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# β-CD based hydrogel microparticulate system to improve the solubility of acyclovir: Optimization through *in-vitro*, *in-vivo* and toxicological evaluation

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ABSTRACT

The prime objective was to enhance the solubility of acyclovir through developing a stable and non-toxic graft structure of β-cyclodextrin-g-poly (AMPS) hydrogel microparticles. Grafting was carried out successfully by applying aqueous free radical polymerization technique. *N, N′*-methylene bisacrylamide (MBA) and ammonium persulfate (APS) were used as crosslinking agent and initiator, respectively. These were characterized for %EE and product yield, solubility studies, FT-IR, DSC, TGA, PXRD, SEM, TEM, EXD, Zeta size and Zeta potential, swelling studies and *in-vitro* release studies. pH independent swelling and release was observed at pH 1.2 and pH 7.4 but slightly better results were seen at pH 7.4. Solubility of ACV was significantly improved, i.e. pH 1.2 (10.66 folds), pH 7.4 (8.90 folds) and in water (9.21 folds). Release data followed first order kinetics with non-Fickian (anomalous) diffusion release. *In-vivo* studies were conducted on developed hydrogel microparticles and compared with pure drug to reveal the *in-vivo* performance of this system. $C_{max}$ (ng/ml) and $AUC_{0\text{-}12}$ (ng/ml.h) were improved while $T_{max}$ (hr) and $t_{1/2}$ (hr) were decreased thus favouring more bioavailability and rapid release. No toxicity was offered from these carriers. A promising tool for solubility and bioavailability enhancement of acyclovir and other hydrophobic drugs was successfully prepared.



## 1. Introduction

Presently, most of emerging and existing chemical substances exhibit solubility problems. These chemicals offer erratic absorption and variable bioavailability. To cope with such issues, development of new technologies or ways for solubility enhancement remained a challenging aspect for pharmaceutical scientists [1].

Acyclovir (ACV) is a synthetic purine nucleoside that inhibits viral DNA replication after activation into triphosphate form by thymidine kinase enzyme within the infected cells therefore, remaining nontoxic to healthy cells. This enzyme is naturally produced by herpes simplex virus (HSV-1), (HSV-2) and varicella-zoster virus (VZV). ACV is an ampholyte having two pKa values i.e. 2.25 and 9.25. It has only 10–30% bioavailability and about 80% of drug is wasted via feces without participating in any pharmacological activity. ACV has very poor water solubility of about 1.2 mg/ml. For therapeutic purposes 200 mg of dose five times a day is orally administered. It has no definite classification in Biopharmaceutics Classification system (BCS); at low dose 200 mg it is confined to BCS-III, but at higher dose of 800 mg it has been placed in BCS-IV [2].

Hydrogels are crosslinked three dimensional polymeric networks that have ability to imbibe large amount of water. Due to their softness, elastic nature and lower interfacial tension in aqueous and biological media; these resemble with natural tissues. These are expansively utilized in pharmaceutical and medical sector due to their outstanding biodegradability, compatibility with living tissues and gel ability [3,4].

β-Cyclodextrin (β-CD) possesses truncated cone like structure with internal hydrophobic cavity and hydrophilic exterior. Cyclodextrins are widely employed in agriculture, chemical, cosmetics and pharmaceutical fields as these modify physicochemical and biological properties of hydrophobic constituents through complexation. Moreover, CD's have evidently proved their significance in improving solubility, dissolution, bioavailability and stability of drugs. β-CD and γ-CD are listed in generally regarded as

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safe list of US FDA [5,6]. β-CD has major utilization in pharmaceutical sector due to its low price, availability and dimensions of cavity. Capability of CD's for enhancing solubility and bioavailability is enhanced when these are incorporated into polymeric networks i.e. Hydrogels.

Depending upon type of monomer i.e. methacrylic acid (MAA), 2-acrylamido-2-methylpropane sulphonic acid (AMPS), acrylic acid (AA) etc. exhausted hydrogel microparticles can be classified into pH responsive, pH independent, ionic responsive etc. These types of microparticulate systems containing β-CD offer more thermal stability to the incorporated chemical moieties; biocompatible with living tissues, prevent drug polymer decomplexation ultimately burst effect, improve solubility to greater extent due to exposure of incorporated drug to β-CD cavities that ultimately promote bioavailability of hydrophobic drugs. While complex systems i.e. inclusion complexes and solid dispersions etc. having weak vander Waals interactions and hydrogen bonding etc. results in rapid dissociation as drug polymer complex that come in come in contact with biological fluids thereby offer burst effect [7,8].

Ammonium persulfate (APS) is an organic compound containing two ammonium cations and one peroxydisulfate anion. It is white, harmless, non-explosive, economical and highly soluble in water. It is used as a potential oxidizing agent in polymer chemistry and also employed in waste water treatment [9,10]. *N, N′*-Methylene bisacrylamide (MBA) is a dual functional monomer having two similar unsaturated double bonds widely applied as cross linking agent in various field during polymerization reactions [11].

2- Acrylamido -2- methylpropane sulfonic Acid (AMPS) is a white crystalline powder that has hydrophilic nature but limited solubility in polar organic solvents. Moreover, it is an acidic polymer that acts as a polyelectrolyte. Swellability of AMPS is highly dependent on ionizable sulfonate groups. AMPS containing products detach over all pH range thus confirming pH independent swelling. AMPS based hydrogels are used in skin sensitive electrodes, packing films, foam stabilizers, photographic materials, water adsorbent and carrier in biomedical engineering, muscle actuators and in drug delivery [12,13].

A large number of techniques have been presented in literature for solubility, permeability, dissolution and bioavailability enhancement i.e. kneading, solvent evaporation, solubilization, salt formation, rapid dissolving tablets, microemulsions, prodrug formation, hydrosols, micronization, addition of surfactants, nanosuspensions, inter penetrating networks, hydrogel microparticles etc. Hydrogels functionalized with β-CD have been prepared in literature by using free radical polymerization and photocrosslinking for solubility enhancement of poorly water soluble drugs i.e. methotrexate etc. and also for delivery of insulin [3,14].

Keeping in view the advantages of crosslinked functionalized microparticulate system in drug delivery, the present work has been focused to fabricate a crosslinked polymeric system suitable to improve the solubility of drug Acyclovir. A complex system of β-CD and AMPS is tuned and optimized to achieve a stable and fast swelling network when get in touch with water. This approach has been successfully employed to enhance the solubility of acyclovir. According to our best knowledge, β-CD-g-poly (AMPS) hydrogel microparticles have not been previously used for solubility enhancement purposes.

## 2. Materials

Acyclovir was received as a generous gift from Brooks Pharmaceuticals (Pvt) Ltd. Karachi, Pakistan. β-Cyclodextrin 97%, ammonium persulfate 99%, 2-acrylamido-2-methylpropane sulfonic acid 99% and *N, N′*-methylene bisacrylamide 99% were purchased from Sigma Aldrich, United Kingdom. Methanol, sodium dihydrogen phosphate, orthophosphoric acid and HCl were purchased from Merck, Germany. Distilled water was freshly prepared in LC/MS lab no. 25 of department.

## 3. Methods

### 3.1. Synthesis of β-CD-g-poly (AMPS) hydrogel microparticles

Aqueous free radical polymerization method was used for preparation of β-CD-g-poly (AMPS) based hydrogel microparticles by using APS as initiator and MBA as crosslinker. Required quantities of β-CD and AMPS as shown in Table 1 were accurately weighed on electronic weighing balance (Shimadzu AUW 220D, Japan). β-CD and AMPS were carefully and separately poured into beakers containing 20 ml distil water in each. Both beakers were subjected to stirring on hot plate magnetic stirrer at 55 °C until clear solutions were formed. Amount of MBA was weighed and poured into AMPS solution with continuous stirring at 250 rpm until clarity of solution. APS was weighed and poured into β-CD solution to initiate polymerization reaction. AMPS and MBA solution was added dropwise into polymer solution with continuous stirring. Whole mixture was sonicated (5min), vortexed (MS2- Minishaker IKA) (3min) and finally purged with nitrogen gas for 20min to assure proper mixing and removal of any dissolved oxygen. Prepared polymeric solution was carefully transferred to clean and dry glass test tubes. Test tubes were sealed with aluminium foils and placed in programmable hot water bath (Memmert) at 55 °C for 4 h, 60 °C for 8 h, 65 °C for 8 h and at 70 °C for 4 h. Upon solidification, test tubes were removed and placed in wooden racks to attain room temperature. Test tubes were broken and hydrogels were cut into small pieces. Resultant particles were washed thoroughly with methanol water mixture (50: 50) to remove unreacted species. Washing was continued until constant pH value of washing solution was attained by pH meter. Particles were separated by using mesh and shifted to petridishes. These were freeze dried in lyophillizer (Christ Alpha 1–4 LD, Japan) at −57 °C for 24 h. Resultant fluffy particles were passed through sieve no.80 to obtain hydrogel microparticles of uniform size. These were stored in air tight containers for further analysis [15]. During preparation different ratios of polymer and monomer with varying concentrations of crosslinker were prepared to check the effect of β-CD, AMPS and MBA on swelling and ACV release.

### 3.2. Drug loading

Diffusion assisted swelling method was adapted for acyclovir loading in microparticles. 1% acyclovir solution was prepared in 0.1 mol/L HCl solution based on solubility of ACV. Dried hydrogel microparticles were accurately weighed, poured into ACV solution and allowed to stand for 24 h at room temperature. Microparticles were separated from solution by using mesh and were flushed with distilled water to remove ACV contents on surface of microparticles. Drying was performed at 40 °C in hot air oven (Memmert, Japan) [2,16].

### 3.3. Micromeritic properties

All prepared formulations (BA-1 – BA-9) were subjected to micromeritic evaluation i.e. angle of repose, bulk density, tapped density, Carr's compressibility index and Hausner ratio. Results were calculated by using following formulas;