent solubilization of CLTZ. The similar study was also performed with all three types of dendrimers to evaluate the effect of dendrimer surface groups on solubility of CLTZ.

Determination of effects of dendrimer concentration on CLTZ solubility

An excess (10 mg) of CLTZ was added into screw capped amber colored vials containing varying concentration (0.05–0.4%, w/v) of G3.5-DCC 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 100rpm in a shaking bath. The vials were allowed to stand for 24 h to attain equilibrium. After equilibration these suspensions were filtered through 0.2µm 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 analyzed for drug content by UV-spectrophotometer at 261nm and are expressed as the molar ratio (drug/dendrimer) and also as drug concentration vs dendrimer concentration. (Figure 1)

Determination of pH-dependent solubility of CLTZ

For the determination of the effect of pH on the solubility of clotrimazole, an excess amount of drug (10 mg) was added in aqueous dendrimer solution (concentration 0.1%, w/v); with a pre adjusted pH to pH4.0, pH7.4 and pH10.0 using 0.1MHCl and 1MNaOH 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 CLTZ.(Figure-2)

Determination of effect of surface groups on CLTZ solubility

For the determination of effect of dendrimer surface groups on the solubility of clotrimazole, 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 10.0, of G3.5-DCC, G4-DCN and G4-DCO dendrimers separately. The rest procedure was similar to the procedure followed to determine the effect of dendrimer concentration and pH on solubility of CLTZ.

In vitro drug release studies

Ex-vivo skin permeation studies were performed on a Franz diffusion cells with an effective diffusion area of 2.54 cm^2 and 45 ml of receiver chamber capacity, using cellophane membrane. The membrane was washed with phosphate buffer before further use. Initially, the donor compartment was empty and the receiver chamber was filled with phosphate buffer. The receiver fluid was stirred with a magnetic rotor at a speed of 600 rpm and the assembled apparatus was placed in a hot air oven where the temperature was maintained at 37 ± 1°C. The whole phosphate buffer saline (PBS) pH 7.4 was replaced with fresh one after every 30 min to stabilize the skin. It was found that the receiver fluid showed a negligible peak area after 2.5 hr and beyond indicating complete stabilization of the membrane. After complete stabilization of the membrane, 2 ml dendrimeric formulation was placed into the donor compartment and sealed with paraffin film to provide occlusive conditions. Samples were withdrawn at regular intervals (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hr), filtered using 0.45 µm membrane filter and analyzed for drug content by UV at 261 nm **.**

Stability studies

The stability testing of a pharmaceutical product is based on the principle of chemical kinetics. In this technique, the degradation rates constant at various temperatures are obtained. Stability of dendritic formulations was carried out at accelerated conditions of temperature and light. The samples were kept in amber colored vials (dark) and in colorless vials (light) at 0°C, room temperature 25°C and 50°C in controlled oven for a period of 5 weeks. The sample were analyzed initially and periodically (by visually) after every week for up to five weeks for any Precipitation, Turbidity, Crystallization, Color change, Consistency Drug leakage and percent drug loss.(Figure-5,6,&7)

RESULTS AND DISCUSSION

Solubility studies

The Solubility enhancement property of dendrimers is explainable by an electrostatic bonding between functional groups of the dendrimers and hydrophobes.[8,9] PAMAM dendrimers with uniform and well defined particle size and shape are of eminent interest in biomedical applications because of their ability to cross cell membranes. Non-polar cavities in PAMAM dendrimers in combination with their hydrophilic exterior surface make them capable of encapsulating hydrophobic drug molecules.[10,11,12]

The solubilization data suggests that higher concentration of corresponding dendrimers was found to be solubilizing more CLTZ compared to lower concentrations (Figure 1).

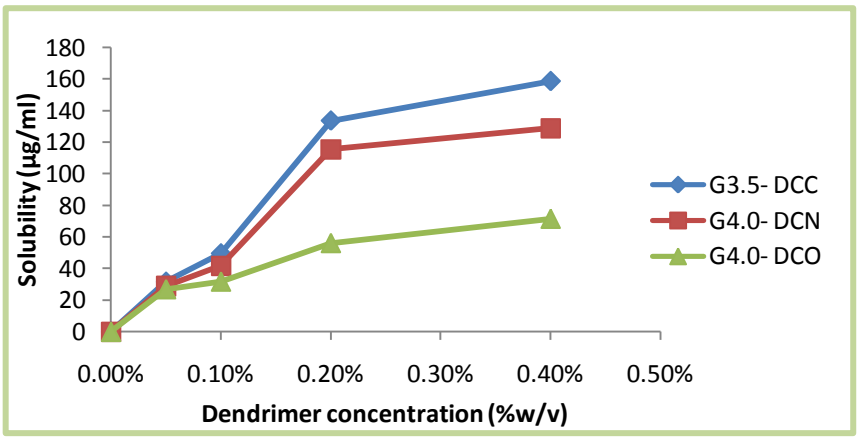

Figure-1: Effect of dendrimers concentration and surface groups (CO_2Na, NH_2, OH)clotrimazole solubility (µg/ml)

Solubility of CLTZ increased linearly as a function of PAMAM concentration. In case of G3.5-DCC dendrimer, the solubility of CLTZ was found to be enhanced approximately 7.6 folds. (from 13.01- 99.20µg/ml) Among the PAMAM dendrimers used in the G3.5-DCC exhibited the highest solubilizing potential for CLTZ followed by G4-DCN, G4-DCO.

The effect of pH on the solubility of CLTZ in the presence of amine dendrimers was also studied (Figure.2). The enhancement in solubility was highest at pH 10, less at pH 7.4 and least at pH 4. The solubility was found to have enhanced nearly 9 times at pH 10, 7.1 times at pH 7.4 and 3.6 times at pH 4. As PAMAM drimers possess empty internal cavities, small organic molecule drugs like CLTZ may be encapsulated into the dendrimer's interior void space, while larger molecules preferably adsorb onto the dendrimer surface. Clotrimazole is a weak base and is reported to be practically insoluble in water[13] is rather encapsulated than attached to dendrimers's amine or hydroxyl end groups. Overall it was found that the increase in CLTZ 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.

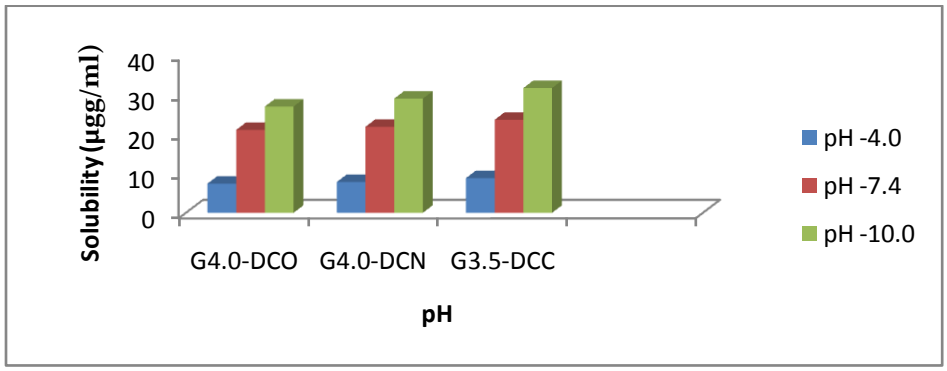

Figure-2: Comparative effect of pH on CLTZ solubility in 0.1% (w/v) G4.0-DCO, G4.0-DCN and G3.5-DCC PAMAM dendrimers solution.

In vitro drug release studies

In-vitro release of clotrimazole with dendrimer formulation was performed in phosphate buffer saline pH 7.4. The effects of different functional group of the formulation on the skin permeation of clotrimazole were evaluated. On the basis of permeation study it was found that dendrimeric formulation G-3.5-DCC_2 exhibited highest permeation profile. The cumulative amount of clotrimazole permeated from G-3.5-DCC_2 was 5.66µg/cm^2 at the end of 24 hrs and the skin permeated rate (flux) of clotrimazole was 0.236µg/cm^2/hr, while all other formulation exhibited lesser skin permeation (Figure-3).

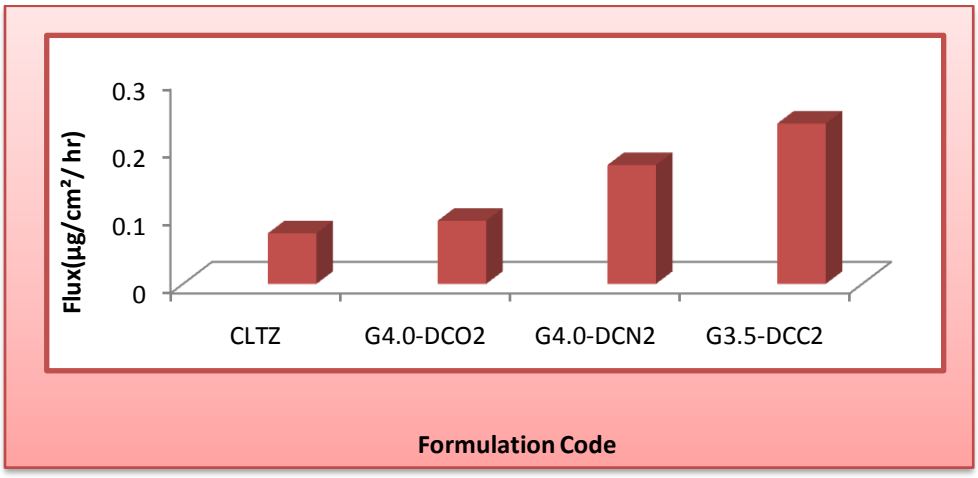

Figure-3: Skin permeation of CLTZ, G4.0-DCO2, G4.0-DCN2 and G 3.5-DCC2.

The concentrations also play an important role and affect the skin permeation rate directly. Dendrimer increase skin permeation by increasing skin partitioning of clotrimazole. Transepithelial water loss, skin resistance studies revealed that dendrimers act by interacting with the skin lipid bilayer. From the above study it can also be concluded that formulation containing more cationic surface i.e. DCC dendrimer has higher transdermal flux.NH_2 dendrimer that contain more cationic in nature show more skin permeation. That may be due to an increase thermodynamic activity of the drug in dendrimeric formulation at more anionic and surface functionality.

The cumulative amount of drug released from formulation G-3.5-DCC was high when compared with release from other formulation (G-4.0-DCN and G-4.0-DCO).(Figure-4) When the cumulative amount of drug permeated from gel through cellophane membrane was plotted against time, the permeation profile of the drug followed first order and then Higuchi release kinetics (r^2=0.946 to 0.964), which indicate the permeation of drug from gel was governed by diffusion mechanism.

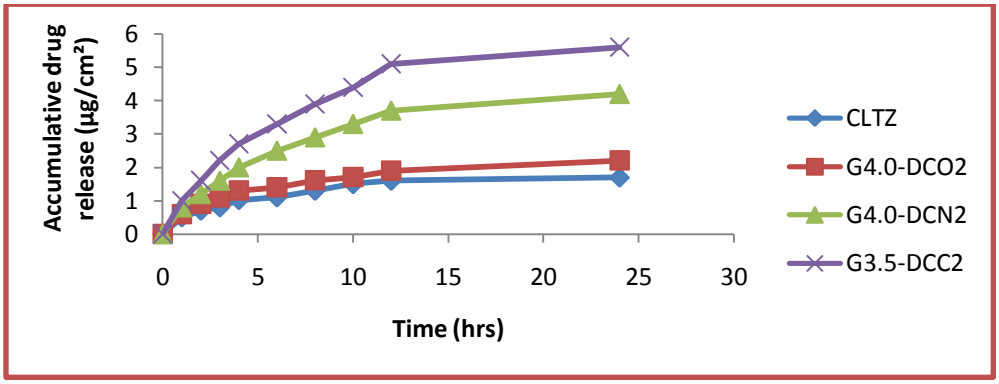

Figure-4: Ex-vivo skin permeation of different dendrimeric formulation

Stability studies

The stability study of all drug dendrimers complex of 0.1% (DCN_2) was performed at different accelerated conditions of temperature (0^0 C, RT and 60^0C).

The stability of G4.0-NH_2 PAMAM dendrimer-clotrimazole complex (G4.0-DCN_2), G4.0-OH PAMAM dendrimers-clotrimazole complex (G4.0-DCO_2) and G3.5-COONa dendrimer-clotrimazole complex (G3.5-DCC_2) was performed at different accelerated conditions of temperature (0 °C, RT and 50 °C). After evaluation of formulations it was observed that the dendrimer based system are stable even at elevated temperature up to 50 °C in if kept in dark (amber color vials). Formulations with hydroxyl (G4.0-DCO_2) and amine surface group (G4.0-DCN_2), were found to be stable in dark, low temperature (0 °C) whereas the dark, RT was suitable storage conditions for formulation with G3.5-COONa PAMAM dendrimer-clotrimazole complex (G3.5-DCC_2). There was change in color, decrease in consistency and precipitation noted after five weeks when kept at 50 °C in presence of light in formulations (G4.0-DCN_2, G4.0-DCO_2, and G3.5-DCC_2). A small change in these parameters was observed at room temperature with, G3.5-DCC_2 formulation. The increase in consistency was also found at dark, low temperature with G3.5-DCC_2 dendrimer. Low turbidity was observed at both low and high temperature with formulation, G3.5-DCC_2 in comparison to G4.0-DCN_2 and G4.0-DCO_2.

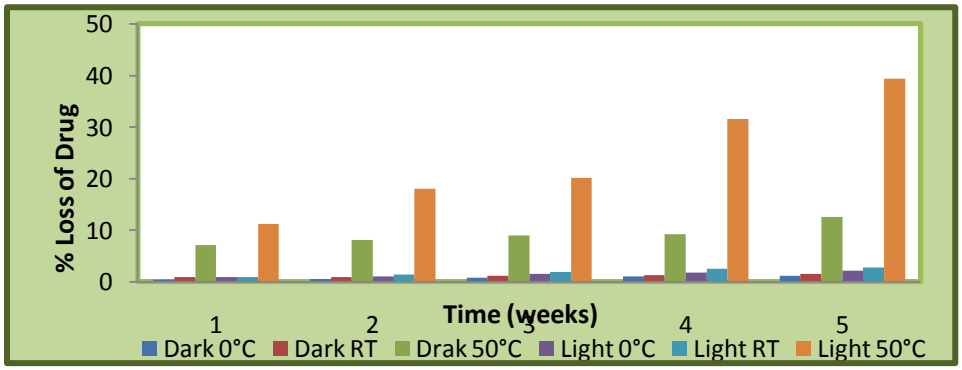

Figure 5: Stability curve for G4 OH PAMAM dendrimer- drug Formulation (DCO_2)

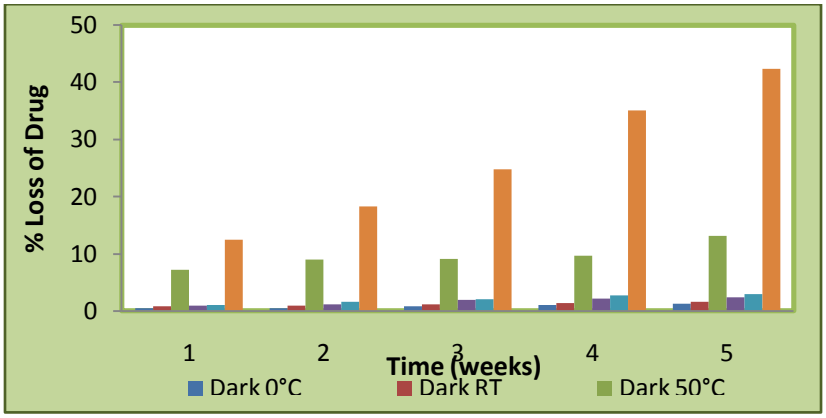

Figure-6: Stability curve for G4 NH_2 PAMAM dendrimer-drug formulation (DCN_2)

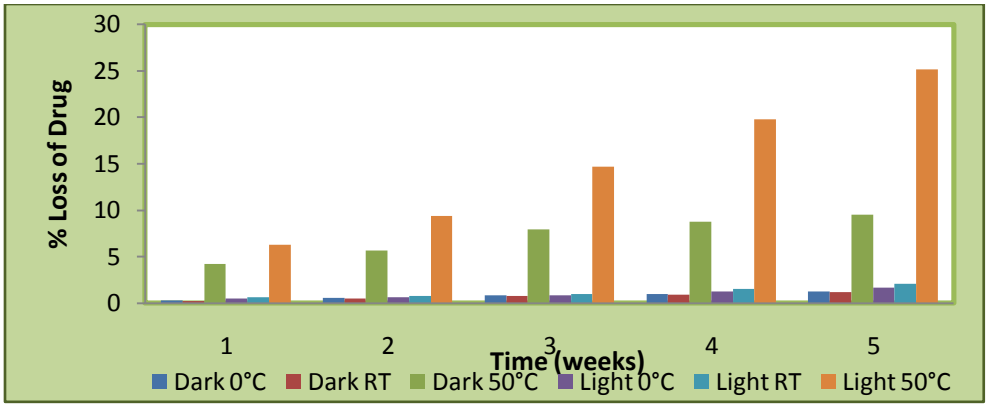

Figure-7: Stability curve for G4 CO_2Na PAMAM dendrimer-drug formulation(DCC_2).

At higher temperature loss of drug observed greater in the presence of light. The dendritic structure is supposed to be more open at higher temperature and this change in surface characteristic 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 (50°C). No change in turbidity, color and consistency were noticed at low temperature G4.0-DCN_2 and G4.0-DCO_2 and room temperature DAP_2 G3.5-DCC_2 therefore, it is concluded that the dendritic formulations can be stored at cool and dark place.

CONCLUSION

PAMAM dendrimers could be used to develop the formulation of a weakly basic and practically water insoluble drug clotrimazole. The dendrimers improve the solubility of clotrimazole, however, the enhancement depends on the concentration of dendrimer, pH of the solution and the surface functional group of the dendrimer. Among the various G3.5 and G4-PAMAM dendrimers, G3.5 PAMAM dendrimers show better in vitro performance.

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