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Research Article

Enhanced dissolution and solubility of Epalrestat with β -Cyclodextrin ternary complex using Arginine

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ABSTRACT

The objective of present is work to achieve improvement in solubility and dissolution using Epalrestat with β -cyclodextrin ternary complex with addition of L-arginine. Kneading method with and without incorporation of L-arginine was used to obtain the solid systems (binary and ternary complex) of Epalrestat with β -cyclodextrin and characterized for DSC, PXRD, SEM. Phase solubility, saturation solubility, dissolution and stability studies were carried out. The effect of L-arginine was investigated on complexation efficiency of β -cyclodextrin towards Epalrestat in an aqueous media through phase solubility according to Higuchi and Connors. Phase solubility studies showed A_L (linear) type of solubility curve in presence of L-arginine, the complexation efficiency and association constant of β -cyclodextrin towards Epalrestat were promoted by L-arginine signifying its use as a ternary component. Dissolution rate of Epalrestat and solubility were significantly improved by complexation with β -cyclodextrin as compared to Epalrestat alone. Ternary complex incorporated with L-arginine proved better than binary complex. Hence, L-arginine could be exploited as a ternary component to improve the solubility of Epalrestat via β -cyclodextrin complexation.

Keywords: Epalrestat, solubility enhancement, inclusion complexes, β -cyclodextrin, L-arginine.

Article Info: Received 26 Sep 2018; Review Completed 24 Oct 2018; Accepted 25 Oct 2018; Available online 15 Nov 2018



Cite this article as:

Jagtap S, Magdum C, Enhanced dissolution and solubility of Epalrestat with β -Cyclodextrin ternary complex using Arginine, Journal of Drug Delivery and Therapeutics. 2018; 8(6):62-67
DOI: <http://dx.doi.org/10.22270/jddt.v8i6.2014>

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1. INTRODUCTION

Epalrestat (EPL), 2-[[5Z]-5-[(E)-2-methyl-3-phenylprop-2-enylidene]-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl]acetic acid is a water-insoluble drug ($14\mu\text{M}$) that inhibits aldose reductase.¹ The most common long-term complication in patients with diabetes mellitus is diabetic neuropathy.² And EPL is used in its treatment.³ in hyperglycemic conditions; EPL reduces intracellular sorbitol accumulation, which can be efficacious in treating diabetic neuropathy. Main complications in patients with diabetes mellitus are neuropathy, retinopathy and nephropathy.^{4,5} Cyclodextrins (CDs) have mainly been used as complexing agents to increase aqueous solubility, bioavailability and stability of drugs by the pharmaceutical industries. CDs are cyclic α -(1,4)-linked oligosaccharides of α -D-glucopyranose containing a relatively hydrophobic central cavity and a hydrophilic external surface.^{6,7} In present research paper, we proposed to improve solubility and the dissolution behavior of EPL by β -cyclodextrin (CD) and its ternary complex with L-arginine (ARG). CDs have the ability to form inclusion complex with many drug molecules entrapping the guest molecule partially or

completely within the cyclodextrin cavity thus resulting in an increase in the solubility of the drug. First, phase solubility studies were performed, which confirmed complexation of β -CD and EPL in aqueous solution. Numerous investigations reported that, incorporation of small quantity of hydrophilic excipients such as organic acids, amino acids, basic compounds, and polymers to an aqueous complexation medium usually give rise to an enhancement in the complexation efficiency of cyclodextrins and ultimately reduce their amounts in pharmaceutical formulations.⁸ The purpose of the current study was to ameliorate the aqueous solubility, dissolution rate and thus bioavailability of EPL and this is achieved by ternary complexation of β -CD with EPL employing amino acids.⁹⁻¹³ The solubility of EPL in absence of β -CD, Intrinsic solubility (S_0) was determined. Complexation with ARG also promoted apparent stability constant (Ks) of ternary as compared to binary complex. The basic amino acid, ARG was proved to be a better option as an auxiliary substance to increase complexation efficiency (CE) of CDs in ternary complex.

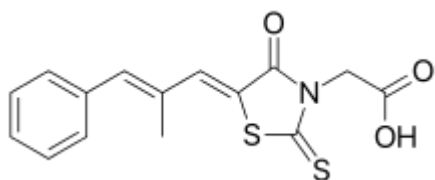


Figure 1: Chemical Structure of EPL

Thus considering the upper hand advantage of adding amino acid as a ternary component during complexation of drug with β -CD, the present investigation was carried out to contribute the promotion of the solubility of poorly water-soluble drug, EPL via β -CD complexation using ARG as an auxiliary substance, which to our knowledge has not been reported yet.

2. MATERIALS AND METHODS

2.1. Materials

Eplrestat was a generous gift from Zydus Cadila Ahmedabad (India). β -CD and ARG were purchased from unique chemicals. All other reagents and solvents used were of analytical grade.

2.2. Analysis of Eplrestat using a UV-visible spectrophotometer

First, 100 μ g/ml stock solution of Eplrestat was prepared in methanol and further dilutions were made in distilled water ranging from 0.3–18 μ g/ml. The calibration curve was obtained by recording the absorbance on a UV spectrophotometer (Shimadzu UV spectrophotometer 1800) at 390 nm.¹⁴ Linearity in the calibration curve was observed with regression $R^2 = 0.994$.

2.3. Phase solubility studies

The solubility behavior of EPL was examined in distilled water at room temperature (25 ± 2 °C) according to the method described by (Higuchi and Connors 1965) [15]. Excess amount of EPL (50mg) was added to 20 mL of aqueous solutions containing various concentrations of β -CD (1mM –10mM) with or without addition of ARG (0.25% w/v). The suspensions were mechanically shaken subsequently on rotary shaker for 72 h at 125 rpm until equilibrium was achieved. The samples were filtered through Whatman filter paper 41, diluted if necessary and analyzed spectrophotometrically (Shimadzu UV spectrophotometer 1800) at 390 nm. The association constant (K_s) of complex and CE of β -CD were calculated according to the equations (1) and (2) respectively^{1, 16}.

$$K_s = \text{Slope} / S_0 (1-\text{Slope}) \quad (1)$$

S_0 is the solubility of EPL in absence of β -CD and slope is obtained from the phase solubility diagram obtained by plotting moles of drug on y-axis and moles of β -CD on x-axis. It gives idea about the linear dependence of drug concentration to β -CD concentration, with slope ratio below one usually assumes 1:1 ratio of the complex and refers to A_L (linear) type of the phase solubility curve.

$$CE = S_0.K_{1:1} = \text{Slope} / (1-\text{Slope}) \quad (2)$$

2.4. Preparation of Binary and kneaded ternary complexes

Binary complex (BC) – For binary complex EPL and β -CD were weighed accurately, mixed thoroughly by triturating in a mortar and sieved through a 120 sieve. Binary complex were stored in a desiccator until further evaluation.

Kneaded complex (KC) – The kneaded complex of EPL, β -CD and amino acid was prepared by wetting the physical mixture in a mortar with a minimum volume of ethanol/water mixture (15/85, V/V) and kneading thoroughly with a pestle to obtain a paste, which was then dried under vacuum at room temperature, sieved through a 120 sieve and stored in a desiccator until further evaluation.

2.5. Fourier transform infrared spectroscopy (FTIR) Analysis

FTIR spectroscopy was performed on a Fourier Transform Infrared Spectrometer (Alpha T Bruker). Spectra were collected in the 4000–400 cm^{-1} range.

2.6. Differential scanning calorimetry (DSC) Analysis

Thermograms of EPL, β -CD, ternary complex were recorded on a Mettler Toledo, Staresw 920. Each sample (2–4mg) was heated in crimped aluminum pans at a scanning rate of 10°C/min in an atmosphere of nitrogen using the range of 100–240°C. The temperature calibrations were performed periodically using indium as a standard.

2.7. Powder X-ray diffractometry (PXRD) Analysis

To obtain the changes in the crystallinity of the ternary complex prepared, the PXRD study was carried out by using X ray diffractometer (Miniflex 600 X-Ray Diffractometer Rigaku Corporation Japan). For this the samples of pure drug, β -CD, and prepared ternary complex were irradiated with monochromatised $\text{CuK}\alpha$ radiation and analyzed between from 5° to 60° (2 θ).

2.8. Scanning electron microscopy (SEM) Analysis

The surface morphological features of EPL, binary, ternary complex were investigated using a scanning electron microscope (VEG A3 TESCAN) operated at an acceleration voltage of 20 kV and obtained microphotographs were examined at X500 and X2000 magnifications.

2.9. Saturation solubility studies

Saturation solubility studies of EPL, binary and ternary complexes were conducted in triplicate. An excess amount of EPL and complexes were added to 20 mL of distilled water in vials sealed with stoppers and shaken in rotary flask shaker at room temperature (25 ± 0.5 °C) for 24 h. A portion of solution was withdrawn, filtered through Whatman filter paper 41 and analyzed spectrophotometrically (Shimadzu UV spectrophotometer 1800) at 390 nm.¹⁶

2.10. Dissolution studies

In vitro dissolution studies for EPL and its binary and ternary complexes were carried out using USP Type-II dissolution test apparatus (EDT 08LX Electrolab). Sample equal to 100 mg of EPL was used in each test. The dissolution tests were performed using 900 ml of phosphate buffer pH 6.8 at 37 ± 0.5 °C with RPM maintained at 100. The release of EPL from each sample was determined by withdrawing 5 ml samples at preset time intervals, and were filtered, appropriately diluted and analyzed spectrophotometrically (Shimadzu UV spectrophotometer 1800) at 390 nm. 5 ml of fresh medium was transferred to maintain sink conditions.

2.11. Stability Study

Stability study binary and ternary complexes of EPL was carried out with the help of (Remi SC-19 Plus) by storing 1gm of each selected complex in an ambered coloured

screw capped glass bottles at accelerated and controlled temperatures 40°C and relative humidities (75%) for a period of 3 months. The complexes were evaluated for physical appearance, drug content and in-vitro dissolution at the end of three months.⁸

3. RESULTS AND DISCUSSION

3.1. Phase solubility studies

The complexing nature of EPL along with β -CD in water was studied by the phase solubility as per standards set by Higuchi and Connors 1965. Fig.2 shows the phase solubility diagrams of EPL with β -CD and that of ARG. A typical Bs-type solubility profile as stated by Higuchi and Connors classification system was shown by the EPL and β -CD, binary complex. Bs-type response stands for development of inclusion complexes of limited solubility within the range of β -CD concentrations examined. The solubility of EPL with β -CD was higher than pure EPL. The phase solubility profile of EPL in aqueous β -CD solution in presence of 0.25% ARG displayed A_L type of solubility curve with linear increase in solubility of EPL with increasing the concentration of β -CD. And slopes curves of phase solubility profile were lesser than 1 characterizing the formation of a 1:1 stoichiometry water soluble complex.¹⁵⁻¹⁸ The values of K_s and CE of complex increased with the incorporation of ARG to the binary complex showing more effectiveness of ternary system over binary. The values of K_s for binary complex and ternary complex were found to be 505.18 and 876.47 respectively. Complexation efficiency of binary complex was found to be 0.24 and for ternary complex it was 0.48 which is almost double of binary complex. Advancement in phase solubility

parameters of EPL by complexation with β -CD in presence of ARG can be attributed to its electrostatic/hydrogen bonding interaction and salt formation with β -CD and EPL.^{19,20}

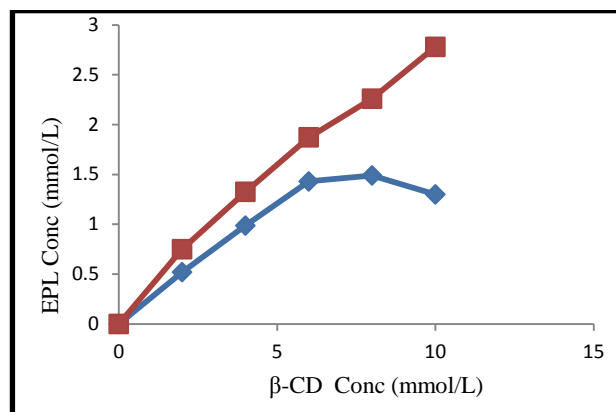


Figure 2: Phase solubility diagrams of EPL: β -CD (Binary complex) and EPL: β -CD: ARG (ternary complex) in water.

3.2. FTIR measurements

The molecular interaction between EPL and β -CD was revealed by FTIR. Fig.3 shows the FTIR spectra of binary and ternary complex. For EPL, principle absorption peaks were found near to 1742, 1673, and 1555 cm^{-1} from carboxyl, amide, and thiocarbonyl stretching vibrations, respectively. All these principle characteristic peaks were retained and did not shift both in binary and ternary complex. As the results, it is concluded that EPL is dispersed uniformly in the β -CD.

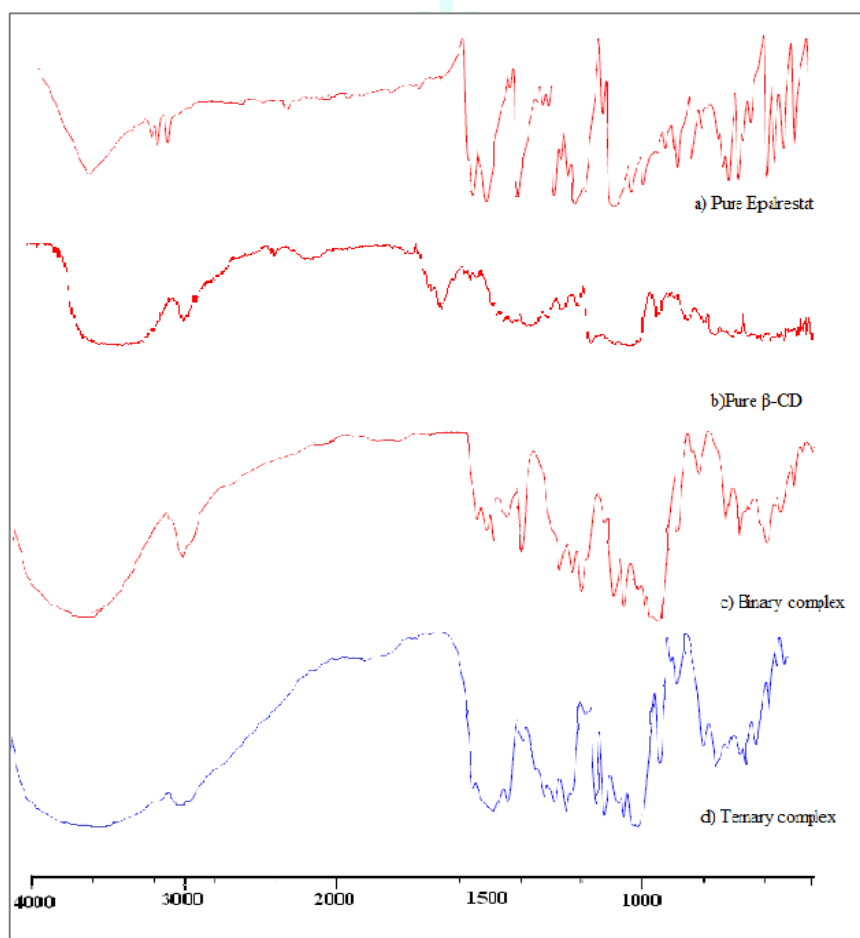


Figure 3: FTIR spectra of A) EPL B) Pure β -CD C) Binary complex D) EPL: β -CD:ARG ternary system

3.3. DSC measurements

DSC analyses were performed to evaluate the thermal behavior of EPL, β -CD and ternary complex, respectively (Fig. 4) and interaction between EPL and β -CD in complex formation. When guest molecule incorporated into cyclodextrin central cavity, their melting point shift to different temperature or it may disappear. One sharp endothermic peak was observed near around 215°C contributed to the fusion of crystalline EPL in the ternary

thermograms broad endothermic peak was observed near around 140°C, which can be attributed to dehydration during the heating process of the β -CD. In the ternary complex, the endothermic peak obtained from EPL decreased or almost disappeared. It may be due to low interaction between the components in the ternary complex. These results suggested that EPL and β -CD were molecularly dispersed in the amorphous form in ternary complex.

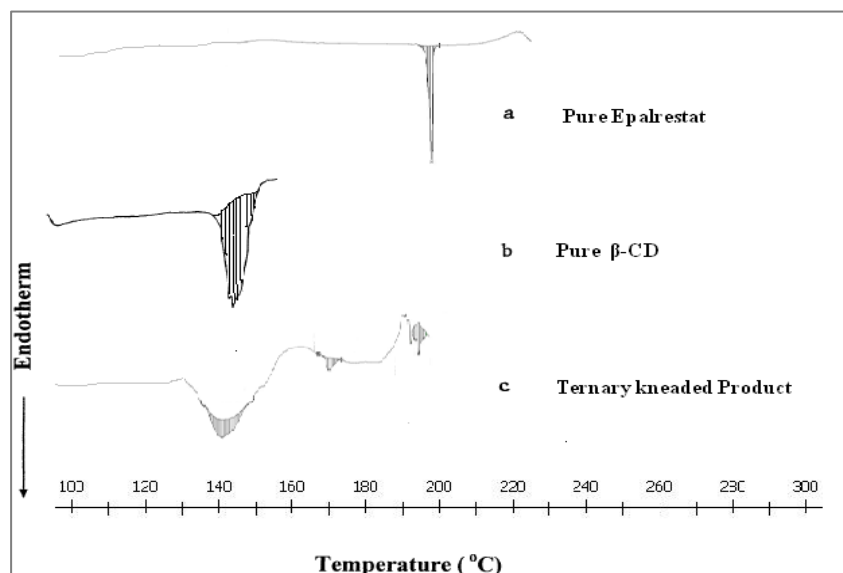


Figure 4: DSC thermograms of A) EPL B) Binary complex C) EPL: β -CD:ARG ternary system

3.4. PXRD measurements

PXRD patterns of EPL, binary and ternary complex (molar ratio 1:1) are represented in Fig. 5. The pattern of the ternary complex could be interpreted as superposition of EPL and β -CD representing absences of crystalline traces of EPL indicating entrapment of EPL in β -CD. In ternary

systems, the overlapping of β -CD and ARG crystalline peaks was observed. The PXRD showed a halo pattern and no diffraction peak occurred when EPL was kneaded with β -CD at a 1:1 molar ratio. Crystalline EPL turned amorphous by kneading with β -CD. It was revealed that the crystalline structure of EPL was distorted and EPL molecules were converted into amorphous state.

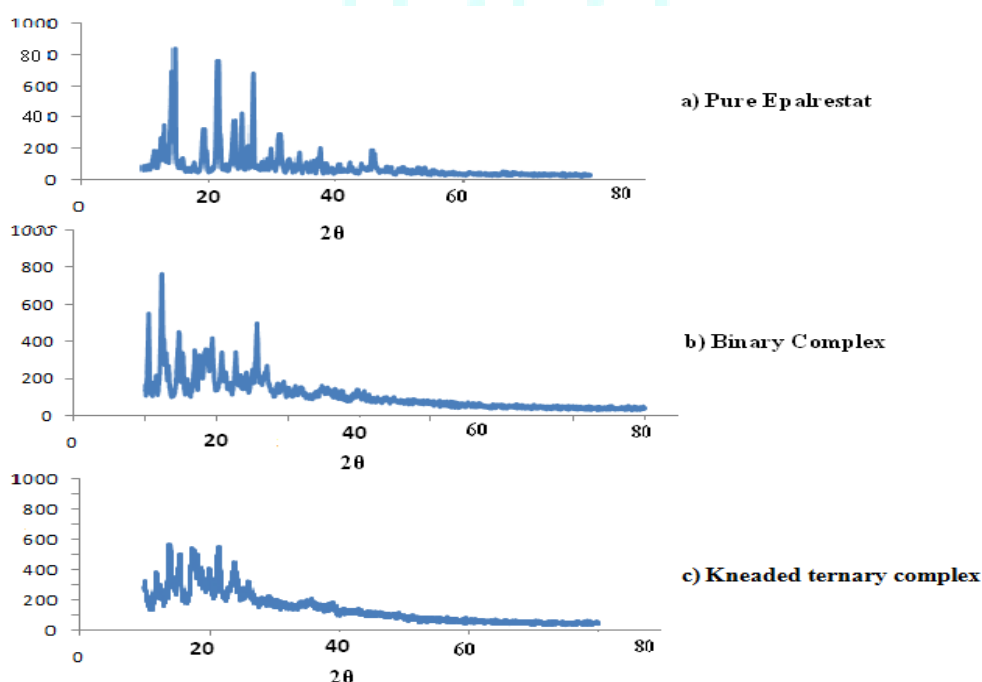


Figure 5: PXRD patterns of A) EPL B) Binary complex C) EPL: β -CD:ARG ternary complex

3.5. SEM analysis

The surface morphological features of EPL and ternary complexes are shown in Fig. 6. Separate entities of amorphous broken particles of Pure EPL were appeared (Fig. 6A). Binary complex particles exhibited altered shape and agglomerate type morphology (Fig. 6B) due to

crystalline nature of β -CD. Change in morphology of ternary complexes was observed due to the crystalline nature of β -CD and ARG (Fig. 6C) showing agglomerated crystalline images. The alerted morphology of kneaded complexes particles indicates existence of a single phase within the complex attaining maximum complexation.²¹

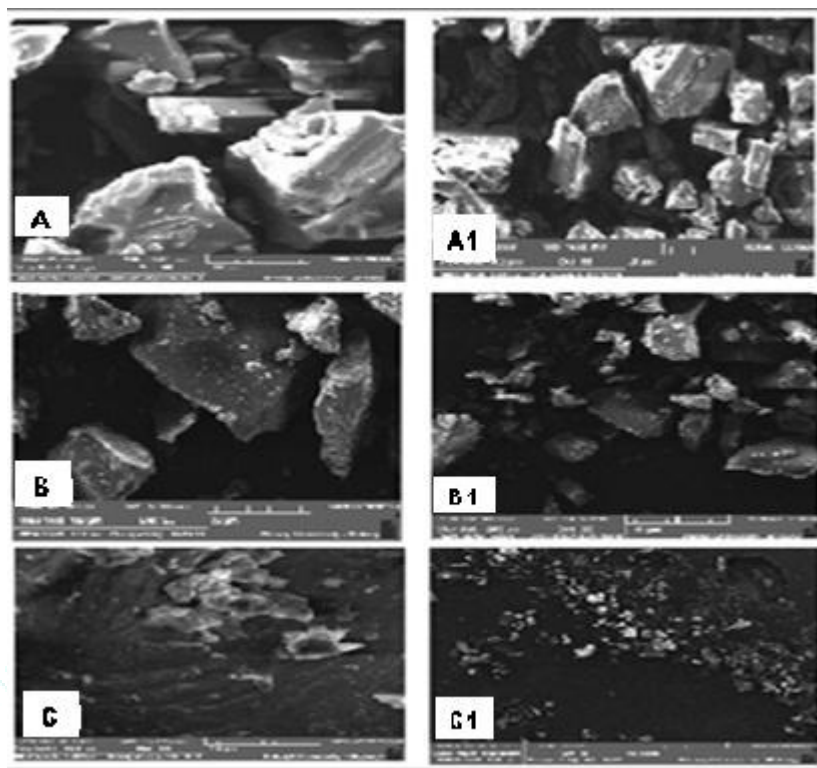


Figure 6: SEM images of A) Pure EPL B) Binary complex C) EPL: β -CD:ARG ternary complex

3.6. Saturation solubility studies

Remarkable enhancement in the solubility of binary and ternary complex as compared to pure EPL was observed in the saturation solubility studies. Pure EPL exhibited a solubility of $47 \pm 0.04 \mu\text{g/mL}$ in distilled water. The binary and ternary complexes have shown solubility of 98.7 ± 0.30 and $163.09 \pm 0.72 \mu\text{g/mL}$, respectively. There was 210% increment in the solubility of binary complex and 347% increment in the solubility of ternary complex was observed which was almost double than binary complex. Formation of stable complex of EPL and β -CD, altered surface morphological features of the complexes due to kneading and inclusion into the hydrophobic β -CD cavity resulted in the enhancement in solubility of complex. ARG imparts significant role as a ternary component resulting in improved performance than binary complex²².

3.7. In vitro dissolution studies

EPL and its corresponding binary and ternary complexes were evaluated for dissolution properties and compared to the pure EPL. The in-vitro dissolution results were assessed on the basis of cumulative percentage drug release, dissolution efficiency and correlation coefficient (r).

The dissolution curves of EPL and ternary complexes are shown in Fig.7. An enhancement in dissolution profile was observed for solid complexes as compared to pure EPL. The binary complex has shown almost 60% drug release in 60min. And ternary complex demonstrated greater

dissolution profile as compared to binary complex with 78.18% drug release within 60 min in dissolution media. Phase solubility parameters such as K_s and CE were greatly promoted and resulted in the greater fruitfulness of ternary complex. Higher release rate was due to positive effect of addition of basic amino acid ARG.^{12,20,23} Hence, we can arrive at a judgment that ternary complex of EPL with β -CD and ARG could be reliable approach for improved dissolution properties.

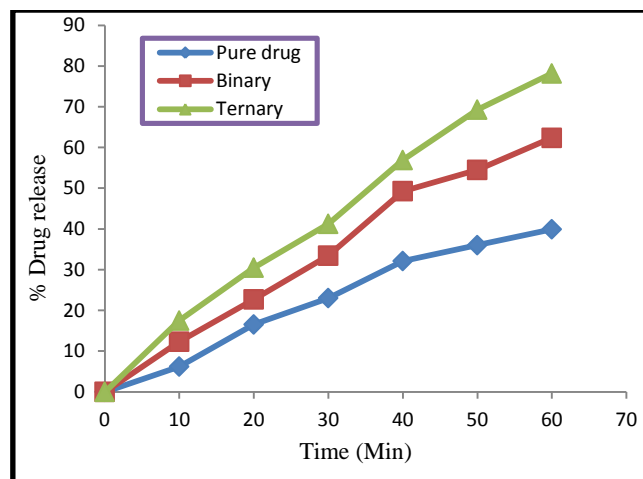


Figure 7: Dissolution profile of pure drug, binary and ternary kneaded complex

3.8. Stability Study

There was no significant change in the physical appearance, drug content and percent drug dissolution in the EPL complexes. A stability results clearly indicate that the complexes were sufficiently stable under accelerated and controlled conditions.

4. CONCLUSION

From the present investigation it is concluded that the formulated complexes revealed the key performance of β -CD and ARG to ameliorate the solubility and dissolution

rate of EPL and also suggests a significant increase in the complexation efficiency between EPL and β -CD by addition of small amounts of amino acid ARG by kneading. Formation of inclusion complexes, greatly enhance the solubility of EPL, which can thus increase its bioavailability and improving pharmaceutical potential of the drug.

ACKNOWLEDGMENT

Authors are thankful to Zydus Cadila Ahmadabad (India) for providing Epalrestat drug as gift sample.

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