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RESEARCH ARTICLE

PREPARATION AND CHARACTERIZATION OF ACETAZOLAMIDE HYDROXY PROPYL- β-CYCLODEXTRIN COMPLEXES PREPARED BY KNEADING METHOD USED IN OCULAR DRUG DELIVERY

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ABSTRACT

Purpose: *Enhancement of the solubility and dissolution of acetazolamide using inclusion complex with HP β-cyclodextrin and polyvinyl alcohol by kneading technique.*

Methods: *Acetazolamide inclusion complexes were prepared using physical mixture method and kneading method. Powder X- RD, Infrared (IR) spectroscopy and Differential Scanning Calorimetry (DSC) were performed to identify physiochemical interaction between the drug and carrier and its effect on dissolution behavior. Morphology of the Inclusion complex was studied using Scanning Electron Microscopy (SEM). A comparative evaluation of the dissolution of acetazolamide-HP-β-CD- PVA inclusion complex, physical mixture and pure drug was carried out.*

Results: *Dissolution of Aceatzolamide (ACZ) improved significantly in inclusion complex as compared to pure drug and physical mixtures. ACZ exhibited better aqueous solubility in presence of both HP-β-CD and PVA. The percentage of ACZ dissolved after 90 min was 48.98%, for pure drug compared with 98.42% for the drug in inclusion complex. IR spectroscopy showed no change in chemical structure of ACZ.* ***Conclusions:*** *Inclusion complex technique of solid dispersion by kneading method can be successfully used for improvement of dissolution of acetazolamide.*

INTRODUCTION

Glaucoma is a group of disease of the eye characterized by damage to the ganglion cells and the optic nerve. If left untreated, these effects may lead to various degrees of loss of vision and blindness. Increased intraocular pressure (IOP) remains the most important risk factor for the development of glaucoma. Different categories of drugs used in treatment of glaucoma include β- blockers, miotics, sympathomimetics and carbonic anhydrase inhibitors [1]. Acetazolimde is widely used CAI for the treatment of glaucoma. Oral acetazolamide reduces IOP in patients suffering from glaucoma. It is used in preoperative management or as an adjuvant therapy in the treatment of open-angle glaucoma. Large oral doses of acetazolamide lower IOP, but usually lead to a multitude of systemic side effects because of the wide distribution of CA enzyme. The most-common side effects are diuresis and metabolic as well as respiratory acidosis. Other side effects of acetazolamide include gastrointestinal upset, lassitude, paraesthesia, anorexia, weight loss, malaise, depression, altered taste, decreased libido, blood dyscrasias, urolithiasis, and rare complications such as renal stones. The occurrence of these side effects results in poor patient compliance. It is estimated that approximately half the patients suffering from glaucoma can tolerate long-term systemic treatment with Acetazolamide[2],[3]. A topical acetazolamide formulation possessing similar efficacy to the oral formulation would be a significant advance in the treatment of glaucoma.

A topical formulation of acetazolamide, when compared with a systemic delivery, can offer the following advantages:
1. Reduction in dose.

2. Faster onset of action.

3. Marked decrease in side effects.

4. Increase in patient compliance.

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Extend of absorption into the eye is severely limited by the physiological constraints such as reflex tearing and blinking. Further drug loss occurs due to tear turnover, solution drainage by gravity and binding of drugs to protein and other components of tear. As a result, typically only 1-2% of the instilled drug is bioavailable [4]. An effective way to achieve slow and prolonged absorption in ophthalmic practice is to incorporate a drug into polymeric film, which when placed in the cul-de-sac of the eye exhibits a prolonged local release for drug action on tissue in the immediate vicinity. Solubility and developing novel formulation are two of the important parameters to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown. This is true for parenterally, topically and orally administered solutions.

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic central cavity. They are generally used to increase the aqueous solubility of the drug by forming water-soluble drug cyclodextrin complexes. They act as true carriers by keeping the hydrophobic drug molecules in solution and delivering them to the surface of the biological membrane [5]. This approach has also been used in ocular drug delivery. The ocular availability of drugs in the aqueous cyclodextrin containing eye-drop solution depends on several factors such as the release of the drug from the cyclodextrin complex and the partitioning of the drug molecules into and through the cornea or the conjuctival epithelium. In general, it is believed that cyclodextrins enhance topical drug delivery by increasing the drug availability at the barrier surface. At the surface, the drug molecules partition from the cyclodextrin cavity into the lipophilic barrier. Thus, the delivery from aqueous cyclodextrin solution is both diffusion and membrane controlled. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) is a nontoxic β -cyclodextrin derivative, which is capable of forming water-soluble drugs .Under normal conditions the large and very hydrophilic HP-β-CD molecules do not penetrate biological membranes but act as penetration enhancers by ensuring a high concentration of dissolved drug at the membrane surface. Therefore, HP-β-CD may improve ocular bioavailability of the drugs by keeping the water- insoluble drug molecules in solution and deliver them to the surface of the corneal barrier where the molecules partition into the eye[6][7].

MATERIALS AND METHODS

Acetazolamide was procured as a gift sample from Dr. Reddys Laboratories, Hyderabad, India. Hydroxypropyl Beta cyclodextrin (β-CD) and polyvinyl alcohol were purchased from gangwal chemicals and SD Fine Ltd., India respectiviely. Other reagents and organic solvents used were of analytical grade. Buffer and its dilutions were prepared with distilled water.

Preparation of inclusion complexes:

Inclusion complexes of acetazolamide were prepared by physical mixture ad Kneading method.

Physical Mixture [9]

The required molar (1:1) quantities of drug, hydroxypropyl betacyclodextrin, and PVA were weighed accurately and mixed together thoroughly in mortar with vigorous trituration for about 1 hr. This mixture was then passed through sieve no. 80 and stored in airtight containers till further use.

Kneading Method [8] [10]

Drug with HP -β-CD in different molar ratios (i.e. 1:1, 1:2) were taken. First cyclodextrin is added to the mortar, small quantity of 50% ethanol is added while triturating to get slurry like consistency. Then slowly drug is incorporated into the slurry and trituration is further continued for one hour. Slurry is then air dried at 25°C for 24 hours, pulverized and passed through sieve No. 80 and stored in desiccators over fused calcium chloride.

Characterization of acetazolamide inclusion complexes [11, 12, 13]

Inclusion complexes of acetazolamide were characterized using following analytical techniques

IR spectral analysis

Infra red spectra of drug complexes were recorded by KBr method using Fourier Transform Infrared Spectrophotometer (Bruker FTIR-8400s).

X – Ray diffraction studies:

X ray diffraction patterns of the selected inclusion complexes were compared with that of the plain acetazolamide. This was done by measuring the 2ø in the range of range 4 to 500 with reproducibility of ±0.0010 on a diffractometer. The XRD patterns were recorded automatically using rate meter with time constant of 2 × 102 pulse/second and with the scanning speed of 20 (2ø)/min.

Differential scanning calorimetric analysis:
The differential calorimetric analysis was carried out using differential scanning calorimeter. For this, the samples were placed in a platinum crucible and the DSC thermograms were recorded at a heating rate of 100c/min in the range of 200c to 2500c.

Scanning electron microscopy (SEM):
The scanning electron microscopy is a type of electron microscopy that images the sample surface by scanning it with a high-energy beam of electrons. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity. SEM was used to investigate solid state physical structure of the prepared ternary complex. S.E.M. photographs of acetazolamide, its physical mixture with hydrotropic agents and its solid dispersions were obtained using a scanning electron microscope model S-3400N, Hitachi, Japan with accelerating voltage from 0.5 to 30 KV.SEM photographs was reported in fig. no.

Saturation solubility studies
Solubility studies were carried out in glass vials. In each of these vials, 10ml distilled water was added. Excess quantities of inclusion complexes were added into each of vials. These vials were shaken continuously for 24 hours on a lab shaker and the resulting solutions were filtered, appropriate dilutions were made and UV absorbances were recorded at 265nm.

Dissolution studies of acetazolamide and its complexes
The quantities of each drug complex equivalent to 20mg of acetazolamide were subjected to dissolution test using USP Dissolution Test Apparatus Type II. Pure acetazolamide was used as control and was subjected to the similar test using 20mg of drug.

Test Parameters
Dissolution medium : - PBS

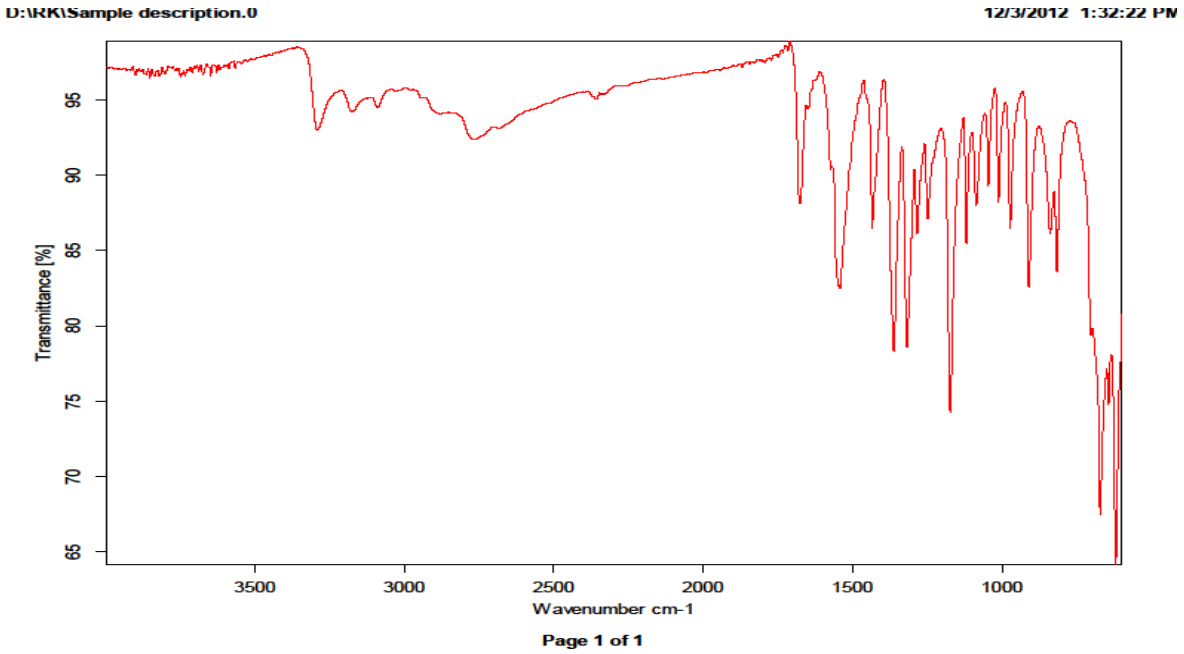
Speed of paddle : - 50 rpm. Temperature of dissolution medium: - 37^0C ± 0.5^0C. Apparatus type : - USP XXII (paddle)

The quantity of drug complex equivalent to 20mg of acetazolamide was placed in dissolution medium and apparatus was run maintaining above stated test conditions 5 milliliters aliquots were withdrawn at time points of 5 min., 10 min., 15 min., 20min., 25 min., 30 min., 40 min., 50 min., 60 min., 75 min., 90 min., 105 min. & 120 min. Every time the equal volume of fresh dissolution medium which was maintained at same temperature was added to the bulk. Samples were filtered through Whatman filter paper (No.41) and the absorbances were recorded at 265 nm. Cumulative percentage of labeled amount of drug released at each time point was calculated. Values of T50 [Time required for 50% dissolution of stated amount of drug.] & T90 [Time required for 90% dissolution of stated amount of drug.] were also calculated.

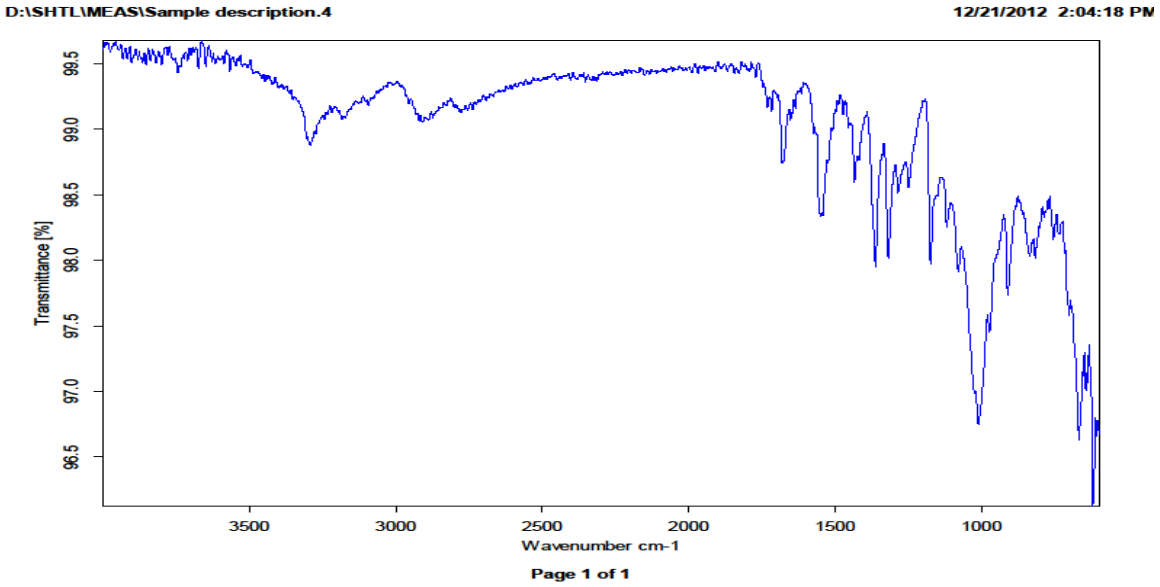
Results:
Following Inclusion complexes were prepared and evaluated

Method	Drug+HP β CD	Polymer	Polymer concentration	Solvent
PM1	**1:5**	**PVA**	**0.25%**	**_**
PM2	**1:5**	**PVA**	**1%**	**_**
K1	**1:5**	**PVA**	**0.25%**	**Water +Ethanol**
K2	**1:5**	**PVA**	**1%**	**Water +Ethanol**

Fourier Transform Infrared (FTIR) Spectroscopy
I.R. Spectra of ACZ and inclusion complex are presented in Figure. Pure ACZ spectra showed sharp characteristic peaks at 3339.14, 1743, 1652.43, 1575.29, 1284.52, 692 cm-1. All the above characteristic peaks appear in the spectra of inclusion complex at same wave number indicating no modification or interaction between the drug and carrier.



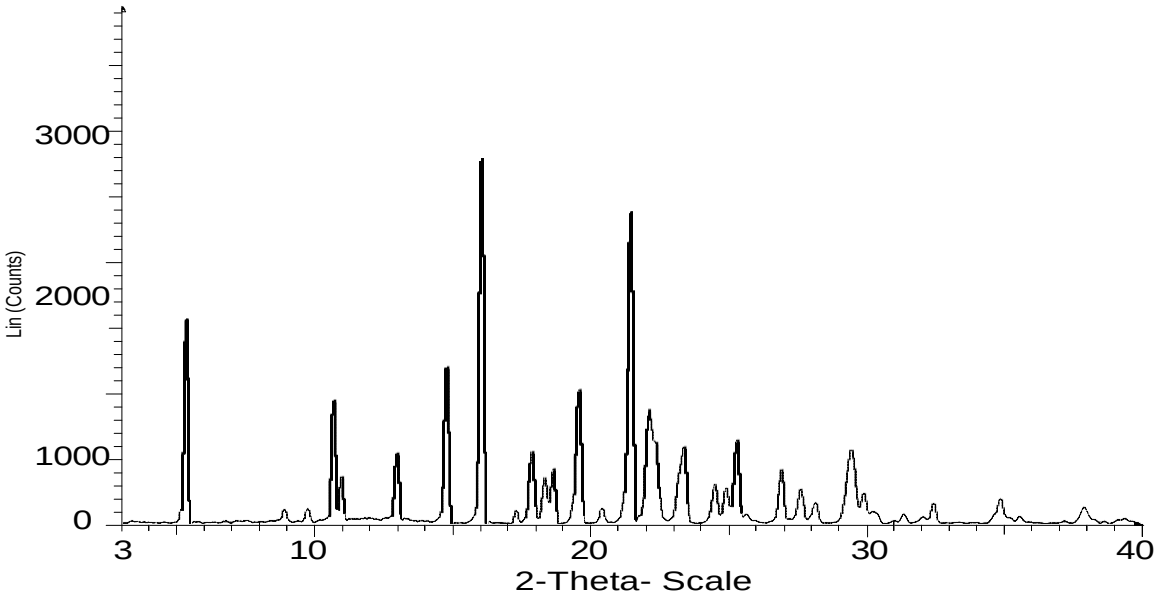
FTIR spectrum of acetazolamide drug sample



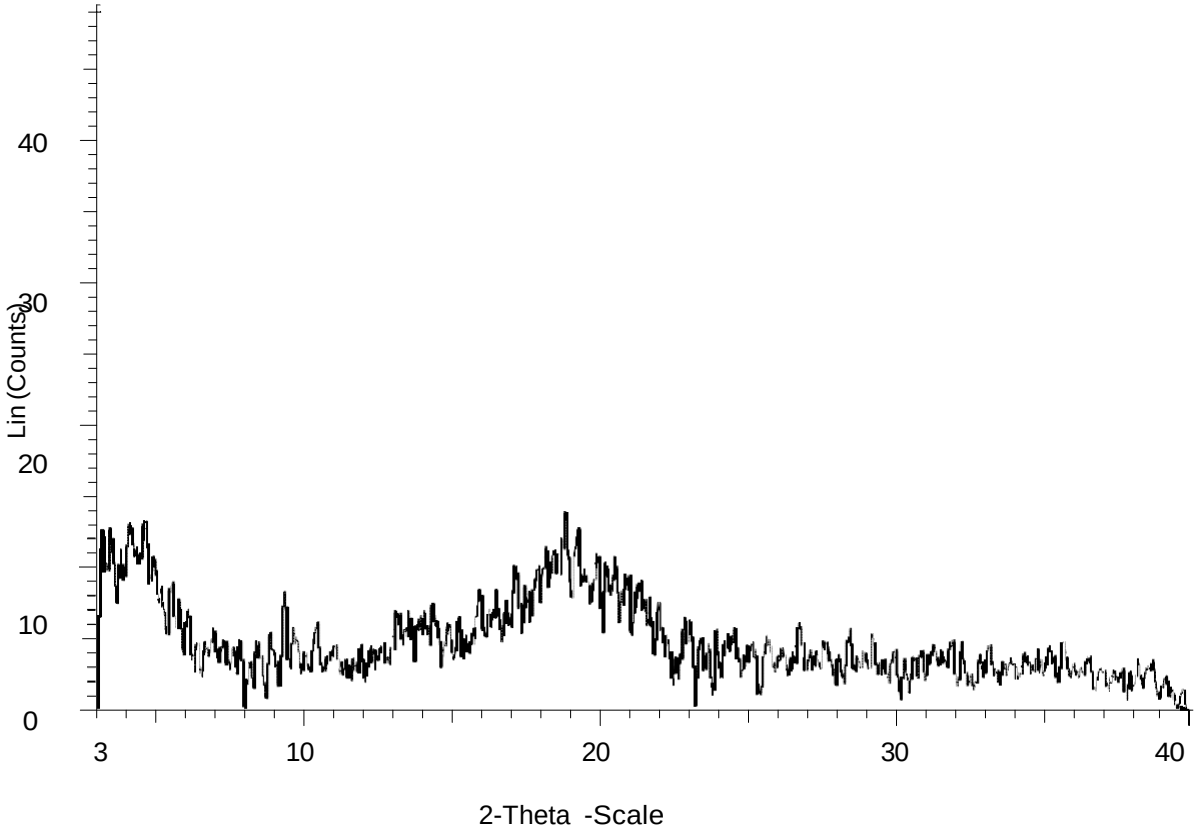
FTIR spectrum of inclusion complex

X-Ray Diffraction Studies:

X-Ray diffraction graphs of pure drug sample and inclusion complexes are shown in below fig. The characteristics sharp peaks in X-ray diffraction graph of drug indicates the crystalline nature of the drug, whereas the X-ray diffraction graph of inclusion complex shows hollow pattern characteristic of amorphous nature. This implies that the ACZ has been transformed to amorphous form from crystalline form.



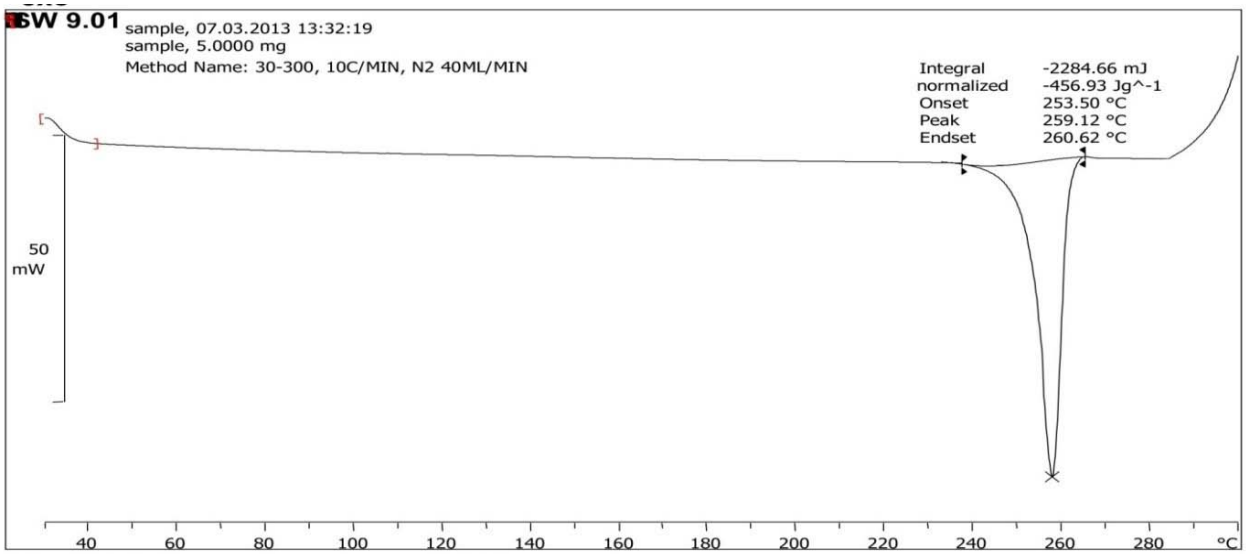
XRD of Acetazolamide drug sample



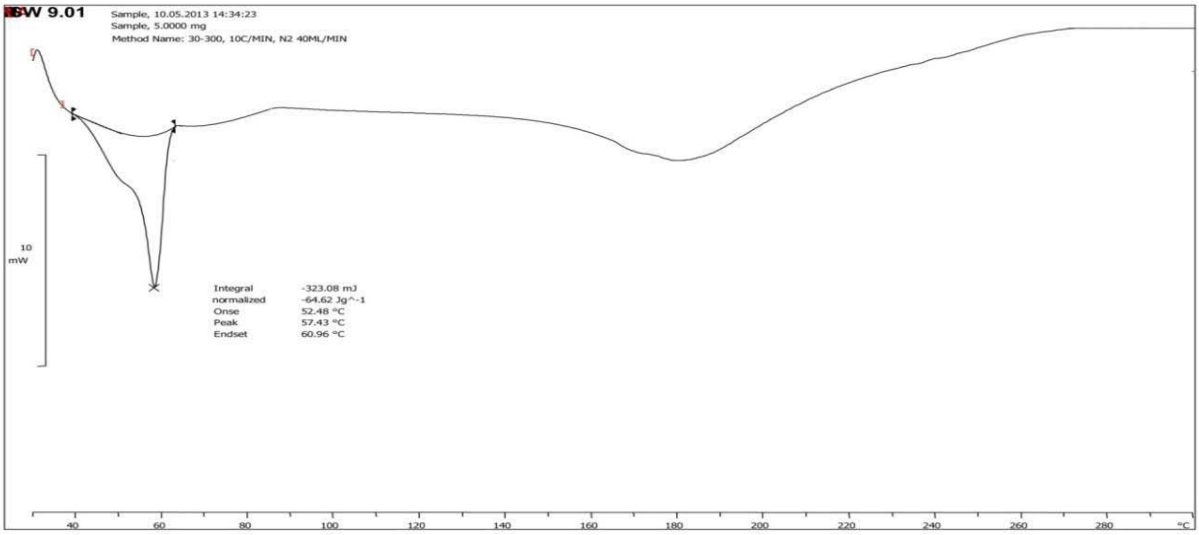
XRD of Inclusion Complex

Differential scanning calorimetric analysis:

Thermal behavior of pure drug and inclusion complex are depicted in below Figure. The DSC curve of DMP profiles a sharp endothermic peak (259.12C) corresponding to its crystalline nature. However the characteristic endothermic peak, corresponding to drug melting was shifted towards lower temperature, with reduced intensity in inclusion complex. This could be attributed to higher polymer concentration and uniform distribution of drug in the mixture of polymers, resulting in complete miscibility of molten drug in polymer. Moreover the data also indicate that there is no interaction between the components of ternary system. DSC pattern shows that kneading process could not induce interaction level and dispersion complex is a physical mixture with highly dispersed drug crystals in carrier.



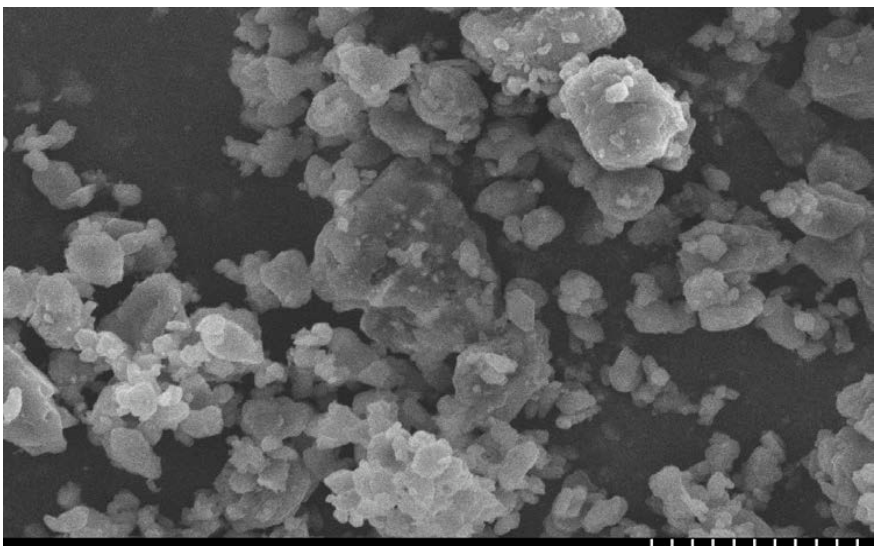
DSC Of pure drug



DSC Of inclusion complex

Scanning electron microscopy (SEM)

The morphology of the dispersion complex was studied by scanning electron microscopy. It was found that ACZ was uniformly dispersed in the mixture of polymers.

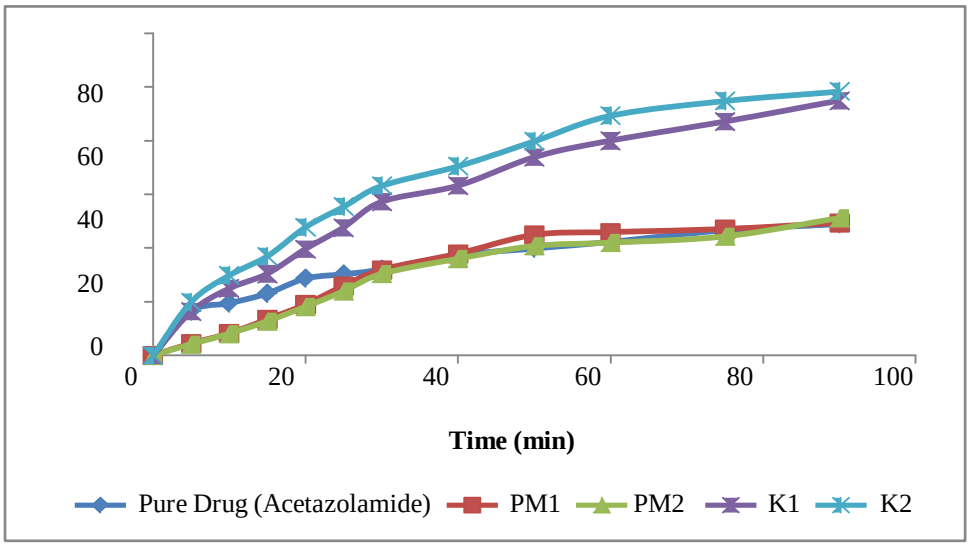


SEM photographs of inclusion complex of Acetazolamide by kneading method

DISSOLUTION STUDIES

Dissolution profiles of original drug crystals and drug-carrier systems are presented in below Figure. It is evident that the inclusion complex has improved the dissolution rate of ACZ to a great extent. Table summarizes % drug dissolved at end of 90 min. The values clearly indicates that the inclusion complexation of ACZ has improved the solubility of drug to a great extend 48.98% for drug alone vs 98.42% for inclusion complex.

Sr. No	Sample Name	% Drug Dissolved at end of 90min
1.	Pure Drug (Acetazolamide)	48.98
2.	Physical Mixture (PM1)	49.41
3.	Physical Mixture (PM2)	51.29
4.	Inclusion Complex (K1)	94.94
5.	Inclusion Complex (K2)	98.42



Dissolution profiles of pure drug, physical mixture and inclusion complex

DISCUSSION

Cyclodextrins increase the aqueous solubility of many poorly soluble drugs by forming inclusion complexes with their apolar molecules or functional groups. In terms of molecular architecture, CyDs can be viewed as hollow truncated conical cylinders with a hydrophilic outer surface inscribing a hydrophobic internal cavity. The cavity sizes for the commonly available α-, β- and γ-CyD are 4.9, 6.2 and 7.9 Å, respectively, dimensions which are ideal for the inclusion of low molecular weight lipophilic drugs. The resulting complex hides most of the hydrophobic functionality in the interior cavity of the CD while the hydrophilic hydroxyl groups on the external surface remain exposed to the environment. The net effect is that a water soluble CD- drug complex is formed. This ability to form inclusion complexes alter the chemical and physical properties of guest (drug) molecules, and effect improved water solubility, prolong in vivo stability, reduce toxicity and irritancy, and improve bioavailability [35]. Cyclodextrin plays the role as dissolution rate promoter due to its ability to solubilize compounds via stabilization of supersaturated drug solutions presumably by inhibition of nucleation and arresting crystal growth. Due to their intrinsic cyclic nature, CyDs are metabolized at much slower rates than their open chain analogs.

The enhancement of dissolution of acetazolamide from drug carrier systems can be described to several other factors also. Lack of crystallinity, i.e. amorphization, increased wettability, dispersibility and particle size reduction are considered to be important factors for dissolution rate enhancement. Kneading results in uniform distribution of drug in the polymer crust in a highly dispersed state. Thus, when such a system comes in contact with an aqueous dissolution medium, the hydrophilic carrier dissolves and results in precipitation of the embedded drug into fine particles, which increase the dissolution surface available. Moreover, other factors such as absence of aggregation and/or reagglomeration phenomenon during dissolution and particle size reduction could be attributed to a better dissolution profile.

CONCLUSION

Acetzaolamide is practically insoluble in water and aqueous fluids. Among the various approaches to improve the dissolution of poorly soluble drugs, the preparation of solid dispersions has often proven to be very

successful; hydrophilic carrier was used in the preparation of inclusion complex and evaluated for their efficiency in increasing the dissolution rate of acetazolamide. Inclusion complexation of acetazolamide in HP-β-CD and PVA was prepared by kneading method. The study shows that the dissolution rate of acetazolamide may be enhanced to a great extent by inclusion complexation technique using kneading method.

REFERENCES

[1] Imam S., Bansal A., 2009. "Novel ocular dosage form in the treatment of glaucoma", the pharma research, Vol. 01: 72-81

[2] Indu Pal Kaur, Mona Kapil, R. Smitha and Deepika Aggarwal 2004 "Development of Topically Effective Formulations of Acetazolamide Using HP-β-CD-Polymer Co- Complexes" Current Drug Delivery 1: 65-72.

[3] Anil K. Singla, Indu P.Kaur, Alka Garg, and Deepika Aggarwal 2002 "Novel Approaches for Topical Delivery ofAcetazolamide"Pharmaceutical technology:24-34.

[4] Rathore K.S., 2010. "In-situ gelling opthalmic drug delivery systems: an overview", International journal of pharmacy and pharmaceutical sciences, Vol. 2: 30-34.

[5] Thoretenin L.,Marcus E.B.,1996."Pharmaceutical Applications of Cyclodextrins. Drug Solubilization and Stabilization" Journal of pharmaceutical sciences Vol. 85:1017-28

[6] Mehta H., Akhilesh D., 2012. "Enhancement of solubility by complexation with cyclodextrin and nanocrystallisation", International research journal of pharmacy: 100-05.

[7] Gerold M.,Thompson Complexation: Cyclodextrins, In: SwarbrickJ. editor, Encyclopedia of aceutical Technology, 3rd ed., Marcel Dekker Inc., New York, USA, p.671-670.

[8] Marcos G., Garnero C., Longhi R.M.2008, "Synthesis, characterization and in vitro release studies of a new Acetazolamide–HP- β-CD–TEA inclusion complex" vol.43issue 3:464-70

[9] Swami G., Koshy M K, PandeyM.1, Saraf A S. 2010," Preparation and characterization of Domperidone- β-cyclodextrin complexes prepared by kneading method" International Journal of Advances in Pharmaceutical Sciences 1: 68-74.

[10] Kalaiselvan R.,Mohanta G.P., Madhusudan S., Manna P.K., Manavalan R. 2007, "Enhancement of bioavailability and anthelmintic efficacy of albendazole by solid dispersion and cyclodextrin complexation techniques" *Pharmazie* 62: 604–7

[11] Saraf S. A., Tripathi G.K., Pandey M.,Yadav P., 2011, "Developement of meloxicam formulations utilizing ternary complexation for solubility enhancement" , Pak. J. Pharm. Sci., Vol.24, Issue 4, :533-38.

[12] Robson A. S., Santos and Rubén D. S., 2011"Pharmaceutical Composition of Hydrochlorothiazide: β-Cyclodextrin: Preparation by Three Different Methods, Physico- Chemical Characterization and In Vivo, Diuretic Activity Evaluation", Molecules J. Pharma, 16, 4482-99;

[13] Chowadary K.P.R., Srinivas V.S., 2012, "Effect of polyvinylpyrillidone on complexation and dissolution rate beta and HP beta CD complexes of Celecoxib", International journal of pharmaceutical sciences, 631- 34.

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