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# Acetazolamide encapsulated dendritic nano-architectures for effective glaucoma management in rabbits

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ABSTRACT

The present investigation was aimed to develop topically effective acetazolamide loaded poly(propylene imine) dendrimer nanoarchitectures and evaluate their intraocular pressure lowering potential. The 5.0G PPI dendrimers were synthesized using ethylendiamine as dendrimer core through divergent approach and characterized and loaded with acetazolamide (ACZ). The developed dendrimeric formulations were characterized for size, loading efficiency. The surface morphology of dendrimer was studied by Transmission Electron Microscopy. The developed dendrimer formulations were evaluated for hemolytic toxicity, ocular irritation index and intra ocular pressure reduction using normotensive adult male New Zealand albino rabbits as in vivo model. The maximum drug entrapment efficiency was found to be 56 ± 2.3%. The in vitro release data of ACZ-5.0G PPI dendrimers showed sustained release of ACZ which was found to be 83.5 ± 1.8 and 80.4 ± 1.6% in phosphate buffer saline (pH 7.4) and simulated tear fluid (pH 7.4), respectively in 24 h. Ocular irritancy, ocular residence time and intraocular pressure lowering effect were performed. The study revealed that in lower concentrations the aqueous solutions of dendrimer formulations were found to be weakly irritant to the eyes. The sustained and prolonged reduction in intraocular pressure suggested that drug entrapped in dendrimers can be used for higher retention in ocular cul-de sac. Further, the PPI dendrimer based formulation seems to enhance the ocular drug residence time and exhibits better intraocular pressure lowering effect for glaucoma treatment, more safely, both in vitro and in vivo.



## 1. Introduction

Development of an effective ocular drug delivery system is one of the most fascinating and exigent task for the nanotechnologists (Zhang et al., 2012). Special anatomic structure of eye and its efficient protective mechanisms, in terms of fast turnover of lachrymal fluid as well as competent drainage system enhance the rapid elimination of ophthalmic solutions, which ultimately decrease the ocular residence time. All these events lead to less than 10% of ocular bioavailability (Das and Suresh, 2011; Natarajan et al., 2011; Urtti and Salminen, 1993). Higher concentrations of drug or increased dosing frequency are adopted to achieve optimal ocular bioavailability of the administered drug but this practice potentiates the possible ocular as well as systemic side effects of the drug (Kaur and Kakkar, 2011; Robinson and Mlynek, 1995; Fogagnolo and Rossetti, 2011).

Glaucoma, the silent thief, is characterized by 'tunnel vision' which is the result of deliberate and progressive degeneration of ganglion cells of retina. Elevated intraocular pressure (IOP) is one of the prime risk factors for the glaucoma (Soltau and Zimmerman, 2002). Statistics of World Health Organization (WHO) indicate that glaucoma is becoming a leading cause of blindness and more than 67 million people worldwide are suffering with glaucoma and by the year 2020, approximately 80 million people are projected to be affected with open angle as well as angle closure glaucoma (Kaur and Kakkar, 2011). It is projected that in India more than 11.1 million people will suffer with bilateral blindness due to glaucoma by 2020 (Quigley and Broman, 2006).

A very small fraction (5%) of administered dose reaches to the intraocular tissues. Accordingly, the exigent purpose is to develop topical ophthalmic drug delivery system which enhances the bioavailability of the drug due to better ocular retention as well as improved corneal absorption and diminished side effects whilst retaining the ease and expediency as eye drops but will serve as slow release depot (Kesavan et al., 2011; Vandamme and Brobeck, 2005; Vandamme, 2002; Granero and Longhi, 2010; Kaur et al., 2010; Gupta et al., 2010; Agnihotri and Aminabhavi, 2007).

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In the arena of ocular drug delivery dendrimers are used as one of the promising nanocarriers because of their nanometric size, easy preparation techniques easily tailored surface functional moieties for the better recognition of biological responses (Vandamme and Brobeck, 2005). Dendrimers, extremely and precisely branched, globular, three dimensional polymeric macromolecular structures with very low polydispersity index (Agrawal et al., 2013) play a pivotal responsibility in the ground of nanotechnology. Dendrimers have found their applications in delivery of various bioactives (Mishra et al., 2009; Tekade et al., 2009; Svenson and Tomalia, 2005; Jain et al., 2013; Mehra et al., 2013), catalysis (Twyman et al., 2002) and electronics (Ma and Jen, 2001). Dendrimers also act as drug solubility enhancer (Gupta et al., 2006, 2007), release modifiers as well as platforms for drug targeting.

The present work was designed for the development and characterization of acetazolamide (ACZ) loaded hyper branched poly(propylene imine) (PPI) dendrimers for the ocular delivery of ACZ, with minimal systemic side effects, for the management of glaucoma.

To the best of our knowledge the present study is a debut report that explores the application of PPI dendrimers for ocular delivery of ACZ in glaucoma management to overcome the systemic side effects of free ACZ by improving the ocular residence time and better IOP lowering effects which further potentiate the patient compliance, and also provide a cost-effective novel drug delivery system.

## 2. Materials and methods

### 2.1. Materials

Ethylenediamine (EDA), Raney Nickel and Acrylonitrile (ACN) were procured from Merck, India and Central Drug House (CDH), Mumbai, India respectively. Drug, Acetazolamide was purchased from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. All the other chemicals were procured from HiMedia Lab, Mumbai, India. All the solvents were of High Performance Liquid Chromatography (HPLC) grade. Triple-distilled water was used throughout the studies.

### 2.2. Synthesis and characterization of poly(propylene imine) dendrimers

PPI dendrimers were synthesized by the divergent approach of synthesis as per previously reported method with slight modification (De Brabander-Van den Berg and Meijer, 1993). In the synthesis of PPI dendrimers the ethylenediamine (EDA) was used as dendrimer core. The double Michael addition reaction between ACN (2.5–4 equiv. per primary amine group) and aqueous solution of EDA produced half-generation [EDA-dendr-$(CN)_{4n}$ (where $n$ is generation or reaction cycle)] PPI dendrimers. After preliminary exothermic effect, the temperature of reaction mixture was raised to 80 °C and maintained for 1 h for the completion of addition reaction. By the vacuum distillation (16 mbar and 40 °C bath temperature), the surplus amount of ACN was recovered as water azeotrope. The hydrogenation of half-generation dendrimer produced full-generation [EDA-dendr-$(NH_2)_{4n}$] PPI dendrimers. Hydrogenation step was accomplished by dissolving half-generation dendrimer in methanol and hydrogenated under 40 atm hydrogen pressure at 70 °C for 1 h. Raney Nickel (pretreated with aqueous solution of hydroxide) was used as catalyst. The resultant mixture was then cooled, filtered and concentrated under reduced pressure. Further extent of solvent was evaporated under vacuum to get dried full-generation PPI dendrimers. By adopting the repetition of all the above mentioned steps with increasing amount of ACN, the PPI dendrimers up to 5.0G were synthesized (De Brabander-Van den Berg and Meijer, 1993).

The synthesized half- and full generations of PPI dendrimers were duly characterized with the help of Fourier Transformed Infrared (FT-IR) spectroscopy by KBr pellet method (Perkin Elmer 783, Pyrogen 1000 Spectrophotometer, USA) and by $^1$H NMR spectroscopy. Half-generations of PPI dendrimers were solubilized in $CDCl_3$ while full generations were solubilized in $D_2O$, and analyzed by NMR Spectrometer (Avance-II, Bruker, Germany) at working frequency of 400.1324008 MHz. Another technique, Transmission Electron Microscopy (TEM) was employed for the characterization of the developed system after drying on carbon grid and negatively stained with phosphotungastic acid (1%) by the metal shadowing techniques (Philips CM-10 TEM, Eindhoven, The Netherlands). The microphotographs were captured at suitable magnifications.

### 2.3. Drug loading and entrapment efficiency

The loading of ACZ in 5.0G PPI dendrimers was accomplished by equilibrium dialysis technique (Dutta and Jain, 2007). Briefly 5 ml of aqueous PPI dendrimer solution (10 μM) was kept in a cellulose dialysis bag [Molecular Weight Cut-off (MWCO) 1 KD; HiMedia Lab, Mumbai, India) and immersed in aqueous solution of ACZ (700 μg/ml; 100 ml). The system was incubated at 25 ± 2 °C for 24 h. The dialyzed formulation was lyophilized (Heto Dry-winner, Heto-Holten, Allerod, Denmark) and used for further characterization. Similarly, the ACZ was loaded in 25 and 50 μM of 5.0G PPI dendrimers. The loading of ACZ in dendrimers was confirmed by FT-IR spectroscopic analysis (Table 1).

The entrapment efficiency of ACZ loaded 5.0G PPI dendrimers was evaluated indirectly by estimating the unentraped ACZ in dialyzing medium employing HPLC method after suitable dilutions ($\lambda_{max}$ 265 nm; LC10-AT vp pump and SPD-M10A vp diode array detector, Shimadzu, Kyoto, Japan) (Dutta and Jain, 2007). The percent entrapment efficiency (EE) was determined by applying following equation:

$$\text{Percent entrapment efficiency} = \frac{(\text{Total amount of ACZ} - \text{Unentraped amount of ACZ}) \times 100}{\text{Total amount of ACZ}}$$

### 2.4. In vitro studies

#### 2.4.1. In vitro drug release

The amount of ACZ released from different dendrimer formulations was estimated by dialysis tube diffusion technique (Dutta and Jain, 2007; Martin et al., 1999; Bhadra et al., 2005). Briefly, ACZ loaded dendrimer formulation (100 mg) kept in pretreated (PBS pH 7.4) cellulose dialysis bag (MWCO 2 KD, HiMedia Lab, Mumbai, India) and dialyzed against sink solution (50 ml; PBS pH 7.4) under strict sink conditions at 37 ± 0.5 °C with continuous stirring at 50 rpm using magnetic stirrer (Remi Equipments Pvt. Ltd., Mumbai, India). For the estimation of ACZ released from dendrimer formulation, 1 ml was withdrawn from external sink solution, which was replenished with fresh PBS (pH 7.4). The amount of released ACZ was determined by HPLC method. Similarly drug release profile was also determined in Simulated Tear Fluid (STF) pH 7.4.

#### 2.4.2. Stability studies

Stability studies were performed under accelerated conditions of temperature and light to ascertain the stability of drug loaded