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PREPARATION AND CHARACTERIZATION OF ALBENDAZOLE MICROPARTICLES PREPARED BY FREEZE-DRYING TECHNIQUE

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من المعروف أن عقار الألبندازول يستعمل كمضاد للديدان وهو شحيح الذوبان في الماء. وقد تناول هذا البحث زيادة معدل الذائبية للعقار باستعمال بعض البوليمرات مثل هيدروكسي بروبيل ميثيل السليلوز وعديد فنيل البيريليدون بواسطة تحضير حبيبات صغيرة من العقار والبوليمرات بطريقة التجفيد. وقد تم تحضير هذه الحبيبات بنسب مختلفة من العقار والبوليمرات وهي كالتالي ١:١ ، ٢:١ و٤:١ وتمت دراسة الخواص الفيزيائية لهذه الحبيبات والمخلوط الفيزيائي بواسطة التحليل الحراري وحيود الأشعة السينية والميكرسكوب الأليكتروني وقد وجد أن العقار قد تحول من الحالة البللورية الي الغير بللورية (المتميئة) في الحبيبات.

وأظهرت النتائج أن معدل ذوبان العقار يتحسن بصورة واضحة في الحبيبات المحضرة مع كلا من هيدروكسي بروبيل ميثيل السليلوز وعديد فينيل البيريليدون عنه في المخلوط الفيزيائي والعقار بمفردة وأن هذا التحسن يعتمد بصورة مباشرة علي نسبة البوليمرات في الحبيبات. ويرجع سبب هذه الزيادة إلي زيادة درجة التبلل للعقار وتحوله إلي الحالة الغير بللورية (المتميئه).

The aim of this work is preparation of albendazole (ABZ) microparticles with certain hydrophilic polymers such as hydroxypropyl methylcellulose (HPMC), and polyvinyl pyrrolidone (PVP) using freeze-drying technique. Microparticles of ABZ with these polymers were prepared in different ratios of 1:1, 1:2, and 1:4. Morphology of the prepared ABZ microparticles was studied using a scanning electron microscope. Spherical microparticles with smooth surface of ABZ were detected by this method. Physicochemical properties of drug alone and its freeze-dried microparticles were investigated using differential scanning calorimetry (DSC) and powder X-ray diffractometry (PXRD). DSC and PXRD analysis showed that ABZ was transformed from the crystalline state to amorphous state by freeze-drying with the chosen polymers as confirmed by disappearance of its melting peak and characteristic crystalline peaks. Dissolution rate of ABZ from

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the prepared microparticles was determined and compared to its corresponding physical mixtures. Results showed that, the dissolution of freeze-dried microparticles was faster than the corresponding physical mixtures and drug alone. This indicates that, the freeze-drying technique improved ABZ dissolution. Moreover, it was found that the dissolution rate of the drug was affected by the polymer type and the ratio of ABZ to polymer.

INTRODUCTION

Albendazole (ABZ), methyl [5-(propylthio)-1-*H*-benzimidazol-2yl] carbamate, is a benzimidazol derivative with abroad spectrum of activity against human and animal helminth parasites[1]. ABZ is effective in the treatment of echinococcosis, hydrated cysts and neurocysticercosis[2]. ABZ is a poorly water-soluble drug (0.2 µg/ml in water at 25°C)[3]. Consequently, it is poorly absorbed from the gastrointestinal tract (<5%)[4] and it has low oral bioavailability[5]. This property is a major disadvantage for the use of ABZ in the treatment of systemic helminthiasis[6]. Furthermore, the lack of water solubility reduces flexibility for ABZ formulation and administration. Therefore, the overcome of the poor aqueous solubility of ABZ is an important goal.

Different efforts have been made to enhance ABZ water solubility and dissolution rate such as preparation of oil in water emulsion[7], incorporation into liposomes[8], complexation with cyclodextrins[9], and preparation of solid dispersions[10]. Moreover, increased systemic bioavailability of albendazole was reported when the drug was co-administered with a fatty meal[11], fruit juice[12], or taken as drug solution with co-solvent[13], or with surfactants[14].

On the other hand, lyophilization is a process in which the solvent is sublimed from the frozen drug solution or suspension containing the drug and the hydrophilic carriers. Glassy amorphous porous structure of the dispersion will be produce with freeze-drying leading to enhancement of the dissolution of the drug[15]. Examples of successfully tested-drugs which their solubility were improved by freeze-drying technique are flurbiprofen[16] and quercetin[17].

The aim of this study was preparation of (ABZ) microparticles and improving the rate of dissolution of the drug, by using freeze-drying technique utilizing two hydrophilic polymers namely; hydroxypropyl methylcellulose (HPMC), and polyvinyl pyrrolidone (PVP). The influence of drug to polymer ratio on the dissolution

profile of ABZ from its freeze-dried microparticles using the previously hydrophilic polymers was conducted. The physical properties of the prepared microparticles of ABZ were characterized by differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD), and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Albendazole (ABZ) was kindly donated from Saudi Pharmaceutical Industries (Riyadh, KSA). Hydroxypropyl methylcellulose (HPMC, Methocel K100) is a water soluble polymer and was kindly donated from DOW (Midland, MI, USA). Polyvinyl pyrrolidone k15 (PVP, MW 10,000) was purchased from Fluka, Chemika AG, Switzerland. All other chemicals and solvents used were of pharmaceutical grades.

Preparation of physical mixtures

Physical mixtures were prepared by grinding ABZ and individual polymeric carriers in a mortar (the ratio of ABZ to polymer used was 1:2 and 1:4).

Preparation of albendazole microparticles by freeze-drying technique

Polymeric solutions of HPMC were prepared by dissolving the polymer in boiled water (0.5%, 1% or 2% w/v according to the ratio desired) followed by immediate cooling to form clear solution[18]. PVP polymeric solutions were prepared directly by dissolving the polymers in water (0.5%, 1% or 2% w/v according to the ratio desired) to form a clear solution. Albendazole was dissolved in ethanol (0.5% w/v) and this ethanolic solution was added slowly to the polymeric solutions with continuous stirring to produce the final solution in (1:1 ethanol : water). Albendazole : polymers ratios were adjusted in the preparations to give 1:1, 1:2 and 1:4. The ABZ solutions (200 ml) were frozen overnight at (- 20°C) and then lyophilized over a period of 30 hrs using freeze-drier (Alpha 1-4 LD-2, Martin Christ, Osterode, Germany) under the following conditions (temperature= - 59°C, vacuum= 0.090 mbar). The dried micro-particles were passed through 250 µm sieve (Endocott Sieve Ltd, London, UK), stored in a dessicator until further investigations.

Morphology of the prepared ABZ microparticles

Samples morphology was examined under scanning electron microscope (Jeol, JSM-6360LV scanning microscope, Tokyo, Japan). Before microscopy, the dried microparticles were mounted at carbon tape and were sputter-coated using gold (Jeol, JFC-1100 fine coat ion sputter, Tokyo, Japan).

The photomicrographies were taken at an acceleration voltage of 15 kV.

Differential scanning calorimetry

Differential scanning calorimetery studies were done for the drug and the prepared microparticles using Universal V4.1D TA Instrument (Q100, TA Instruments, Delaware, USA) and they were carried out under the following conditions: sample weight 3-5 mg, scanning speed 10°C/min, in the 25-300°C temperature range. The samples were heated in hermetically sealed aluminum pans and Indium was used as standard.

Powder X-ray diffractometry

Powder X-ray diffraction patterns of the prepared freeze-dried microparticles were carried out using a wide-angle X-ray diffractometer (Siemens D-500, Bruker AXS, Coventry, UK). The instrument was operated in 2-Theta scale. The angular range was 5° to 40° (2θ) and counts were accumulated for 1 second at each step.

Dissolution study

Dissolution measurements were carried out in a USP dissolution test apparatus (Caleva Ltd., Model 85T, Philips, UK). The dissolution profiles of ABZ from microparticles were studied in 0.1 N HCl (pH= 1.2). The drug-loaded microparticles containing 20 mg of ABZ were dispersed on the dissolution medium (900 ml) under 100 rpm stirring rate, thermostated at 37±0.5°C. At schedule time intervals, the samples (5 ml) were withdrawn and replaced immediately with fresh dissolution medium. The samples were assayed spectrophotmetrically at 291 nm for dissolved drug[13], where samples were automatically filtered before measuring the absorbance. The dissolution experiments were conducted in triplicate and the means of the absorbance were calculated.

Statistical analysis

All data are expressed as mean standard deviations (±SD). Statistical analysis was performed using student t-test at 0.05 level of significant.

RESULTS AND DISCUSSION

Scanning electron microscope studies (SEM)

Scanning electron microscope (SEM) of the prepared ABZ microparticles is shown in Figure 1. ABZ powder has an irregular crystalline shape (Fig. 1-A). In contrast, a change in the morphology and shape of the freeze-dried microparticles of ABZ, either in the presence of HPMC or PVP was occured. They are nearly spherical particles (Fig. 1-B, 1-C). SEM showed smooth surface of ABZ microparticles which indicated the complete disappearance of ABZ crystals.

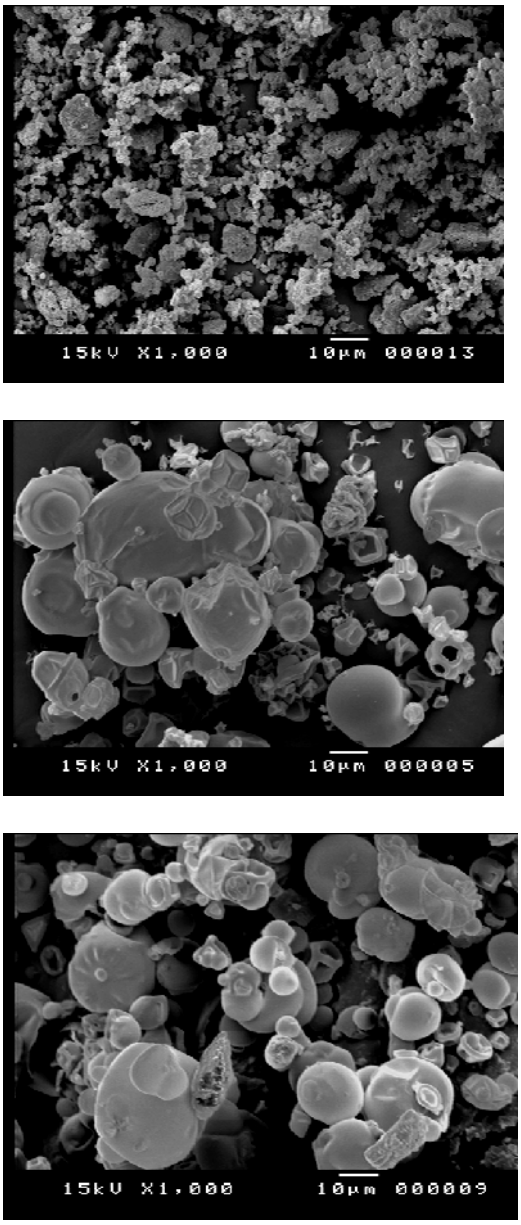

Fig. 1: Scanning electron micrographs of ABZ particles (A), ABZ:HPMC 1:4 (B), ABZ: PVP 1:4 (C).

Differential scanning calorimetry (DSC)

The thermal behavior of ABZ alone and its freeze-dried micro-particles as well as its physical mixtures with the used polymers are shown in Figures 2-4. The DSC thermograms of pure ABZ (Fig. 2) show an endothermic peak at 218°C with a shoulder at 198°C which is due to the melting of the drug. The weakness of the endothermic peak of the physical mixtures (Fig. 2) in the presence of HPMC or PVP might be attributed to the dilution effect of the polymers. Also, the physical mixtures show a disappearance of the shoulder and shifted to lower temperature (197°C). In the DSC thermograms of the prepared microparticles using HPMC (Fig. 3) the endothermic peak of drug disappeared completely in the all ratios of HPMC due to change of the drug to amorphous form. In the DSC thermogram of pure PVP (Fig. 4), a broad endothermic peak ranging from about 65 to 115°C was observed. This broad endothermic peak may be due to the evaporation of the adsorbed water. The endothermic peak of the drug disappeared completely for the prepared Freeze-dried micro-particles of ABZ using the two polymers suggesting that ABZ was converted to an amorphous form by Freeze-drying process.

X-ray powder differactometry (XRD)

The powder X-ray diffraction (XRD) patterns of pure ABZ, pure polymers and freeze-dried microparticles are shown in Figures 5,6. The crystalline peaks located at 7.3°, 11.8°, 18.5° and 24.7° (2θ) corresponding to albendazole crystals were observed[10]. The intensity of crystalline diffraction peaks of the drug in its freeze-dried products was decreased gradually by increasing the polymer ratio until completely disappeared in the ratio of 1:4 (Figs. 5,6). This indicates that transformation of ABZ occurs from crystalline state into the amorphous state by freeze-drying with the used polymers. This is in good agreement with DSC results. It has been known that transforming the crystalline state of the drug to the amorphous state leads to a high-energy state and high disorder, resulting in enhancing solubility and dissolution rate[18]. Accordingly, this will improve the dissolution rate of ABZ noticeably.

In-vitro dissolution study

The dissolution rate of ABZ in the form of powder, physical mixtures and freeze-dried particles in HPMC or PVP was examined in 0.1 N HCl (pH 1.2) (Figs. 7-9). The dissolution rate of pure ABZ was extremely low, only about 20% of

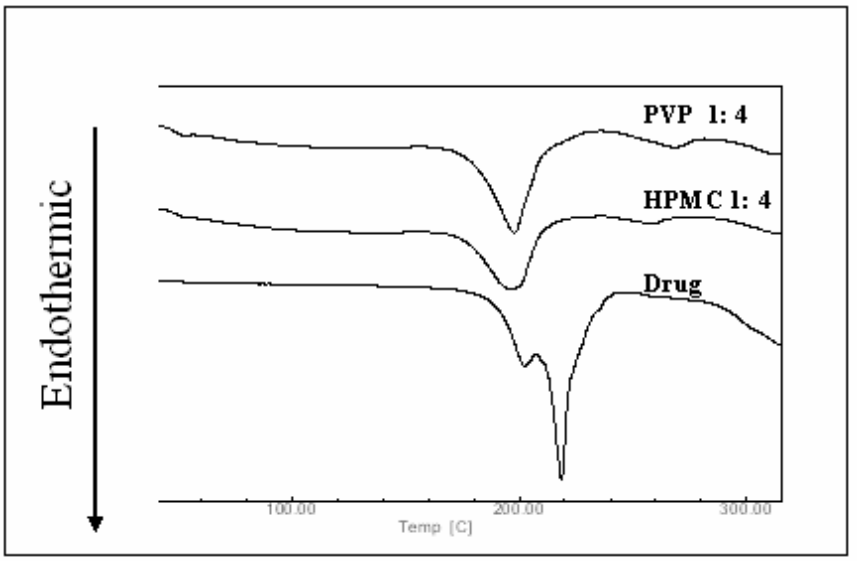

Fig. 2: DSC curves of ABZ alone and its physical mixtures with HPMC and PVP in 1:4 ratios.

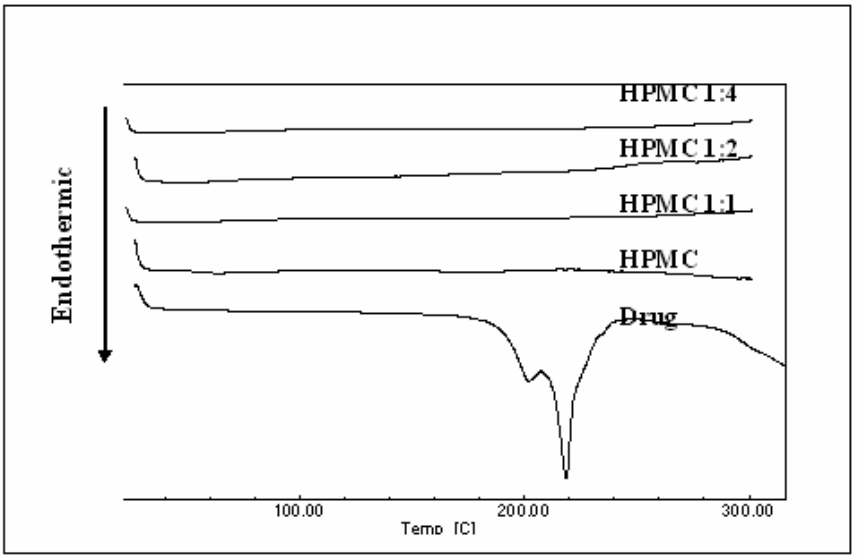

Fig. 3: DSC thermograms of albendazole as well as HPMC microparticles in different ratios.

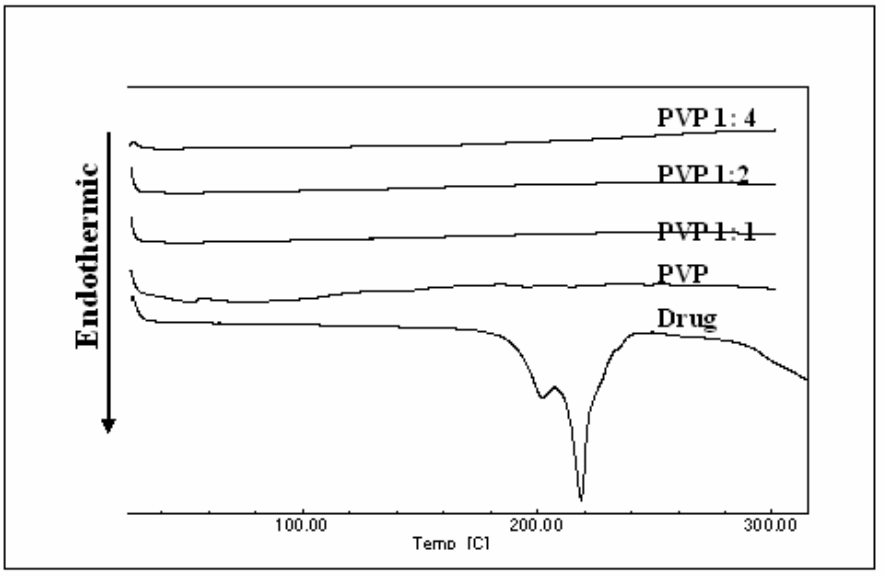

Fig.4: DSC thermograms of albendazole as well as PVP microparticles in different ratios.

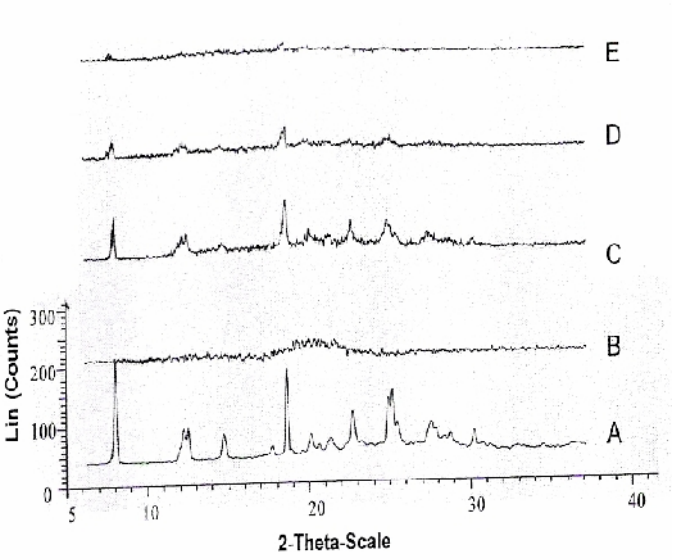

Fig. 5: Powder X-ray diffraction patterns of albendazole as well as HPMC microparticles prepared by freeze-drying. (A) drug, (B) HPMC, (C) 1:1, (D) 1:2, (E) 1:4.

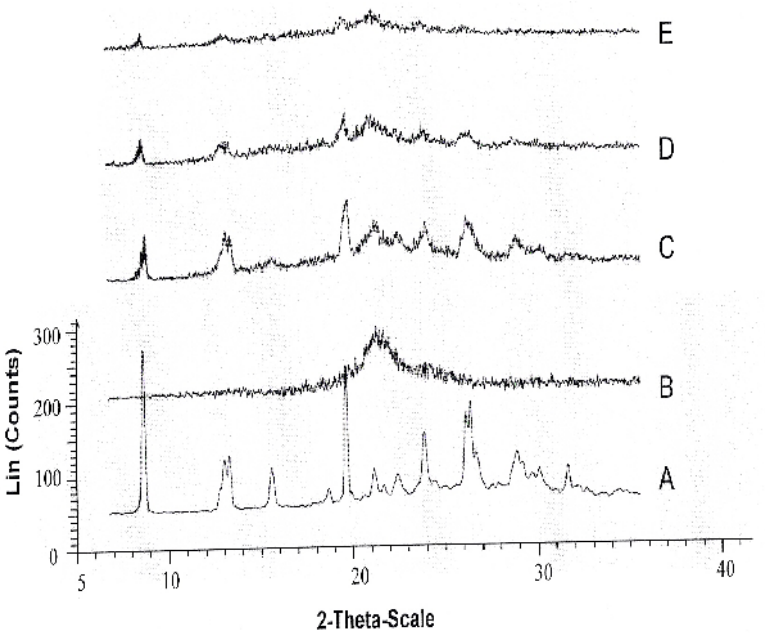

Fig. 6: Powder X-ray diffraction patterns of albendazole as well as PVP microparticles prepared by freeze-drying. (A) drug, (B) PVP, (C) 1:1, (D) 1:2, (E) 1:4.

drug released during 120 min. of the dissolution run in 0.1 N HCl. This might be attributed to poor solubility of ABZ[3]. The dissolution rate of ABZ from the physical mixtures with HPMC or PVP was increased more than that of pure drug (Fig. 7), this is due to an increase the hydrophilicity of the drug in the presence of these polymers. Figure 8 represents the effect of HPMC on ABZ dissolution rate from its freeze-dried microparticles. The percent dissoluted of the drug from its freeze-dried microparticles with HPMC was 90% and 95% after one hour and two hours respectively, while that from its physical mixture was 45% after two hours. Therefore, the dissolution rate of ABZ has been enhanced remarkably from its microparticles with hydrophilic HPMC prepared by freeze-drying technique. The improvement in dissolution rate of ABZ could be attributed to amorphization of drug by freeze-drying with HPMC as confirmed from DSC and PXRD data. It was noted that the release rate of ABZ is reduced with increasing the HPMC

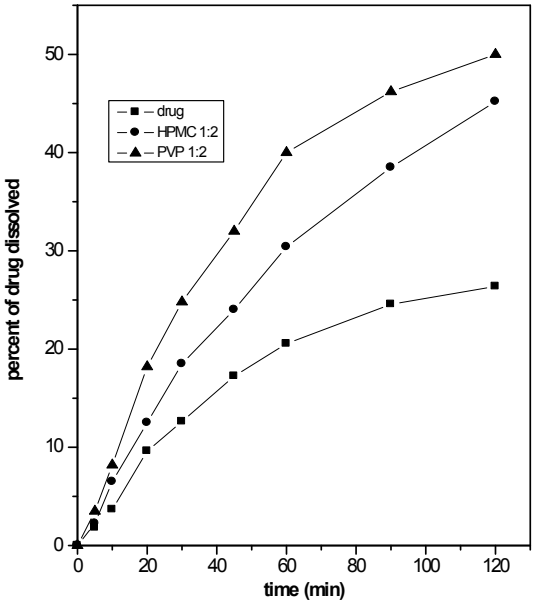

Fig. 7: Dissolution profiles of ABZ, in 0.1 N HCl at 37 ± 0.5 ^{o} C, from its Powder and physical mixture with HPMC or PVP at the ratio 1: 2.

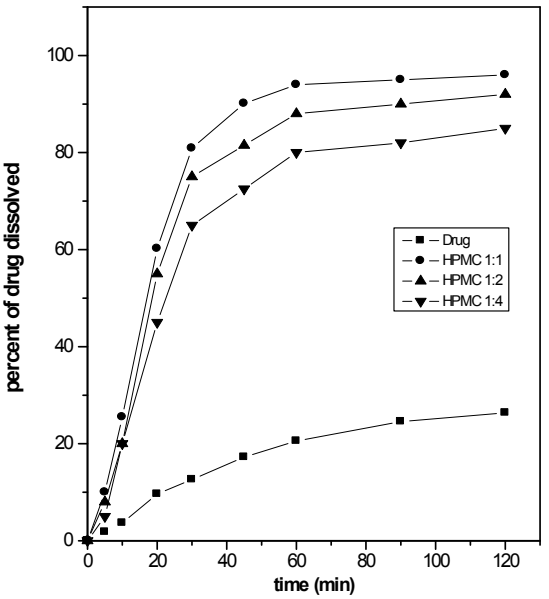

Fig. 8: Dissolution profiles of ABZ–HMPC microparticles in pH 1.2 at 37±0.5°C prepared by freeze-drying technique.

content in the microparticles prepared by freeze-drying. The highest ABZ dissolution rate was achieved from the microparticles of HPMC in 1:1 ratio ($P< 0.05$). This is in agreement with our previous study in which the highest dissolution of indomethacin from its microparticles achieved with the lower HPMC content[19]. This finding can be explained by the fact that the microparticles with low polymer content were expected to be more porous than those with high polymer content, which might facilitate the release of the residual drug from the microparticles[20]. Also, the thickness of the hydrogel layer increases with high polymer content due to polymer swelling and forming a gel layer which retarded drug diffusion[21].

The effect of PVP on ABZ dissolution rate from its freeze-dried microparticles is presented in Figure 9. The amount dissolved of drug from the microparticle of PVP was 90% after two hour dissolution interval, as compared to 48% from its physical mixture with PVP in 1:2 ratios after the same time. Again, the dissolution rate of ABZ has been enhanced extremely from its microparticles with PVP, which confirm the success of freeze-drying technique as well as the presence of this polymer for improvement the drug dissolution.

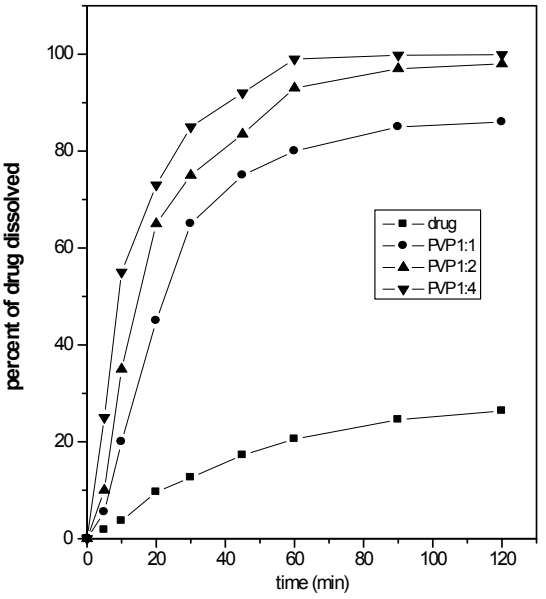

Fig. 9: Dissolution profiles of ABZ–PVP microparticles in pH 1.2 at 37±0.5°C prepared by freeze-drying technique.

It was observed that increasing in PVP content in the system increased the fraction of ABZ released from microparticles prepared by freeze-drying. The highest ABZ dissolution was achieved from the sample of ratio 1:4 ($P< 0.05$), with the increase in the ratio of PVP, the dissolution rate increased. This is in agreement with Sekikawa *et al.*[22] who proposed that the presence of PVP in the medium may lower the surface tension and facilitate the wetting, thus, the dissolution rate of drug increases. EL-Badry and Fathy[23] reported that, the increase in dissolution rate of meloxicam was dependent on the ratio of drug : PVP in the solid dispersion.

Conclusion

In conclusion, spherical microparticles with smooth surface of ABZ with the used polymers were produced by freeze-drying technique. ABZ was transformed from crystalline state to amorphous state, that is confirmed by DSC and PXRD results. The dissolution rate of ABZ was improved from freeze-dried microparticles as compared to its physical mixtures. Dissolution rate of ABZ was influenced by the type of the polymer and drug: polymer ratio. In case of HPMC the release rate of ABZ is reduced with increasing polymer content whereas in case of PVP the release rate increased concurrently with the polymer content. Also, PVP is preferable to prepare ABZ microparticles than HPMC.

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