

## Research Article



## PEGYLATED DENDRITIC ARCHITECTURE FOR THE DEVELOPMENT OF A SUSTAINED DRUG DELIVERY SYSTEM FOR AN ANTIVIRAL

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Accepted on: 13-01-2012; Finalized on: 20-03-2012.

### ABSTRACT

The purpose of the present study was to develop, optimize and characterize PEGylated citric acid dendritic architecture for the delivery of an anti-viral drug, Nevirapine. Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Nevirapine is structurally a member of the dipyridodiazepinone chemical class of compounds. Dendrimers are highly branched, monodisperse, polymer macromolecules. It is composed of a multifunctional core molecule attached to polymer branches. Drugs can be inserted between the branches and held until 'interactions' release it. Various physicochemical parameters like solubility, drug entrapment and *in vitro* release studies of PEGylated systems were determined and compared. Further, analytical parameter like DSC was confirming the structure drug entrapment. The PEGylation of the systems was found to have increased their drug-loading capacity, reduced their drug release rate. The systems were found suitable for sustained delivery of Nevirapine.

**Keywords:** PEGylated citric acid dendrimer, Nevirapine Solubilization, *In vitro* release.

### INTRODUCTION

Dendrimers are a new class of three-dimensional, man-made molecules produced by an unusual synthetic route which incorporates repetitive branching sequences to create a unique novel architecture. Exceptional features of the dendritic architecture include a high degree of structural symmetry, a density gradient displaying an intra-molecular minimum value and a well defined number of terminal groups which may be chemically different from the interior. The combination of these features creates an environment within the dendrimer molecule facilitates an avenue to developing reliable and economical fabrication and manufacturing of functional nanoscale materials that would have unique properties (electronic, optical, opto-electronic, magnetic, chemical, or biological) that could be the basis of new nanoscale technology and devices<sup>1</sup>.

Poly ethylene glycol (PEG) is typically a clear, colorless, odorless substance that is soluble in water, stable to heat, inert to many chemical agents, that does not hydrolyze or deteriorate, and is generally non-toxic, PEG is considered to be biocompatible, which is to say that PEG is capable of coexistence with living tissue or organisms without causing harm, as reviewed earlier<sup>2,3</sup>. Citric acid is widely used in variety of Pharmaceutical formulations and food products, primary to adjust the pH of solution. It has also been used experimentally to adjust the pH of tablet matrices in enteric coated formulations for colon specific drug delivery<sup>4</sup>.

### MATERIALS AND METHODS

#### Materials

Nevirapine was a gift sample from hetero drugs, India. Pegylated citric acid dendrimers was synthesized. Polyethylene glycol (PEG) 400 was purchased from S.D. Fine Chemicals Ltd, Mumbai, India. All other solvents and reagents used were of analytical grade.

#### Drug loading in formulations

The Pegylated citric acid dendrimers so synthesized were dissolved in Dimethyl Formamide (DMF) and mixed with 100 molar times of Nevirapine and allowed the Nevirapine to dissolve. The mixed solution was allowed to incubate with slow magnetic stirring (50 rpm) using teflon beads for 45 hr. This solution was twice dialyzed by using Cellulose dialysis bag (MWCO 12-14 KDa) under strict sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically ( $\lambda_{\text{max}}$  290 nm) to determine indirectly the amount of drug encapsulated with the system. The dialyzed formulations were dried and used for further characterization.

#### Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed to study the thermal stability and changes in crystallinity over a range of temperatures. The Pegylated citric acid dendrimers and manufactured drug particles and Nevirapine loaded citric acid dendrimers 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



(DSC TA 6000, TA Instruments, USA). The temperature of the DSC module was equilibrated at 35°C and then increased at a rate of 10°C/min under a N<sub>2</sub> 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.

### Solubility study

Solubility study was performed using the Higuchi rotating bottle method with slight modification<sup>5</sup>. The solubility of Nevirapine and Nevirapine loaded Pegylated citric acid dendrimers was determined by adding an excess amount of samples into 3ml of double distilled water in 5ml screw capped, amber colored vials. The vials were rotated at 60 rpm while being kept at room temperature. The present method involved 24 h shaking followed by 6 hr equilibration period. Thereafter, samples were filtered through a 0.45 mm polycarbonate filter (Millipore), diluted appropriately with the distilled water and analyzed in a UV spectrophotometer.

### In vitro release

Drug release from known amounts of Nevirapine loaded Pegylated citric acid dendritic Nanostructure was determined using a modified dissolution method. The medium comprised of a 0.05 mol phosphate buffer solution (PBS) (pH 7.4). The dialysis bags were filled with a known mass of Nevirapine Nevirapine loaded pegylated citric acid dendritic Nanostructure (MWCO 1000 Da) and the dialysis bags were placed in 50 ml of PBS (pH 7.4) at 37°C 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 290nm.

## RESULTS AND DISCUSSION

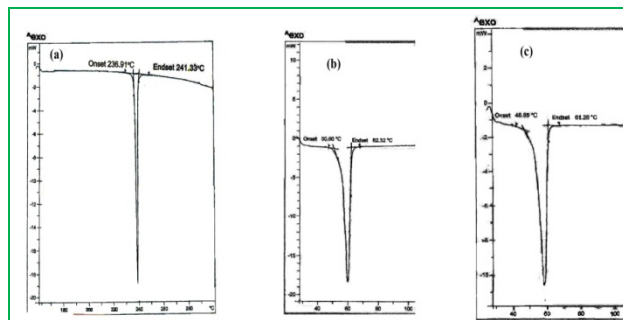
### Differential scanning calorimetry

Differential scanning calorimetry (DSC) curves suggests that Nevirapine loaded 3<sup>rd</sup> generation PEGylated citric acid dendrimer were not a physical mixture. Upon heating to 241.33°C, nevirapine experienced as an endothermic transition. This was previously described as melting of the nevirapine. Broad endothermic peaks between 50.80°C and 62.32°C were found for 3<sup>rd</sup> generation PEGylated citric acid dendrimer. The characteristic peaks of nevirapine and blank 3<sup>rd</sup> generation PEGylated citric acid dendrimer almost disappeared in the curve for nevirapine loaded 3<sup>rd</sup> generation citric acid dendrimer<sup>6</sup>.

### Solubility study

Before the drug release studies were conducted, the solubility of nevirapine and nevirapine in the formulations was determined. The dendrimers could function as solubility enhancer by virtue of electrostatic interactions in addition to hydrogen bonding and molecular encapsulation in the crevices of the dendrimeric

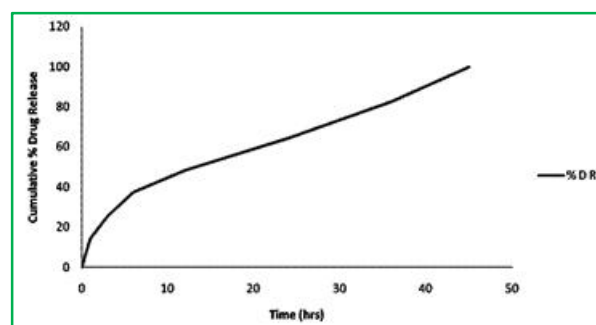
network<sup>7,8</sup>. The 3<sup>rd</sup> generation PEGylated citric acid dendrimer increased the aqueous solubility of nevirapine.



**Figure 1:** DSC analysis of (a) Nevirapine (b) Pegylated Citric acid dendrimer (c) Drug Loaded Pegylated Citric acid dendrimer.

### In vitro release

*In vitro* release studies of nevirapine loaded 3<sup>rd</sup> generation PEGylated citric acid dendrimer was carried out using 7.4pH phosphate buffer. There was a slow increase in drug release in buffer phase, which has shown 98±0.948% of drug release in 45 hours. It was observed that the formulation showed sustained release of the drug. This may be due to greater hydrophobic interaction between the drug and the core of 3<sup>rd</sup> generation PEGylated citric acid dendrimer.



**Figure 2:** *In vitro* study release of Nevirapine in 7.4 pH phosphate buffer solution

## CONCLUSION

The present work establishes suitability of novel pegylated citric acid dendritic nanostructure as sustained delivery system for nevirapine. From the present study, it can be concluded that 3<sup>rd</sup> generation pegylated citric acid dendrimer units increased the aqueous solubility of nevirapine.

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