

Formulation and *In-Vitro* Evaluation of Spherical Crystal Agglomerates of Ebastine by Quasi Emulsion Solvent Diffusion Method

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Abstract

Ebastine (EBS) is a poorly water-soluble antihistaminic drug; it belongs to the class II group according to the biopharmaceutical classification system (BCS). This work aims to enhance the solubility, dissolution rate and micrometric properties of EBS, by formulating it as spherical crystal agglomerates by Quasi Emulsion Solvent Diffusion (QESD) method. Spherical crystal agglomerates (SCAs) were prepared in the presence of dichloromethane (DCM), water and chloroform as a good solvent, poor solvent and bridging solvent respectively. Agglomeration of EBS involved the use of some hydrophilic polymers like polyethylene glycol 4000 (PEG 4000), polyvinyl pyrrolidone K30 (PVP K30), D- α -tocopheryl polyethylene glycol 1000 succinate (TPGS) and β -cyclodextrin. (EBS) and its agglomerates (with and without polymers) were characterized for their drug content, percentage yield, solubility, *in vitro* drug release study and micromeritic property as well as by optical microscope, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and X-ray Diffraction (XRD). The results showed that there was a marked enhancement in the solubility with improvement in dissolution rate, physiochemical properties, and decrease in crystallinity and alteration in the crystal habit of the drug especially in the presence of polymers. The best results were obtained with formula prepared by the combination of PEG 4000 and β -cyclodextrin in the agglomeration process of (EBS).

Keywords: Ebastine, Spherical crystal agglomerates, Quasi emulsion solvent diffusion method.

صياغة وتقييم التكتلات البلورية الكروية لدواء الايباستين

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الخلاصة

دواء الايباستين هو عقار مضاد للهستامين ضعيف الذوبانية في الماء، وهذا الدواء ينتمي إلى المجموعة الثانية من نظام تصنيف المستحضرات الصيدلانية الحيوي. كان الهدف من هذا العمل هو تحسين الذوبانية، ومعدل الذوبان والخواص الدقيقة للدواء، عن طريق صياغته بشكل تكتلات بلورية كروية باستخدام طريقة انتشار المذيبات من شبه مستحلب. تم تحضير التكتلات البلورية الكروية في وجود ثلاثة مذيبات وهي ثنائي كلورو ميثان، ماء وكلوروفورم كمذيب جيد، مذيب ضعيف ومذيب رابط على التوالي. تضمنت عملية التحضير استخدام بعض البوليمرات القابلة للذوبان في الماء مثل البولي ايثيلين جلايكول ٤٠٠٠، بولي فينيل بيروليدين كي ٣٠، دي-الفا توكيفريل بولي ايثيلين كلاكول ١٠٠٠ سيكسنت وبيتا سيكلودكسترين. تم وصف العقار النقي (ايباستين) وتكتلاته مع وبدون بوليمرات لمحتواها من الدواء، وعائد الانتاج المنوي، والذوبان، وتحرر الدواء خارج الجسم، والخاصية الدقيقة وكذلك من خلال المجهر الضوئي، المسح المجهر الإلكتروني، والدراسات الطيفية، المسح التفرقي للمسعات وحيد الأشعة السينية. أظهرت نتائج هذا العمل تحسن ملحوظ في قابلية الذوبان مع تحسن كبير في قابلية تحرر الدواء والخصائص الفيزيائية والكيميائية مع انخفاض في التبلور والتغير في اشكال بلورات الدواء. وخاصة في وجود البوليمرات وان أفضل النتائج تم الحصول عليها باستخدام الصيغة التي تم إعدادها عن طريق الجمع بين بولي ايثيلين كلاكول ٤٠٠٠ وبيتا ساكلوديكسترين في عملية التكتل لدواء الايباستين.

الكلمات المفتاحية: دواء الايباستين، التكتلات البلورية الكروية، طريقة انتشار المذيبات من شبه مستحلب.

Introduction

The oral route of drug administration is the most common and preferred one over the other routes due to its convenience and ease of administration. However, drug absorption from

the gastrointestinal tract (GIT) can be limited by a variety of factors with the most significant contributors being its aqueous solubility and/or its membrane permeability⁽¹⁾.

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Direct compression method is frequently and preferably used for manufacturing of tablets. In the direct tableting method, it is necessary to increase the flowability and compressibility of the bulk powder⁽²⁾. On the other hand, it is also important to increase bioavailability of the drug by improving the solubility of the bulk drug powder⁽³⁾. Spherical crystal agglomeration (SCAs) method directly transforms the fine particles produced in the crystallization process into a spherical shape⁽⁴⁾. Thus; it improves the secondary characteristics like flowability and compressibility so that direct tableting is possible without further processing⁽⁵⁾. It is the particle engineering technique by which crystallization and agglomeration can be carried out simultaneously in one step to transform crystals directly into compacted spherical form which has been successfully utilized for improvement of solubility and dissolution. Spherical crystallization is carried out by four methods: Spherical agglomeration method, Quasi-emulsion solvent diffusion method (QESDM), Ammonia diffusion method, and Neutralization method⁽⁶⁾. Ebastine (EBS) A piperidine derivative, is a long-acting, non-sedating, second-generation histamine receptor antagonist that binds preferentially to peripheral H₁ receptors. It is metabolized to active metabolite, carebastine. It has antihistaminic, antiallergic activity and prevents histamine-induced bronchoconstriction. This drug presents very low water solubility and a high hydrophobicity with partition coefficient (log P) of 7.64, therefore, and according to the biopharmaceutical classification system (BSC), this drug belongs to the class II group of pharmaceuticals; for which the rate – limiting step in the absorption process is drug dissolution,

Table 1. Composition of SCAs

Formula Number	EBS gm	PEG4000 gm	PVPK30 gm	β. cyclodextrin gm	TPGS gm	DCM ml	Water ml	Chloroform ml
EBS1	2					10	100	2
EBS2	2	2	-	-	-	10	100	2
EBS3	2	-	2	-	-	10	100	2
EBS4	2	-	-	2	-	10	100	2
EBS5	2	-	-	-	2	10	100	2
EBS6	2	2	-	2	-	10	100	2

Evaluation of SCAs of EBS

Percentage yield of SCAs

The product yield of SCAs was determined by calculating the weight of resulted SCAs after drying with respect to initial weight of drugs and polymers used in formulation of SCAs⁽¹⁰⁾ as in equation:

because they can permeate well through the gastrointestinal tract⁽⁷⁾. The objective of this work was to enhance the solubility, dissolution rate and micromeritic property of EBS by SCAs method.

Materials and Method

Material

Ebastine, β. cyclodextrin and TPGS were purchased from Hyperchem, china. PEG4000 and PVPK30, chloroform and dichloromethane were purchased from (BDH chemicals, Ltd., Liverpool, England) All other reagents and chemicals used were of analytical grade

Method

Preparation of EBS spherical crystal agglomerates

SCAs prepared by emulsion solvent diffusion method. 2g EBS was dissolved in 10ml good solvent (DCM), this solution was poured drop by drop in to 100ml distilled water (poor solvent) containing either no, single or combination of polymers in presence of 0.1 g Aerocil 200; stirred by mechanical stirrer (propeller type stirrer). Then 2ml chloroform (bridging solvent) was added drop by drop to the mixture with continuous stirring at stirring speed of 900 rpm for one hour. The selection of these solvent was according to solubility of drug in each of the above solvents and miscibility of these solvent with each other^(8,9). As the good solvent diffused to the poor solvent, droplet gradually solidified. The formed spherical agglomerate filtered by vacuum filtration and dried at room temperature.

Different formulas were prepared (table 1) as a trial to get the required physicochemical properties regarding shape, solubility, dissolution and flow properties

$$\text{Yield\%} = \frac{\text{Practical weight of SCAs}}{\text{Theoretical weight of drug and polymers}} \times 100$$

Drug Content

The percentage of drug content of the SCAs can be determined by crushing and

dissolving fixed amount of the agglomerates in 100ml methanol and sonicated for 30 minutes. The resulting solution was then filter through 0.45Mm Whatman filter paper and analyzed for EBS by UV spectrophotometer at 253nm⁽¹¹⁾.

Optical microscope

Optical microscope was used to determine the change in shape SCAs. Comparison between formulas and pure drug was made. Samples of pure drug and SCAs were placed on glass slides and examined under the microscope at magnification of 40 ×100^(12, 13).

Scanning electron microscope (SEM)

To evaluate surface topography of SCAs of the selected formulas and that of pure drug (crystal habit) scanning electron microscope SEM was used after coating with gold^(14, 15).

Solubility study of EBS and SCAs

An excess amount of EBS and prepared formulas were added to 10ml of distilled water in a closed glass tube. The tubes were placed in shaker water bath for 48 hr. at 25°C. After that samples were filtered through filter membrane of 0.45 µm, and the concentration of the dissolved EBS was determined by UV-spectrophotometer at 258nm⁽¹⁶⁾.

Dissolution study of SCAs

The release profile of EBS from formulated SCAs was determined by using (paddle) type II USP dissolution test apparatus. A weighed amount of SCAs equivalent to 10 mg drug was add to (1000) ml of 0.1 N HCl (pH 1.2) media kept at 37±0.5 °C at rotation speed of 100 rpm. Sink condition was maintained throughout the study. At predetermined time interval 10 ml sample was withdrawn and replaced by equal volume of fresh sample maintained at the same temperature. The samples were then filtered through 0.45 Mm filter membrane and analyzed spectrophotometrically at 258nm^(17, 18).

Micromeritic property of SCAs

The flow properties of, SCAs were determined by measuring angle of repose, Carr's index and Hausner's Ratio for each formula and compared with those of pure drug as follow:

Angle of repose

Angle of repose is an expression or measurement for the flowability of powder or granules and can be determined by using funnel method. The tan⁻¹ of the height of the pile/radius of its base gave the angle of repose⁽¹⁹⁾.

$$\tan(\theta) = h/r$$

Where: (θ) is angle of repose, **h** is the height of the resultant powder cone and **r** is the radius of the powder cone

Bulk density

The volume occupied by the accurately weighed SCAs was measured, which gave bulk volume. The bulk density of SCAs was determined using the following formula⁽¹⁹⁾

$$\text{Bulk density} = \frac{\text{Total weight of SCAs}}{\text{Bulk volume of SCAs}}$$

Tapped density

An accurately weighed quantity of crystals was introduced into a measuring cylinder and tapped until no further change in volume was noted, which gave the tapped volume. The tapped density was determined by the following formula⁽¹⁹⁾.

$$\text{Tapped density} = \frac{\text{Total weight of SCAs}}{\text{Tapped volume}}$$

Compressibility index

Compressibility index was determined according to Carr's index^(19, 20)

Carr's index

$$= \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100$$

Hasnurs ratio

Hasnurs ratio gave an indication on flow property of powder and can measure from ratio of tapped density to bulk density^(19, 20).

$$\text{Hasuners ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Fourier transform infrared spectroscopy (FTIR)

The drug-polymer interactions were studied by FTIR^(21,24). The FT-IR spectra of pure drug, PEG4000, β. cyclodextrin, SCAs (EBS1), physical mixture, SCAs (EBS6) were obtained by preparing the above samples in potassium bromide discs and scanned within scanning range of 4000 cm⁻¹ to 400 cm⁻¹.

Powder X-ray diffraction (PXRD)

Powder X-ray diffraction (PXRD) study was performed to evaluate changes, in the crystalline nature of the drug^(22,24). PXRD analysis was performed for pure drug and SCAs (EBS1) and physical mixture of selected formula and SCAs (EBS6) using an X-ray diffractometer, the samples were irradiated with the monochromatized CuKα radiation and analyzed between 2° and 50° theta.

Differential scanning calorimetry studies (DSC)

To detect the degree of crystallization and drug-polymer interactions ; DSC studies were carried out for pure drug, PEG4000, β. Cyclodextrin, SCAs (EBS1) physical mixture and

SCAs (EBS6) by using differential scanning calorimeter. All approximate weighed samples (about 3 mg) were placed in sealed aluminum pans, before heating under nitrogen flow (100mL/min) at a scanning rate of 10 °C min⁻¹, from 25 °C to 400 °C. An empty aluminum pan was used as reference^(23,24).

Statistical analysis

The results were analyzed by two tailed Student's t-test using Microsoft Excel 2010. For all analyzed results and the level of significance was set at a p-value of 0.05:

A P value of more than 0.05 was considered to be non-significant.

A P value of less than 0.05 was considered to be significant

The results obtained from the dissolution studies were statistically validated using similarity factor (*f*₂) .

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{j=1}^n |R_j - T_j|^2 \right]^{0.5} \times 100 \right\}$$

n is the sampling number, *R_j* and *T_j* are the percent dissolved of the reference and test products at each time point *j*.

The similarity factors fits the result between 0 and 100. It is 100 when the test and reference profile identical (*f*₂ higher than 50 show the similarity of dissolution profile) and tend to 0 as the dissimilarity increased this method is more adequate to dissolution profile comparison when more than three or four dissolution points are available⁽²⁵⁾.

Result and Discussion

Preparation of EBS SCAs

Ebastin SCAs were prepared by using QESDM. The principle of this method is the use three solvents; good solvent, bridging solvent and poor solvent the selection of these solvents depends on the miscibility of the solvents and the solubility of drug in individual solvent⁽²⁶⁾.

DCM was used as good solvent due to good solubility of EBS and rapid removal of DCM from crystallization system due to its low boiling point^(27,28,29) water used as dispersion phase that's because of poor solubility of EBS in water so that EBS rapidly crystalize in water . Chloroform was used as bridging solvent since it is immiscible with poor solvent (water). When the organic drug solution was added to the stirring water, emulsion droplets were formed due to the interfacial tension between the two solvents, the good solvent gradually diffuses out of the emulsion droplets into the outer poor solvent phase, and the poor solvent diffuses into

droplets, which reduced the solubility and eventually caused drug crystallization inside the droplets⁽³⁰⁾, the bridging liquid would then wet the precipitated crystals. And as a result of interfacial tension effects and capillary forces, the bridging liquid will be gathering the crystals to one another resulting in the formation of larger size agglomerates⁽³¹⁾.

Aerosil acts as a separating agent and mass compactor, because coacervation droplets formed from the drug-polymer droplets during the solidifying period were sticky and readily coalesced, so, introduction of Aerosil efficiently prevented coalescence and produced compact spherical crystals⁽³²⁾.

Percentage yield

The percentage yield of the SCAs that were prepared without polymers (EBS1) was 99.982%, while the percentage yield of SCAs prepared in the presence of polymer ranges from 78.8% to 53.4%. Highest yield was obtained from the formula EBS4 which was prepared by using β. cyclodextrin which have the lowest water solubility in comparison with other polymers used in this study. In addition β.cyclodextrin practically insoluble in DCM so that β.cyclodextrin rapidly precipitate and solidified on the surface of SCAs⁽²⁷⁾. On the other hand, lowest yield was obtained from SCAs prepared with PEG4000 (EBS 2) due to the high solubility of PEG4000 in water and DCM so that, PEG4000 lost in dispersion phase and only traces amount of polymer precipitate with agglomerated crystals⁽³³⁾ The above results are shown in table 3.

Drug content

Percent drug content of all the prepared formulas was found to be in the range of 98.44±0.708 to 99.8±0.54 that indicated there was negligible loss of drug during crystallization process resulting from sticking on vessels and impeller table 3.

Optical microscope

Optical microscope showed the great difference between the shape of untreated EBS which have needle and irregular crystals and agglomerated crystals which have compact spherical form (Figure1).

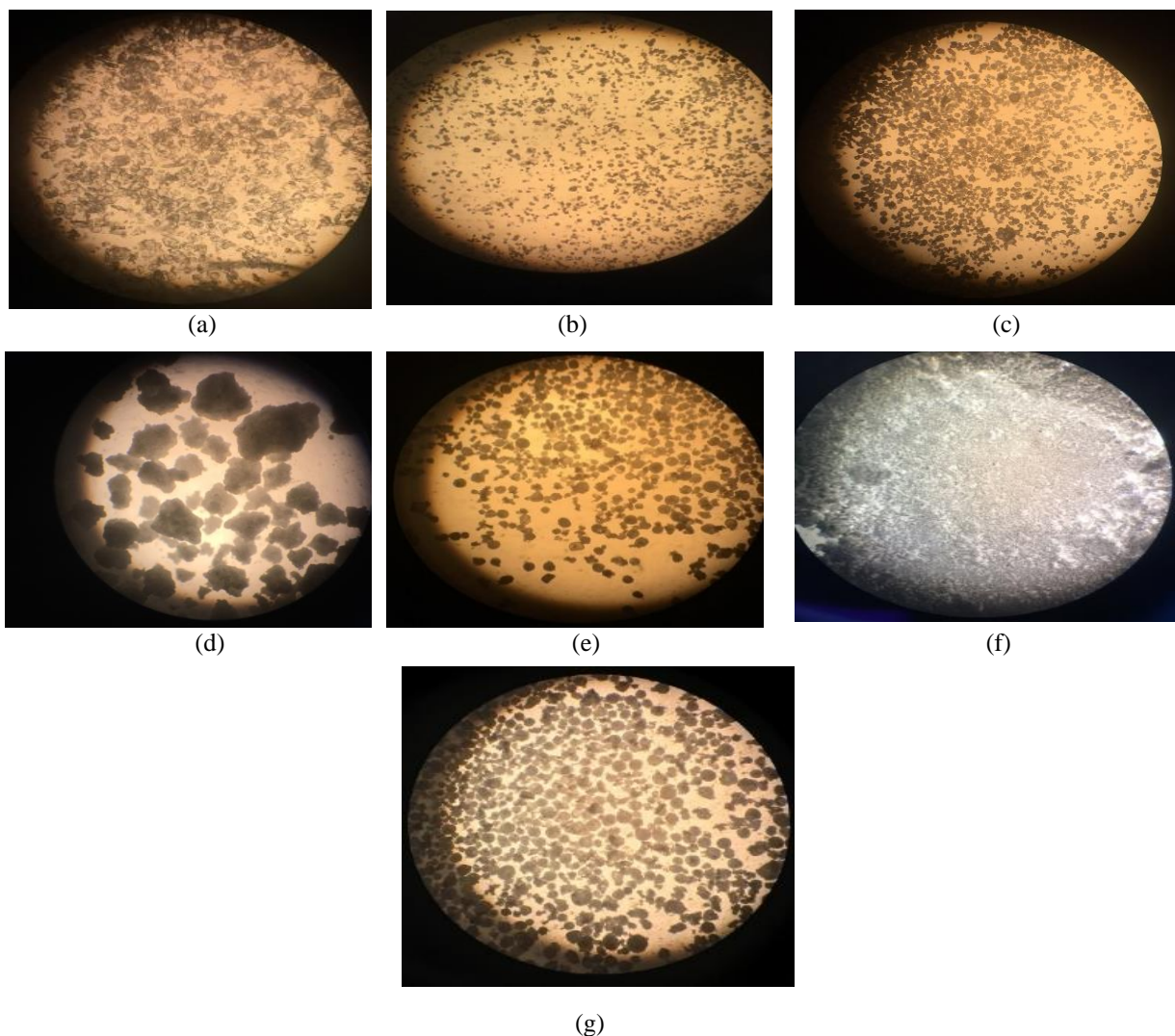


Figure 1. Optical microscope of pure drug and formulated SCAs (a) pure drug, (b) EBS1, (c) EBS2, (d) EBS3, (e) EBS4, (f) EBS5, (g) EBS6

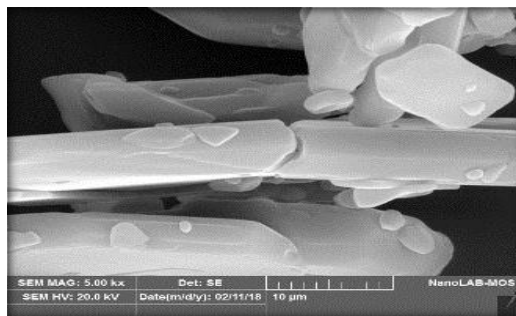
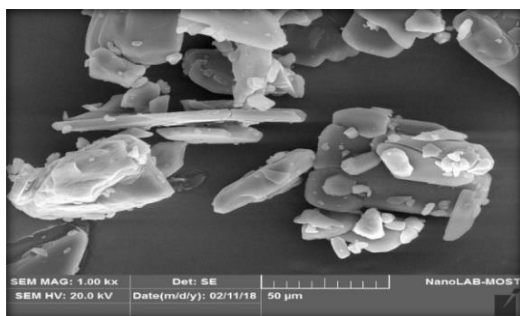
Scanning electron microscope (SEM)

The results of surface morphology studies are shown in (figure5). The SEM results revealed that the untreated EBS has irregular needle crystal while SCAs have spherical structure. The surface morphology studies also revealed that the agglomerates were formed by very small crystals, which were closely compacted into spherical form (figure2).

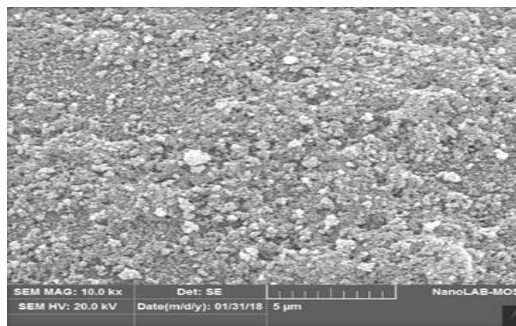
Solubility study

Solubility profile shows that the solubility of SCAs (EBS1) was dramatically more than that of pure drug which can be explained to be due to partial amorphism of drug in agglomerates and increase in surface area of

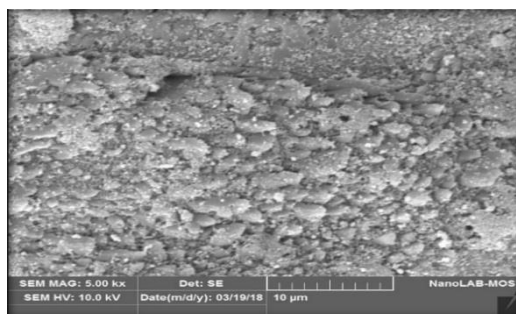
particles as a result of spherical shape. Furthermore, the formulas prepared with use of polymers (EBS2, EBS3, EBS4, and EBS5) showed higher solubility as compared to EBS1. This expected result was due to wetting effect of the hydrophilic polymers⁽³⁴⁾, while, the formula prepared by the use of combination of polymers (EBS6) showed greatest improvement in solubility than formulas prepared by single polymer since the use of hydrophilic polymer (PEG4000) survive the spherical shape of particle and enhanced the solubility effect of (β -cyclodextrin)^(27,45) (table 2).



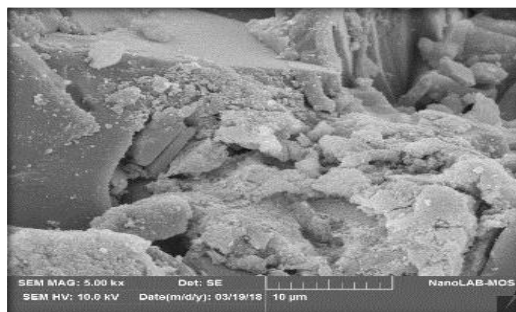
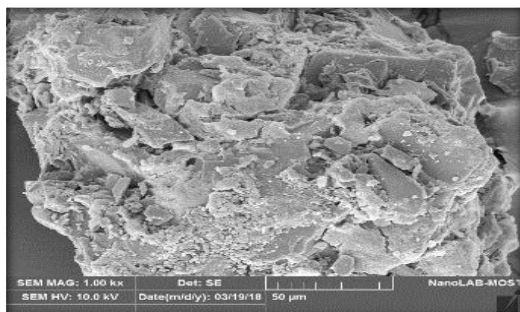
a



b



c



d

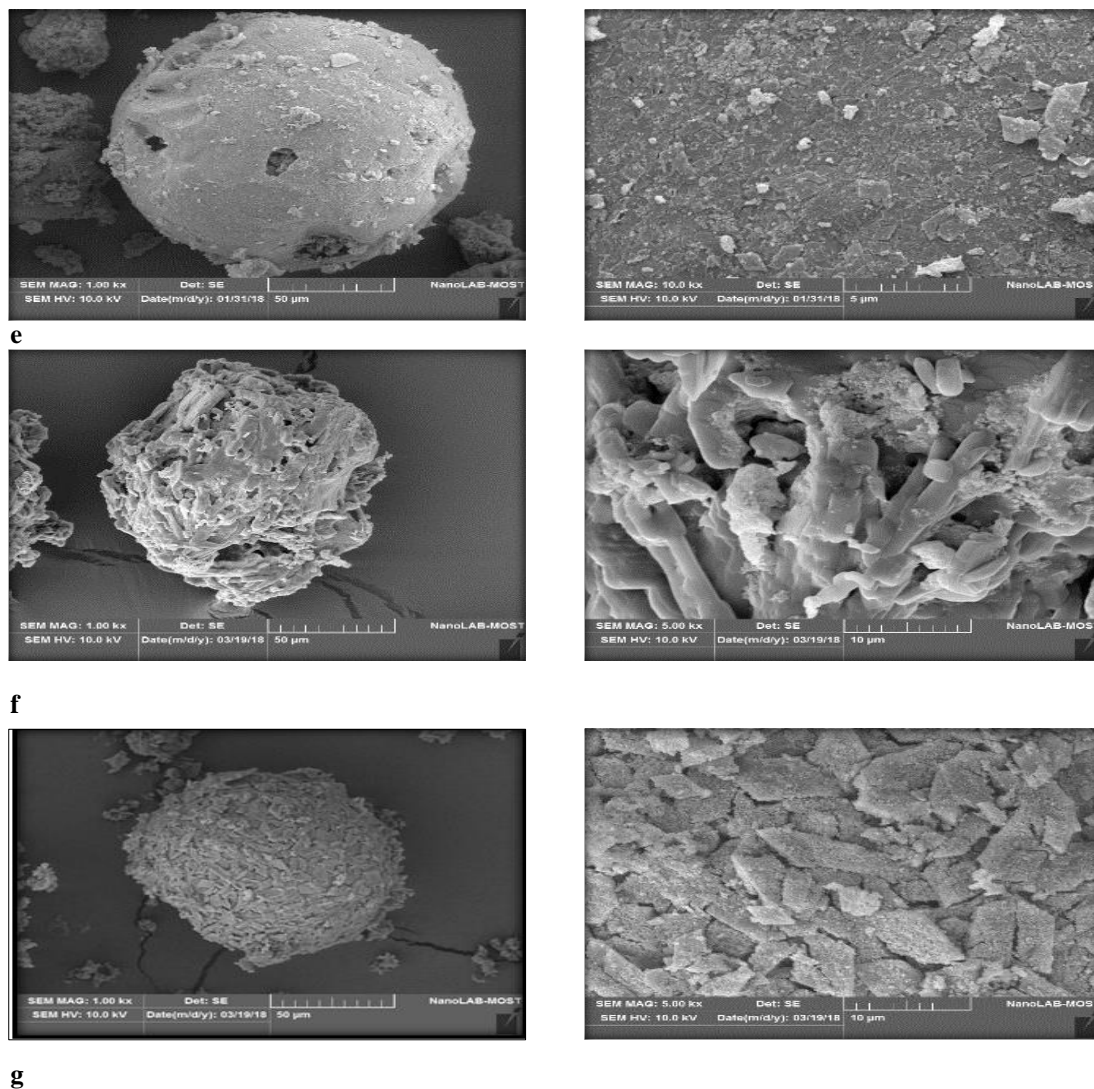


Figure 2. Scanning electron microscope of pure drug and formulated SCAs (a) pure drug, (b) EBS1, (c) EBS2, (d) EBS3, (e) EBS4, (f) EBS5, (g) EBS6 under 1KX and 10KX magnification.

In-vitro dissolution study

The dissolution profiles of (EBS1) in 0.1 N HCl (pH 1.2) at 37° C; and that of pure drug were non similar ($f_2=35.485$). There was an enhancement in the rate of dissolution in comparison to the pure drug as a result of improved porosity resulting from diffusion of solvents during solidification process^(34,35) (figure 3). In vitro release study of SCAs with different single polymer were studied and the results indicated that, there was a dramatic improvement in the rate of dissolution in comparison with pure EBS. However, the increase in dissolution rate was in order of EBS2 > EBS4 > EBS5 > EBS1 > EBS3 > EBS as shown in (figure 4). This increase was mainly related to spherical shape of SCAs

and by increasing in wettability due to the presence of different polymers. PEG 4000 show highest increase in rate of dissolution ($f_2=20.014$) in comparison with pure drug dissolution rate that's because PEG 4000 have important effect on maintaining the spherical shape of agglomerated crystal⁽³⁶⁾ and also it enhances the physical and chemical stability of drugs and prevents aggregation of the drug particles as a result of the steric hindrance and/or masking of charges provided through formation of a conformational cloud⁽³⁷⁾ so, higher surface area will be available for dissolution. β -cyclodextrin and TPGS increase in dissolution

of EBS ($f_2 = 24.07294$) and ($f_2 = 39.46175$) respectively due to improvement in solubility. PVP K30 shows similar dissolution profile as that of pure drug ($f_2 = 80.563$) in spite of increase in solubility which may be due to increase diffusion layer of particle would slow the release of drug since this water soluble polymer at high (about 33% w/w of the formula) amount swell to form concentrated continuous layer between solid surface and bulk medium on dissolution medium, with high viscosity in diffusion layer that caused retardation in dissolution of drug^(38,39).

(EBS6) formulated by combination of β -cyclodextrin and PEG 4000 a very fast rate of drug release ($f_2 = 8.955621$) with reduction in time required for complete the release in comparison with that of pure drug (figure 5) Therefore, incorporation of PEG 4000 caused increase in solubility as well as in rate of drug release. This result can be attributed to the properties of β -cyclodextrin. It has a low water solubility and rigid structure due to high number of intramolecular hydrogen bonds among secondary hydroxyl groups. Thus, it prevents hydration by water molecules and cause retardation on drug release. By addition of PEG 4000 which binds to the rigid structure of β -cyclodextrin; the wettability and solubility of SCAs increased which results in increase the rate of drug release. so the effect of combination on enhancing solubility and dissolution can be utilized to reduce the amount of β -cyclodextrin used in formulations to avoid side effect of large amount of β -cyclodextrin consumed by patient which may cause osmotic diarrhea^(33,40,41).

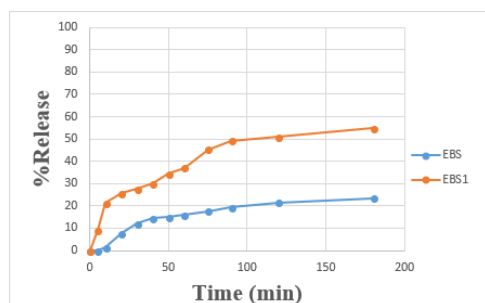


Figure 3. The release profile of pure drug (EBS) and SCAs (EBS1) in 0.1 N HCl at 37°C

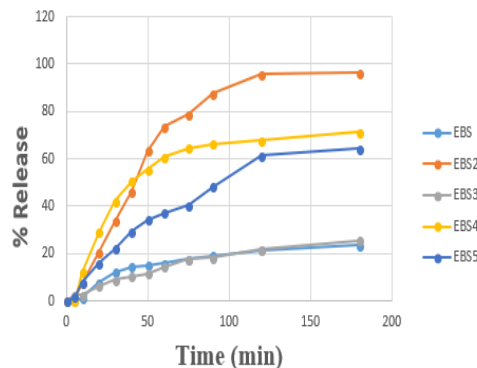


Figure 4. The release profile of pure drug and SCAs (EBS2), (EBS3), (EBS4), (EBS5) in 0.1 N HCl at 37°C

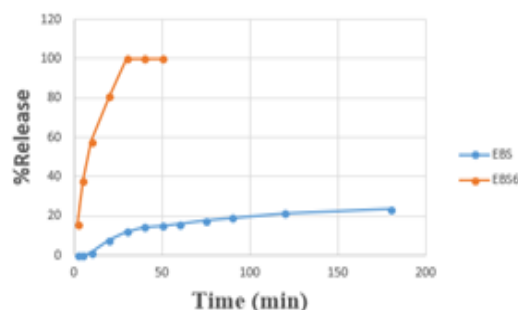


Figure 5. The release profile of pure drug (EBS) and SCAs (EBS6) in 0.1 N HCl at 37°C

Micromeritic property

The values of angle of repose, Carr's index and Hausner ratio of pure drug and SCAs of EBS are shown in table 3. High value for angle of repose of untreated EBS ($47.27^\circ \pm 0.047$) indicated poor flowability of the drug due to its irregular crystals which hindered their flowability from funnel in comparison with the formulated SCAs. The significant improvement ($p < 0.05$) in flowability for SCAs of each formula in comparison with untreated EBS as indicated by the low values of angle of repose was due to their spherical shape and smooth surface, which results in reduction in the interparticle friction since the area of contacts for spherical shape was smaller than that for other particle shape⁽⁴²⁾. Carr's index and Hausner's ratio were of highest value for untreated EBS compared to the prepared SCAs that's mean poor compressibility and packability pure drug. These properties were also improved by formulation of SCAs due to their spherical shape⁽⁴³⁾.

Table 2. Drug content, %yield and micromeritic property of pure and SCAs of ebastine

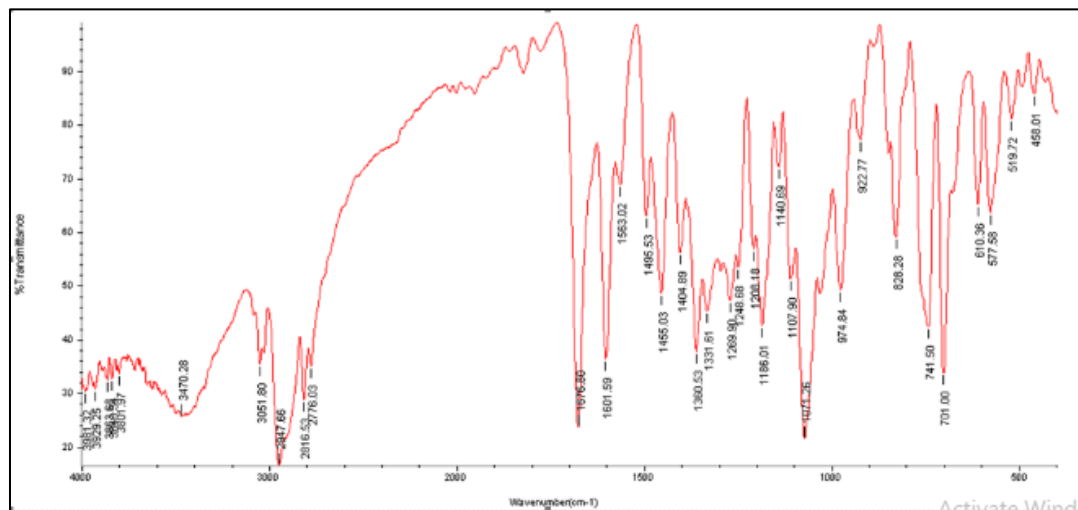
Formula Number	Type of polymer	Drug content %	Yield %	Angle of repose (degree)	Carr's index%	Hasnurs ratio	Aqueous solubility mg/ml
EBS	-----	100±0.0 (Pure drug)	-----	47.27° ±0.04	32.46467±1.41	1.481567±0.03	0.00293 ±0.001
EBS1	No	99.8±0.54	99.982	25.82 °±0.09	8.9533±1.78	1.0986±0.021	0.007851 ±0.001
EBS2	PEG4000	99.56±0.29	53.4	24.94° ±0.29	9.0133±2.29	1.0997±0.028	0.0106 ±0.003
EBS3	PVPK30	98.61±0.49	59.042	25.09° ±0.39	5.0323±1.73	1.053±0.019	0.012 ±0.003
EBS4	β. CD	98.44±0.70	78.8	27.73°±0.37	11.559±3.58	1.110±0.018	0.0433 ±0.004
EBS5	TPGS	98.72±1.11	55.22	32.82°±0.62	16.2276±1.48	1.17163±0.12	0.0136 ±0.002
EBS6	β. CD + PEG4000	98.84±1.38	60.216	27.75±0.63	13.0366±1.81	1.131±0.13	0.055 ±0.004

FTIR study

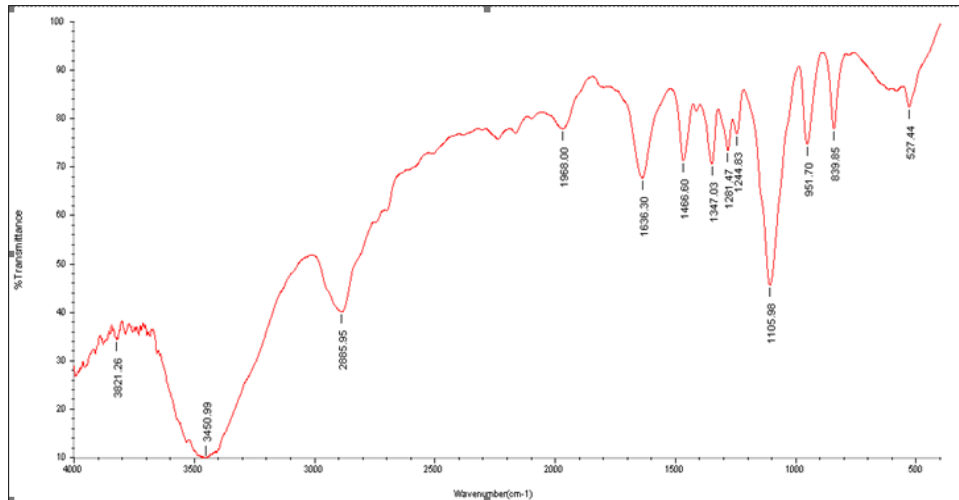
The FTIR spectrum of EBS show C-H stretching of the ring at 3051cm⁻¹, C-H stretching of (CH3) at 2947cm⁻¹, C=C stretching aromatic ring at 1450cm⁻¹, C-N stretching at 1267cm⁻¹ and C=O stretching band at 1677cm⁻¹, the broad peak at 3470 cm⁻¹ may be due to the molecular water present (42,43). FTIR spectrum of β.cyclodextrin shows a characteristic peak at 3371 cm⁻¹ due to the O-H group stretching, an intense peak at 2924 cm⁻¹ due to C-H stretching, in addition, peak at 1644 cm⁻¹ represented H-O-H band of water present in β.cyclodextrin and also at 1156 cm⁻¹ of C-O stretching band, and 1028 cm⁻¹ of C-H stretching overtone.

On other hand, FTIR spectrum of PEG 4000 show broad band at 3450.99 cm⁻¹ due to O-H group stretching, intense peak at 2885.95 cm⁻¹ due to C-H stretching in addition to an intense band at 1105.98 cm⁻¹ of C-O group (43,44).

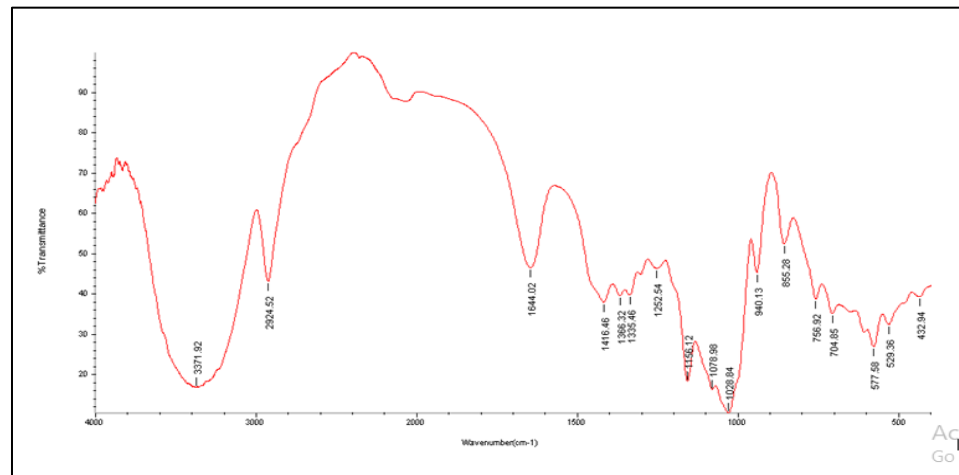
FTIR spectrum of the physical mixture of drug and polymers, spherical agglomerated SCAs (EBS1, EBS 6) show no significant shift or reduction in intensity of peaks of EBS, and of β. cyclodextrin, which indicate there was no interaction between drug and polymer. The disappearance of PEG 4000 peaks may be due to a small amount of PEG4000 entrapped within the formula (figure 6: a through f)



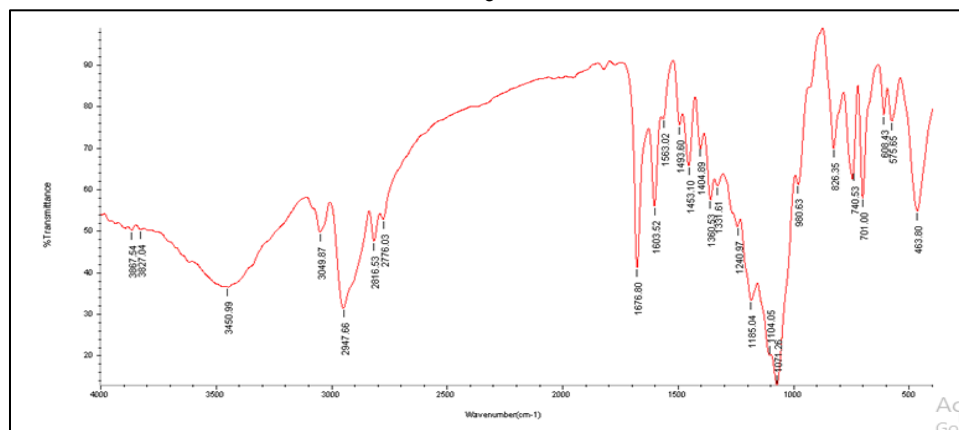
a



b



c



d

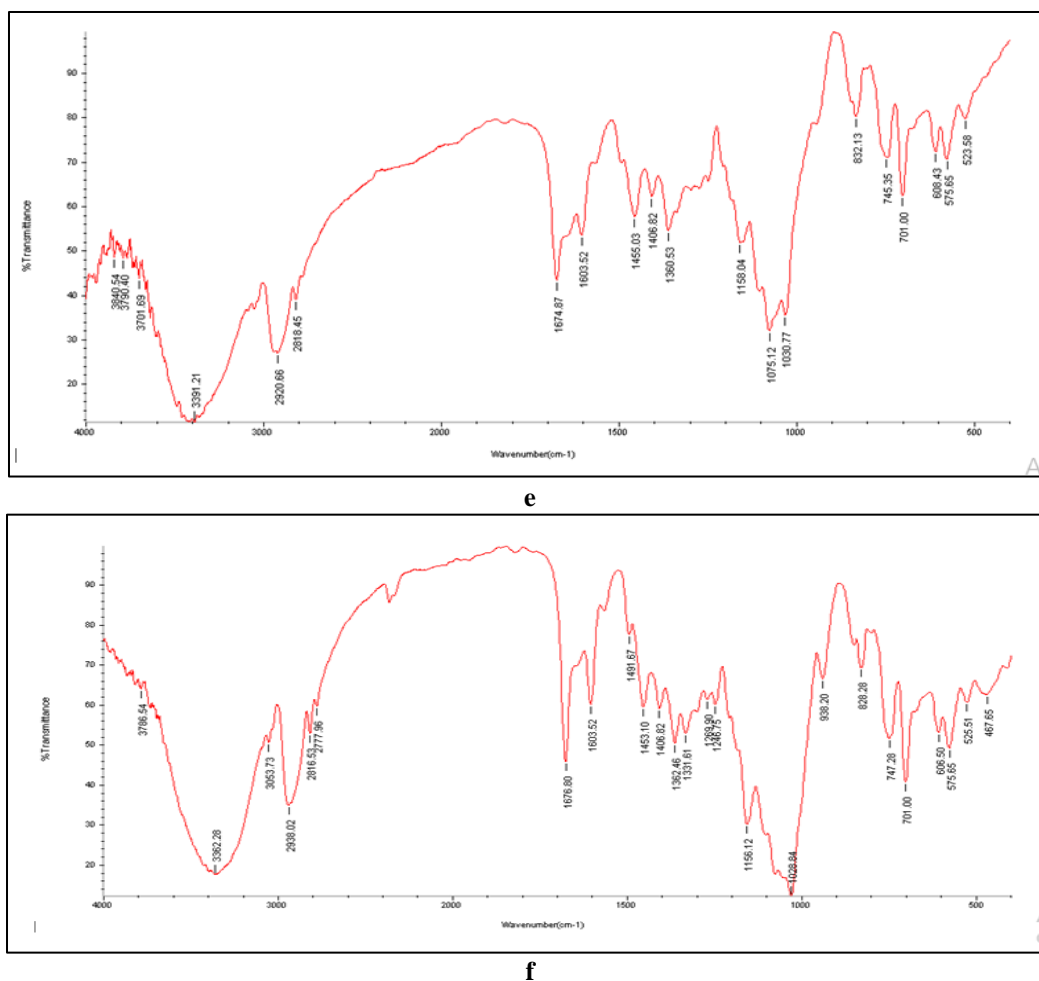


Figure 6. FTIR of: (a)EBS (b)PEG4000 (c) β . cyclodextrin (d)EBS1, (e)PM (f)EBS6

X-ray diffraction (X. RD)

The diffractogram for EBS, EBS1, physical mixture of drug and polymers used in the preparation of the EBS6, and EBS6 are viewed in (Figure 7 a through d). The diffractogram of pure drug exposed several diffraction peaks with high intensities at 16.4° , 18.9° , and 19.3° at 2θ indicating the crystal nature of the drug. Nearly the same three strongest peaks at 16.9° , 18.73° , and 19.3° were also shown for EBS1 confirming the success of the crystallization process.

Physical mixture of EBS6 showed three strongest peaks at 14.3° , 19.2° , and 23.3° , in addition to other minor peaks, while (EBS6) displayed three strongest peaks at 18.4° , 18.8° , and 19.4° and other minor peaks detected in untreated EBS and also appearance of several new peaks related to

polymers added to formula. The difference in XRD patterns of the physical mixture from that the EBS6 may be attributed to change in crystal arrangements of EBS in its spherical crystal form.

Since all the samples showed similar peak positions (2θ) in X-ray diffraction, so, the formation of different polymorphous of EBS was ruled out. However relative intensities of XRD peaks were modified. This could be attributed to the markedly different crystal habits of the samples. Therefore, the related abundance of the planes exposed to the X-ray source would have been changed, producing the variations in the relative intensities of the peak or may be due to the reduction in crystallinity (partial amorphism) of particle cause reduction in intensity of peaks ^(45,46).

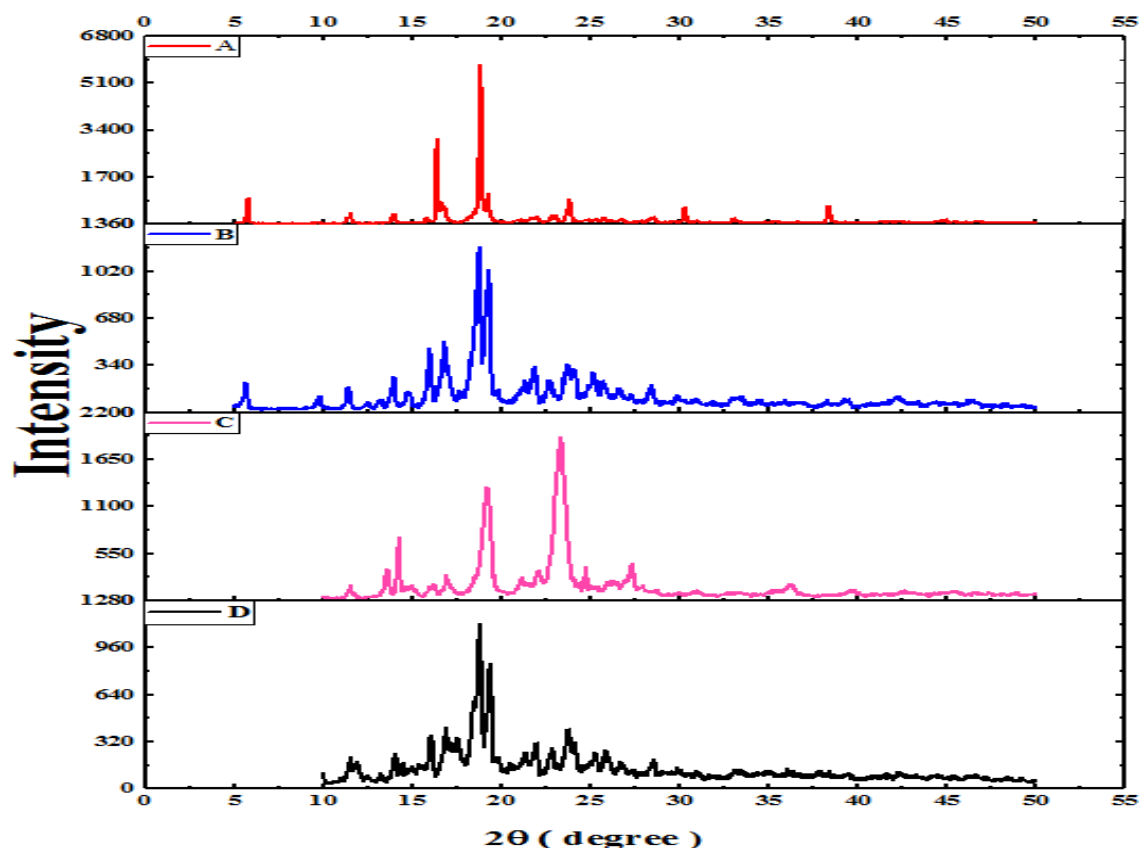


Figure 7. X.RD of : (A) EBS (B) EBS1 (C) PM (D)EBS6

Differential scanning calorimetry (DSC):

DSC analysis was used to observe the effect of polymers used in the preparation of SCAs on the melting point of EBS and also on the crystal habit of the resultant products^(47,48). The thermograms of pure EBS, PEG4000, β -cyclodextrin, EBS1, physical mixture of drug and polymers used in the preparation of the EBS6 and EBS6 are shown in (figure 8 a through f) respectively. Pure drug showed sharp endothermic peak at 89 °C corresponding to its melting point⁽²⁷⁾. Thermogram of PEG also showed sharp endothermic peak at 65.54°C related to its melting point^(31,49), while β -cyclodextrin showed a broad peak at 114.44°C which corresponding to the loss of the water molecules existing in the form of residual humidity as well as those included in the cavity by evaporation, while the peak at 312.43°C corresponds to its melting point⁽⁵⁰⁾. So, the above results indicated the purity and crystallinity of the

used materials. Furthermore, the DSC analysis of (EBS1) gave a sharp peak at 86.62 C which is slightly below the melting point of the pure drug which may be due to little amorphousim resulting from the spherical shape of SCAs⁽⁵¹⁾ as well as resultant from the suppression effect due to presence of polymer which act as impurity. Physical mixture showed characteristic peaks for the drug, PEG, and β .cyclodextrin which revealed there was no interaction between drug and polymers and maintaining the crystallinity. (EBS6) gave a sharp peak at (87.12°C) which corresponding to the melting point of EBS crystals form (EBS1) and weak broad band at (351.15°C) related to the melting point of β . cyclodextrin indicating that, β . cyclodextrin was entrapped within the formula. The disappearance of PEG 4000 peak may be due to the small amount of polymer trapped with SCAs. These results confirm that there was no interaction between drug and polymers and there was no change in crystal nature of drug⁽⁵²⁾ (figure 8 f) and also, these results ensure the result obtained from x.ray diffraction.

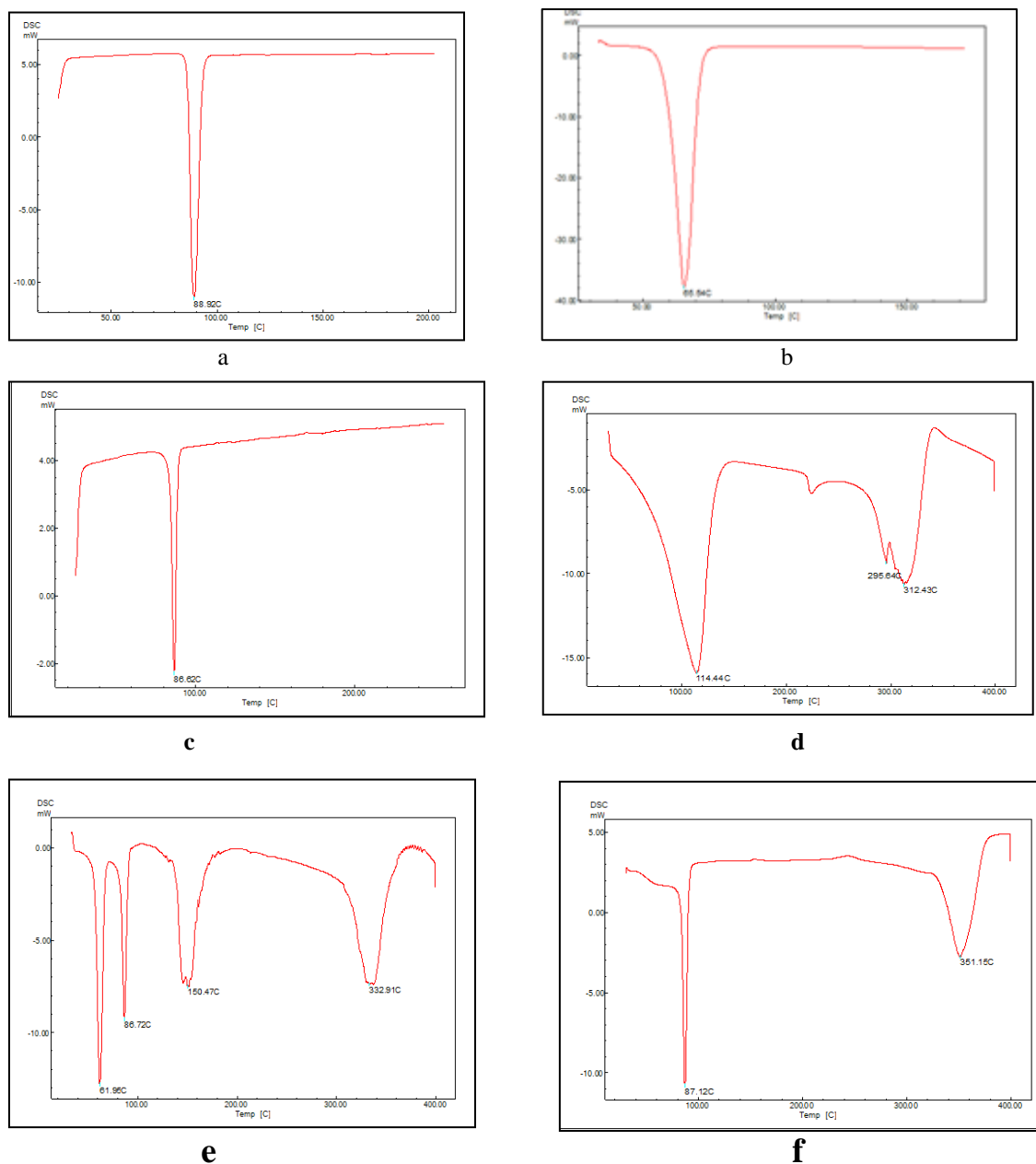


Figure 8. DSC analysis of (a)EBS, (b)PEG4000 (c) β -cyclodextrin, (d)EBS1, (e)PM, (f)EBS6

Conclusion

SCAs were successfully prepared by QESD method. The resultant crystals show enhancement in solubility and dissolution rate of ebastine except that formula prepared by PVP K 30. And also all formulas have the desired micromeritic properties, such as flowability and packability. Dramatic improvement in solubility and dissolution rate of ebastine was obtained by the combination of β -cyclodextrin and PEG4000. However in vivo bioavailability studies are required to ensure whether the results obtained in this investigation can be extrapolated to the in vivo conditions.

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