

CENTRAL COMPOSITE DESIGN FOR ENHANCEMENT OF ETODOLAC SOLUBILITY VIA INCLUSION COMPLEXATION WITH PVP K-30 AND HYDROXYPROPYL β -CYCLODEXTRIN^{1,2}Shaikh Amir A.*, ³Chaudhari Praveen D. and ²Lavte Pravind K.¹Research Scholar, CRD, PRIST University, Vallam, Thanjavur, TN, India.²Dept. of Pharmaceutics, SCES's Indira College of Pharmacy, Pune, MS, India.³PES's Modern College of Pharmacy, Pune, MS, India.***Corresponding Author: Shaikh Amir A.**

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ABSTRACT

Background: Today, 35-40% all new chemical entities suffer from poor aqueous solubility. The biopharmaceutical classification system (BCS) classifies them as class II substances. Different carriers were used to increase solubility of these class II drugs. **Objective:** The objective of the present investigation was to study the effect of co-precipitation and fusion method on drug release of etodolac (ETO) using polyvinyl pyrrolidone K30 (PVP K30) and hydroxypropyl β -cyclodextrin (HPB). **Methods:** PVP K30 and HPB were used to prepare ternary system of ETO. Central composite design (CCD) was used for preparation and optimization of ternary system. Effect of co-precipitation and fusion method was investigated by evaluating dissolution efficiency (DE), mean dissolution time (MDT) and percent drug release of ETO. Characterization of drug and polymer interactions were done using differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and X ray diffraction (XRD) study. **Results:** Results of *in vitro* drug release showed that various combinations of PVP K30 and HPB prepared using CCD approach by both the methods showed greater dissolution rate of ETO than pure ETO (* $p < 0.05$). Optimized formulation (run 3) of co-precipitation and fusion method gave 85.5% and 73% drug release in 10 min, respectively. Results of DSC, SEM and XRD revealed the interaction of drug with carriers and formation of amorphous ternary system of ETO. **Conclusion:** Data obtained by comparing both methods suggest that co-precipitation method is superior in increasing dissolution of ETO than fusion method. Use of central composite design in preparation of ternary system of ETO was an effective approach for dissolution enhancement of ETO.

KEYWORDS: Etodolac, co-precipitation, fusion method, polyvinyl pyrrolidone K30, hydroxypropyl β -cyclodextrin, ternary system.

INTRODUCTION

One of the challenging problems for pharmaceutical industry in recent years was to develop formulation of poorly water soluble drugs.^[1,2] For chronic conditions oral route is preferred for drug therapy. However, due to poor aqueous solubility numerous lipophilic drugs exhibit low oral bioavailability. Amidon et al defined this class of compounds as 'low solubility/high permeability class'.^[3] In order to increase clinical efficacy of lipophilic drugs efforts are going on to enhance their oral bioavailability. For improving solubility of poorly soluble drugs several conventional methods have been reported. Some of the methods among these are solubilization using cosolvents, solid dispersion, co-precipitation and micronization.^[4-8]

Etodolac, [(±)-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid] is an indole analog and it shows anti-inflammatory and an analgesic (selective COX-2

inhibitor) activity. The drug especially used in the treatment of arthritis. It has been reported that, mainly because of prostaglandin biosynthesis inhibition etodolac shows anti-inflammatory activity.^[9] The recommended oral dose of etodolac is 200 to 400 mg given every 6 to 8 h to a maximum of 1200 mg daily.^[10] Due to very poor water solubility of this drug, its use is reduced in oral solid dosage forms. Clinical studies on various human carcinoma cell lines revealed that etodolac possess anticarcinogenic effect against chemically induced cancer.^[11,12] Etodolac is having 10 fold COX 2 selectivity over COX 1.^[13] This recent development induced interest of various scientists in etodolac to produce better NSAID drug delivery systems for multiple benefits.

The formation of inclusion complex with nontoxic agents is one of the methods which are used to improve the dissolution properties of drugs.^[14] Cyclodextrins (CD), the cyclic oligomers of dextrose or its derivatives have

been used extensively for this purpose. They increase drug solubility by forming an inclusion complex with the non polar region of the drug molecule (guest) being inserted into the cavity of the CD molecule (host).^[15] In some cases, large amounts of CD are required to solubilize the small amounts of drug therefore addition of different concentrations of hydrophilic polymers like polyvinyl pyrrolidone K30 (PVP- K30) is employed to increase the complexation efficiency of the system.^[16-18]

MATERIALS AND METHODS

Materials

Etodolac (ETO) and PVP K30 were obtained from Lupin Research Park, Pune, India, Hydroxypropyl β Cyclodextrin (HPB) was obtained from Roquette Pharma, France. All other reagents and chemicals used were of analytical grade.

Table 1: Central composite design.

| Run | ETO mg | PVP K30 (A) mg | HPB (B) mg |
|-----|--------|----------------|------------|
| 1 | 100 | 200 | 200 |
| 2 | 100 | 300 | 300 |
| 3 | 100 | 200 | 341.421 |
| 4 | 100 | 58.5786 | 200 |
| 5 | 100 | 341.421 | 200 |
| 6 | 100 | 200 | 200 |
| 7 | 100 | 200 | 200 |
| 8 | 100 | 100 | 100 |
| 9 | 100 | 200 | 200 |
| 10 | 100 | 200 | 200 |
| 11 | 100 | 100 | 300 |
| 12 | 100 | 200 | 58.5786 |
| 13 | 100 | 300 | 100 |

ETO: Etodolac; PVP: Polyvinyl pyrrolidone; HPB: Hydroxypropyl β -cyclodextrin.

Preparation of inclusion complexes

Different methods namely co- precipitation and Fusion were used for preparation of drug and polymers complexes in various concentrations as shown in Table 1.

Co precipitation method

Accurately weighed drug, hydroxypropyl β -cyclodextrin (HP- β -CD) and PVP k30 (As shown in table 1) were dissolved in 1:1 solution of methanol:water to get a clear solution. The resulting solution was stirred at ambient temperature until complete evaporation of the solvent occurs. This solution was kept in desiccator for 48 h and then grounded in a ceramic mortar for size reduction; and it was subsequently passed through sieve # 100, which resulted in the formation of a powder-like complex.

Fusion method

The solid dispersion systems (SDs) were prepared using the fusion method. Calculated amounts of each of the carriers were left to melt over a thermostatically controlled magnetic stirrer, each at its respective melting point. After the complete melting, the calculated amount of ETO was suspended in the corresponding molten mass; the blend so obtained was then heated at a

Methods

Design of Experiments (DoE)

Design Expert V10 software was used for designing of experiments. The amounts of PVP K30 (A) and HPB (B) were selected as experimental factors and studied at three levels each. A CCD with $\alpha=1$ was employed as per standard protocol.^[19,20] The central point (0,0) was studied in quintuplicate. All other formulation ingredients and processing variable were kept constant throughout the study. As depicted in Table 1 Thirteen experimental runs with different combinations of factors were obtained by design expert software. Percent dissolution efficiency (%DE) and mean dissolution time (MDT) were taken as response variables.

temperature 10°C above their melting point with continuous stirring for 10 min. The molten mixtures were then immediately put in a freezer for 24 hours. Afterwards, the resulting frozen masses were crushed, milled and passed through suitable sieve and stored in closed containers away from the light until use.

Determination of Content Uniformity

An equal amounts of each solid dispersion (equivalent to 100 mg of ETO), were accurately weighed and dissolved in 100 ml volumetric flask containing SSF (pH 6.8). The one ml of solution was withdrawn and diluted to 100 ml with SSF and then absorbance of it was measured spectrophotometrically at the known λ_{max} , 279 nm. In each case, the mean value of triplicate estimate was used in calculating the required amount for dissolution experiment.

In- vitro drug release study

The dissolution studies were performed in USP dissolution test apparatus type II (rotating paddle type). Accurately weighed complexes equivalent to 100 mg of ETO were spread over 900 ml of dissolution media (phosphate buffer pH 6.8). The temperature was maintained at 37°C \pm 0.5°C and the stirring speed

employed was 50 rpm. At various time intervals, 5 ml aliquots of dissolution media were withdrawn and replaced by 5 ml of fresh dissolution media maintained at same temperature. The collected samples at different intervals were analyzed spectrophotometrically at 279 nm. All the determinations were performed three times.

Statistical analysis

MDT and % DE were calculated using PCP Disso V3 software. Data of MDT and % DE were fed to design of experiment software (DoE) as response variables. To generate various models, multivariate linear regression was used. Central composite design (CCD) was used for preparing different combinations of drug and carriers. For testing the significance and validity of the models, analysis of variance (ANOVA) was applied.

Flow property measurement

Flow properties were evaluated for pure drug and optimized ternary system. Angle of repose, compressibility index and Hausner's ratio were calculated to study flow of each material.

Characterization of plain drug and prepared solid dispersion

Plain drug and prepared solid dispersion were characterized by differential scanning calorimetry (DSC), X-ray diffraction (XRD) and scanning electron microscopy (SEM).

a) Differential scanning calorimetry (DSC)

DSC thermogram of plain ETO and optimized sample of ternary system were performed on Mettler-Toledo (Mettler-Toledo, Switzerland), which was calibrated using indium standard and equipped with a refrigerated cooling system. Flow rate of nitrogen gas was kept at 50 ml/min. The program temperature was set from 30-300°C and increased at a rate of 10°C/min. Melting points of the samples and onset temperature were automatically calculated using the software provided (STARe Ver. 12.1 Mettler Toledo, Switzerland).

b) X-ray diffraction (XRD)

Powder X-ray diffraction patterns for all samples were determined using X-ray diffractometer (Bruker, D8 Advance, Germany) under the following conditions: voltage 35 kV; current 30 mA; receiving slit 0.2 inches; Ni filtered Cu k (α) radiations as source. Samples were analyzed over 2 θ range for stability interpretation.

c) Scanning electron microscopy (SEM)

The surface characterization of various samples was analyzed with a scanning electron microscope (JEOL, JSM-6360A Japan). Auto fine coater JFC-1600 (JEOL, Japan) was used to coat the samples with gold ion sputtering. The sample was kept on the sample holder and the scanning electron micrograph was taken.

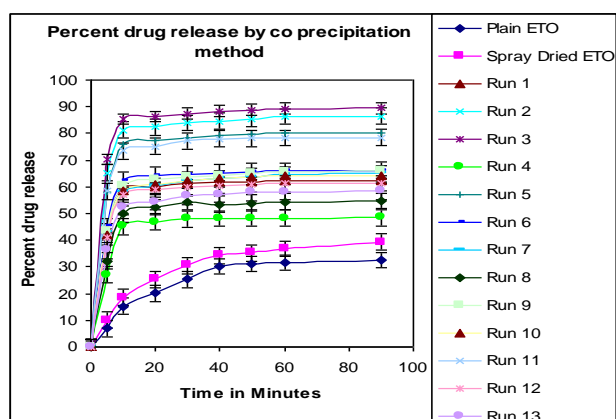
RESULT AND DISCUSSION

Determination of Content Uniformity

Drug content of prepared solid dispersions by both the methods were found in the range of 95.12% to 101.41%.

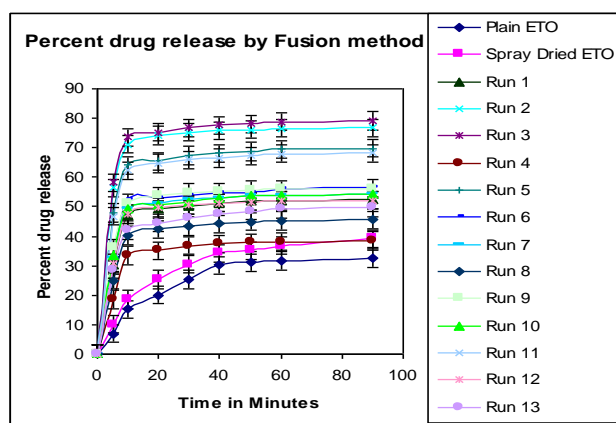
In-vitro drug release study

The release profiles of ETO by co precipitation and fusion method were shown in figure 1 and 2. From the comparative study between two methods i.e. co-precipitation and fusion method; it can be noted that co-precipitation method is superior in increasing dissolution of ETO (Figure 3). It showed that the inclusion complex of ETO (Run 3) prepared by the co-precipitation method released 85.5% drug in 10 min while the inclusion complex (Run 3) prepared by the fusion method showed 73% drug release in 10min. Pure drug exhibited the release of only 15.2% in 10min and 32.6% drug release in 90min. It was evident that the complex exhibited the faster dissolution rate than ETO alone. The increase in ETO dissolution rate through their inclusion complexes might be due to several reasons like the formation of soluble complex, the amorphization of drug and the reduction of particle size, which consequently leads to the better wettability.



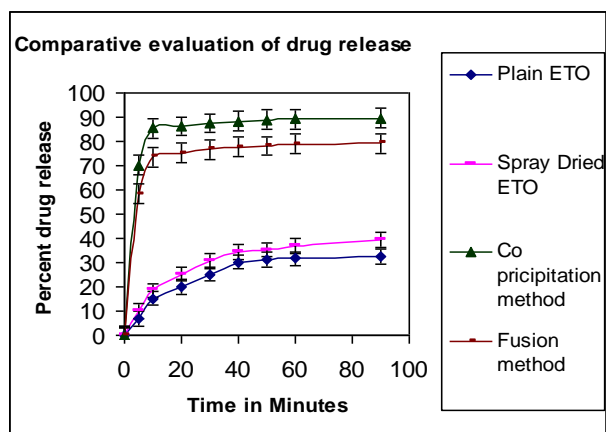
Data given as mean \pm S.D. (n=3).

Fig.1 Percent drug release of ETO by Co precipitation method



Data given as mean \pm S.D. (n=3).

Fig.2 Percent drug release of ETO by fusion method



Data given as mean \pm S.D. (n=3).

Fig. 3 Comparative evaluation of drug release from optimized formulation (run 3) of co precipitation and fusion method.

Statistical analysis

Table 3 depicts overall dissolution efficiency at 90 min. and mean dissolution time of Co precipitation and fusion

Table 3: Dissolution efficiency (DE) and Mean dissolution time (MDT) of Co precipitation and Fusion method in pH 6.8.

| Run | Co precipitation method | | Fusion method | |
|-----|-------------------------|-----------|---------------|-----------|
| | DE (%) | MDT (min) | DE (%) | MDT (min) |
| 1 | 62.45 | 13 | 52.3 | 15 |
| 2 | 86.54 | 4 | 76.8 | 6 |
| 3 | 89.5 | 4 | 79.2 | 5 |
| 4 | 48.42 | 17 | 38.4 | 19 |
| 5 | 80.12 | 12 | 69.8 | 13 |
| 6 | 66.24 | 14 | 56.4 | 15 |
| 7 | 64.9 | 14 | 54.21 | 15 |
| 8 | 54.7 | 16 | 45.6 | 18 |
| 9 | 65.9 | 13 | 56.2 | 15 |
| 10 | 64.3 | 13 | 54.1 | 15 |
| 11 | 78.4 | 9 | 68.2 | 10 |
| 12 | 61.3 | 18 | 52.1 | 19 |
| 13 | 58.4 | 15 | 49.7 | 16 |

Data given as mean \pm S.D. (n=3) DE: Dissolution efficiency; MDT: Mean dissolution time.

Mathematical modeling

As per ANOVA results, all the polynomial equations were found statistically significant (* $p < 0.05$). The values obtained showed HPB has more effect on both responses (%DE and MDT) compared to PVP K30.

Response surface analysis

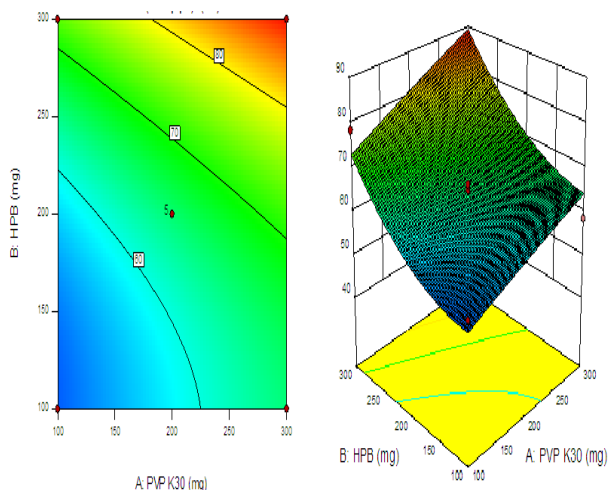
Figures 4A, 5A, 6A and 7A represents the counter plots, while 4B, 5B, 6B and 7B are the corresponding 3-dimensional response surface plots for each response (%DE and MDT) by co precipitation and fusion methods.

Figures 4A, 4B, 5A and 5B exhibited that dissolution efficiency vary in an ascending order but in a non-linear manner with an increase in amount of each polymer. The

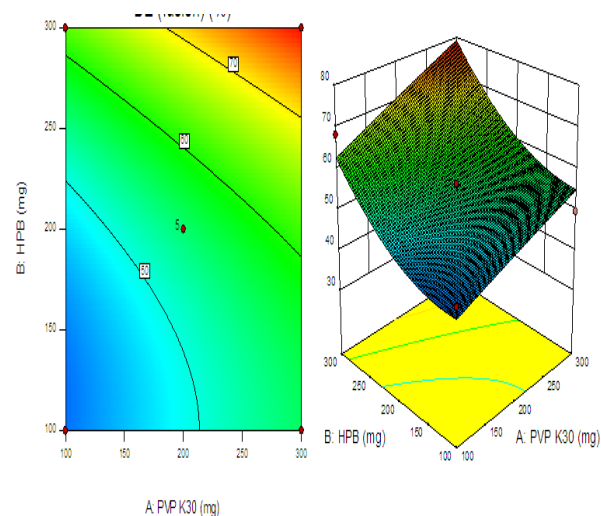
method samples at pH 6.8. These results revealed that Co precipitated ETO products in presence of various combinations of PVP K30 and HPB shows greater solubility compared to fusion method samples. The results were analyzed by ANOVA (* $p < 0.05$). In case of co-precipitation method, Run 3 showed maximum dissolution efficiency of 89.5% and mean dissolution time of 4 min. Dissolution efficiency and mean dissolution time of fusion method sample (run 3) were found to be 79.2% and 5 min, respectively; which is possibly due to highest concentration of HPB that forms complex with ETO, reduction in drug particle size, formation of hydrophilic layer over drug particles by PVP K30 and formation of inclusion complex by HPB with drug. DSC and XRD results also confirmed the formation of amorphous ternary system.

counter plot showed that HPB has comparatively greater influence on dissolution efficiency than PVP K30.

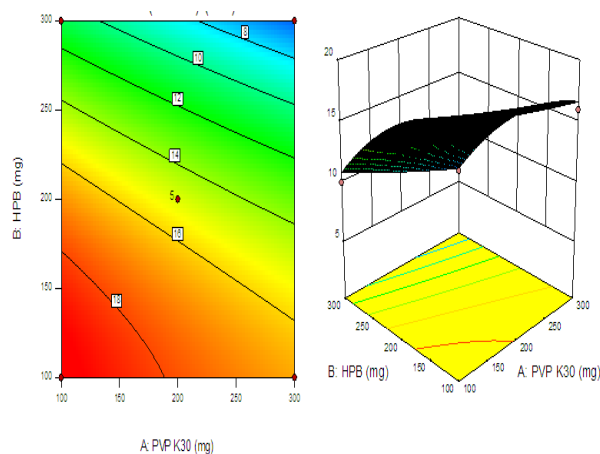
Effect of PVP K30 and HPB on MDT is depicted in figure 6A, 6B, 7A and 7B. Inverse relation was observed on mean dissolution time by use of PVP K30 and HPB. High concentration of HPB showed minimum MDT; whereas high level of PVP K30 increased MDT slightly.



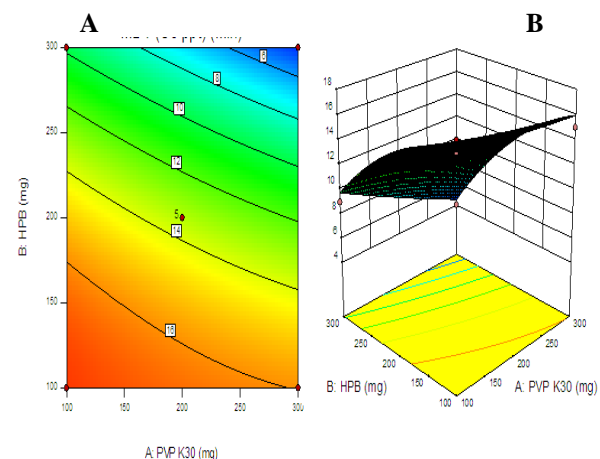
PVP: Polyvinyl pyrrolidone; HPB: Hydroxypropyl β -cyclodextrin
Fig. 4: Effect of PVP K30 and HPB on %DE of Co precipitation method, Contour graph (left), 3D Graph (right).



PVP: Polyvinyl pyrrolidone; HPB: Hydroxypropyl β -cyclodextrin
Fig. 5: Effect of PVP K30 and HPB on %DE of Fusion method, Contour graph (left), 3D Graph (right).



PVP: Polyvinyl pyrrolidone; HPB: Hydroxypropyl β -cyclodextrin
Fig. 6: Effect of PVP K30 and HPB on MDT of fusion method, Contour graph (left); 3D Graph (right).



PVP: Polyvinyl pyrrolidone; HPB: Hydroxypropyl β -cyclodextrin
Fig. 7: Effect of PVP K30 and HPB on MDT of co precipitation method, Contour graph (left), 3D Graph (right).

Flow property measurement

From the results of flow property measurement it can be noted that there was improvement in flow property of ETO in optimized ternary system as compared to plain ETO (Table 4). This might be due to size reduction of particles and formation of more uniform particles.

Table 4. Flow property study

| Sr. No. | Parameters | ETO | Optimized formulation Co precipitation method | Optimized formulation fusion method |
|---------|--------------------------------|-------|---|-------------------------------------|
| 01 | Compressibility index (%) | 34.94 | 27.25 | 30.12 |
| 02 | Hausner's ratio | 01.55 | 01.28 | 01.33 |
| 03 | Angle of repose ($^{\circ}$) | 36.30 | 21.40 | 25.56 |

ETO: Etodolac.

Characterization of optimized ternary system

To confirm the formation of solid complex and its amorphous nature; ternary system of ETO-PVP-HPB

obtained by co-precipitation process was characterized by DSC, XRD and SEM analysis.

a) Differential scanning calorimetry (DSC)

DSC thermogram of pure ETO and optimized ternary systems by co precipitation method were presented in figure 7. The DSC thermo gram of ETO exhibited an endothermic peak at 153°C corresponding to its melting point. The endothermic peak at 153°C was not observed in case of optimized ETO-HPB-PVP K30 ternary system which indicates inclusion complex was formed and drug was converted to amorphous form.

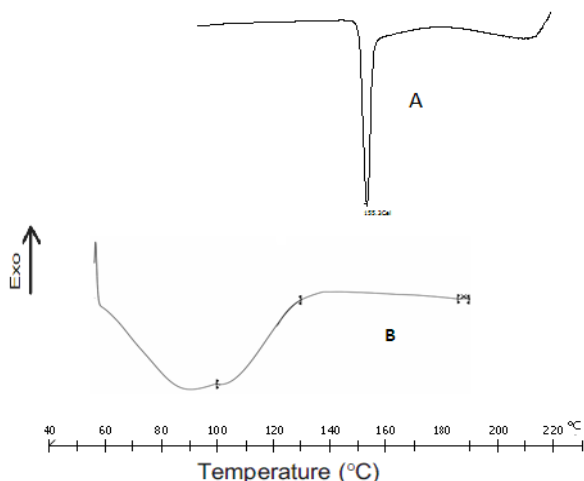


Fig. 7: DSC thermogram of A: Pure ETO, B: Optimized ternary system by co precipitation method

b) Scanning electron microscopy (SEM)

Morphological features of the ETO solid complex were examined by scanning electron microscopy. Figures 8 show the scanning electron microscopic picture of optimized ternary system obtained by co-precipitation method. SEM photographs depicted relatively rough surface, suggesting that drug might be attached and

dispersed onto the surface of the solid carriers. This observation suggested the existence of interaction between ETO, HPB and PVP K30. Such molecular dispersion of the drug on the solid carrier may account for the enhanced drug release and absorption.

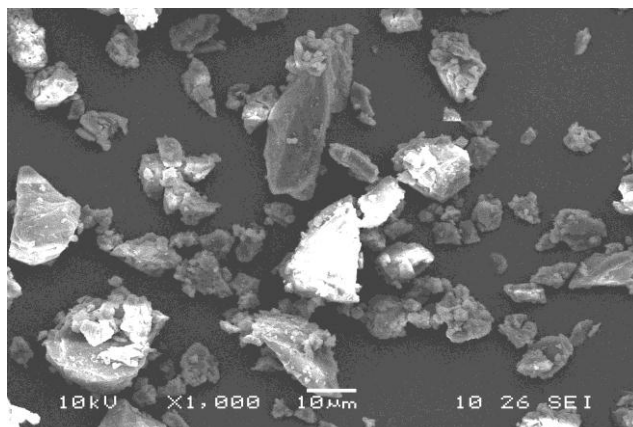


Fig. 8: SEM of optimized complex (ETO-PVP K30-HPB) by co precipitation method.

c) X-ray diffraction (XRD)

X-ray powder diffraction patterns of ETO and optimized formulation (run 3) by co precipitation method were shown in Fig. 9. The pure ETO was in crystalline form as demonstrated by the sharp and intense diffraction peaks (Figure 5A). X-ray powder diffraction patterns of optimized formulation (run 3) by co-precipitation method showed less intense peaks. The observed variation in peaks in the x-ray diffraction patterns indicates formation of complex between drug HPB and PVP K30.

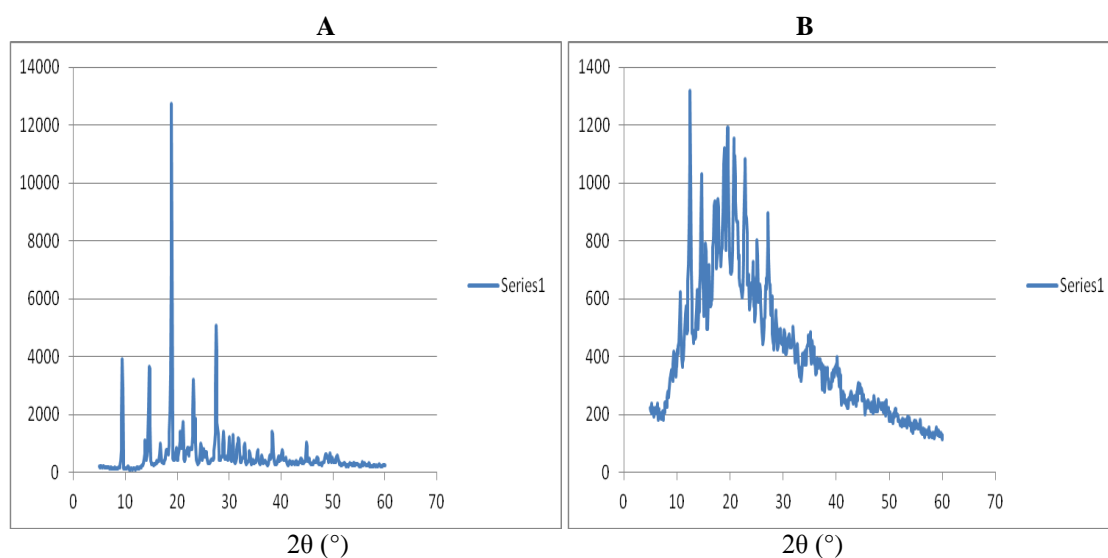


Fig. 8: X-RAY diffraction of A: Pure ETO, B: Optimized ternary system (co precipitation method).

CONCLUSION

Ternary systems composed of ETO, HPB and PVP were prepared using co-precipitation and fusion methods. Effects of PVP K30 and HPB on DE and MDT of ETO

can be evaluated and optimized effectively using DoE approach. The release rate of the drug from the ternary systems revealed that co-precipitation method gives product with better release rate of drug as compared to

fusion method. Run 3 of fusion method and co-precipitated ternary system given drug release of 73% and 85.5% respectively in 10 min. DSC, XRD and SEM analysis exhibited formation of complex of drug with HPB and PVP K30 which probably increased solubility and dissolution of the drug. Thus, the present study has demonstrated the potential utility of combine use of HPB and PVP K30 for development of ternary system of ETO using systemic design of experimentation approach.

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CONFLICT OF INTEREST

Authors explicitly declare that there is no conflict of interest.

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