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Formulation and Evaluation of Silymarin as Microcrystals by *In- Situ* Micronization Technique

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Abstract

Silymarin (SM) is a plant extract obtained from *Silybum marianum*(milk thistle). It is hepatoprotective drug class II type according to Biopharmaceutics Classification System. It is practically insoluble with low bioavailability.

Micro/nanonization during crystallization, surface modification and crystal structure modification may improve the solubility and dissolution rate of poorly water-soluble drugs.

The aim of this study was to enhance the water solubility and dissolution rate of SM by in-situ micronization using solvent change either by stirring or ultrasonic method. Polymers like gelatin, PVP-K30, HPMC15, pululan were used to stabilize the prepared ultra-fine crystals. Effect of type and concentration of hydrophilic polymer, solvent: anti-solvent volume ratio and the effect of ultrasonic irradiation were studied. The prepared microcrystals were evaluated for their percent yield, water solubility, crystals structure by XRD, DSC, and SEM. Particle size and dissolution rate were also determined. Silymarin microcrystals prepared by ultrasonic method and stabilized by 0.1% w/v gelatin using 1:2 solvent: anti-solvent volume ratio showed the best results with percent yield 93.4±2.7% and particle size reduction from mean diameter of 1.5µm (untreated SM) to 0.43µm with uniform morphology and enhanced solubility for about four folds with improvement in dissolution.

Keywords: Silymarin, crystallization, in-situ micronization, solvent change method.

تصييغ وتقييم السليمارين كبلورات مايكروية بتقنية التصغير الآني

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الخلاصه

السليمارين هو مستخلص نباتي مستحصل عليه من عشبة سليبيوم مارينيوم (شوك الحليب). هو دواء يقي من امراض الكبد من ضمن الصنف الثاني (نظام تصنيف المستحضرات الصيدلانية الحيوي) والذي لايذوب في الماء عمليا, ويملك توافر حيوي منخفض بسبب انخفاض ذوبانيته. التصغير الميكروي /النانوي أثناء التبلور وتعديل سطح وبنية البلورة قد يحسن من ذوبانية ومعدل انحلال الأدوية ضعيفة الذوبان في الماء.

الهدف من هذه الدراسة هو زيادة الإذابة ومعدل انحلال السليمارين من خلال تقنية التصغير الآني بإستخدام تغيير المذيب إما عن طريق التحريك أو طريقة الموجات فوق الصوتية. تم إستخدام المثبتات مثل الجلاتين, بولي فينيل بيروليدون- ك٣٠، هيدروكسي بروبل ميثل سيليلوز- ي١٥،والبوليولان لتحقيق الإستقرار في البلورات المتناهية الصغر المحضرة. حيث تم دراسة تأثير نوع وتركيز البوليمر المائي، نسبة حجم المذيب:المذيب المعاكس، وتأثير الإشعاع بالموجات فوق الصوتية. وقد تم تقييم البلورات الدقيقة المحضرة من حيث نسبة الإنتاجية %، ذوبانيتها في الماء، وبنيتها البلورية بواسطة إختبار حيود الأشعة السينية، والمسح التفريقي للحرارة، والمسح المجهري الإلكتروني. كما تم إختبار حجم الجسيمات ومعدل انحلالها .

ان بلورات السلمارين المكروية المحضرة بواسطة طريقة الموجات فوق الصوتية والمستقرة بواسطة الجيلاتين بنسبة ٠،١ % (وزن\حجم) بإستخدام نسبة ١:٢ من المذيب للمذيب المعاكس أظهرت أفضل النتائج من حيث نسبة الانتاجية ٩٣,٤±٢،٧% وتخفيض حجم الجسيمات من متوسط قطر ١,٥ مايكرون لمسحوق السلمارين الى ٠,٤٣ مايكرون مع شكل متجانس وزيادة في الذوبانية لحوالي اربعة أضعاف مع تحسن في معدل الانحلال.

الكلمات المفتاحية : سليمارين، التبلور، التصغير الآني، طريقة تغير المذيب.

Introduction

Many new drugs are poorly water-soluble, with low dissolution and bioavailability [(1)]. One way for improving their dissolution rates is by decreasing the particle size. Decreasing the particle size increases the surface area, which results in an increase in the rate of dissolution of these drugs in aqueous media such as body fluids and enhance their absorption and bioavailability[(2)].

Micro / nanonization during crystallization, surface modification and crystal structure modification may improve the dissolution rate of poorly water-soluble drugs. Several studies have shown that modification of the crystalline form of the drug, by the inclusion of specific additives increases the rate of dissolution which can strongly affect the physicochemical and kinetic properties of the drug from the final product.

Micro/nanoparticles can be produced by several techniques such as crystallization/precipitation and solvent evaporation and by spray drying methods[(3)].

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In situ micronization process; it is a technique in which microcrystals are prepared using a precipitation technique of a drug from solution by either pH change or by solvent change process (using anti-solvent) in the presence of protective hydrophilic polymers[4]. This technique is beneficial in these aspects as it does not require any external processing conditions except mild agitation using magnetic stirrer and the processing time is less compared to the other techniques[5].

The use of the anti-solvent in crystallization reduces the solubility of a solute in the solution and generates high supersaturation that subsequently induces nucleation and simultaneous growth by condensation and coagulation.to induce rapid crystallization followed by agglomeration in case of uncontrolled growth [6].

Silymarin a flavonolignans extracted from the fruits and seeds of milk thistle *Silybum marianum* (L.). (Asteraceae), it is an annual herb, native to the Mediterranean and North African regions; it is wildly grow throughout Europe, North Africa, Americas and Australia[7,8].

Silymarin (SM) is is a hepatoprotective class II type drug according to Biopharmaceutics Classification System, which is practically insoluble in water, and soluble in organic solvents like methanol, ethanol, and acetone [8].

It is absorbed when given orally and the peak plasma concentration is achieved in 6-8 hr. The oral absorption of silymarin is only about 23-47% resulting in low bioavailability [9].The aim of this study was to enhance the solubility of silymarin by preparing microcrystals by in- situ micronization technique using solvent change process in presence of protective hydrophilic polymers.

Materials and Methods

Materials

Silymarin (SM) purchased from Hyperchemical Limited,China. gelatin, polyvinylpyrrolidone (PVP-k30) from Riedel De Haen AG Seelze, Honnover, Germany, HPMC15 and pullulan from Hyperchemical Limited,China, methanol from GCC Analytical reagent, UK, all other reagents used were of analytical grade.

Methods

Determination of SM saturated solubility

The solubility was measured in distilled water, by addition of an excess amount of SM powder to 10ml of distilled water in test tube and incubated in water bath shaker at 25 °C for 48 hrs.

After that, the samples were centrifuged at 500 rpm for 5 min using a high-speed centrifuging machine to remove the excess insoluble SM, and then filtered through a 0.45 µm filter membrane and assayed spectrophotometrically for SM at 288nm λ_{max} [10,11]. Results were recorded as the mean value of three determinations.

Preparation of SM microcrystals

Microcrystals of SM were prepared by solvent change method.

In this method; organic phase was preapared by dissolving 3% of SM in methanol. Aqueous phase was prepared by dissolving specific amount of polymer as stabilizing agent in distilled water (as anti-solvent).

The aqueous phase was poured rapidly into the drug solution under constant stirring (400rpm) by magnetic sterrier [12] and stirring was continued for 60 min. Microcrystals were formed spontaneously, collected after filtration through whatman filter paper (grade 1, 110mm diameter) by using vacuum filtration, followed by washing for three times with cold diatilled water to remove non-adsorbed excipients if any, and dried in an oven at 40°C for 1hr[13]. Several factors were studied to optimize the best conditions for micronization .

Factors affecting SM in situ micronization

To select the best conditions for obtaining microcrystals with maximum yield, solubility and crystallinity the following factors were studied.

Effect of polymer type

To select the best polymer for preparation of SM microcrystals, four formulas were prepared; MC1, MC2, MC3 and MC4 using gelatin, PVP-K30, HPMC15 and pullulan respectively as stabilizing agent with 0.1% (w/w) concentration for each as shown in table.1

Effect of polymer concentration

To estimate the minimum concentration of stabilizing agent (gelatin or PVP k30) required to get the maximum crystals yield and solubility, three concentrations of each stabilizing agent 0.05% (MC5 and MC7),0.1% (w/v)(MC1 and MC2) and,0.2% (w/v) (MC6 and MC8) were used respectively as shown in table.1.

Effect of solvent: anti-solvent ratio

Gelatin and PVP k 30 were selected as stabilizers to study the effect of solvent: anti-solvent ratio on the properties of the prepared SM Microcrystals. Table 1 shows that three solvent: anti-solvent ratios 1:2(MC9and MC11), 1:4(MC1 and MC7) and 1:6(MC10 and MC12) were used for the selection of most appropriate solvents ratio for each polymers.

Effect of ultrasound

Sonocrystallization method was used to study the effect of ultrasound irradiation on the preparation of SM microcrystals (MC9s, MC1s, MC10s) using 0.1% (w/v) gelatin as stabilizing agent with different solvent: anti-solvent ratios as shown in table.1

The properties of the resultant microcrystals are to be compared with those of the corresponding formula prepared by stirring method.

By this method, the aqueous phase was poured to the organic drug solution as the previous experiments but the stirring was changed to

sonication by ultrasonic bath for 20 min. Ultrasonic power used was 200 W and the frequency of ultrasound was 40 kHz [14]. The concentration of SM in methanol was kept constant (3%)(w/v).

Table (1) Composition of SM microcrystals formulas.

F. No.	SM. Conc. %w/v	Organic solvent	Polymer	Polymer. conc. %w/v	Solvent: anti-solvent ratio	Method
MC1	3	Methanol	Gelatin	0.1	1:4	Stirring
MC2	3	Methanol	PVP-K30	0.1	1:4	Stirring
MC3	3	Methanol	HPMC15	0.1	1:4	Stirring
MC4	3	Methanol	Pullulan	0.1	1:4	Stirring
MC5	3	Methanol	Gelatin	0.05	1:4	Stirring
MC6	3	Methanol	Gelatin	0.2	1:4	Stirring
MC7	3	Methanol	PVP-K30	0.05	1:4	Stirring
MC8	3	Methanol	PVP-K30	0.2	1:4	Stirring
MC9	3	Methanol	Gelatin	0.1	1:2	Stirring
MC10	3	Methanol	Gelatin	0.1	1:6	Stirring
MC11	3	Methanol	PVP-K30	0.05	1:2	Stirring
MC12	3	Methanol	PVP-K30	0.05	1:6	Stirring
MC9 s	3	Methanol	Gelatin	0.1	1:2	Sono-crystallization
MC1 s	3	Methanol	Gelatin	0.1	1:4	Sono-crystallization
MC10 s	3	Methanol	Gelatin	0.1	1:6	Sono-crystallization

Method

Evaluation of the SM microcrystals

Determination of percent crystals yield

Percentage crystal yield was calculated by comparing the practical yield with respect to initial weight of drugs and polymers used in formulation of micro crystals to know about efficiency of any factors and thus it helps in the selection of appropriate micro crystallization condition. The final weights of the dried microcrystals of SM were taken and percentage crystal yield was calculated by using the formula as follows [15].

$$Percentage\ yield = \frac{Actual\ weight\ of\ silymarin\ microcrystals}{Total\ weight\ of\ drug\ and\ stabilizer} \times 100$$

Drug content

A weighed quantity of the samples was dispersed in 10 ml of methanol. It was sonicated for 10 min and the samples were filtered and diluted with suitable quantity of methanol and analyzed spectrophotometrically for SM at 288nm λ_{max}, the experiment was carried out in triplicate [16].

Solubility

An excess amount of the resulted crystals was added to 10 ml of water in test tubes. All samples were placed in the shaking water bath, which was thermostatically controlled at 25±0.5°C, for 48 hours. After that the samples were withdrawn from the supernatant by the use of a syringe ;filtered through a 0.45-µm membrane and assayed spectrophotmetrically for SM at 288nm. Results were recorded as the mean value of three determinations. [11]

X-ray diffraction

X-rays diffraction patterns (diffractograms) can be used to study the crystalline nature of a sample. Therefore, this information was used to confirm whether the drug was in crystalline or amorphous form.

The x-ray diffractograms of SM, and the selected microcrystal formulas were obtained by x-ray diffractometer (XRD-6000,Shimadzu,Japan) at continuous scan range between 10 - 80º (2θ); The operating voltage was 40 kV and 30 mA current [4] .

Differential scanning calorimetry (DSC)

DSC can be used to determine the compatibility between the drug and polymer and also used to evaluate the crystalline state of drug especially when micronized to microparticles or nanoparticles. Thermal characteristics of the samples determined by an automatic thermal analyzer system (Shimadzu, DSC–60,Japan). An approximately 5mg sample of SM, gelatin as a selected polymer, or selected microcrystals; were heated at a scanning rate of 10 °C/min in an aluminum pan under a nitrogen atmosphere at a temperature range of 50-300 ˚C. A similar empty pan was used as the reference [17].

Scanning electron microscope (SEM)

The morphology of pure powder SM and the selected microcrystals was observed by SEM. Air dried microcrystals were mounted onto metal stubs using double-sided adhesive tape, vacuum-coated

with gold and directly analyzed under SEM at magnifying power of (5000×,10000×,25000×)[18].

Particle size

A sample of SM and the selected crystals suspension were obtained after precipitation by solvent change was sonicated at 37 ˚C for 30 min and put in a 3 ml cell of ABT-9000 Nano Laser Particle Size Analyzer. The average particle size, and the polydispersity index (PDI), for the selected samples were measured.[17, 18]

In-vitro dissolution

Dissolution study was carried out using the USP type II dissolution apparatus (paddle). The dissolution media was either 900 ml 0.1N HCl (pH 1.2) or phosphate buffer (pH6.8) at 37° C± 0.5 and stirring at 100 rpm. Dissolution studies were performed on untreated SM (100mg) and the microcrystals of the selected formula containing an equivalent amount of the drug.

Aliquots of 5 ml. were withdrawn periodically and were replaced with 5 ml of fresh dissolution medium at selected time intervals (2,5, 10, 20, 30, 40, 50, 60, 90, and 120 min). The aliquots were filtered and analyzed spectrophotometrically at 288nm . Each in vitro release study was performed in triplicate. The percentage drug release versus time graph was obtained and enhancement in dissolution with respect to pure drug was calculated.[19] . The dissolution data were analysed using dissolution efficiency up to 120 min (DE120) which was calculated according to :

$$DE\ (\%) = \frac{\int_{t1}^{t2} y\ .dt}{y100 \times (t2-t1)} \times 100$$

where y is the drug percent dissolved at time t [20], and dt is equal to t_2-t_1 .

Fourier transforms infrared spectroscopy (FTIR)

The (FTIR) spectra were obtained using FTIR Shimadzu 8300 Japan. Samples of untreated SM, gelatin , and microcrystals of selected formulas, compressed with potassium bromide. The spectrum was obtained within scanning range of 4000-400 cm^{-1} [17,18] .

Statistical analysis

Using Microsoft Excel 2010, the results of the experiments were given as a mean of triplicate samples ± standard deviation and were analyzed according to the analysis of variance (ANOVA) test and t-test. The level of significance was set at a p-value of 0.05:

P value of more than 0.05 (P> 0.05) was considered to be non-significant.

P value of less than 0.05 (P< 0.05) was considered to be significant

Results and Discussion

SM saturated solubility

The saturated solubility of SM in water was found to be equal to 53 ±3.1 µg/ml, so the SM considered as practically insoluble in water according to USP [21].

Factors affecting SM in situ micronization :

Effect of polymer type:

Gelatin, PVP-K30, HPMC 15 and pululan stabilizers were tested by measuring percent of crystals yield. Table (2) shows that gelatin and PVP K30 were the best polymers among the other, which may be due to their high affinity to the newly created drug particle surface [22], therefore they were used to study the other factors affecting the preparation of SM microcrystals

Table (2) Effect of polymer type on SM microcrystals formulas

F. No.	SM. conc. %w/v	Organic solvent	Polymer	Polymer conc. %w/v	Solvent :antisolvent ratio	Percent yield %
MC1	3	Methanol	Gelatin	0.1	1:4	93.9± 3.3
MC2	3	Methanol	PVPk30	0.1	1:4	83.45±1.7
MC3	3	Methanol	HPMC15	0.1	1:4	no yield
MC4	3	Methanol	Pullulan	0.1	1:4	54 ± 4.5

Effect of polymer concentration

Table 3 shows that, the minimum concentration of the stabilizer gelatin required for obtaining maximum yield with significant increase in the solubility (P <0.05) of the drug was 0.1% (w/v) . Increasing the solubility of SM pure powder can be explained to be due to good adsorbed layer of hydrophilic stabilizer which induces the water solubility [23]. It was also observed that as the concentration of gelatin decreased to (0.05%) (w/v), low yield was obtained with lower solubility than that obtained from 0.1% (w/v), which may be attributed to that the amount of gelatin was not enough to form a hydrophilic stabilizer layer which leads to crystal growth with high drug content [24].

Furthermore, increasing the concentration of gelatin solution to 0.2% (w/v), the dried crystals stick with gelatin on whatman filter paper and no yield was obtained even by drying at room temp or using nylon filter paper. This can be explained to be due to that increasing gelatin concentration might cause efficient stabilization of drug particles until a certain concentration. Further increase in concentration would lead to increase the thickness

and viscosity of the gelatin layer around each particle that may led to aggregation of many particles which were stick by drying [25].

On the other hand, a fluffy powder was obtained with a significant increase ($p<0.05$) in solubility related to drug using PVP-k30 as stabilizer in concentration of 0.05% (w/v), and no further increase in solubility was obtained by using 0.1% (w/v) .While using 0.2% (w/v), low yield was obtained with lowest solubility than other concentrations. These results were in agreement with that obtained by Jain SA and Kurup NS [13].

From these results, 0.1% (w/v) of gelatin and 0.05% (w/v) of PVP-K30 were the selected concentrations for further studies.

Table (3) Effect of polymer concentration on SM microcrystals formulas

F. No.	Polymer	Polymer conc.% w/v	Percent yield	Drug content %	Solubility µg/ml
MC5	Gelatin	0.05%	73.36±2.4	93.2±1.8	95± 2.5
MC1	Gelatin	0.1	93.9 ± 3.3	83.95± 2.1	101.33±3.5
MC6	Gelatin	0.2%	0	-	-
MC7	PVPk30	0.05%	90.2±1.4	94.6± 1.3	122± 1.5
MC2	PVPk30	0.1	83.45±1.7	90.7±1.1	128.3± 4.1
MC8	PVPk30	0.2%	85.7±1.7	87.8±2	75.5± 3.2

Effect of solvent: anti-solvent volume ratio

No significant difference in percent yield was obtained for all solvent : anti-solvent ratios of both polymers PVP-K30 and gelatin ($p> 0.05$) which can be explained that the lowest volume of aqueous phase was enough to cause efficient change in polarity and bring out complete crystallization of both drug and polymer. While the low drug content obtained with increasing the volume of aqueous phase in both polymers may be attributed to enhance the solubilization of the drug in high aqueous phase volume in presence of hydrophilic polymer [26]. The differences in solubility between batches of both polymers may be due to the different amount of the hydrophilic polymer that adsorbed on the precipitated drug particles [27].

Table (4) Effect of solvent: anti-solvent ratio on SM microcrystals formulas.

F. No.	Polymer	Polymer conc. %	Solvent :anti-solvent ratio	Percent yield	Drug content %	Solubility µg/ml
MC9	Gelatin	0.1	1:2	92.3 ±2.3	95.6 ±2.2	148.66±4
MC1	Gelatin	0.1	1:4	93.9 ±1.6	83.95 ±2.1	101.33±3.5
MC10	Gelatin	0.1	1:6	96.3 ±2.1	82 ±2.5	134.66±3.5
MC11	PVPk30	0.05	1:2	91.8 ±1.7	94.6 ±1.5	83.33±4.1
MC7	PVPk30	0.05	1:4	90.2 ±1.4	94.6 ±2.1	122±1.5
MC12	PVPk30	0.05	1:6	94.8±1.7	82.2 ±1.8	79±3

Effect of sonication

Since microcrystals prepared using 0.1 % (w/v) gelatin as stabilizer for the three solvent: antisolvent ratios have higher solubility than those prepared by using PVP . Therefore, MC1s, MC9s and MC10s were prepared to be compared with the corresponding formulas prepared by stirring.

Table 5 shows that all batches prepared by sonication have nearly the same percent yield and drug content to that prepared by stirring method. On the other hand, the solubility of the sonicated batches was higher than those prepared by stirring method which may be explained to the action of ultrasound waves that create microscopic turbulence within interfacial film surrounding crystals, as a result, ultrasound intensified external mass transfer and adsorption rate of gelatin which diffused rapidly and covered the surface of the SM crystals. So the polymer would be quickly reached adsorption equilibrium that would increase the solubility of SM [25].

Table (5) SM microcrystals formulas prepared by two method using gelatin polymer

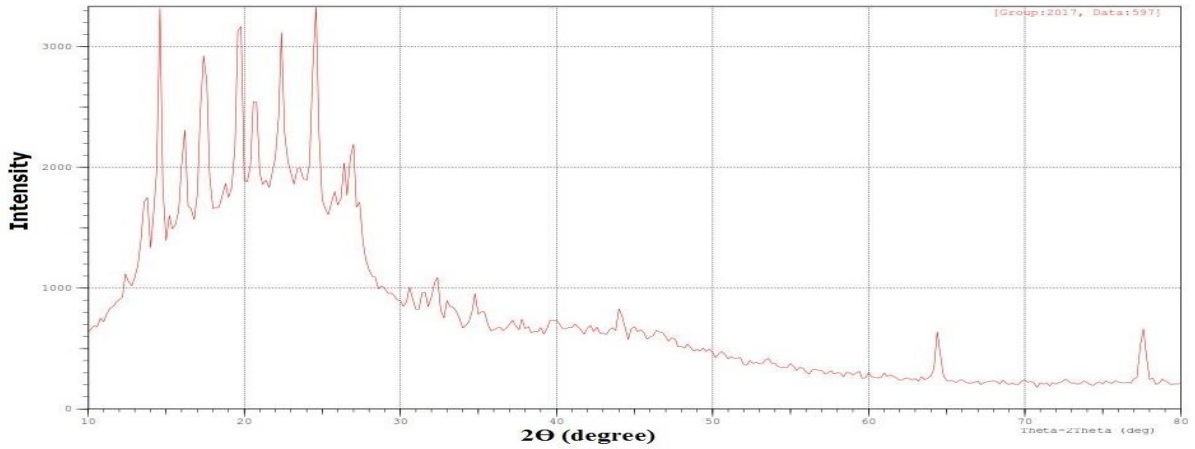
F. No.	Solvent :anti-solvent ratio	Method of preparation	Percent yield	Drug content %	Solubility µg/ml
MC9	1:2	Stirring	92.3 ±2.3	95.6 ±2.2	148.66±4
MC1	1:4	Stirring	93.9 ±1.6	83.95 ±2.1	101.33±3.5
MC10	1:6	Stirring	96.3 ±2.1	82 ±2.5	134.66±3.5
MC9 s	1:2	Sono- crystallization	93.4±2.7	94.6±4.5	220±2.7
MC1s	1:4	Sono- crystallization	95.5±2.5	85.3±3.2	120.6±1.5
MC10s	1:6	Sono- crystallization	97.4±3	84.5±2.1	155±4.5

X-ray diffraction

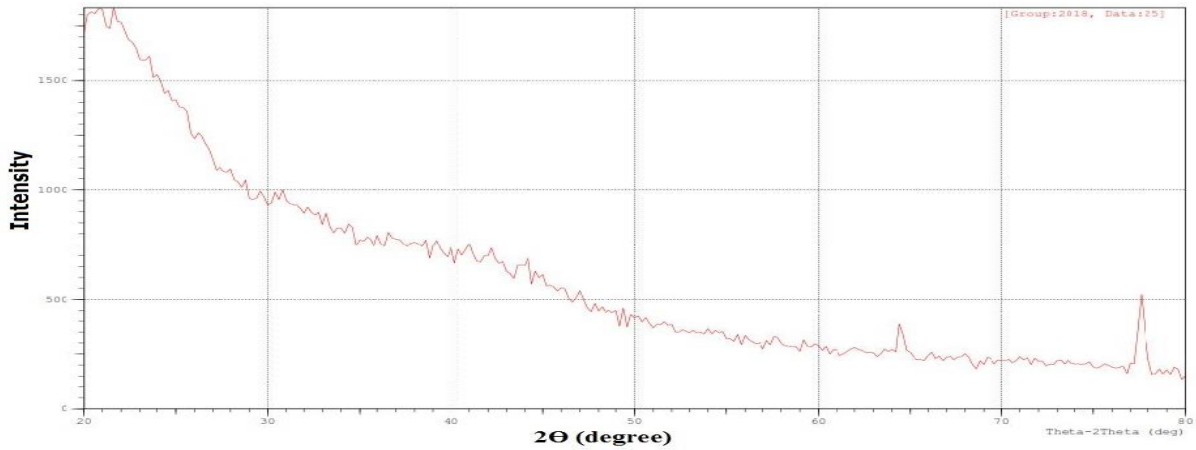
The X-ray patterns of the SM pure powder (Figure.1:a) displayed the presence of numerous narrow and characteristic diffraction peaks (17°, 19°, 24°, 44°, 77°) with high intensity indicating the crystalline structure of the drug. Generally, the positions of the most characteristic peak 24º of SM and microcrystals preparations were identical, although the peaks for the untreated SM crystalline powder were of highest intensity than those of the SM microcrystals. Since, the peak height is affected by crystal size and crystallinity. Therefore, the peaks height or intensity of the microcrystals was lower than those of SM, due to smaller size of the prepared microcrystals compared to that of SM crystals as it will be confirmed by measuring their particle size [17, 28].

In addition, XRD shows that the crystallinity of the products was affected by solvent: anti solvent ratio used in the preparation of the microcrystals. Microcrystals prepared with 1:2 solvent: anti solvent (Figure.1:c) ratio gave peaks with highest intensity than those prepared using (1:4 or 1:6) solvent: anti-solvent ratio (Figure.1:d,e).

Furthermore, peaks with higher intensity were obtained with microcrystals prepared by sonication method (Figure.1:f,g,h) compared with those prepared by stirring method, this phenomenon was observed with all solvent : anti-solvent ratios. This may be explained to ultrasound irradiation can intensify the mass transfer, accelerate molecular diffusion and, thus, reduce the induction time of crystallization, increase the nucleation rate. These effects bring considerable benefits to the crystallization process [25].



(A)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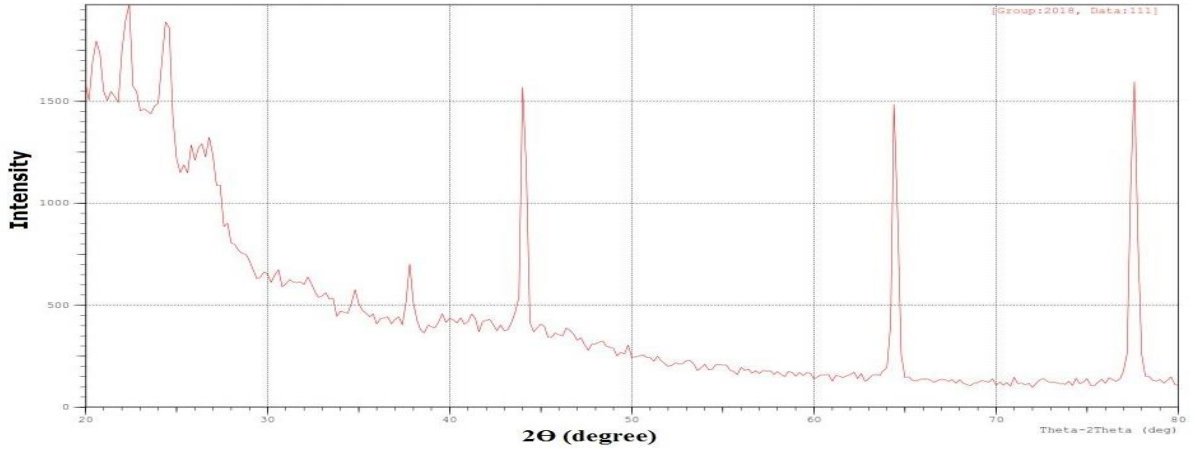
Theta-2Theta (deg)

(B)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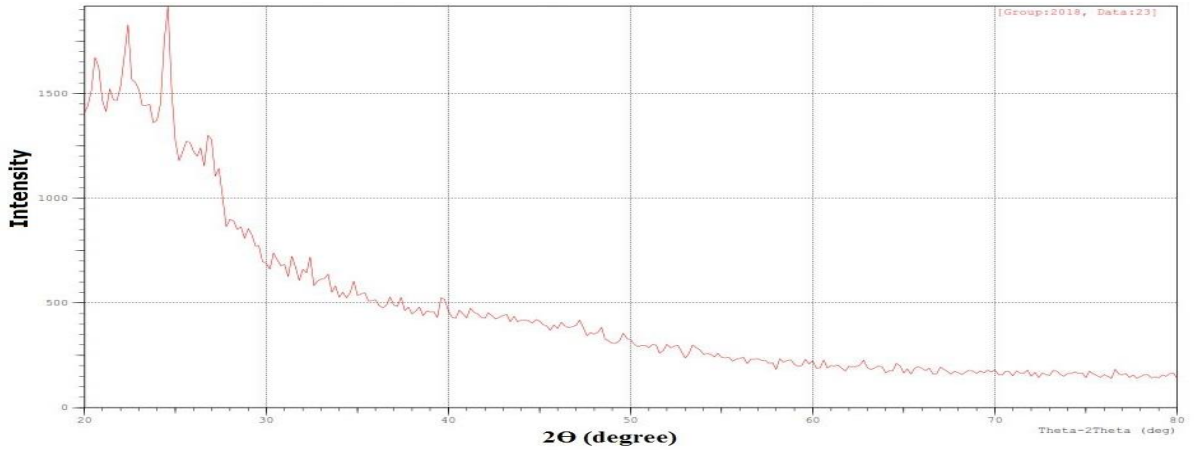
(C)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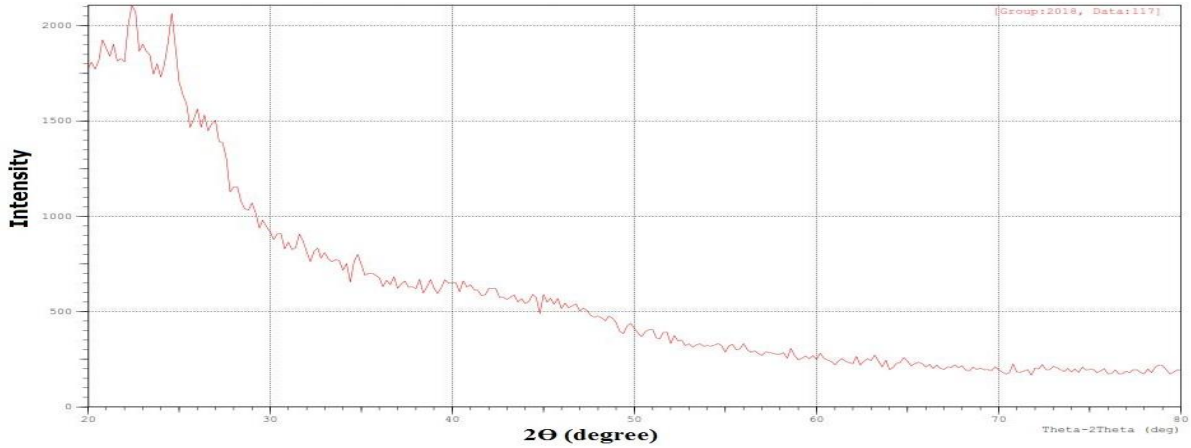
Theta-2Theta (deg)

(D)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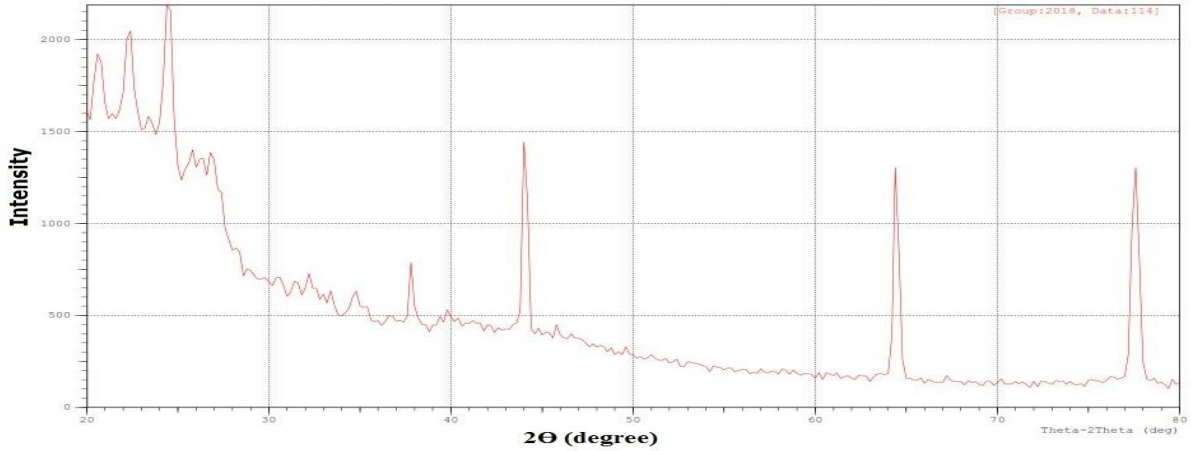
Intensity
2Θ (degree)
Theta-2Theta (deg)

(E)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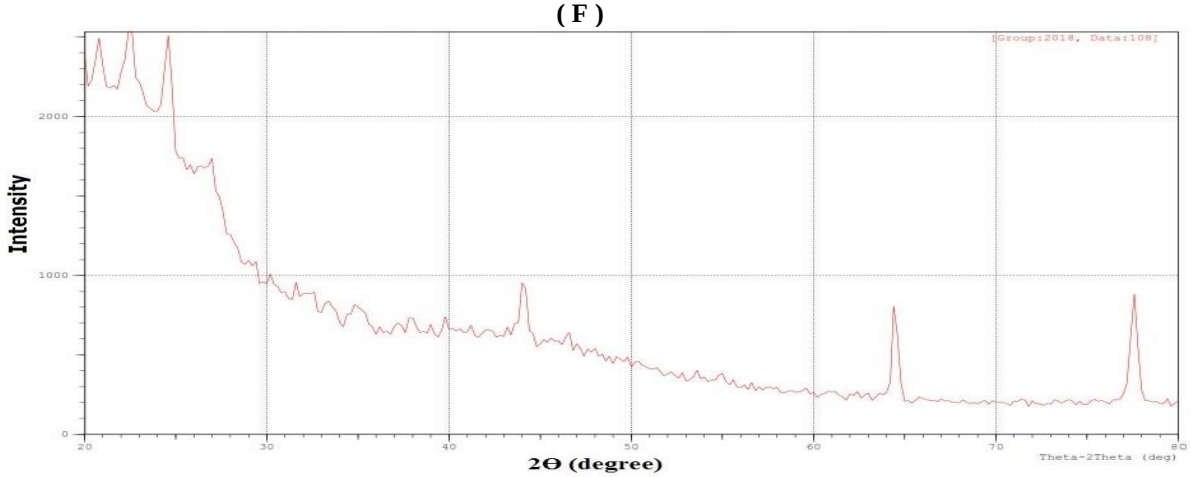
2Θ (degree)
Theta-2Theta (deg)

(F)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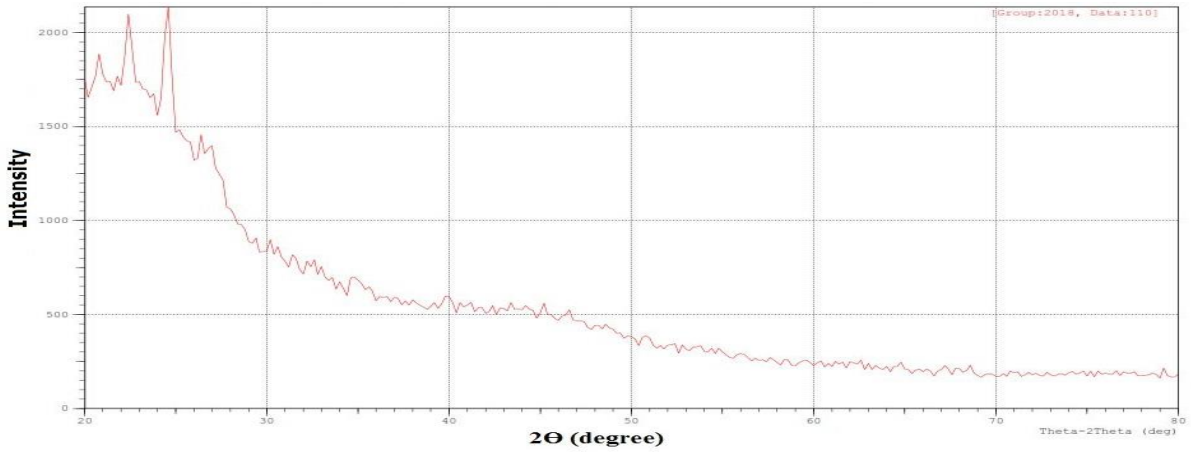
Intensity
2Θ (degree)
Theta-2Theta (deg)

(G)



(H)

Figure (1) X-ray diffraction patterns of silymarin (A) , gelatin(B), MC9 (C), MC1 (D), MC10 (E),MC9s (F), MC1s (G), MC10s (H)

Differential scanning calorimetry (DSC)

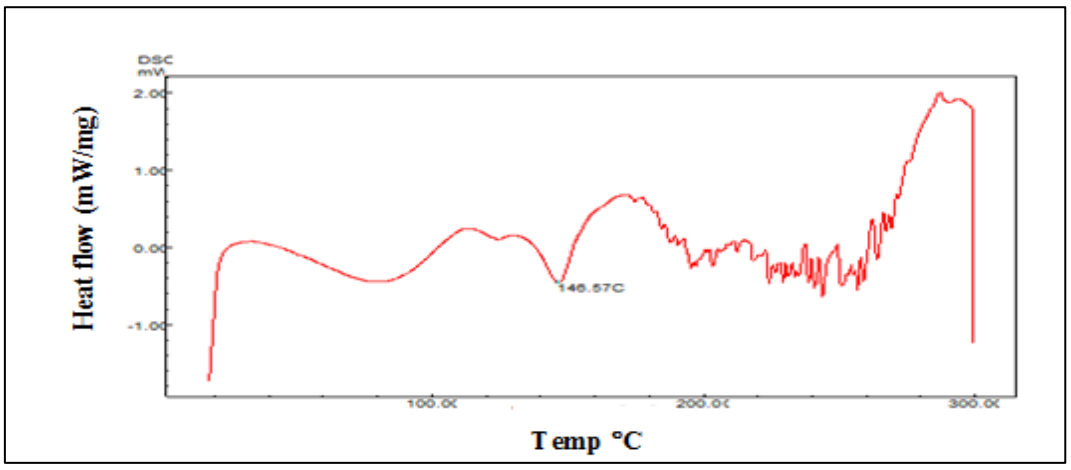
DSC thermogram of untreated SM shows an endothermic peaks about 200ºC corresponds decomposition of drug compounds (Figure.2:A). In addition broad endothermic event between 50°C and 120 °C corresponds to melting peak of silybin, an active constituent of SM complex [29].

The thermogram of gelatin (Fig.2:B) showed an intense glass transition temperature located around 98ºC. The thermogram of the selected microcrystals with different solvent: anti-solvent ratio MC9, MC1, MC10 stabilized by gelatin are shown in (Figure.2:C,D,E). They reveal an induction in the intensity of endothermic peaks of SM in batches prepared with 1:2 solvent: anti-solvent ratio in comparison to that of untreated SM, and microcrystals prepared with 1:4 and 1:6 solvent: anti-solvent ratio although they were available relatively at the same temperature and this may be due to increase in crystallinity due to ultrasound effect on nucleation [25,30].

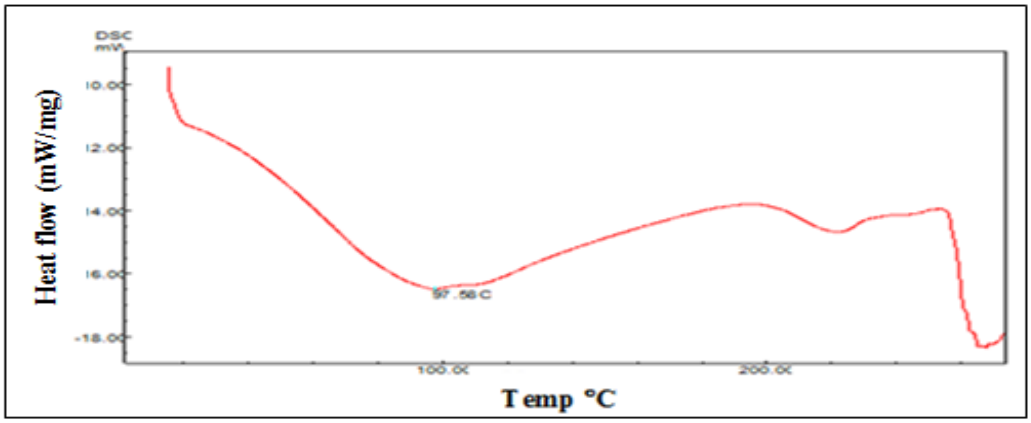
Batches prepared by using 1:6 solvent: anti-solvent volume ratio (MC10) showed a reduction in the intensity of endothermic peaks that may be due to the conversion of the drug from crystalline to amorphous state. This result agreed with increased solubility of this preparation, and confirms what was obtained from XRD results.

The crystals prepared by sonication (Figure.2: F,G,H) showed higher peak intensity for all solvent: anti- solvent ratio in comparison to those prepared by stirring method.

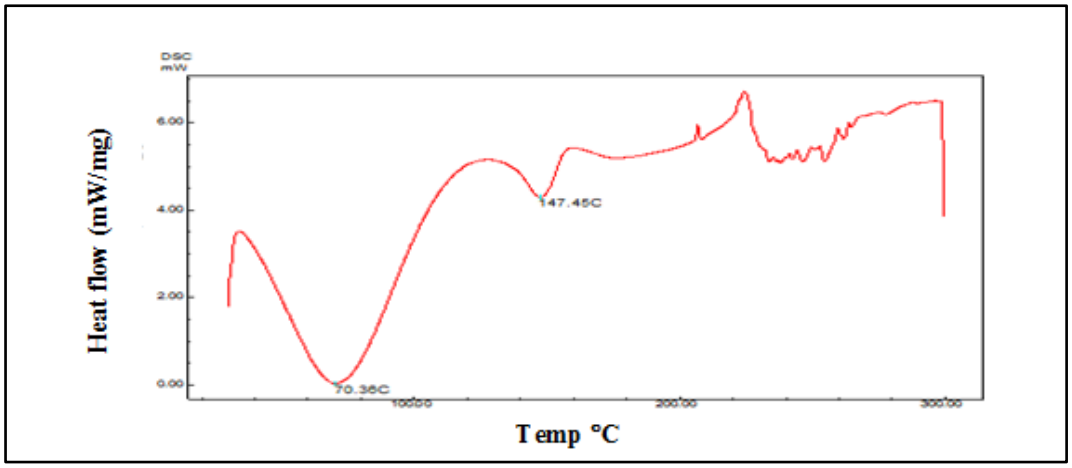
The higher crystallinity obtained by using 1:2 solvent: anti-solvent and by sonication method may be due to rapid nucleation rate with reduction in the induction time of crystallization [25,30]. On the other hand, the disappearance of gelatin peaks in both XRD and DSC patterns of micronized SM, indicating a high drug load in these preparation [24].



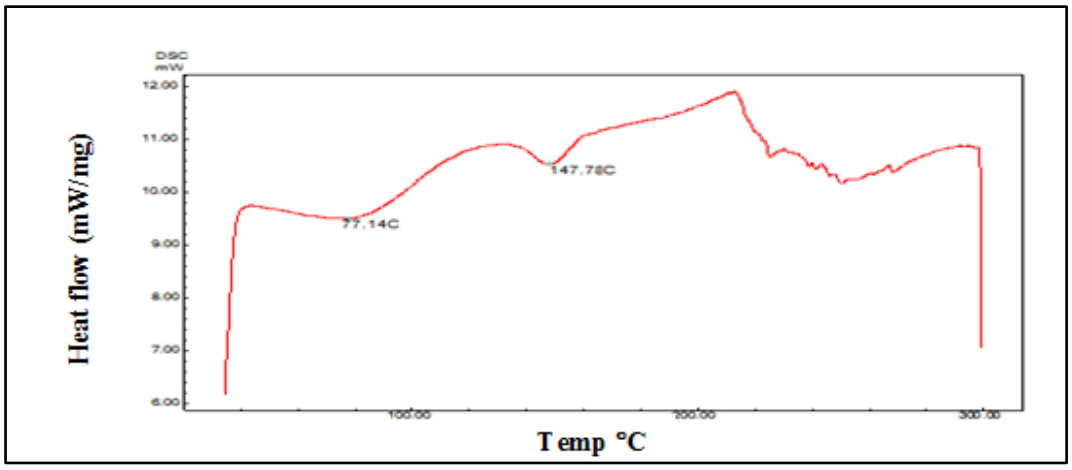
(A)


DSC
mW
10.00
12.00
14.00
16.00
-18.00
97.58C
100.00
200.00
Heat flow (mW/mg)
Temp °C

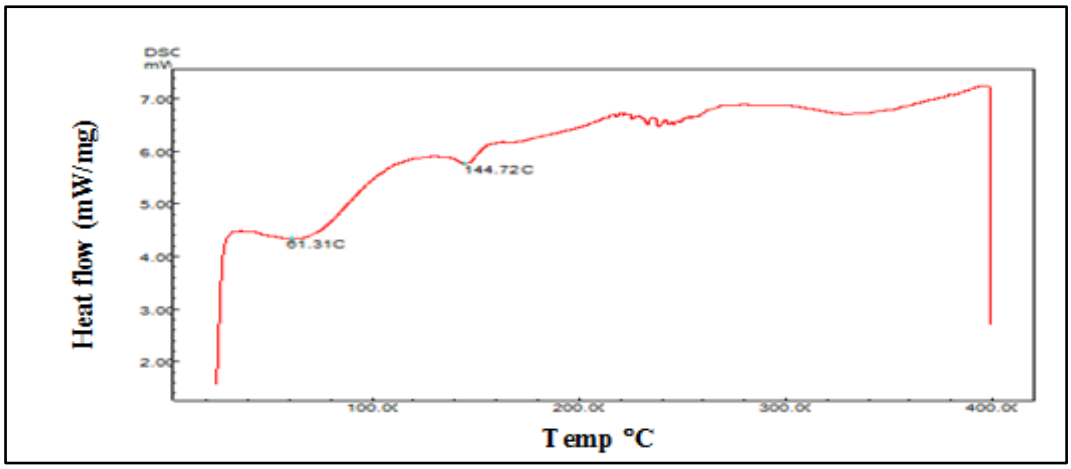
(B)


DSC
mW
6.00
4.00
2.00
0.00
70.36C
147.45C
100.00
200.00
300.00
Heat flow (mW/mg)
Temp °C

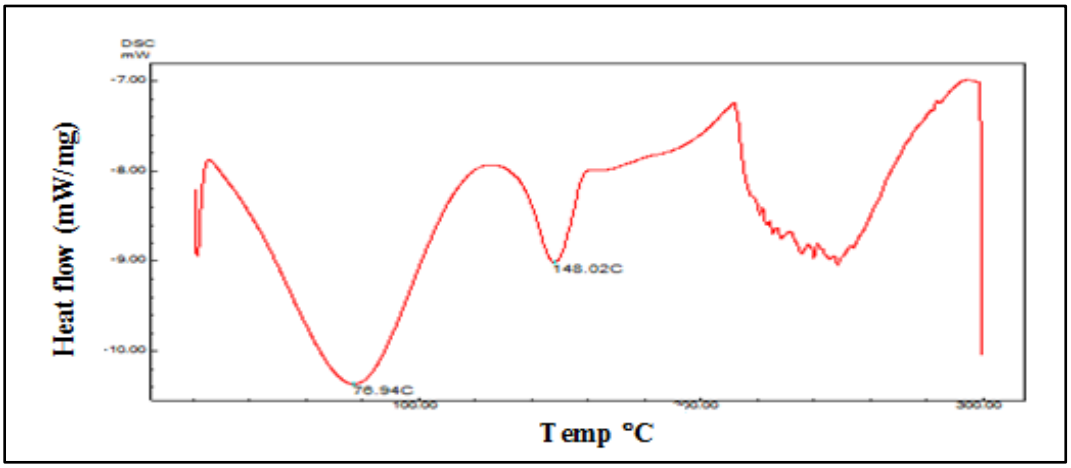
(C)


DSC
mW
12.00
11.00
10.00
9.00
8.00
7.00
6.00
77.14C
147.78C
100.00
200.00
300.00
Heat flow (mW/mg)
Temp °C

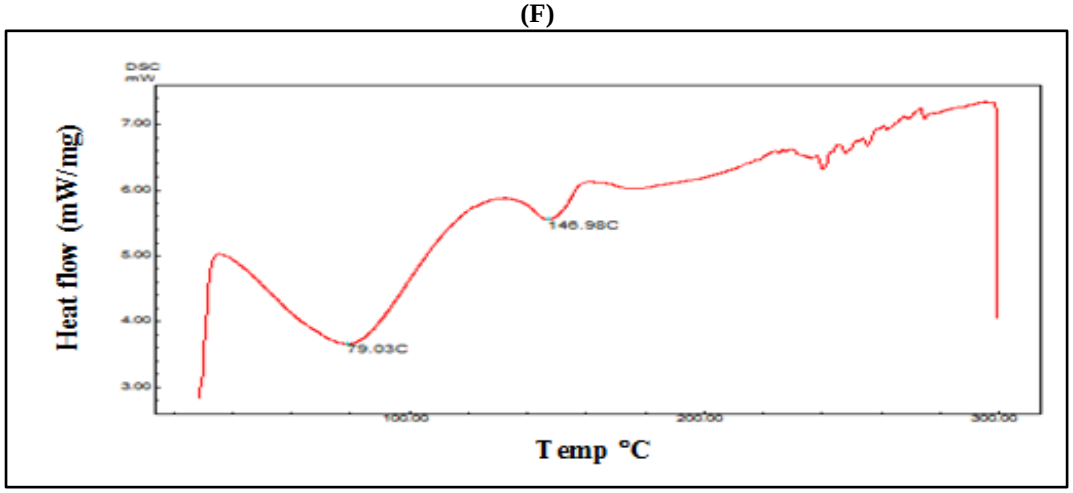
(D)


DSC
mW
7.00
6.00
5.00
4.00
3.00
2.00
144.72C
61.31C
100.0C
200.0C
300.0C
400.0C
Heat flow (mW/mg)
Temp °C

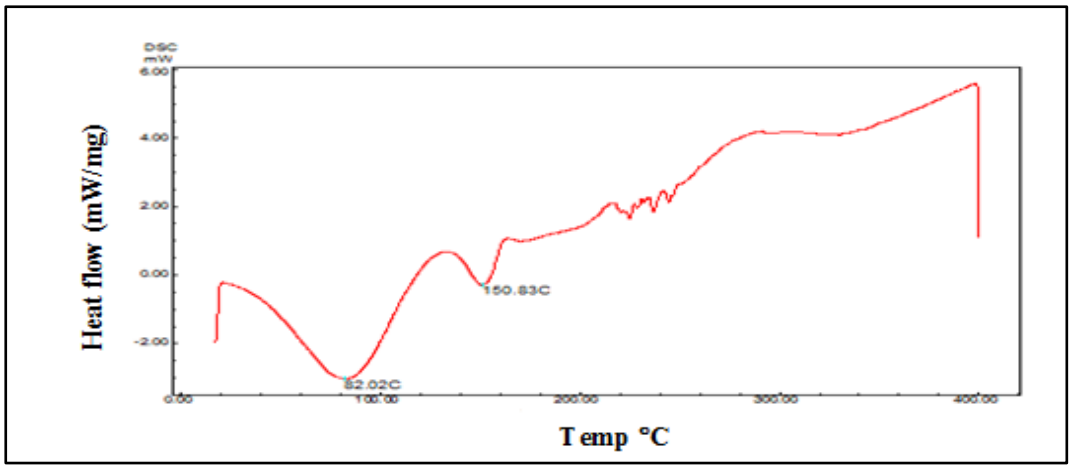
(E)


DSC
mW
-7.00
-8.00
-9.00
-10.00
148.02C
76.94C
100.00
300.00
Heat flow (mW/mg)
Temp °C

(F)


DSC
mW
7.00
6.00
5.00
4.00
3.00
146.98C
79.03C
100.00
200.00
300.00
Heat flow (mW/mg)
Temp °C

(G)

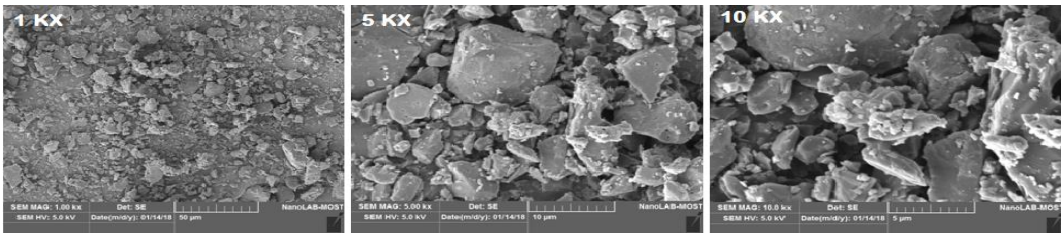


(H)

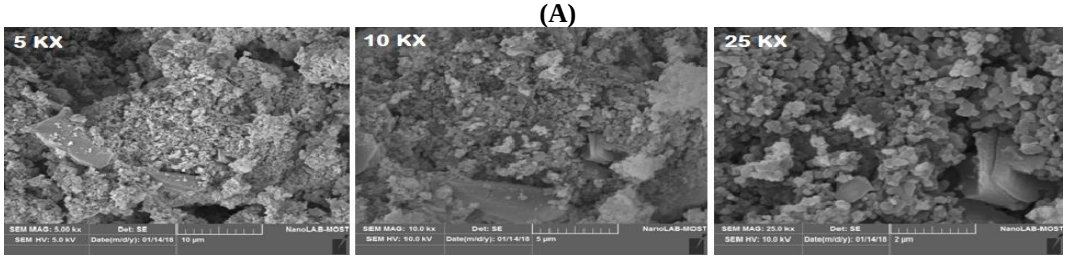
Figure (2) DSC thermogram of SM (A), gelatin (B), MC9 (C), MC1 (D), MC10 (E), MC9s (F), MC1s (G), MC10s (H).

Scanning electron microscope (SEM)

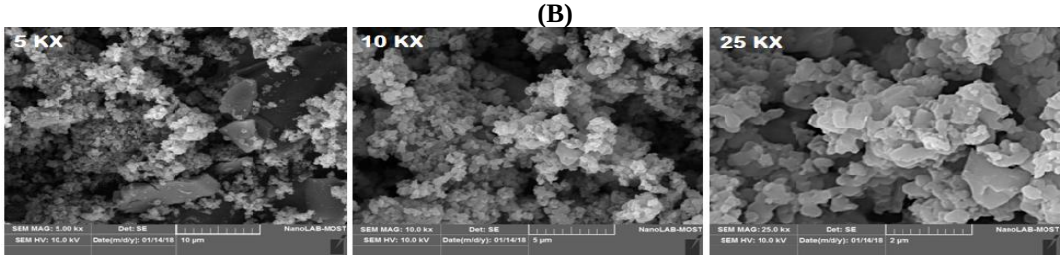
Micrographs of the untreated SM crystalline powder and microcrystals of the optimum formulations prepared by two methods are shown in (Figure.3). The untreated SM particles are of irregular shape, whereas the microcrystal particles MC9 which were prepared by stirring method have uniform particles nearly spherical shapes with smooth and regular surface. The microcrystals prepared by sonication (MC9s) were also homogeneous with larger crystals. Obviously, the microcrystals prepared by any method were more uniform shape than that of the pure silymain drug.



(A)



(B)



(C)

Figure (3) SEM micrographs of untreated SM (A), MC9 (B), and MC9s (C)

Size distribution of microcrystals

The particle size and polydispersity index (PDI) for the untreated SM and the prepared microcrystals were measured by laser particle size analyzer (Brookhaven instrument), the crystals size were the largest for standard SM crystalline powder with mean diameter of 1.53µm and PDI of 0.36. The mean diameter for the crystals prepared by in situ micronization technique using stirring method (MC9) was markedly decreased to 0.43µm with 0.33 PDI, while ultrasonic irradiation method produced

crystals (MC9s) with mean diameter of 0.61 µm and PDI of 0.14.

The size distribution obtained by application by sonication was more uniform as it had low PDI value which confirmed the SEM. The mean size was slightly increased which can be explained to be due to the large ultrasonic energy that accelerates effectively the mass transfer in the mixture and thus enhances the driving force of crystallization .With large kinetic energy and speed, the solute molecules will have an increased opportunity to collide with each other and increase the crystals size.[31]

In-vitro dissolution

The In-vitro dissolution, results showed an increase in dissolution rate of both MC9 and MC9s compared to the untreated drug (figure 4,5). This effect can be explained by an increased specific surface area with hydrophilic nature due to the adsorbed hydrophilic polymers [25].

Significant increase (P<0.05) in dissolution efficiency was obtained by MC9s as it increased from 84% to 91.09% in HCl and from 88% to 91.02% in phosphate buffer of pH6.8 in comparison to MC9. The increase in DE of MC9s may be due to its higher solubility, therefore, the formula MC9s was selected as the best formula.

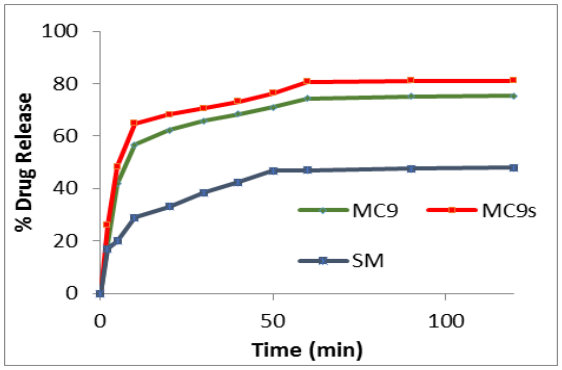


Figure (4) Dissolution profile of microcrystals MC9,MC9s and untreated powder of drug in HCl pH 1.2 at 37°C

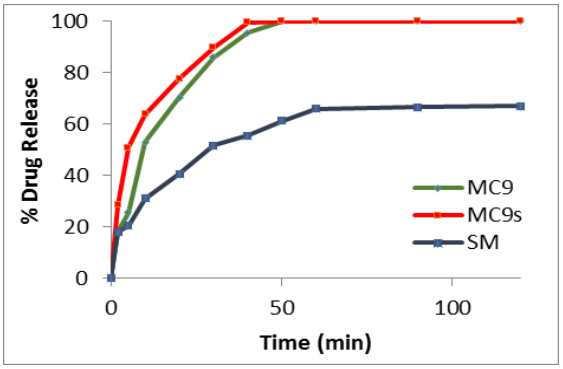
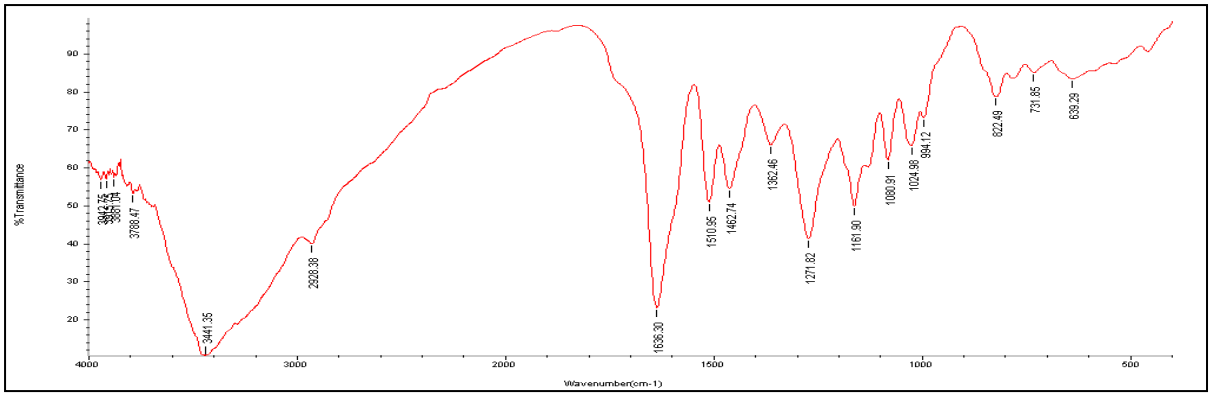


Figure (5) Dissolution profile of microcrystals MC9, MC9s, and untreated drug in phosphate buffer pH 6.8 at 37°C.

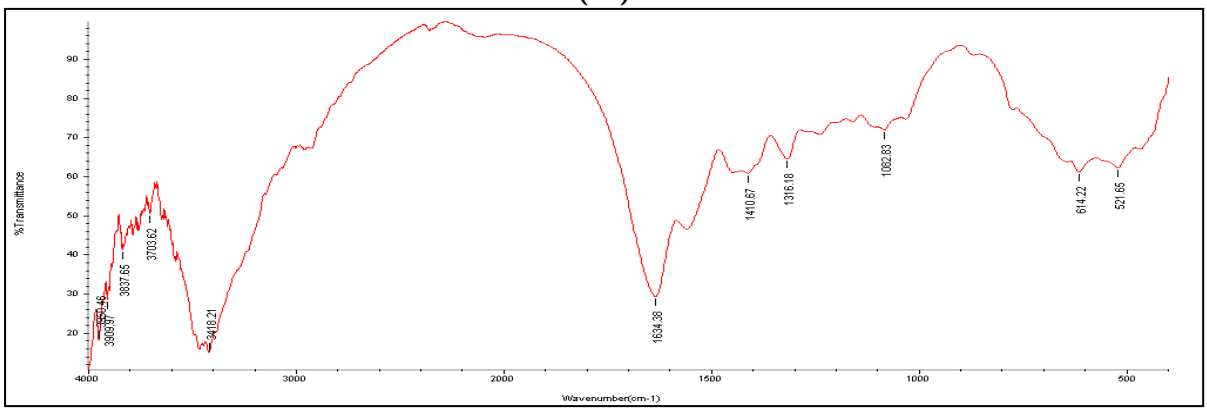
Fourier transforms infrared spectroscopy (FTIR):

The FTIR of untreated SM, gelatin and the selected formula MC9s were studied by FTIR spectroscopy using KBR disc. The data were presented in the (figure.6:a,b,c) respectively. Characteristic peaks of SM appeared at 3441.35 cm-1 (-OH stretching vibration), 2928.3 cm-1 (O-H stretching), 1636.3 cm-1 (C=O stretching), 1510.95,1462.74 cm-1 (skeleton vibration of aromatic C=C ring stretching),1362.46 cm-1 (-OH in plane bending),1271.82 cm-1 (C-O-C stretching),994.12 cm-1 (O-H out plane bending) and 1030.91-161.9 cm (in plane= C-H bending) [31].

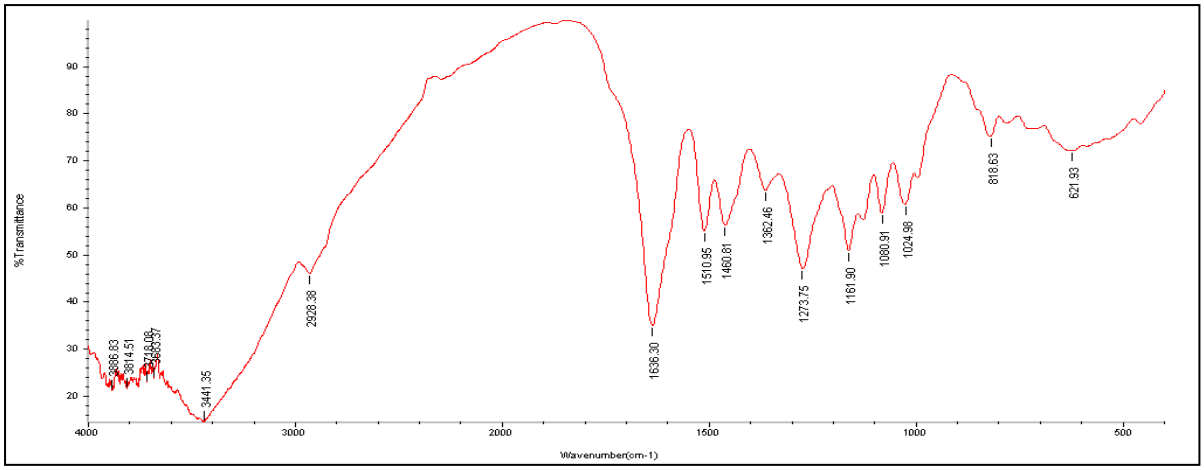
The results revealed that the FTIR spectra of untreated SM and selected microcrystals were identical and the main absorption bands of SM still appeared in the spectra of MC9s which confirmed that no chemical modification of the drug had been taken place during in-situ micronization process [33].



(A)



(B)



(C)

Figure (6) FTIR of SM (A), Gelatin (B), MC9s (C)

Conclusion

In the light of the results, it has been concluded that solubility and dissolution rate can be increased by *in- situ* micronization by solvent change method with using 0.1% (w/v) gelatin as hydrophilic stabilizer by sonication irradiation method. The resulted crystal in reduced size with the hydrophilic surface contribute enhance solubility and dissolution rate.

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