PREPARATION AND EVALUATION OF PIROXICAM - PLURONIC SOLID DISPERSIONS

M. Fathy and M. El-Badry

Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt

من المعروف أن عقار البيروكسيكام وهو من مضادات الالتهاب الغير ستيرويدية أنه شحيح الذوبان فى الماء و أن هذه الخاصية تقلل الاتاحة الحيوية له. و قد استهدفت هذه الدراســـة التوافق الفيزيائى و الكيميائى بين هذا العقـــار و بعض البلورونك و هى بوليمرات تحتوى على عديد الاوكسى ايثلين و عديد الاوكسى بروبيلين. وقد تم تحضير الخليط الفيزيائى و المتراسب من العقار و بعض البلورونك مثل (ف ، ف ، ف) بنسب مختلفة : للخليط الفيزيائى و (: : :) للمتراسب. وأيضا استخدام هذه البوليمرات لتحسين الذوبان المائى للعقار. وقد تم دراسة هذا التوافق بواسطة التحليل الحرارى و الأشعة تحت الحمـــــراء و حيود الأشعة السينية. وقد أتضح من الدراسة أنه يوجد توافق بين العقـــار و هذه البوليمرات. و قد تمت دراسة ثبات العقار فى المخلوط المتراسب (:) لفترة قدرها أربعون أسبوع فى درجة حرارة درجة مئوية بواسطة المسح الحرارى و قد بينت الدراسة ثبات العقار تحت هذه الظروف و لهذه الفترة. و قد أظهرت النتائج أن ذوبان العقار فى الماء قد تحسن بصورة ملحوظة عند استخدام هذه البوليمرات و هذا التحسن كان معتمدا على زيادة تركيز البوليمر. وقد أمكن ترتيب البوليمرات طبقا لقدرتها على اذابة العقار وعلى معامل توزيع العقاربين جزيئات هذه المواد و بين الماء كالاتى: بلورونك ف ﴾ف ، ﴾ ف ، . وأشارت النتائج أيضا أن معدل أذابة العقار قد تحسن بشكل ملحوظ فى وجوده سواء فى المخلوط الفيزيائى أو وجوده فى صورة المخلوط المتراسب وان كان معدل الاذابة كان أكبر فى حالة المخلوط المتراسب عنه فى حالة المخلوط الفيزيائى. وأن معدل الاذابة للعقار فى وجود بلورونك ف كان أكبر منه سواء فى وجود ف ، أو ف ، .

With an objective of enhancing the dissolution rate of piroxicam (PXM), solid dispersions (SDs) of piroxicam were prepared using different grades of pluronic F-68 (Pl. F-68), pluronic F-98 (Pl. F-98), and pluronic F-127 (Pl. F-127) adopting the solvent evaporation technique. The solid dispersions were prepared at different (drug: polymer) ratio (1:1, 1:3 and 1:5). Piroxicam- pluronic solid dispersions were investigated by inferared spectroscopy (IR), x-ray diffraction (XRD) and differential scanning calorimetry (DSC). The IR spectra prevailed no chemical interaction between the drug and copolymer. The powder x-ray diffraction (XRD) patterns and differential scanning calorimetry (DSC) thermograms demonstrated that piroxicam was existed in a solid solution state in the SD. Phase diagram for solid dispersion of piroxicam in pluronic F-98 indicated that the solid solution of piroxicam was obtained at a concentration of PXM less than 35% in the SD. The stability study was carried out for the solid dispersion prepared at 1:1 (piroxicam/ Pl. F-98) at 40 ° for 40 weeks prevailed that; the drug is stable during the storage period in the dispersion. The solubility study of piroxicam was carried out at different concentrations of pluronics, also the dissolution rate of the drug was improved either by physical mixture or the solid dispersions.

INTRODUCTION

Currently, in the field of pharmaceutical technology, great efforts are being directed towards the solving problems related to poor-water solubility and poor bioavailability of drugs. This trend of working has led to development of different techniques. Sekiguchi and Obi[1] first introduced the concept of solid dispersions as a drug delivery system, where urea is used as the carrier for sulphathiazole as a poorly water-soluble drug. Subsequent research works were done to improve the dissolution and bioavailability of poorly

soluble drugs.[2-4] Solid dispersions can be obtained by three procedures: by melting, by using solvents or by combined process including melting and solvents.[5]

Piroxicam is a non-steroid anti-inflammatory compound with analgesic and antipyretic effects, used for the treatment of rheumatoid arthritis, ankylosing spondylitis, acute gout, acute musculoskelatal disorders, primary dysmenorrhea, osteoarthritis and traumatic contusions.[6, 7] The drug is practically water-insoluble.[8] However, solid dispersions of piroxicam by some water-soluble carriers were found to enhance drug dissolution.[9-12] Of the amphiphilic carriers, which have attracted significant attention in pharmaceutical fields, are pluronic block copolymers. They are (poly (ethylene oxide)-block-(poly (propylene oxide)-block poly(ethylene oxide), $EO_{m/2}$-PO_n-$EO_{m/2}$.[13-15] Pluronic polyols are chemically similar but physicochemically diverse surface-active agents composed of polymers of hydrophilic polyoxyehylene and lipophilic polyoxypropylene. Many researches have focused on these copolymers as transdermal delivery systems utilizing its gelling properties in gels formation.[16-18]

The aim of this article was to formulate and to study the interaction of piroxicam and different grades of pluronics, namely PL-F-68, PL F-98 and Pl-F-127 using different tools such as infrared spectroscopy (IR), X-ray diffractometry (XRD) and differential scanning calorimetry (DSC). The stability test for 1:1 solid dispersion of PL-F-98 was carried out and detected using (DSC). Phase diagram of solid dispersion of piroxicam and PL F-98 was studied at different weight ratios. The solubility of the drug in different pluronics was carried out, also the dissolution of the drug from its solid dispersion as well as its physical mixture was investigated.

EXPERIMENTAL

Materials and methods

Piroxicam was kindly donated by Sedico, Egypt. The characteristics of the Pluronic block copolymers Pl. F-68 & Pl. F-127 (BASF, Aktiengesellschaft, Ludwigshafen, Germany) and Pl. F-98 (C.H.Erbsloeh, Krefeld, Germany)

were presented in Table 1. All other chemicals used were of analytical reagent grade.

Preparation of solid dispersions

Solid dispersions containing piroxicam and each copolymer in different weight ratios (1:1, 1:3 and 1:5 drug: polymer) were prepared employing solvent evaporation method. The dug was dissolved in methylene chloride and the copolymer in a least amount of ethanol. The two solutions were mixed together and the organic solvents were removed by evaporation at room temperature with the aid of gentle stirring. The dried mass was pulverized, sieved and the 100-250 µm particle size fractions were used in this study. The samples were kept in a desiccator till further investigations. Physical mixtures of piroxicam and different pluronics were prepared in the same ratios as in solid dispersions by gentle mixing for the drug and copolymer in a mortar.

Infrared spectroscopy (IR)

IR spectra were recorded with an IR spectrophotometer (IR-470, Shimadzu, Japan) by KBr disc method. The samples were scanned over the range of 4000 to 400 cm^{-1}.

Powder x-ray diffraction

The x-ray diffraction (XRD) patterns of the samples were obtained using an automated x-ray diffractometer (Model FW 1700 series, Philips, Netherlands) using CuK_α radiation (CuK_α =1.5418 Å), voltage 40 kV; current 30 mA and scanning speed 10 mm/sec.

Thermal analysis

Differential scanning calorimetry (DSC-50 Shimadzu, Japan) was used under the following conditions: sample weight 3-5 mg, scanning rate 10°/min, N_2 purge 30 ml/min. The samples were heated in hermetically sealed aluminum pans in the temperature range 25-250°. Thermal analysis data were obtained using a TA 50I PC system with Shimadzu software programs.

Stability test

Solid dispersion of pluronic F-98 prepared at 1:1 weight ratio was stored at 40° for 40 weeks. The melting point and the heat of fusion (J/g) were determined every 2 weeks for 40 weeks and at the end of study using (DSC).

Table 1: Characteristics of the pluronic block copolymers used in this study

	Pluronic F-68	Pluronic F-98	Pluronic F-127
Generic Name	Poloxamer 188	Poloxamer 288	Poloxamer 407
Molecular weight	8400	13000	12600
Average No of PO units	29	44.8	65.2
Average No of EO units	152.7	236.4	200.4
PO/EO ratio	18.99	18.95	32.53
HLB	29	28	22

PO = Propylene oxide, EO = Ethylene oxide, HLB = Hydrophilic-lipophilic balance.

Solubility study

Solubility of the drug in water in presence of different concentrations of pluronics (0.03, 0.0625, 0.125 and 0.25 mM) was studied. Solubility was studied using the following procedure: an excess amount of piroxicam powder was added to 10 ml of each solution in a 120 ml glass stoppered bottle. The bottles were firmly closed and placed into the mechanical shaking water bath (SBS Instruments, Germany) previously adjusted at 37 ºC ±0.5 for 24 h. One ml aliquot sample was withdrawn by pipette and diluted to an appropriate volume with simulate gastric secretion pH 1.2. The absorbance was measured at 331 nm (UV. 1601- Shimadzu Co. Japan) against blank. The concentration of the drug was calculated.

Dissolution rate studies

The dissolution rate of the drug from the prepared solid dispersions and some selected physical mixtures as well as the untreated drug was studied using USP dissolution apparatus (Erwecka Co., Germany). Accurately weighed amount equivalent to 10 mg of piroxicam were dispersed over the dissolution medium (900 ml of simulate gastric secretion pH 1.2) at 37º ±0.5 which immediately stirred at 50 rpm. At time intervals, five ml sample was withdrawn and diluted to suitable volume with the dissolution medium and the absorbance was measured at 331 nm against blank. Equal volume of fresh dissolution medium, was replaced into the dissolution medium to keep the volume of the dissolution medium constant. The experiment was carried out in duplicate and the average of absorbance was calculated.

RESULTS AND DISCUSSION

IR spectra for piroxicam, pluronics, physical mixtures and solid dispersions were displayed in Figs. 1-3. The most characteristic bands of PXM are the band of N-H and enolic O-H stretching at 3385 cm^{-1} for the needle form, 3330 cm^{-1} for the cubic form and the band for amid carbonyl stretching at 1637 cm^{-1}. These values were comparable with those reported by Mihalic and Vrecer et al.[19-20] The IR spectra of pluronic copolymer displayed a broad peak at 3355-3610, 3250-3625, 3245-3745 cm^{-1} for Pl. F-68, Pl. F-98, Pl. F-127 respectively. This broad peak did not prevent the appearance of N-H or O-H stretching vibration of piroxicam at 3395 cm^{-1}. The position of the characteristic peaks for both of physical mixtures or solid dispersions are not affected but show broading and weakening in the higher ratio of the copolymer. This could be due to the dilution effect caused by copolymer. The results of IR spectra suggested the absence of interaction between piroxicam and pluronic copolymers. This result was in consistent with Fernandez et al.[9] who found no chemical interaction between piroxicam and PEG 4000, which possess similar groups of pluronics.

The XRD patterns of PXM, Pluronics (Pl. F-68, Pl. F-98 and Pl. F-127), PMs and SDs were depicted in Figs. 4-6. PXM was a highly crystalline powder with characteristic diffraction peaks appeared at a diffraction angle of 2θ at 8.99, 15.75, 19.73, 22.99 and 25.84°. These values were comparable with those reported for needle form of piroxicam[19] (9.18, 15.78, 19.46, 22.85, 25.22). The pluronic copolymers produced two characteristic peaks
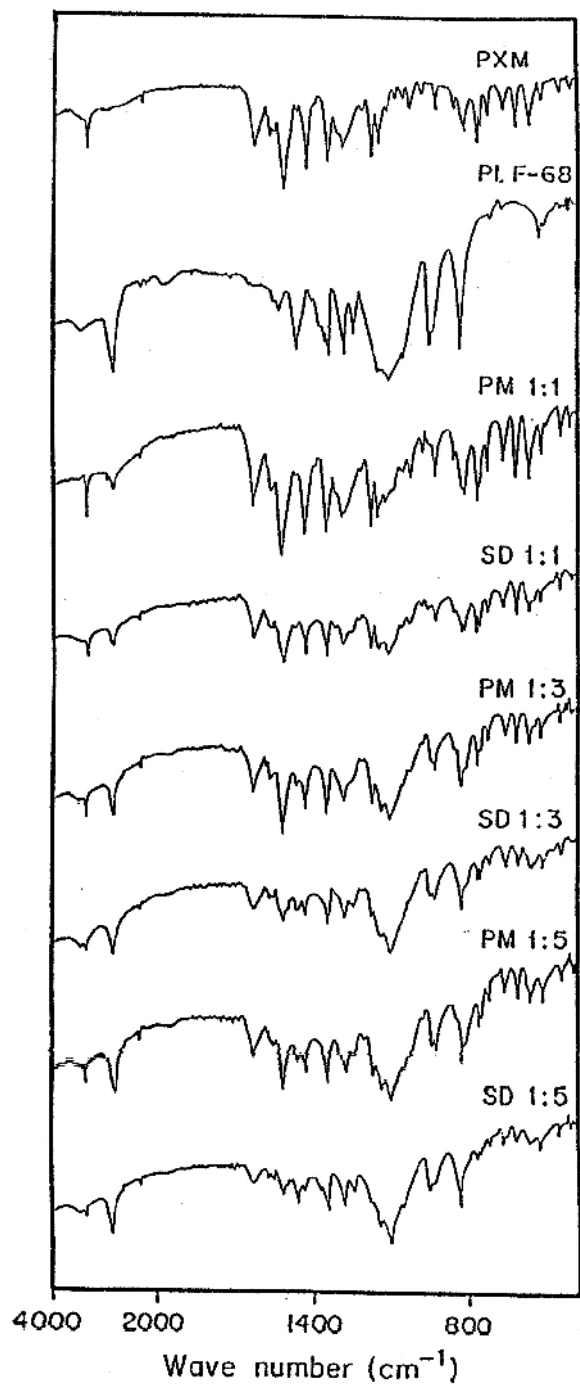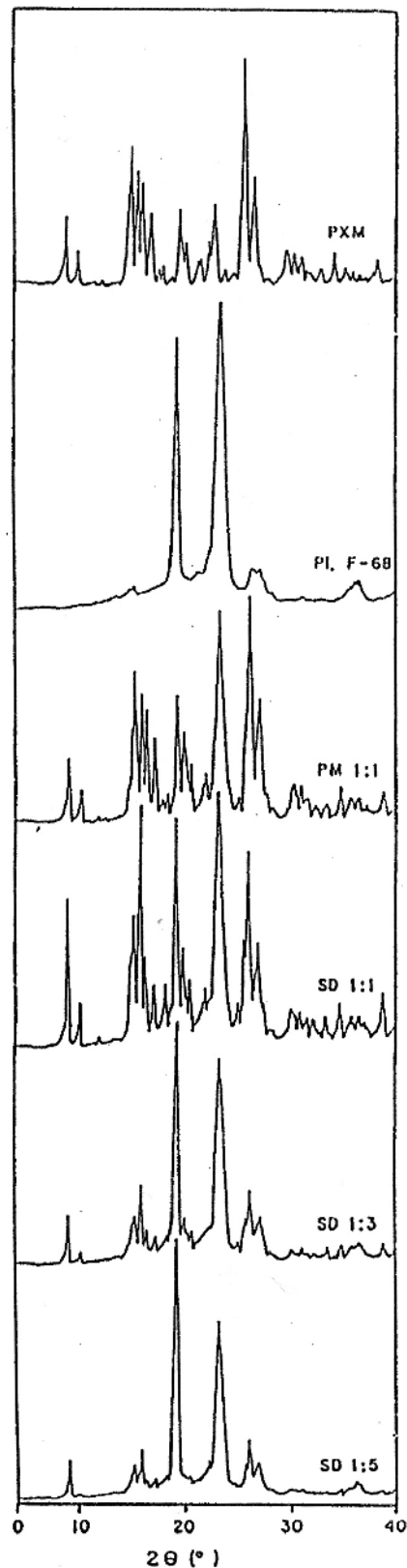
Fig. 1: Infrared spectrograms of piroxicam (PXM), pluronic F-68 (Pl. F-68), physical mixture (PM) and solid dispersion (SD) in different drug polymer ratios.
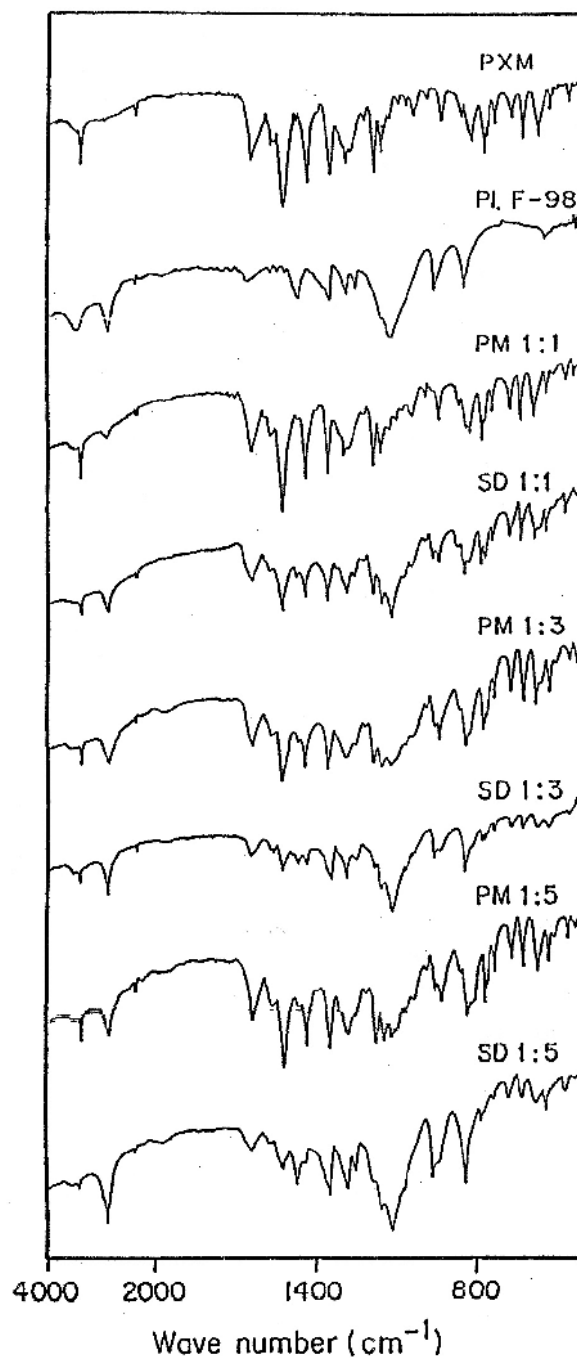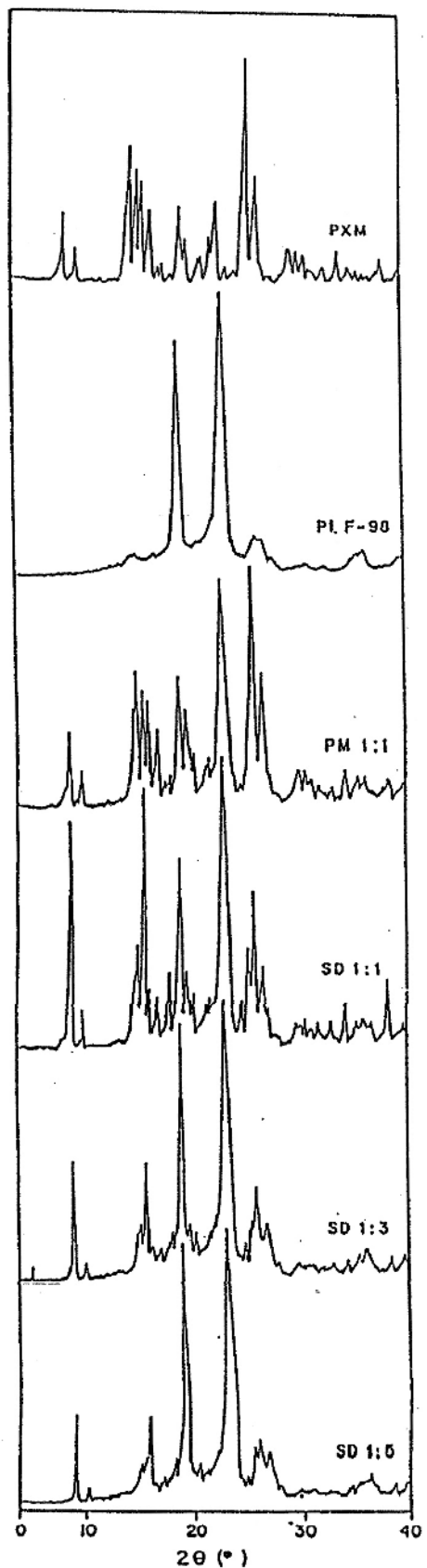
Fig. 2: Infrared spectrograms of piroxicam (PXM), pluronic F-98 (Pl. F-98), physical mixture (PM) and solid dispersion (SD) in different drug polymer ratios.
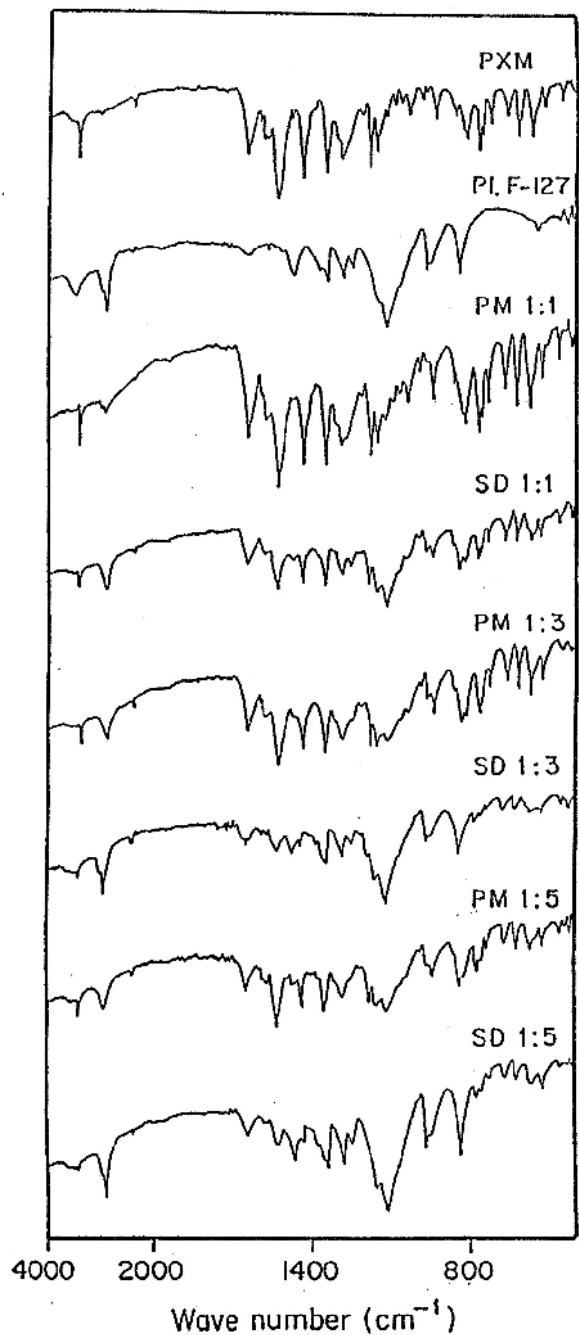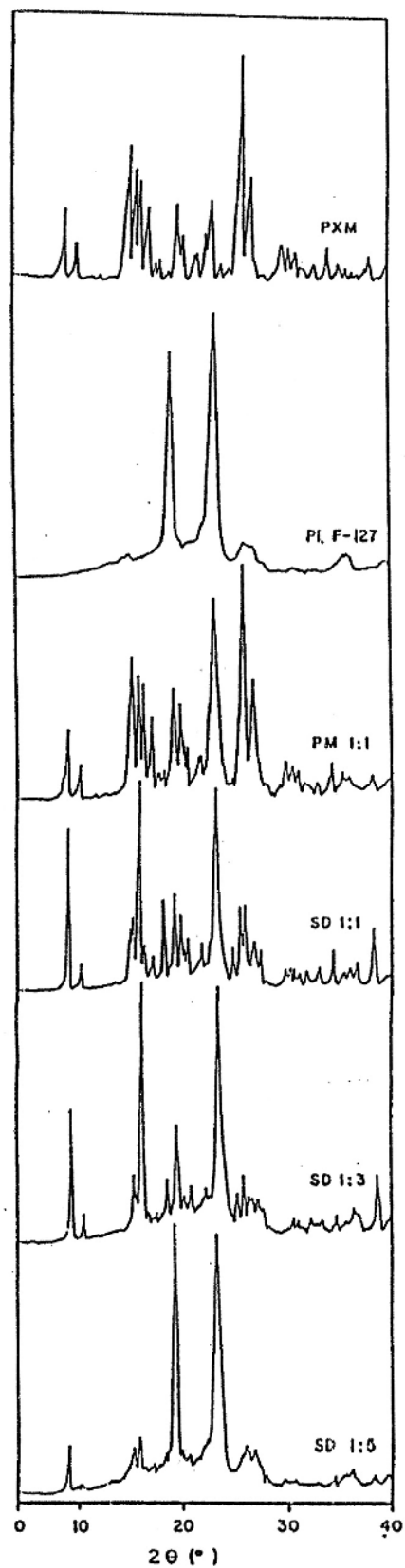
Fig. 3: Infrared spectrograms of piroxicam (PXM), pluronic F-127 (Pl. F-127), physical mixture (PM) and solid dispersion (SD) in different drug polymer ratios.

Fig. 4: X-ray diffraction patterns of piroxicam (PXM), pluronic F-68 (Pl. F-68), physical mixture (PM) and solid dispersion (SD) in different drug polymer ratios.

Fig. 5: X-ray diffraction patterns of piroxicam (PXM), pluronic F-98 (Pl. F-98), physical mixture (PM) and solid dispersion (SD) in different drug polymer ratios.


Fig. 6: X-ray diffraction patterns of piroxicam (PXM), pluronic F-127 (Pl. F-127), physical mixture (SD) in different drug polymer ratios.

102

of high intensity at 19.13, 23.24°; 19.10, 23.21°; 19.13, 23.25° for Pl. F-68, Pl. F-98 and Pl. F-127 respectively. The intensity of the major peaks of drug in case of SDs (1:1 ratio) was less than that of PMs prepared at the same ratio. The decrease in the intensity of PXM's major peaks in SDs was in the following order: Pl. F-68< Pl. F-98 <Pl. F-127. This difference could be attributed to the difference in lipophilicity and molecular weight of pluronics (Table 1), which might affect the dispersibility of PXM as well as the degree of it's mixing with the copolymer. The nonpolar hydrophobic moiety might promot solid solution formation.[21] In contrast, Yuasa et al.[22] found that the dispersibility of flurbiprofen (FP) in the SD of hydroxypropyl cellulose (HPC) is higher with increasing HPC composition ratio. By increase in copolymer concentration, the piroxicam diffractograms were almost completely masked by the signals from the copolymer. The results indicated that, the less of crystalline properties of the drug or the drug may be found in a solid solution state at low concentration.
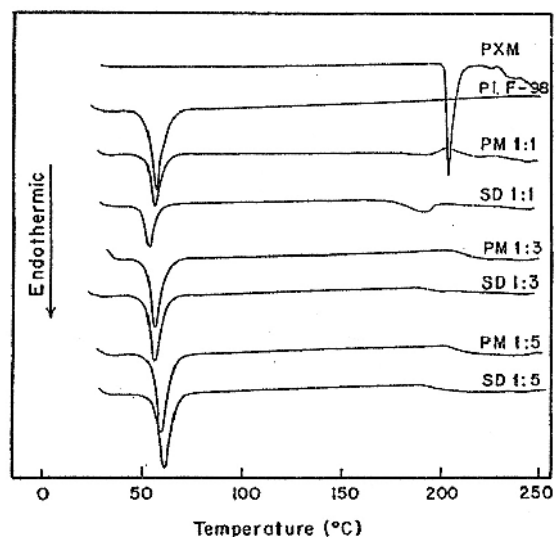
The DSC thermograms of PXM, Pluronics, PMs and SDs were shown in Figs. 7-9. Piroxicam showed a single fusion endotherm with a melting point at 201.5°. The melting points of pure polymers were 51.8, 56.0, 53.2° for Pl. F-68 (Fig. 7), Pl. F-98 (Fig. 8), Pl. F-127 (Fig. 9), respectively. All PMs and SDs exhibited sharp endothermic peaks due to the fusion of pluronics at the same position as the pure copolymers. This revealed the existence of pluronics in the crystalline state that was in consistent with the appearance of the diffraction peaks in the corresponding XRD patterns. The melting of PXM was observed in the physical mixtures and in the SDs at 1:1 ratios as broad endotherms at a melting point less than that of pure drug.

The peak of PXM in SDs, prepared at 1:1 ratio, occurred at 191.7, 192.6, 193.9° for Pl. F-68, Pl. F-98, Pl. F-127 respectively. The disappearance of PXM melting endotherms was observed in all PXM-pluronic solid dispersions of higher than 1:1 ratio. The results might suggest that, the drug is much less crystalline or it is in amorphous form. Moreover, physical mixtures of PXM and different types of pluronics showed the same thermal behavior as the solid dispersions of the same compositions. These similarities between the DSC data of solid dispersion and the physical mixture indicated the absence of the chemical interaction between PXM and this copolymer. Yuasa et al.[22] reported that the reason for the disappearance of the endothermic peak in the PMs was presumably that the crystalline state of drug (flurbiprofen) became the amorphous state because of the enhancement in molecular mobility during the heating process.
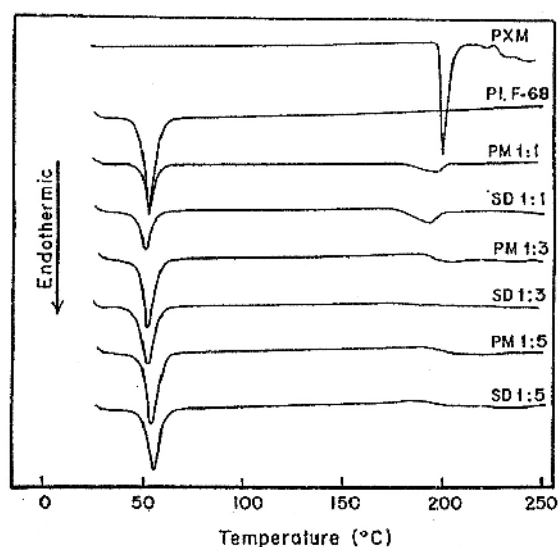
Fig. 7: DSC thermograms of piroxicam (PXM), pluronic F-68 (Pl. F-68), physical mixture (PM) and solid dispersion (SD) in different drug polymer ratios.

Fig. 8: DSC thermograms of piroxicam (PXM), pluronic F-98 (Pl. F-98), physical mixture (PM) and solid dispersion (SD) in different drug polymer ratios.

103
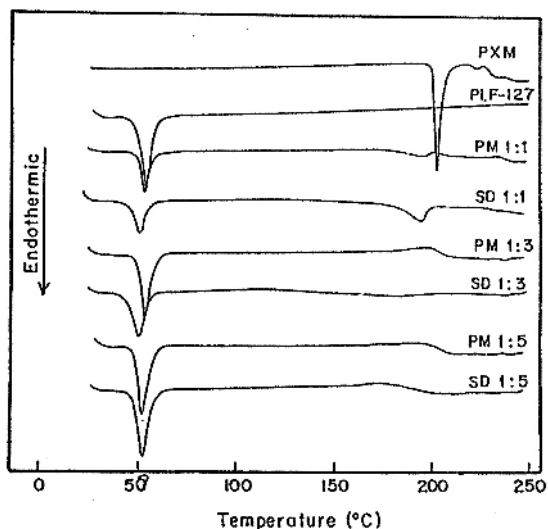
Fig. 9: DSC thermograms of piroxicam (PXM), pluronic F-127 (Pl. F-127), physical mixture (PM) and solid dispersion (SD) in different drug polymer ratios.

Construction of phase diagram of a drug and its prospective carrier from thermal analysis data is generally regarded as an important preliminary exercise in evaluating solid dispersion.[23] Thermograms of various SDs prepared at different concentrations of PXM (0, 20, 40, 50, 60, 80, 100 %w/w) was used to construct PXM: Pl. F-98 binary phase diagram (Fig. 10). The resulting diagram showed that with an increase in the copolymer concentration, the melting point peak was decreased. Fourty percent w/w PXM gave an endothermic peak with a melting point 190.2°, while the concentrations less than 40 % w/w gave no peak (Fig. 8). That means, the solid-state solutions were obtained if the concentration of PXM was less than 25% in the mixture. The heat of fusion as a function of the weight percent of PXM: Pl. F-98 was calculated and was plotted in Fig. 11.[24] The plotted heat of fusion values were the corrected ones (ΔH_{corr}) and were obtained using the following formula:

$$\Delta H_{corr} = (\Delta H_{obs}/ \%PXM \text{ in sample}) * 100 \text{ .. (1)}^{24}$$

Fig. 11 shows that, there is a linear relation between the composition of the solid dispersion and the heat of fusion values (r=0.988). The intercept on the abscissa did not cross the origin, however, the heat of fusion was zero, at less than 35 % w/w.
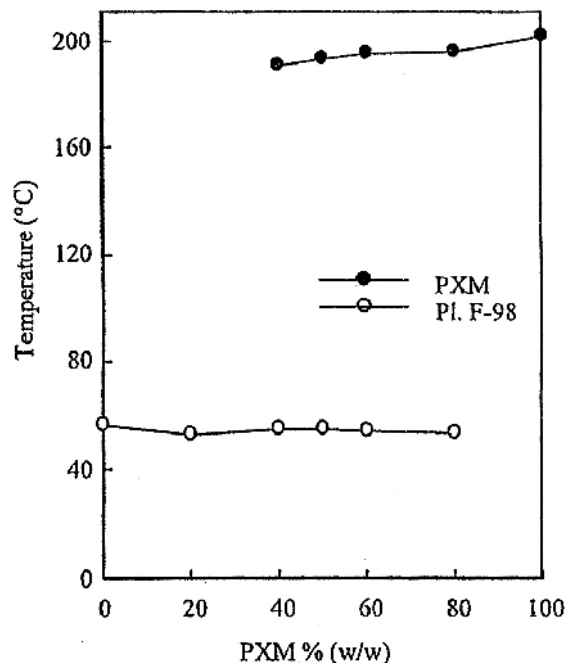
Fig. 10: Phase diagram for solid dispersion of piroxicam (PXM) in pluronic F-98 (Pl. F-98).
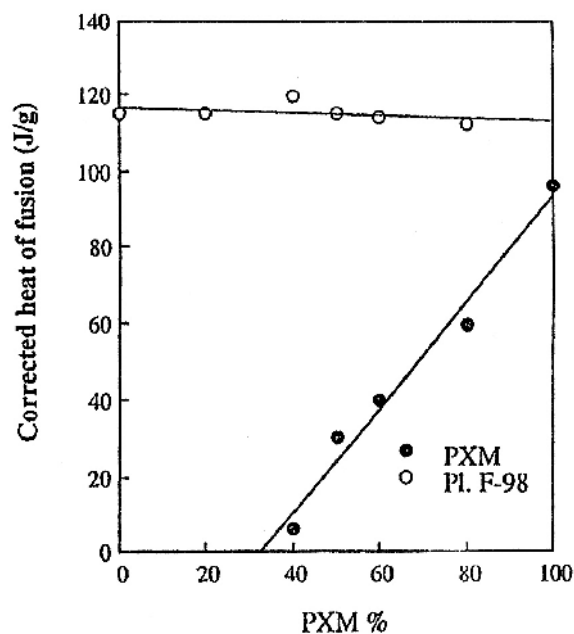
Fig. 11: Relation between piroxicam (PXM) concentration in solid dispersion of pluronic F-98 (Pl. F-98) and correct heat of fusion.

Figs. 10 and 11, indicated that, both of melting point and heat of fusion values did not change with the change of copolymer (Pl. F-98) concentrations which ascertained the previous conclusion that, pluronics in the crystalline

state in the SDs in despite of its presence in low concentration (20% w/w is lowest concentration used).

Table 2 demonstrated the melting point and the heat of fusion of PXM in the SD of pluronic F-98 prepared at 1:1 weight ratio after storage at 40° for 40 weeks. The melting point of piroxicam in the solid dispersion was not changed. Moreover, the heat of fusion was barely affected. This indicated that, piroxicam in solid dispersion of pluronic F-98 was stable during aging.

Solubility of piroxicam in pluronic solutions

Different concentrations of different pluronics were studied for their solubilizing effect on piroxicam. The solubilization of piroxicam in different pluronic solutions at 37° is shown in Figure 12. The tested concentrations were used above their respective critical micelle concentration (CMC). The solubility of the drug in these solutions was increased linearly by increasing the pluronic concentration.

The solubilization of a slightly water-soluble compound can be treated in terms of association equilibrium between the solutes and the micelles in a micellar solution[25] Thus:

$$D_w + M \xrightleftharpoons{K_m} D_m \dots \dots \dots \dots \dots \dots (2)$$

Where D_m and D_w are the drug solubilized in the micellar and bulk water, respectively. M is the micellar concentration and km is the distribution coefficient of the drug between the micellar and the bulk aqueous phases.
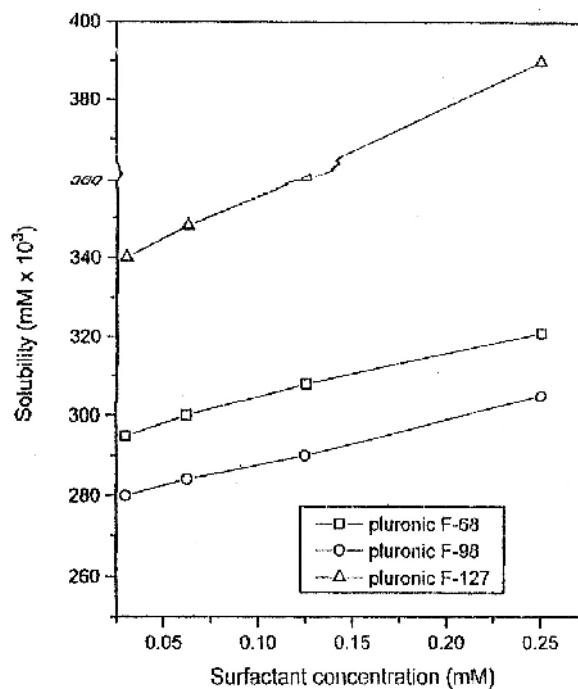

Fig. 12: Solubility or piroxicam in aqueous micellar solution of different pluronics at 37°.

Table 2: Melting points (m.p; °) and heat of fusion (ΔH; J/g) for piroxicam and pluronic F-98 after storing their solid dispersion, prepared at 1:1 ratio, for 40 weeks at 40°.

Storage time (Weeks)	Pluronic F-98 peak		Piroxicam peak	
	m.p (°)	ΔH (J/g)	m.p (°)	ΔH (J/g)
0	51.9	50.66	191.0	15.24
2	50.3	53.42	192.5	16.49
4	54.9	54.90	192.6	15.16
6	54.6	60.77	192.2	18.41
8	49.4	54.22	191.3	15.07
40	55.3	60.3	190.1	16.35

Accordingly, the following relationships were drived:

$$K_m = S_m / S_w M \dots \dots \dots \dots (3)$$

and $S_t = S_m + S_w = S_w + K_m S_w M \dots \dots (4)$

Where S_m and S_w are the solubility of the drug in the micellar and bulk aqueous phase, respectively. M is the molar concentration of the pluronic and St is the total molar solubility of the drug in the micellar solution. Therefore, a plot of St versus M will produce a straight line with an intercept of S_w and a slope of K_m S_w, which is the solubilizing capacity of the micelles. The slopes were determined by linear regression analysis. The distribution coefficient (k_m) can be calculated from the slope ($k_m S_w$) and S_w.

Table 3 lists the solubilizing capacity of the tested pluronics and the distribution coefficient of the drug between micellar and bulk aqueous phases.

Table 3: Solubilizing capacity of different pluronics and the distribution coefficient of piroxicam between the micellar and aqueous phases.

Pluronic	Solubilizing capacity x 10^2	Distribution coefficient (mM) x 10^2
Pluronic F-68	10.7	36.3
Pluronic F-98	10.3	37.0
Pluronic F-127	20.6	61.1

The solubilization of piroxicam in different pluronic solutions Fig. 12, it was found that pluronic F-127 was more efficient as a solubilizer than pluronic F-68 and pluronic F-98. In addition Pluronic F-127 exhibited a higher solubilization capacity and distribution coefficient than other pluronics.

Dissolution rate studies

Figures 13-15 show the dissolution profiles of piroxicam, its solid dispersions as well as the selected physical mixture with different pluronics block copolymer. From Figures 13-15, it was found that, the improvement of dissolution rates of piroxicam by different pluronics was ranked as Pluronic F-127 > F-68 ≥ F-98.
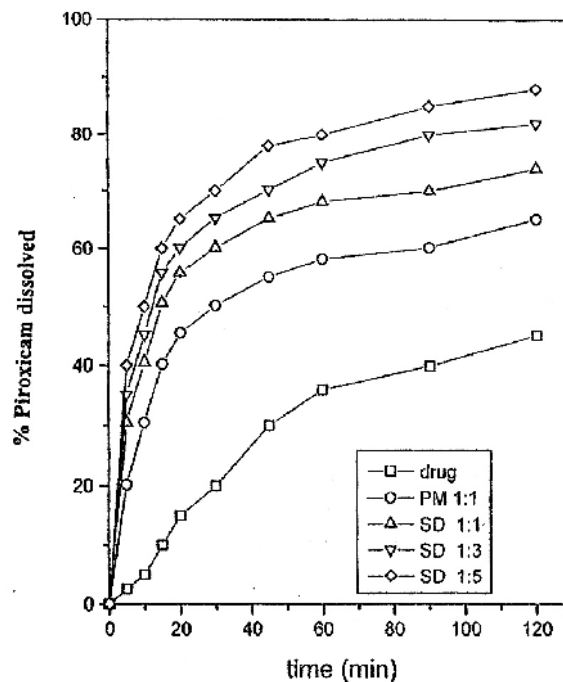

Fig. 13: Dissolution profile of piroxicam, its physical mixture and solid dispersion with pluronic F-98 at different drug polymer ratios.
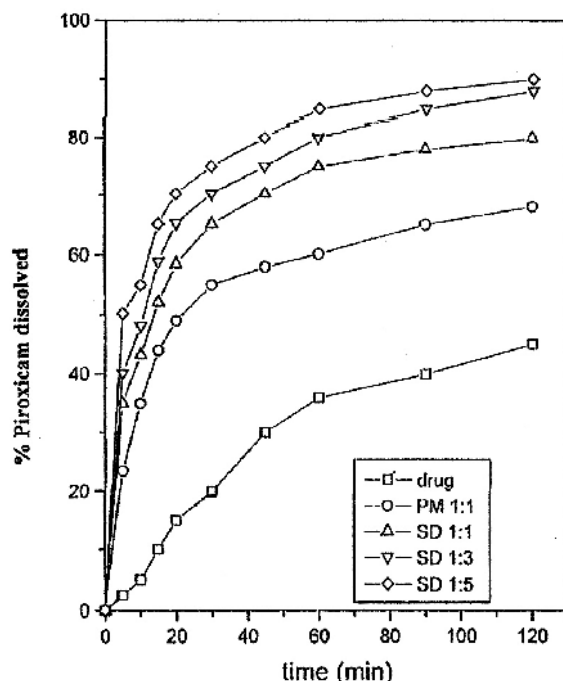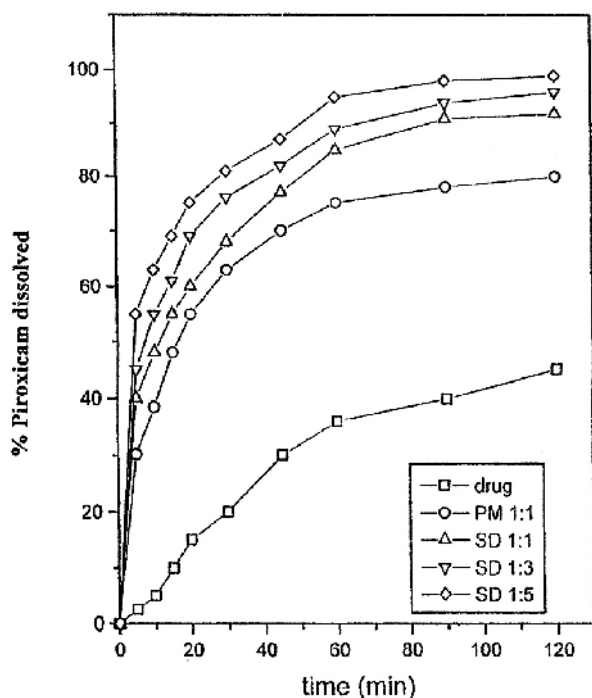

Fig. 14: Dissolution profile of piroxicam, its physical mixture and solid dispersion with pluronic F-68 at different drug polymer ratios.

Fig. 15: Dissolution profile of piroxicam, its physical mixture and solid dispersion with pluronic F-127 at different drug polymer ratios.

It was found that, the physical mixtures exhibit a significant increase in the dissolution rate of the drug than the untreated drug. However, the solid dispersion displayed faster and higher dissolution rates than the corresponding physical mixture. Also, the dissolution rate of piroxicam was increase by increase the ratio of the carrier in the tested system. The dissolution rate of piroxicam from physical mixtures was greater than the drug itself. This may be explained in terms of the possible lowering of the surface tension of the medium by pluronics, resulting in better wetting of the drug surface. The improvement of the dissolution rate of the drug from its solid dispersion might be due to the conversion of its crystalline form into the amorphous one.[26]

In conclusion, IR spectroscopy, X-ray diffraction and DSC detected no chemical interaction between piroxicam and Pluronics. Pluronics keep their crystallinity in SD even at low concentration. Solid-state solutions were obtained for combinations containing less than 35% piroxicam. The solid dispersion for PXM was stable and no change in its form along 40 weeks at 40°. The aqueous solubility of the drug has been improved by using different pluronics. The dissolution rates of piroxicam was markedly improved either by physical mixture or the solid dispersion of different pluronics and this improvement can be ranked in a descending order as Pluronic F-127 > F-68 ≥ F-98.

REFERENCES

1- K. Sekiguchi, and N. Obi, Chem. Pharm. Bull., 9, 866 (1961).

2- M. Sugimoto, T. Okagaki, S. Narisawa, Y. Koida, and K. Nakajima, Int. J. Pharm., 160, 11 (1998).

3- K. P. R. Chowdary, R. O.Srinivasa, and S. K. Comm, Drug Dev. Ind. Pharm., 26, 1207 (2000).

4- S. D. Yoo, S-H. Lee, E. Kang, H. Jun, J-Y, Jung, J. W. Park, and K-H. Lee, Drug Dev. Ind. Pharm., 26, 27 (2000).

5- S. L. Law, W.Y. Lo, F.M.; Lin, and C. H. Chaing, Int. J. Pharm., 84, 161 (1992).

6- M. E. A. Dalmora, and A. G. Oliveira, Int. J. Pharm., 184, 157 (1999).

7- E. Swanepoel, W. Liebenberg, M. M. de Villiers, and T. G. Dekker, Drug Dev. Ind. Pharm., 26, 1067 (2000).

8- Parfitt, K., Martindale-the extra pharmacopoeia. 2002, 33rd edition, the pharmaceutical press, London, p. 79.

9- M. Fernandez, I. C. Rodriguez, M. V. Margarit, and A. Cerezo, Int. J. Pharm., 84, 197 (1992).

10- M. Fernandez, M. V. Margarit, I. C. Rodriguez, and A. Cerezo, Int. J. Pharm., 98, 29-35 (1993).

11- V. Tantishaiyakul, N. Kaewnopparat, and S.Ingkatawornwong, Int. J. Pharm., 143, 59 (1996).

12- V. Tantishaiyakul, N. Kaewnopparat, and S. Ingkatawornwong, Int. J. Pharm., 181, 143 (1999).

13- A. V. Kabanov, and V. Y. Alakhov, Crit. Rev. Ther. Drug Carrier Syst., 19, 1 (2002).

14- P. Alexandridis, Colloid & Interface Science, 2, 478 (1997).

15- E. Batrakova, S. Lee, S Li, A. Venne, V. Alakhov, and A. Kabanov, Pharm. Res., 16, 1373 (1999).

16- S. C. Shin, C. W. Cho, and H. K. Choi, Drug Dev. Ind. Pharm., 25, 273 (1999).

17- S. C. Shin, E. Y. Shin, and C. W. Cho, Drug Dev. Ind. Pharm., 26, 563 (2000).

18- S. C. Shin, C. W. Cho, I. J. Oh, Int. J. Pharm., 193, 213 (2000).

19- M. Mihalic, In Florey, K., (Ed.), Analytical profiles of Drug Substances, Vol. 15, Academic press, London, 1986, pp. 509-531.

20- F. Vrecer, S. Srcic, and J. Smid-Korbar, Int. J. Pharm., 68, 35 (1991).

21- R. Kaur, D. J. W. Grant, and T. Eaves, J. Pharm. Sci., 69, 1317 (1980).

22- H. Yuasa, T. Ozeki, H. Takahashi, Y. Kanaya, and M. Ueno, Chem. Pharm. Bull., 42, 354 (1994).

23- W. L. Chiou, and S. Riegelman, J. Pharm. Sci., 60, 1281(1971).

24- T-T. Yang, and J. Swarbrick, J. Pharm. Sci., 75, 53 (1986).

25- J. T. H. Ong and E. Manoukian, Pharm. Res., 5, 704, (1988).

26- Y. Chiba, N. Kohri, K. Iseki and K. Miyazaki, Chem. Pharm. Bull., 39, 2158 (1991).