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PREPARATION AND EVALUATION OF MICROCRYSTALS OF CEFUROXIME AXETIL

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ABSTRACT: Cefuroxime axetil (CA) is an orally absorbed pro-drug of cefuroxime; having poor aqueous solubility. Aim of the present study was to prepare its microcrystals by emulsion solvent diffusion method with various surfactants (HPMC E-5 and E-15; PVP K-30 and K-90; β-cyclodextrin; HP-β-cyclodextrin) to foster its solubility and dissolution rate. Manufacturing of microcrystals implies creation of additional surface area and hence interface. Microcrystals were screened for apparent solubility, dissolution rate, morphology (SEM) and crystallinity (XRD and DSC). Microcrystals prepared with HPMC E-15LV showed highest solubility and dissolution rate than the untreated drug and the crystals prepared with other surfactants. C-6 crystals (Prepared with HPMC E-15LV) were passed through accelerated stability and wettability studies and observed to be improved.

KEY WORDS: Cefuroxime axetil, microcrystals, emulsion solvent diffusion.

1. INTRODUCTION

Cefuroxime is the first commercially available second generation cephalosporin to be widely used in the therapy; it is a semi-synthetic cephalosporin obtained from the 7-cehalosporanic acid nucleus of cephalosporin C[1]]. Since cefuroxime is not absorbed orally, cefuroxime axetil (CA) (1-acetoxyethyl ester of a β-lactamase-stable cephalosporin), an orally absorbed pro-drug of cefuroxime is used in the treatment of common community acquired infections because of its in-vitro antibacterial activity against several gram-positive and gram-negative organisms[2-4]]. It has a carbamoyl group at position 3, which gives it a considerable metabolic stability and in position 7 it has a methoxy-imino group which makes it more stable against β-lactamase attack. Together with the furyl ring these groups contribute to the antibacterial properties of the molecule by enhancing its activity against gram-negative bacteria[5]. Despite the potential of CA as an antibacterial agent, some problems have been derived from its poor aqueous solubility and instability represents some challenges for pharmaceutical researchers[6,7].

Numerous approaches have been employed to enhance the dissolution profile and, in turn, the absorption efficiency and bioavailability of poorly water soluble drugs. Use of water-soluble salts, polymorphic forms, water-soluble molecular complexes, solid dispersion, co-precipitation, lyophilization, micro-encapsulation, and inclusion of drug solutions or liquid drugs into soft gelatin capsules are some of the major formulation tools which have been shown to enhance the dissolution characteristics of water-insoluble drugs[8-11].

A common method for increasing the dissolution rate is formation of high specific surface area by micronizaton. It implies additional surface area and hence interfaces; which increases Gibb's free energy of the system and results in aggregation of the particles formed[12]. In these cases surfactants plays an important role as stabilizer. In the present study, microcrystals of CA were prepared by emulsion solvent diffusion process to enhance its solubility and dissolution rate using various surfactants. Surfactants were selected on the basis of their properties like aqueous solubility, low cost and less toxicity. Prepared microcrystals were screened for solubility, dissolution rate, morphology, crystallinity, wettability and stability.

2. MATERIALS AND METHODS

2.1 Materials

Cefuroxime axetil (CA) was procured as a gift sample from Okasa Pharma (Satara, India). Methocel (HPMC, E-5LV and E-15LV) were supplied by Colorcon, (Goa, India). Povidone (PVP, K-30 and K-90), β-cyclodextrin (β-CD) and Hydroxypropyl-β-Cyclodextrin (HP-β-CD) were procured from Alembic (Vadodara, India). Acetone

and hydrochloric acid (HCl) were of AR grade (S. D. Fine, Mumbai, India).

2.2 Preparation of Microcrystals

Microcrystals of CA were prepared by emulsion solvent diffusion method. Briefly, a weighed quantity of CA (1 g) was dissolved in 5 ml of acetone. This phase was added at room temperature, under constant mechanical stirring (2000 rpm, Remi Stirrer, Mumbai, India) to 100 ml aqueous solution of surfactants (Table I). Stirring was continued for 30 min. Crystals were collected by filtration and dried at room temperature.

2.3 Drug Content

A weighed quantity of the crystals was dispersed in 10 ml of 0.07 N HCl. It was sonicated for 10 min and centrifuged at 2000 rpm for 10 min. The supernatant was diluted with suitable quantity of 0.07 N HCl and analyzed by UV-Visible Spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) at 278 nm.

2.4 Apparent Solubility

Saturation solubility measurements were assayed through ultraviolet absorbance determination at 278 nm using UV-Visible Spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). To 10 ml of the simulated intestinal fluid excess quantity of samples (50 mg) were added. Apparent solubility study was performed by standardized shake flask method at 37 ^{0}C[13]. After shaking for 48 hrs, the samples were filtered through 0.2 µm membrane filters (PALL life Sciences, Mumbai, India) and the filtrate was analyzed for drug content. Each sample was tested in triplicate.

2.5 In-Vitro Dissolution Studies

A LabIndia Disso 2000 dissolution test apparatus type II (Paddle) at rotation speed of 100 rpm was used for the study. Dissolution of the untreated drug and microcrystals was carried out on an equivalent of 250 mg of the CA. HCl (0.07 N) was used as dissolution media. The volume and temperature of the dissolution media were 900 ml and 37 ± 0.2 ^{0}C, respectively. After fixed time intervals, 5 ml of samples were withdrawn (sink condition was maintained) and assayed through ultraviolet absorbance measurement at 278 nm using UV-Visible Spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) by an analytically validated method (r^2 = 0.9997). Each sample was tested in triplicate.

2.6 Scanning Electron Microscopy (SEM)

Morphological evaluation of the untreated drug and microcrystals was carried out by JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan). Samples were fixed on aluminum stubs with conductive double sided adhesive tape and coated with the gold by sputter coater at 50 mA for 50 s.

2.7 Powder X-Ray Diffraction (PXRD)

Physical state of the raw material and the crystals was characterized by Philips Analytical X-RD (Model: PW 3710, Holland) with copper target. The conditions were: 40 kV voltage; 30 mA current; at room temperature. The scanning angle ranged from 5 to 60 ^{0} of 2θ, steps were 0.02 ^{0} of 2θ and the counting rate was 0.4 s/step.

2.8 Differential Scanning Calorimentry (DSC)

Phase transition of the untreated drug and the crystals were analyzed by DSC (Universal V2.4F TA Instruments, USA, Model: SDT 2960). The samples were heated in a hermetically sealed aluminum pans. Temperature range for each sample was set from 30 to 350 ^{0}C at a heating rate of 10 ^{0}C/ min, using nitrogen as purging gas.

2.9 Stability studies

Accelerated stability study of an optimized sample (C-6) was carried out as per ICH guidelines. C-6 crystals (each 10 mg, n=3) were kept for stability studies at 40 ± 2 ^{0}C and 75 ± 5 % RH in an environmental test chamber (HMG INDIA, Mumbai, India) for a period of 3 months. These samples were kept in glass vials without rubber plugs. After 30, 60 and 90 days, the samples were taken out and analyzed for the drug content.

2.10 Wettability

Wettability of the drug and C-6 crystals was carried out by powder bed hydrophilicity test[14]. The untreated drug and C-6 crystals were placed (1 g) on a sintered glass disk forming bottom of glass tube on which methylene blue crystals were placed. The whole device was brought in contact with water. The time taken for the capillary rising of water to the surface so as to dissolve methylene blue crystals was noted.

3. RESULTS AND DISCUSSION

3.1 Preparation of Microcrystals

Microcrystals of CA were prepared by emulsion solvent diffusion method using acetone. Same process was tried by using ethanol, methanol and methylene chloride; however in entire cases drug becomes sticky and gets adhered to the stirrer blades whereas with acetone this problem was less evident. Concentration of the surfactants was optimized to reduce sticking of the drug to the stirrer blades. The lowest concentration at which problem of sticking reduced to remarkable extent were determined and used in formulation. Optimized concentrations of the surfactants are given in Table 1. HPMC was observed to be efficient stabilizer; at lower concentrations it has reduced sticking of crystals to stirrer blades. It may be due to the higher affinity of HPMC towards newly formed surfaces; at lower concentration it covers the surface of these particles completely and reduces stickiness of the crystals formed. Crystal growth was inhibited by adsorbed stabilizers[7]. Stirring was carried out at constant rate of 2000 rpm. The process was carried out using baffles which have increased its efficiency by creating a turbulent flow and avoided vortex formation due to high stirring rate.

3.2 Drug Content, Solubility and In-Vitro Release Study

For untreated drug the drug content was considered to be 100 %; however drug content of the microcrystals was found to be in the range of 98.02 ± 0.22 to 99.11 ± 0.15 %. Results of the solubility studies are given in Table 1. CA/HPMC E-15LV microcrystals (C-6, 0.253 ± 0.004 mg/ml) showed highest solubility as compared to the

untreated drug (0.084 ± 0.003 mg/ml) and the microcrystals prepared with other surfactants.

Fig. **1** dictates the results of dissolution studies. CA/HPMC E-15LV microcrystals (C-6) showed fastest dissolution rate, with approximately 50 % of the drug being released within 20 min compared to the microcrystals prepared with other surfactants and just 18.32 % for the control (untreated drug). More than 90 % of the drug from C-6 crystals was dissolved in 75 min and; however the untreated drug (52.93 %) did not achieve complete dissolution up to the end of 90 min testing period. Microcrystals have shown higher dissolution rate than the raw material used. It may be due to the surfactants used; which covers the newly formed surfaces spontaneously. For microcrystals, dissolution creates a local surfactant concentration in the boundary layer surrounding the drug particles, providing a lower energy pathway for drug dissolution[15]. HPMC concentration of 0.1 % w/v relative to other surfactants was found optimal. HPMC E-15LV (C-6 crystals) presented a dramatic increase in the solubility and dissolution rate of the drug. Cyclodextrin[16] is mostly used to form an inclusion complex whereas povidone[16] and HPMC[17] are the well known stabilizers in the solubility and dissolution rate enhancement experiments. HPMC of low viscosity grades were used for the microcrystals stabilization as these water soluble polymers offers adequate surface active properties when compared to other commonly used surfactants. Reduced particle size of the crystals, increases their specific surface area as well as the adsorption of the surfactants on the surface of these crystals enhancing its wettability. Moreover, the solubility results advocate to the output of dissolution studies. Based on Noyes-Whitney equation an increase in saturation solubility leads to an increase in dissolution velocity[18] .

3.3 SEM, XRD and DSC Analysis

The SEM photomicrographs of the untreated drug and the crystals are shown in Fig. **2**. Pure CA was characterized with larger particle size (Fig. **2**, CA). In contrast, microcrystals were with rough surfaces indicating adsorption of the surfactants on their surfaces. CA/HPMC microcrystals (Fig. **2**, C-6) indicate smaller particle size with rough surface area. According to the Rasneck, if a hydrophobic substance is inserted to the stabilizer molecule, the drug particle size decreases[12] . Rough surface area of the crystals is due to the presence of surfactants which result in higher dissolution rate.

Powder X-ray diffraction pattern confirmed physical nature of the raw material and microcrystals (Fig. **3)**. X-ray diffractogram of raw material did not show any peak indicating its amorphous nature (Fig. **3**, CA). Similarly, C-1, C-2 and C-4 crystals have shown less intense peaks at 8.855, 9.35 and 45.225 (2θ angles) respectively dictating retention of the partial amorphous form of the drug. In contrast, C-3 (2θ = 21.185, 18.29, 15.865, 22.465), C-5 (2θ = 21.205, 18.445, 24.855, 22.735) and C-6 (2θ = 18.245, 18.965, 20.385, 21.120, 22.535, 25.355) crystals exhibit well defined characteristic peaks manifestating conversion to crystalline forms. This difference was attributed to the presence of HPMC. These results confirm that the change in the nature of the particles is due to the dilution of the particles with surfactants rather than from particle size.

Thermal properties of the drug and the samples were determined by DSC (Fig. **4**). The untreated drug showed a slight bending of the line at 115.64 ^{0}C corresponding to its glass transition temperature indicating its amorphous nature. However, C-1, C-2 and C-4 microcrystals showed endothermic peaks at 95.19, 104.28 and 131.54 ^{0}C corresponding to melting of the drug whereas C-3 crystals displayed a small diffuse endothermic peak at 52.08 ^{0}C. In contrast, C-5 and C-6 crystals did not show any endothermic peak. These modifications are clearly attributed to the presence of HPMC.

3.4 Wettability and Stability Studies

The drug and optimized crystals were passed through the powder bed hydrophilicity test to determine its wetting properties. For C-6 crystals methylene blue crystals wet after 31 min. In contrast, up to the same time methylene blue crystals were not wet for the untreated drug, indicating an increased wetting property of the C-6 crystals.

CA/HPMC E-15LV (C-6) crystals were screened for accelerated stability studies and did not show any physical changes during the study period. The drug content was observed to be (n = 3, mean ± S.D.): 99.07 ± 0.04 % after 30 days; 98.56 ± 0.11 % after 60 days and 98.14 ± 0.07 % after 90 days indicating that C-6 crystals are quite stable at accelerated storage conditions. This improved stability and wettability of the drug are ascribed to the presence of surfactants on its surface.

4. CONCLUSION

Results of the present investigation lead to a conclusion that reduced size of the drug particles with presence of the surfactants on the surface of CA microcrystals are responsible for meteoric rise in solubility and dissolution velocity along with stability. HPMC E-15LV was found to be the best surfactant in solubility and dissolution rate enchantment of CA.

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Table 1: Surfactants used with its concentration, the drug content and apparent solubility of the drug and microcrystals

Batch	Batch ID	Concentration of Surfactants (% w/v)	Drug Content* (%)	Solubility* (mg/ml)
CA	CA	-	100.00 ± 0.00	0.084 ± 0.003
CA/β-CD	C-1	0.20	98.30 ± 0.08	0.121 ± 0.002
CA/HP-β-CD	C-2	0.20	98.02 ± 0.22	0.152 ± 0.003
CA/PVP-K30	C-3	0.20	98. 45 ± 0.12	0.138 ± 0.018
CA/PVP-K90	C-4	0.20	99.11 ± 0.15	0.146 ± 0.004
CA/HPMC E-5	C-5	0.10	98.08 ± 0.14	0.203 ± 0.002
CA/HPMC E-15	C-6	0.10	98.53 ± 0.19	0.250 ± 0.004

CA- Cefuroxime axetil, * n = 3, mean ± S.D.

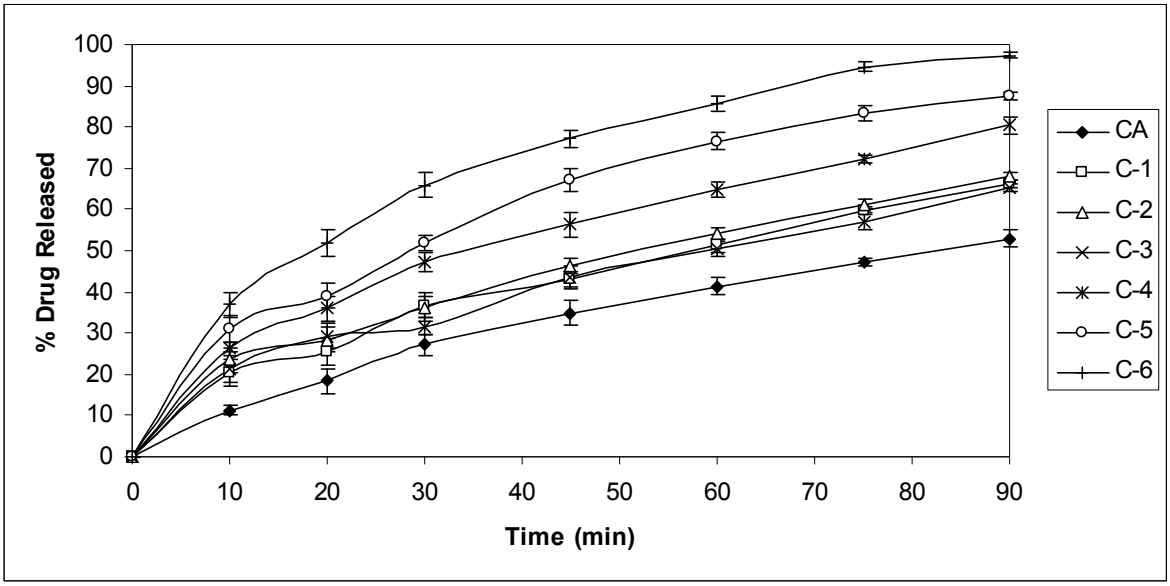

Figure 1: Dissolution profile of (CA, ⊣◆⊢) cefuroxime axetil and crystals prepared with (C-1,⊣□⊢) β-CD, (C-2, ⊣△⊢) HP-β-CD, (C-3, ⊣×⊢) PVP K-30, (C-4, ⊣✳⊢) PVP K-90, (C-5, ⊣○⊢) HPMC E-5LV and (C-6, ⊣+⊢) HPMC E-15LV.

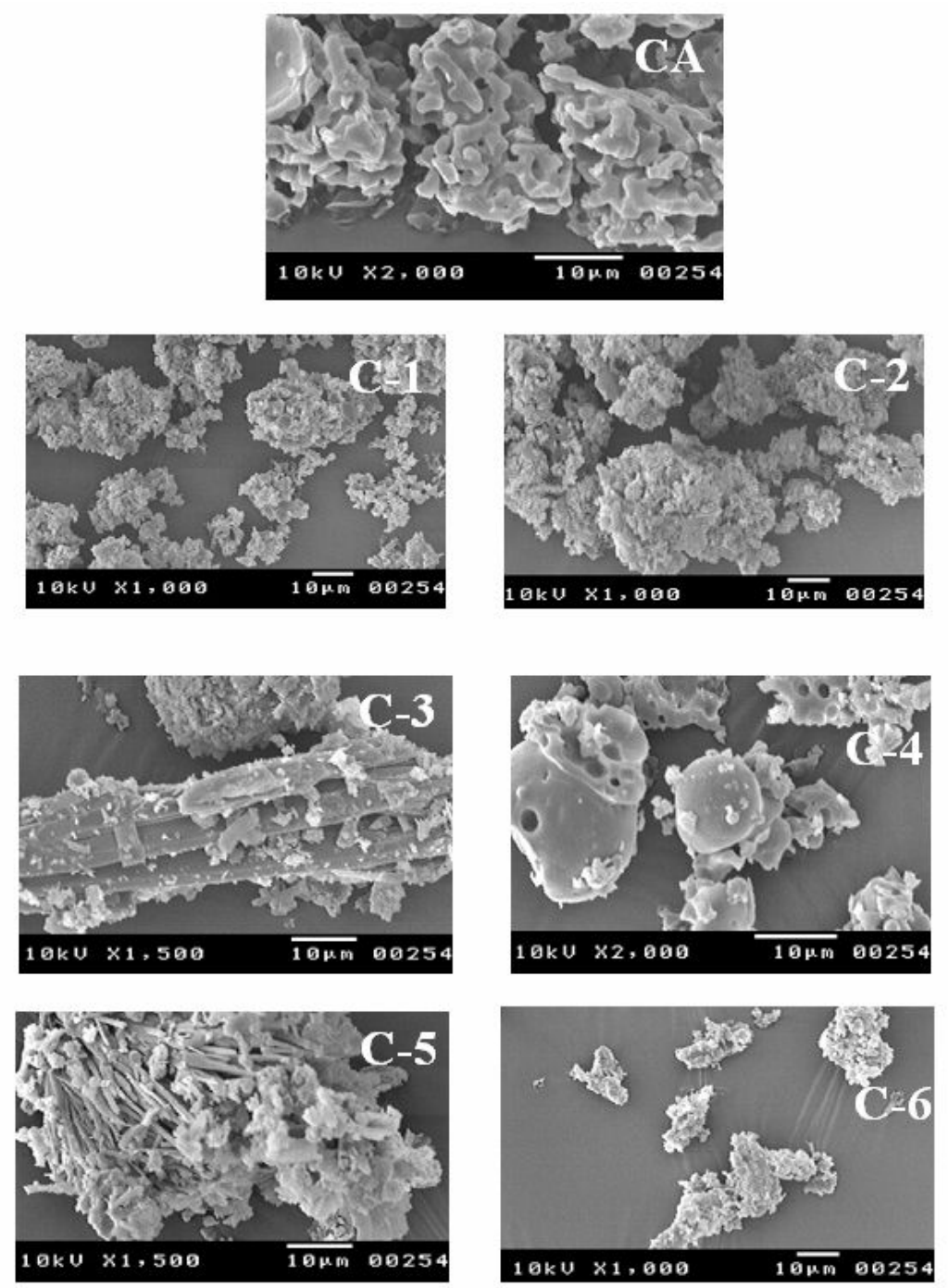

Figure 2: SEM photomicrographs of (CA) cefuroxime axetil and crystals prepared with (C-1) β-CD, (C-2) HP-β-CD, (C-3) PVP K-30, (C-4) PVP K-90, (C-5) HPMC E-5LV and (C-6) HPMC E-15LV

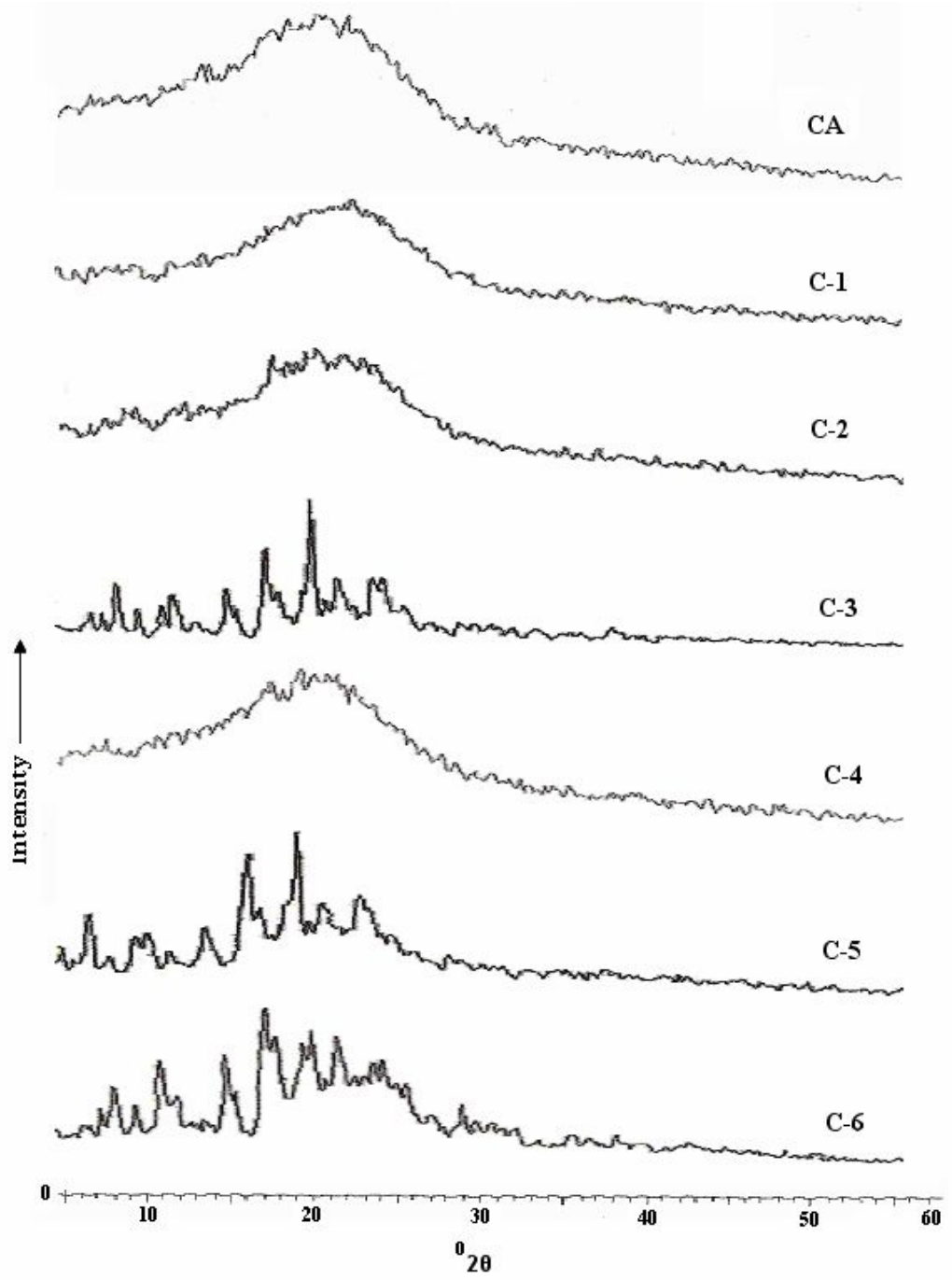

Figure 3: X-ray diffraction pattern of (CA) cefuroxime axetil and crystals prepared with (C-1) β-CD, (C-2) HP-β-CD, (C-3) PVP K-30, (C-4) PVP K-90, (C-5) HPMC E-5LV and (C-6) HPMC E-15LV.

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