Original Research Article

Polyethylene glycolated PAMAM dendrimers-Efavirenz conjugates

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Abstract

Aim: The preparation of novel PEGylated PAMAM (poly-amidoamine) dendrimers for delivery of anti-HIV drug Efavirenz is reported. **Method and Materials:** About 5.0 G PAMAM dendrimers are prepared by ethylene diamine core via Michael addition by divergent method. PEGylation is done by polyethylene glycol 600 using epichlorhydrin as linker. PEGylated 5.0 G PAMAM dendrimers loaded with Efavirenz (EFV) are evaluated for FTIR, DSC, SEM, drug release, and stability studies. **Results and Conclusion:** From the results it is proved that this method is less time consuming, inexpensive, and reproducible. Drug-release studies indicate, PEGylated 5.0 G PAMAM-EFV dendrimers have shown prolonged drug-release property.

Key words: Dendrimers, efavirenz, epichlorhydrin, Michael addition, PEGylation

INTRODUCTION

Dendrimers are defined as highly branched macromolecular structures. Dendrimers have unique properties like molecular uniformity, narrow molecular weight distribution, specific size, and shape and highly functional terminal surfaces.[1] Tomalia and coworkers reported, PAMAM dendrimers are formed by polyamide branches with tertiary amines.[2] Later in 2001, Milhem and coworkers found that the solubility of ibuprofen significantly enhanced in aqueous solution of PAMAM dendrimers.[3]

PEGylation of dendrimers is done mainly to decrease the cytotoxicity. Jevprasesphant and coworkers reported that reduction or shielding of the positive charge on the dendrimer surface by the attached chain was one of the reasons for the decrease in cytotoxicity.[4] Zhu and coworkers later found that increase in PEGylation degree decrease the cytotoxicity.[5]

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Due to the properties like biocompatibility and hydrophilicity polyethylene glycol is widely used as conjugating agent.[6-12] In the present work, a new approach has done for PEGylation of dendrimers using epichlorhydrin as linker.[13]

Treatment for HIV infection is very complex, prolonged and expensive. Generally treatment includes multiple combinations of antiretroviral drugs i.e., highly active antiretroviral therapy (HAART). Disadvantage of all these drugs are their resistance and adverse affects hence they are recommended to administrated in small dose. Targeted drug release is one of the best methods to delivery such drugs.

Efavirenz belongs to the class of antiretroviral drugs under the sub class of non-nucleoside reverse transcriptase inhibitor. EFV is freely soluble in methanol, ethanol and isopropanol, practically soluble in water. Marzolini and Nunez in 2001 performed studies of EFV on HIV-infected patients and have shown adverseaffects and high EFV plasma concentration, respectively.[15,16] Hence administration of EFV in small dose may results in the decrease of side effects and minimizes drug action on non-infected cell.

The purpose of the study is to synthesis, PEGylate 5.0 G PAMAM dendrimers loaded with EFV and also to study characterization of the same.

MATERIALS AND METHODS

Materials

Efavirenz, Ethylene diamine, Polyethylene glycol, Epichlorhydrin, Methanol, Sodium hydroxide, Potassium dihydrogen phosphate

Method

Conjugation of 5.0 G PAMAM dendrimers

Synthesis of PAMAM dendrimers was performed by divergent method.[13] Construction of an ethylene diamine EDA core PAMAM dendrimers consist of consecutive steps: Michael addition of primary amine (EDA in very first step) to methyl acrylate followed by amidation of formed multiester (tetra ester at very beginning) of EDA.

The conjugation of polyethylene glycol (PEGylation) was done using epichlorohydrin as a cross-linking agent. Epichlorhydrin used was 1.8 mM (1.8 g) and PEG used was also 1.8 mM (10.8 g), that is, in 1:1 ratio. One hundred milligram (6.3 lM) of lyophilized 5.0 G PAMAM dendrimer was dissolved in methanol. Sixteen molar times of polyethylene glycol (PEG-600) was mixed with epichlorohydrin in separate container and stirred vigorously for 2 h and incubated for 36 h at room temperature (rt) in dark, now in this mixture the 5.0 G dendrimer solution was added and shaken properly and kept a side for 24 h, which facilitate the linking of PEG with 5.0 G dendrimer using epichlorohydrin as a linker. The final product was dialyzed to remove byproducts.

Identification of dendrimers was done by subjecting the plain and PEGylated dendrimers to reaction of copper sulfate aqueous solution (1% w/v) in (0.1% w/v) methanol. The formed PEGylated system were subjected to IR spectroscopy analysis; various peaks were interpreted for different groups. The sample was analyzed by NMR spectroscopy. The PEGylated dendrimers were solubilized in D_2O using methanol as co-solvent and analyzed at 300 MHz various shifts in the peaks were observed, which were interpreted for different groups present in PEGylated system.

Drug Loading in Formulation

The known molar concentrations of PEGylated 5.0 G EDA-PAMAM dendrimers were dissolved in methanol and mixed with methanolic solution of EFV (100 mol).[14] The mixed solutions were incubated with slow magnetic stirring (50 rpm) using teflon beads for 24 h. These solutions were twice dialyzed in cellulose dialysis bag against double distilled water under sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically (λmax 247 nm) to determine indirectly the amount of drug loaded within the system. The dialyzed formulation were lyophilized and used for further characterization.

Drug-release studies[14]

Drug release from known amounts of EFV-loaded PEGylated 5.0 G EDA-PAMAM-dendrimers were determined using a modified dissolution method. The dialysis bags were filled with a known mass of EFV-loaded PEGylated dendritic architectures (MWCO 1000 Da) and the dialysis bags were placed in 200 ml of PBS (pH 7.4) at 37ºC with slow magnetic stirring under sink conditions. Aliquots of 1 ml were withdrawn from the external solution and replaced with the same volume of fresh PBS. The drug concentration was detected in a spectrophotometer at 247 nm λmax.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed to study the thermal stability and changes in crystallinity over a range of temperatures. The carriers and manufactured drug particles were studied by this method. A known mass of powder was placed in an aluminum pan and a lid was crimped onto the pan. The pan was then placed in the sample cell of a DSC module. The temperature of the DSC module was equilibrated at 35°C and then increased at a rate of 10°C/min under a N_2 gas purge until the material began to degrade. The temperatures were obtained for each peak in the resulting curve and provided indications of temperature stability and phase transitions.

Scanning electron microscopy

Scanning electron microscopy (SEM) was performed to study the particle size and morphology of PEGylated 5.0 G EDA-PAMAM dendrimers.

Stability

After storage for 3 months at 40°C the drug content and release rate of EFV-loaded PEGylated 5.0 G EDA-PAMAM dendrimers were determined.

RESULTS

Conjugation of 5.0 G PAMAM dendrimers

Synthesis and conjugation of 5.0 G PAMAM dendrimers with PEG 60 were done by the procedure reported by Suman Ramteke.[13] PEGylation of 5.0 G PAMAM dendrimers was confirmed by Figure 1 IR peaks for N-H stretch 3334.63 cm^{-1}, confirming conversation of nitrile terminal group to amine terminal; C=O stretch of carbonyl group 1727.20 $cm^{-1}$and C-H stretch 2875.03 cm^{-1}. Further confirmed by Figure 2 ^{1}H NMR peaks for (-CH_2-CH_2-) at 3.56; amide group N-H at 2.71.

Drug loading and entrapment efficiency

EFV entrapment was found in the hydrophobic core of the molecule due to the hydrophobic interaction among EFV and PEGylated 5.0 G PAMAM dendrimers. The entrapment efficiency of the system was calculated as a ration of amount

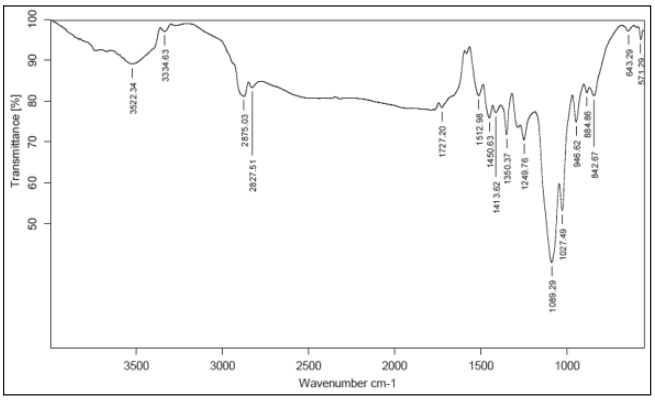

Figure 1: IR spectrum of PEGylated 5.0 G PEG PAMAM dendrimers loaded with EFV

of drug entrapped by the system to the amount of drug taken, expressed in percentage. EFV entrapment efficiency in 5.0 G PAMAM dendrimers was found to be 65.71%.

Drug-release studies

Comparative evaluation of two formulations PEGylated 5.0 G PAMAM dendrimers and 5.0 G PAMAM dendrimers loaded with EFV was performed for 120 hours is presented in Figure 3 Drug-release rate of EFV in PEGylated 5.0 G PAMAM dendrimers is 83.28% in 120 hours while non-PEGylated 5.0 G PAMAM dendrimers released 93.7% IN 48 hrs. Between two formulations, it is clearly indicates that PEGylated dendrimers shows relatively slow release compared with non-PEGylated dendrimers.

Differential scanning calorimetry

DSC Curves of 5.0 G PEGylated PAMAM dendrimers Figure 4, clearly evident that it is not a physical mixture by exothermic and endothermic peaks. Pure EFV shows it characteristics peaks at 137°C [Figure 5]. Absence of peak in [Figure 4] 5.0 G PEGylated PAMAM dendrimers indicates drug encapsulation.

Scanning electron microscopy

SEM images Figure 6 clearly show EFV-loaded PEGylated 5.0 G PAMAM dendrimers are in nano scale and mostly individual. Surface morphology of particles found to be having smooth surface and spherical in shape.

Stability

After storing the EFV-loaded PEGylated 5.0 G PAMAM dendrimers at 40±2°C for 3 months it has been found no change either in appearance or in its drug release [Figure 7].

DISCUSSION

5.0 G EDA PAMAM dendrimers were synthesized and PEGylated using PEG 600 using epichlorhydrin as cross-linking agent. From the results it is proved that this method is less time consuming, inexpensive, and reproducible. PEGylated 5.0 G EDA PAMAM dendrimers loaded with EFV, showing prolonged and targeted drug release hence it shows better therapeutic efficacy at a lower dose. A further careful *in vivo* study of these dendrimers is necessary.

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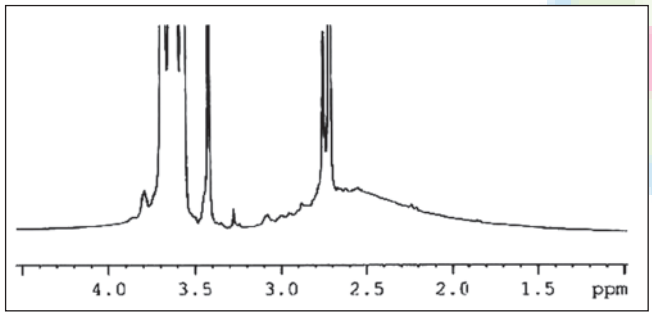

Figure 2: 1H NMR spectrum of PE Gylated 5.0G PAMAM dendrimers loaded with EFV

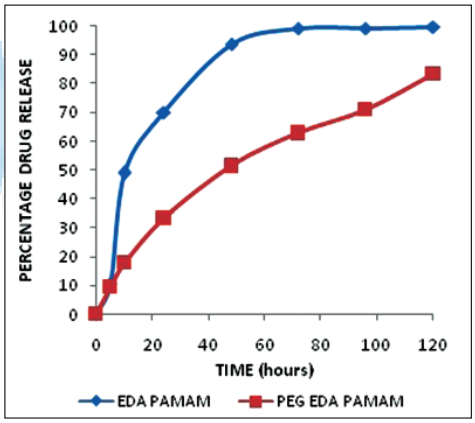

Figure 3: Drug release in phosphate buffer 7.4

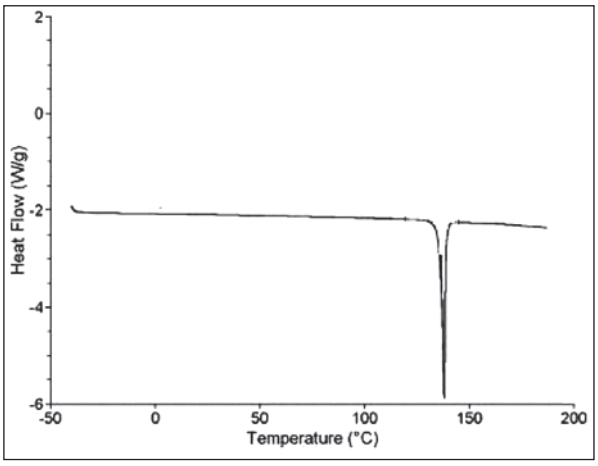

Figure 4: DSC peaks of PEGylated 5.0 G PAMAM dendrimers loaded with EFV

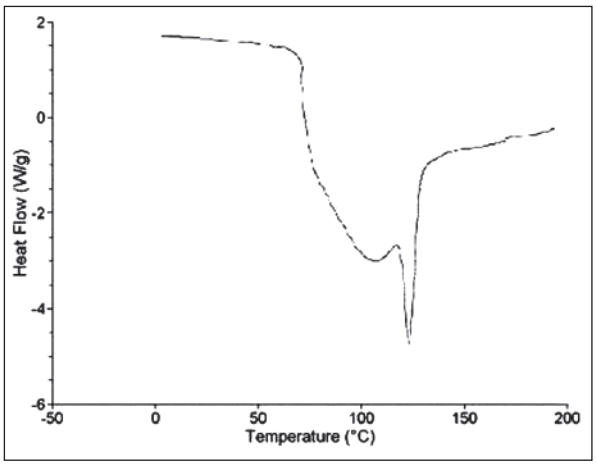

Figure 5: DSC peak of EFV

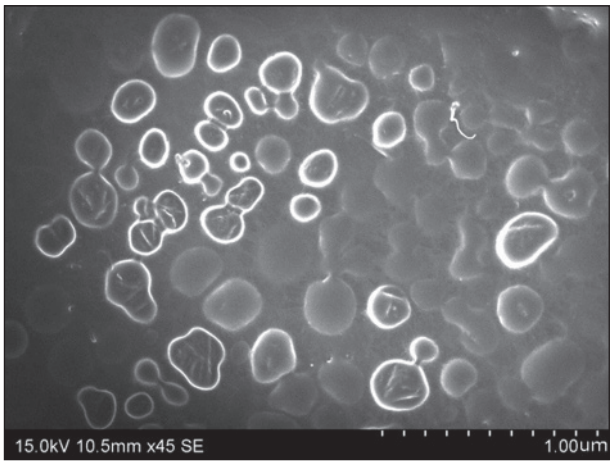

Figure 6: SEM image of PEGylated 5.0 G PAMAM dendrimers loaded with EFV

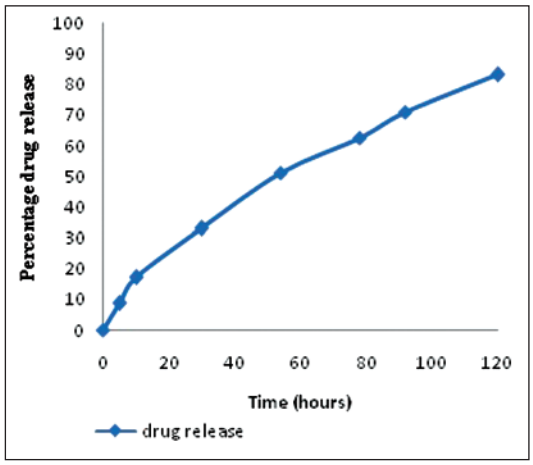

Figure 7: Drug release of PEGylated 5.0 G PAMAM dendrimers loaded with EFV after storage for 3 months

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