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Preparation and *In vitro* Characterization of PCL Microspheres of Acetazolamide

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ABSTRACT

Drug micro-particle formulation is a very good technique leading to a product called microsuspension. Acetazolamide is used orally for the reduction of Intra Ocular Pressure (IOP) in patients suffering from glaucoma. Acetazolamide is practically insoluble in water and in the aqueous tear fluid, and this limits its ocular bioavailability. Several attempts have been made to formulate topically active acetazolamide in order to minimize its systemic side effects. Poly-ε-caprolatone (PCL) is a biocompatible and biodegradable polymer and does not generate an acid environment, also it possesses high hydrophobicity. So acetazolamide loaded PCL microsphere was prepared and optimized by physical parameters. Microsphere prepared with rotation speed of 1500 rpm having less size and smooth surface morphology, further faster rate of addition of drug polymer mixture did not get sufficient time to diffuse in aqueous PVA solution, so leads to formation of larger size of microsphere and vice versa. Dissolution studies of different batches of acetazolamide loaded PCL microsphere reveals that there is 87% of the drug release at the end of 12 hrs.

Keywords: Herbal medicines, Ayurveda, Medicinal plants, potential medicinal value.

1. INTRODUCTION

Drugs poorly soluble aqueous and non-aqueous media are challenging problem in drug formulation. This is the problem leads to poor bioavailability and absorption of drugs to site of action. There are lot of attempts have been made by researchers to increase the bioavailability of poorly soluble drugs [1]. Drug micro-particle formulation is a very good technique leading to a product called microsuspension. Acetazolamide is used orally for the reduction of Intra Ocular Pressure (IOP) in patients suffering from glaucoma. It is used in the pre-operative management of closed angle glaucoma, or as an adjunct therapy in the treatment of open-angle glaucoma. To obtain the desired lowering in IOP, large oral doses of acetazolamide are used, and this usually leads to systemic side effects. Acetazolamide is practically insoluble in water and in the aqueous tear fluid, and this limits its ocular bioavailability. Several attempts have been made to formulate topically active acetazolamide in order to minimize its systemic side effects. These included surfactant-gel preparations [2] contact lenses [3] and aqueous solutions containing cyclodextrins. Poly-ε-caprolatone (PCL) is a biocompatible and biode -toxicity and high permeability to low molecular weight drugs [4]. Therefore, many investigations have focused on its application for controlled delivery of various drugs [5,6]. So a suitable plan has been designed to encapsulate acetazolamide into PCL microspheres by solvent evaporation technique and secondly to characterize the microspheres in terms of particle size, morphology, encapsulation efficiency and drug release.

2. MATERIALS AND METHODS

2.1 Material

Acetazolamide was procured from Intas Pharmaceuticals, Ahmedabad as gift sample.

Poly-ε-caprolactone (MW 50,000) was supplied by Sigma Aldrich, Banglore. Polyvinylalcohol (PVA, 88% hydrolyzed) was provided by Sigma Aldrich, Banglore, as emulsifier. Tween 80 and dichloromethane were obtained from SD fine chemicals, Mumbai.

2.2 Preparation of microspheres

Acetazolamide loaded PCL microspheres were prepared by solvent evaporation technique. 1gm of micronized drug was dispersed in 10ml of dichloromethane containing PCL 1gm. 1% aqueous solution of PVA was prepared with water. The dispersed drug was added into 50 ml of PVA aqueous solution. Then the emulsion was stirred with high speed homonizer for 40 min followed by another 20 min under reduced pressure. The speed of rotation was varied in different batches. (Guinedi et al., 2005)Then the resulting mixture was filtered to collect the microspheres. Further the microspheres were washed with deionized water and dried in a vacuum desiccator at room temperature.

2.3 Particle size analysis

Particle size analyzer (SALD-2201, Shimadzu) was used for measurement of particle size of microspheres. About 20 mg of microsphere was taken in 5 ml of filtered distilled water. Then 2% w/v of tween-80 was added followed by sonication in water bath to prevent aggregation. The particle size was expressed as the volume mean diameter in micrometer.

2.4 Morphology of microspheres

The prepared microspheres were subjected to surface morphology study with the help of a scanning electron microscope (SEM) (Jeol JSM-1600, Tokyo, Japan). Dried microspheres were mounted onto stubs using double-sided adhesive tape with conductive effect and analyzed with SEM.

2.5 Analysis of drug content

About 30 mg of acetazolamide loaded microspheres of each batch were dissolved in 1.5 ml of acetonitrile followed by addition of 8.5 ml of purified water to precipitate the polymer matrix. The resulting solution was filtered to separate the precipitated polymer. The concentration of acetazolamide was determined in the solution by measuring the UV absorbance at 265 nm. Drug loading and entrapment efficiency were calculated as follows: Drug loading = Mass of drug in microspheres/ Mass of microspheres × 100% and Encapsulation efficiency = Actual drug loading/ Theoretical drug loading × 100%

2.6 *In vitro* release behavior

Fifty milligram of acetazolamide loaded microspheres were taken in 5ml of 0.05M phosphate buffer of pH 7.4 and incubated in a horizontal-shaker at 37°C. Then microspheres were centrifuged for 2 min at 500 rpm. 0.1 ml of sample was collected at 0 min, 15 min, 30 min, 60 min, 2 hr, 6 hr and 12 hr intervals. To the 0.1 ml of sample 4.9 ml of buffer was added and the absorbance was measured.

3. RESULT & DISCUSSION

Primarily this study was designed to investigate the influence of some process parameters on the physical characteristics of the microspheres. Acetazolamide loaded PCL microspheres were prepared by solvent evaporation technique. PCL is used as a polymer due to its biocompatibility and biodegradability and does not generate an acid environment also it possesses high hydrophobicity, non-toxicity and high permeability to low molecular weight drugs. The rotation speed applied are 800, 1000, 1200 and 1500 rpm and 2000rpm, in which 1500 rpm was found to be optimum. Particle size of various batch formulations were measured with the help of SALD-2201, Shimadzu particle size analyzer, the result shown in table-1.The rotation speed directly influence the size of microsphere, further addition of drug, PCL mixture in organic phase to the PVA aqueous solution also influence the size of microsphere[8]. This reveals that faster rate of addition of drug polymer mixture did not get sufficient time to diffuse in aqueous PVA solution, so leads to formation of larger size of microsphere and vice versa. Surface morphology of pure acetazolamide and the optimized microspheres were studied with the help of SEM. The surface morphology of pure acetazolamide was identified with irregular shape, rough surface and large particle size. Microspheres prepared with PCL are spherical with smooth surface, shown in figure-1.

Table 1: Influence of rotation rate on particle size, entrapment efficiency of microspheres (n=3)

Batch	Drug: Polymer	Rotation Rate (RPM)	Particle size(µm)	Entrapment Efficiency %
F-I	1:1	800	75.23±1.36	63.23±1.25
F-II	1:1	1000	69.43±2.69	69.56±1.09
F-III	1:1	1200	61.21±1.45	78.36±1.35
F-IV	1:1	1500	50.12±2.10	80.50±2.12
F-V	1:1	2000	55.25±2.15	65.12±1.63

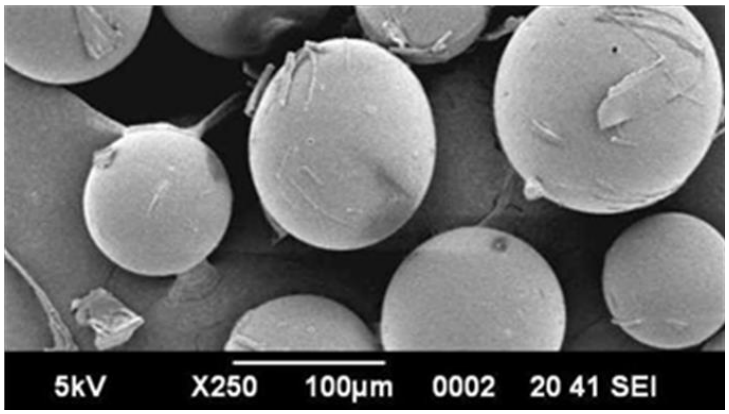

Figure.1: SEM image of optimized formulation.

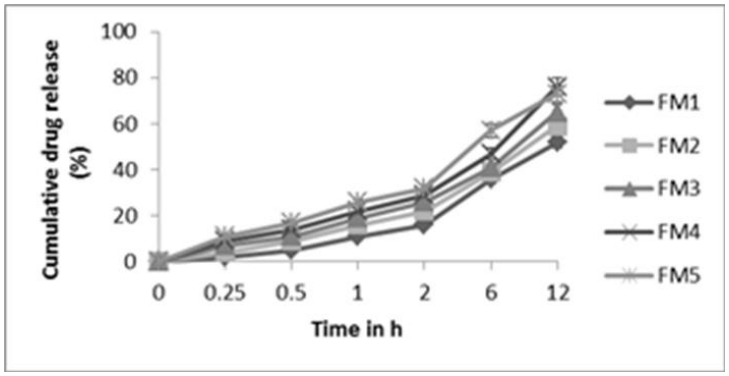

Figure.2: Drug release profile of different formulations.

Results of the drug content and entrapment efficiency are given in Table 1. This reveals that with increase in rotation drug content and entrapment efficiency increases, and found to be optimum at 1500 rpm having 80.50% entrapment efficiency (El-Gazayerly et al., 1997), but with much higher rotation rate there is formation of very fine particles, this leads to aggregation.

Dissolution studies of different batches of acetazolamide loaded PCL microsphere reveals that there is 67% of the drug release at the end of 12 hrs. Drug release from the microsphere took place in pH 7.4 might have due to mechanism of diffusion and erosion. Figure-2 shows the cumulative % release of acetazolamide from microspheres prepared in different batches with respect to rotation. This figure clearly demonstrate that formulations prepared with low rotation rate released the drug more rapidly as compared to formulations prepared with high rotation rate, in which the drug is released in a more controlled manner.

4. ACKNOWLEDGEMENT

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REFERENCES

1. Liversidge GG, Cundy KC, Bishop JF and Czekai DA. Surface modified drug nanoparticles. 1992, US Patent No., 5; 145: 684.
2. Tous SS and Nasser KAE. Acetazolamide topical formulation and ocular effect, S.T.P. Pharmaceutical Sciences. 1992; 2: 125-131.
3. Friedman Z, Allen RC and Steven MR. Topical acetazolamide and methazolamide delivered by contact lenses, Arch Ophthalmol., 1985; 103: 936-966.
4. Pitt CG. Poly (ε-caprolatone) and its copolymers. In: Chasin MR. (Eds), Biodegradable polymers as drug delivery systems, Marcel Decker Inc., New York, 1990; 71-120.
5. Kim SY and Lee YM. Taxol-loaded block copolymer nanospheres composed of methoxy poly (ethylene glycol) and Poly (ε-caprolactone) as novel anticancer drug carriers, Biomaterials, 2001; 22: 1697-1704.
6. Dhanaraju MD, Vema K, Jayakumar R. and Vamsadhara C. Preparation and characterization of injectable microspheres of contraceptive hormones, Int. J. Pharm., 2003; 268: 23-29.
7. Guinedi AS, Mortada ND, Mansour S and Hathout RM. Preparation and evaluation of reverse phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide, Int. J. Pharm., 2005; 306: 71-82.
8. El-Gazayerly ON and Hikal AH. Preparation and evaluation of acetazolamide liposomes as ocular delivery systems, Int. J. Pharm., 1997; 158: 121-127.