

EFFECT OF NATURAL POLYMERS ON THE PHYSICO-CHEMICAL PROPERTIES AND ULCEROGENIC ACTIVITY OF PIROXICAM

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عقار البيروكسيكام هو أحد مجموعة مضادات الإلتهاب الغير استيرويدية ونظرا لطبيعته الحمضية وكذلك شحة ذوبانه في الماء فعادة ما يتسبب عن إستعماله تكون قرحة في المعدة. وقد تمت دراسة تأثير مشتتات من العقار مع بعض البلمرات الطبيعية مثل البكتين ، الليسيثين ، الجينات الصوديوم والجيلاتين. وقد أدى خلط العقار مع هذه البلمرات الطبيعية بتركيزات مختلفة إلى الإقلال من ذوبان العقار إلا في حالة الجيلاتين والجيلاتين الصوديوم فقد أدى تكون مشتت منه مع العقار إلى زيادة ذوبان العقار. وتم دراسة التفاعل بين العقار من ناحية وكل من تلك البلمرات الطبيعية من ناحية أخرى بإستخدام الأشعة السينية والأشعة تحت الحمراء. وقد تم دراسة تأثير مشتت العقار مع الجيلاتين المتناول عن طريق الفم لفتران التجارب على الغشاء المبطن للمعدة وعلى القابلية لإحداث القرحة. وقد أوضحت الدراسات الهستولوجية على معدة الفتران المعاملة بالعقار منفردا أو مشتتة مع الجيلاتين أن خلط العقار مع الجيلاتين في صورة مشتت أدى إلى الإقلال بشكل واضح من التأثير على المعدة حيث ظهر الغشاء المبطن لجدار المعدة سليما متكاملا وكذلك الغدد المعدية ظهرت سليمة. لذلك فإن خلط عقار البيروكسيكام مع مادة الجيلاتين في صورة مشتت يؤدي إلى تحسين ذوبان العقار مع تقليل تأثيره على جدار المعدة من حدوث القرحة المعدية.

Piroxicam belongs to the class of acidic non-steroidal antiinflammatory drugs. Under the acidic conditions in which its absorption takes place, its low solubility and dissolution rate has been well documented. The effect of natural polymers such as pectin, lecithin, sodium alginate and gelatin on the physicochemical properties of piroxicam has been studied. Dispersions of these polymers with the drug were prepared in different weight ratios by the kneading method. The dissolution rate of the drug decreased in presence of all these polymers except gelatin. Gelatin showed enhancing influence on piroxicam dissolution. A slight increase in dissolution was also obtained with 1:2 drug-pectin ratio. The solubility of piroxicam decreased in the presence of different concentrations of either pectin or lecithin but increased in the presence of different concentrations of gelatin and sodium alginate. The interaction of these polymers and piroxicam was traced by X-ray diffraction and IR spectroscopy. In addition, piroxicam-induced gastric lesions in rats, disappeared by dispersing the drug in gelatin. Histological studies showed intact surface epithelium and fundic glands in case of piroxicam-gelatin dispersion. So, piroxicam-gelatin dispersed mixture is recommended to increase the dissolution of the drug and protect against ulcerogenic activity.

INTRODUCTION

Piroxicam has analgesic, antiinflammatory and antipyretic effect. It is used in rheumatic disorders and in acute gout.¹ Through its action

by inhibition of the cyclooxygenase pathway of arachidonic acid metabolism,² and prostaglandin synthesis and release of oxygen radicals. It leads to impaired gastric mucus synthesis, vasoconstriction of the gastric mucosa, gastric

ulceration and haemorrhage.³ Piroxicam shows poor aqueous solubility and dissolution.

The use of natural polymers as drug carriers has attracted considerable attention in pharmaceutical formulation due to their inert nature and safety.^{4,5}

Reviewing the literature showed that from natural polymers, gelatin and egg albumin solid mixtures with nifedipine resulted in significant increase in the rate of dissolution of the drug.⁶ The dissolution rate and bioavailability of indomethacin from its alginate dispersions were greatly enhanced.⁷ The interpolymer complex formation of chitosan with pectin or acacia on the release behaviour of chlorpromazine HCl was reported.⁸

The aim of this work was to investigate the possible effect of some natural polymers on the solubility and ulcerogenic activity of piroxicam.

MATERIALS AND METHODS

Materials

Piroxicam (piroxicam) supplied from CID, Egypt; gelatin, from E. Merck, Darmstadt, Germany; lecithin, BDH Chemicals Ltd, Poole, England; sodium alginate, the General Chemical and Pharmaceutical Co. Ltd. Sudburg Middlesex, England. All chemicals and solvents were of pharmaceutical or analytical grade and used without further purification.

Methods

Preparation of piroxicam dispersions

The calculated amounts of (piroxicam) and each of sodium alginate, lecithin, pectin or gelatin to form physical mixtures of ratios (1:1 and 1:2) drug : polymer were weighed and mixed. Each mixture was thoroughly kneaded on cold with little water using a mortar and a pestle for a period of half an hour. The prepared mixture was left to dry in a vacuum oven at 40° for one day then transferred to a vacuum desiccator till used.

Dissolution rate studies

The dissolution rate of piroxicam powder (20 mg) and an equivalent weight of its

dispersions and physical mixtures with the respective polymers in 1:1 or 1:2 ratio filled into gelatin capsules (size 1) was determined. The method applied was a revised method of USP XXII dissolution test for piroxicam.⁹ The particle size of drug dispersions used was confirmed to the mesh size of 45-63 μm . The dissolution medium was 900 ml of simulated gastric fluid TS without pepsin (pH 1.2); maintained at 37° \pm 0.1 and stirred at a rate of 50 rpm using the paddle. At appropriate time intervals, 5 ml samples were removed, filtered and assayed spectrophotometrically at 286 nm. Corrections were applied for cumulative dilution caused by replacing the sample by equal volumes of the same medium kept at the same temperature. Each dissolution experiment was performed in duplicate and the mean absorbance values were used for calculation.

Solubility studies

Excess amounts of piroxicam were added to aqueous solutions containing different concentrations of each polymer and shaken at 25° \pm 0.1. After equilibrium was attained, an aliquot was pipetted, filtered and diluted with water and analyzed spectrophotometrically at 286 nm.

IR spectroscopy

A Shimadzu IR-470 spectrometer was used. The spectra were obtained using potassium bromide discs for the dried solid products.

X-ray diffraction

A Philips PW 1710 powder X-ray diffractometer with Nickel filter, Cu-K radiation was used under the following conditions: voltage 40 KV, current 30 mA, scanning speed 2 θ = 0.06 degree/sec.

Partition coefficient determination

The apparent partition coefficient (P.C.) of piroxicam between octanol and phosphate buffer (pH 7.4) was determined. In a stoppered glass bottle, octanol (10 ml) and buffer (10 ml) containing piroxicam (0.8 mg/ml) or its equivalent concentration from different

dispersions of piroxicam with natural polymers were shaken (40 rpm) in a thermostatically controlled water bath at 37° till equilibrium was attained (24 h). From the aqueous phase, samples were withdrawn, suitably diluted, and estimated spectrophotometrically at 286 nm for piroxicam content. The concentration of the drug in the organic phase, was determined by subtraction of the drug concentration in the aqueous phase from the initial concentration of the drug. The apparent P.C. was calculated as:

$$\text{Apparent P.C.} = C_o/C_w$$

where C_o and C_w are the concentrations of the drug in octanol and aqueous phase respectively.

Evaluation of ulcerogenic activity Protocol

Twelve male Wistar rats (180-200 g), were divided into three equal groups, each of 4 rats. Either piroxicam suspension (0.5 mg/ml) in distilled water in a 1 ml dose or its equivalent amount of piroxicam-gelatin dispersion were administered orally, followed by 1 ml water. This treatment continued for 4 days.¹⁰ The third group acted as a control and was given distilled water instead. The animals were put into individual cages without access to food 12 hr, and to water for 3 hr before the first administration and throughout the entire experiment. At the fifth day, the animals were sacrificed and the stomachs were rapidly removed, opened along the length of the greater curvature, washed with physiological saline and preserved in formalin.

Histological studies

At least two mucosal strips were obtained from each stomach, either from regions with apparent mucosal damage or at random from stomachs without macroscopic damage. The strips were embedded in paraffin. Sections were prepared and stained with H&E, then examined by light microscopy.

RESULTS AND DISCUSSION

Dissolution studies

Figures 1,2 illustrate the percent of piroxicam dissolved against time from capsules

containing the drug alone, its physical mixtures or drug dispersions with the different polymers. It is obvious from the figures that dispersing the drug with gelatin enhances its dissolution rate, while using other polymeric carriers viz., pectin and lecithin decreases the dissolution of piroxicam. Sodium alginate dispersion releases the drug in almost the same pattern and a rate that is nearly equal or little bit smaller than the drug alone. It is obvious from the figures that the percent dissolved of piroxicam from its dispersion with gelatin far exceeds drug dissolution from the corresponding physical mixture (93% to 62%), this indicates that a sort of interaction is created during dispersing the drug into the carrier. Pectin dispersion of the drug (1:2) showed high dissolution of piroxicam (not included in Figs. 1&2).

Solubility studies

The influence of these polymers on the solubility of piroxicam was studied and the results are shown in figures 3&4. It is clear that both sodium alginate and gelatin exhibit solubilizing effect on the drug. The effect of gelatin is more pronounced and the influence of higher concentrations could be performed, but due to the low solubility of alginate, concentrations till 1 mg/ml only could be adopted. Moreover, gelatin is known to absorb about ten times its volume of water and at 30° (the temperature of the solubility experiment), gelatin begins to dissolve and this property is responsible for its solubilizing effect. Moreover, the linear structure of gelatin is a promoting factor for solubilization.

Both pectin and lecithin (Fig. 4) decrease the solubility of piroxicam at the concentration levels applied. It is concluded that these carriers interact with piroxicam and the interaction product seemed to hinder drug solubility and dissolution.

Elucidation of interactions

Two dispersion systems of piroxicam and each of gelatin and pectin were chosen for further studies to elucidate the expected interactions between piroxicam and these carriers.

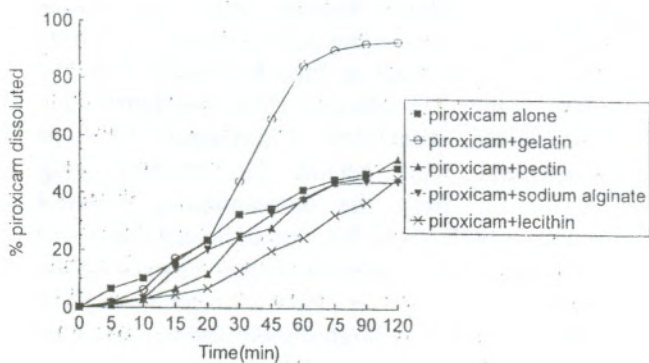


Fig. 1: Dissolution of piroxicam dispersion 1:1 drug : polymer ratio with different natural polymers.

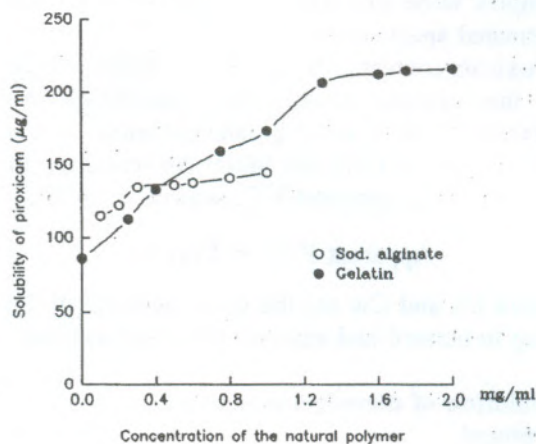


Fig. 3: Effect of different concentrations of gelatin, and sod. alginate on the solubility of piroxicam.

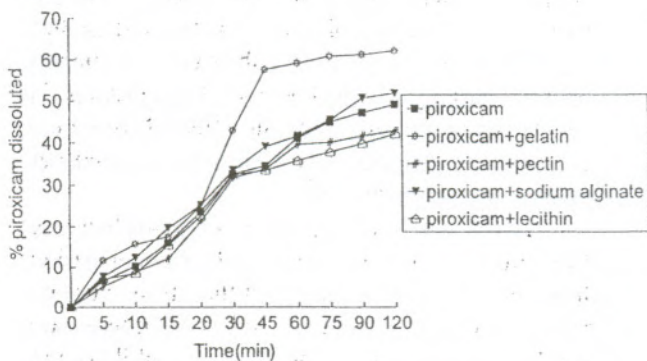


Fig. 2: Dissolution of piroxicam physical mixtures 1:1 drug : polymer ratio with different natural polymers.

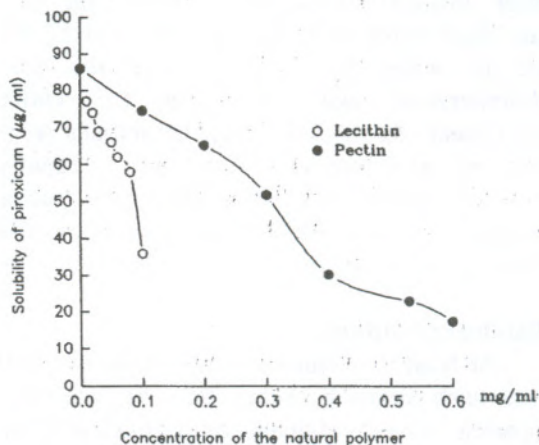


Fig. 4: Effect of different concentrations of pectin and lecithin on the solubility of piroxicam.

IR studies

Figures 5 and 6 show the IR spectra of piroxicam, the carriers, their respective physical mixtures and dispersions in 1:1 ratio. The IR spectra of the two physical mixtures revealed that each is a superimposition of the spectra of its respective components. As for the dispersion system, distinctive changes in the finger print bands had been observed (Figs. 5&6). The OH and -NH stretching bands at 3475 cm^{-1} were shifted to higher frequency. The bands of piroxicam spectrum at 1523 , 1573 and 1635 cm^{-1} was coupled together especially in case of gelatin dispersion. The asymmetric stretching produces a change in dipole moment (μ) of the group and therefore change in position of bands was observed. Thus interactions between piroxicam and each carrier revealed to be through intermolecular hydrogen bonding which alters the force constant of groups. Thus, the frequencies of both stretching and bending vibrations are altered. This led to lower frequency (longer wavelength). The stretching frequency of the acceptor group, for example, C=O is also reduced but to a lesser degree than the proton donor.

X-ray studies

The X-ray diffraction pattern of piroxicam dispersions and the equimolar physical mixtures and the drug alone are shown in figures 7&8. The diffraction pattern of piroxicam-gelatin and piroxicam-pectin physical mixtures differed slightly from the pattern of the drug alone. Some peaks decreased in heights or shifted a little, indicating less crystallinity. But the corresponding pattern of the dispersion system of piroxicam with the corresponding carrier differed significantly from the pattern of the drug alone or its physical mixture. In case of gelatin dispersion the peaks at $2\theta = 9.047$, 10.143 and 12.011 were drastically decreased in height. Moreover, all the peaks behind $2\theta = 27.97$ were completely disappeared. All these changes provided evidence of interaction between piroxicam and each of gelatin and pectin with formation of amorphous dispersions.

The partition coefficient (PC) of piroxicam between octanol and buffer (pH 7.4) were calculated and the influence of the different

polymers on PC was studied (Table 1). The PC seemed to be not changed on dispersing piroxicam with either pectin or lecithin but its value is slightly increased in case of sodium alginate and gelatin. That is due to the solubility decreasing influence of the two former polymers.

Table 1: Partition coefficient determination of piroxicam and its dispersion with the different natural polymers.

Type of carrier	P.C. of piroxicam (octanol/buffer)
-	1.60
gelatin	1.77
pectin	1.62
lecithin	1.53
sodium alginate	1.86

Histological examination

The histological examination of strips of rat stomachs which were treated with gelatin dispersion with piroxicam revealed absence of morphological damage and were indistinguishable from stomachs of untreated rats. Thus, the interaction through dispersion of piroxicam with the natural carrier gelatin, affords protection of the gastric mucosa from the ulcerogenic effect of piroxicam (Figs. 9&10). As the histological examination of the stomachs in case of piroxicam alone (Fig. 9B) showed the presence of multiple superficial ulcers and deep erosions. The basal part of fundic glands showed marked cystic dilatation in relation to the ulcers. The submucosa showed marked oedema, congestion and polymorphs infiltration with infiltration of the mucosal glands resulting in their destruction.

Since piroxicam- β -cyclodextrin complex had been reported in a previous work,¹⁰ to exhibit the lowest value of ulcer index, compared to the drug alone or piroxicam- α -cyclodextrin complex. The slides including strips of stomachs treated with piroxicam- β -cyclodextrin showed only erosions, and mild inflammation in the submucosa related to the areas of erosions (Fig. 10B). For purpose of

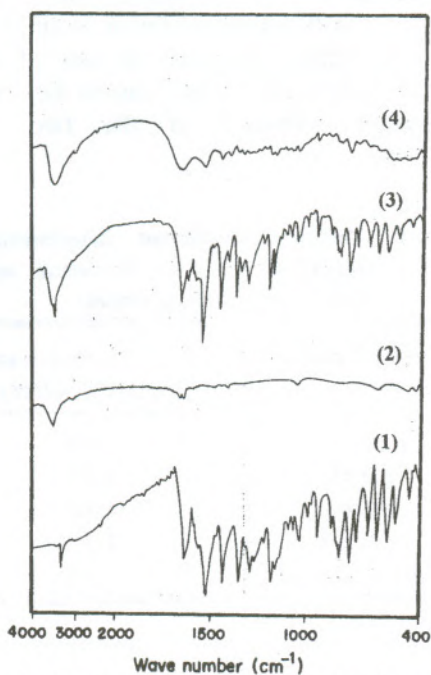


Fig. 5: Infrared spectroscopy of piroxicam (1), gelatin (2), their physical mixture (3) and their dispersion (4).

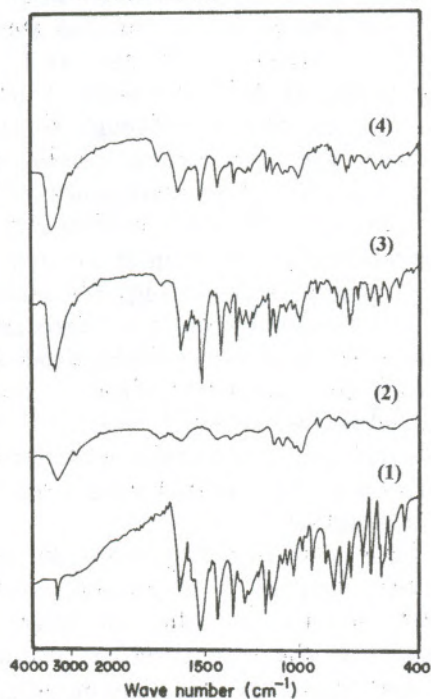


Fig. 6: Infrared spectroscopy of piroxicam (1), pectin (2), their physical mixture (3) and their dispersion (4).

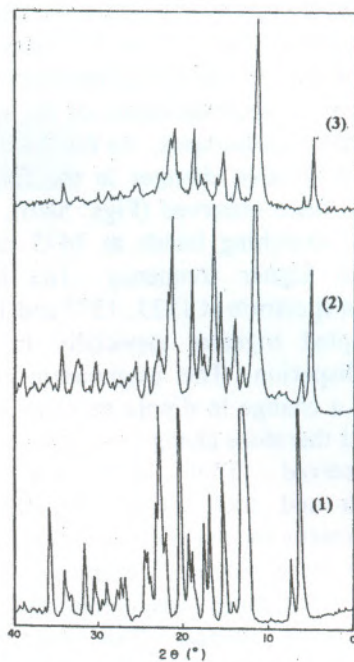


Fig. 7: Powder X-ray diffraction pattern of piroxicam (1), physical mixture with gelatin (2), and its dispersion (3).

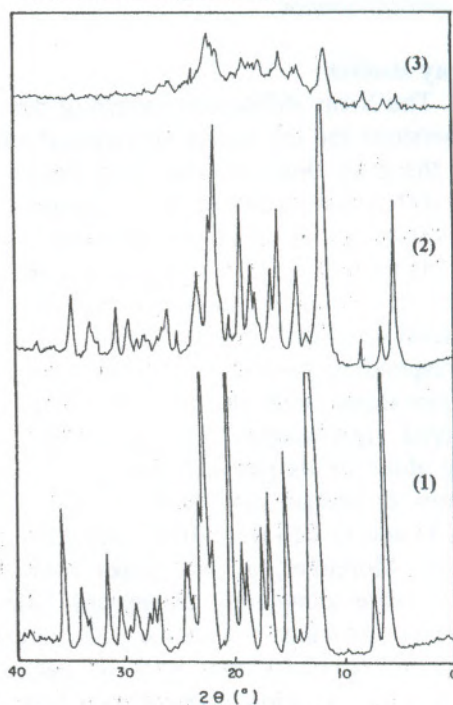


Fig. 8: Powder X-ray diffraction pattern of piroxicam (1), physical mixture with pectin (2), and its dispersion (3).

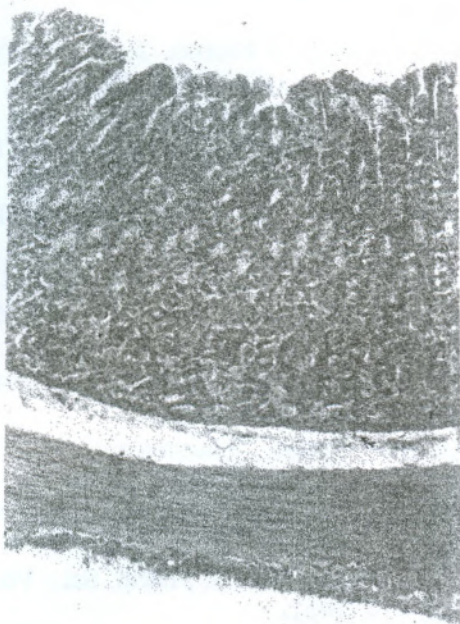


Fig. 9A: Section in the stomach fundus of a rat treated with piroxicam-gelatin dispersion.

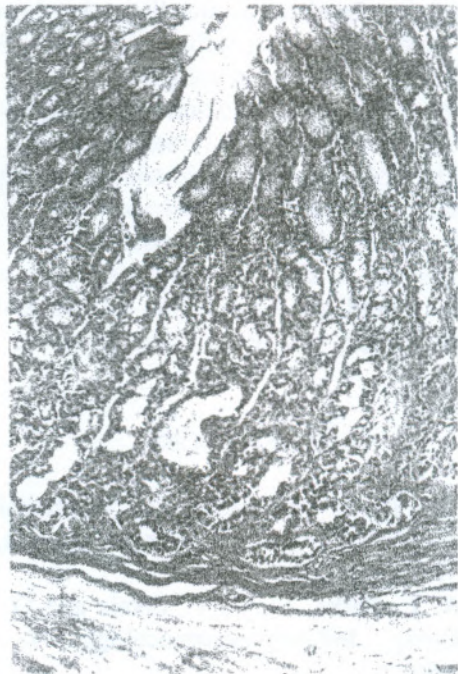


Fig. 9B: Section in the stomach fundus of a rat treated with piroxicam alone.

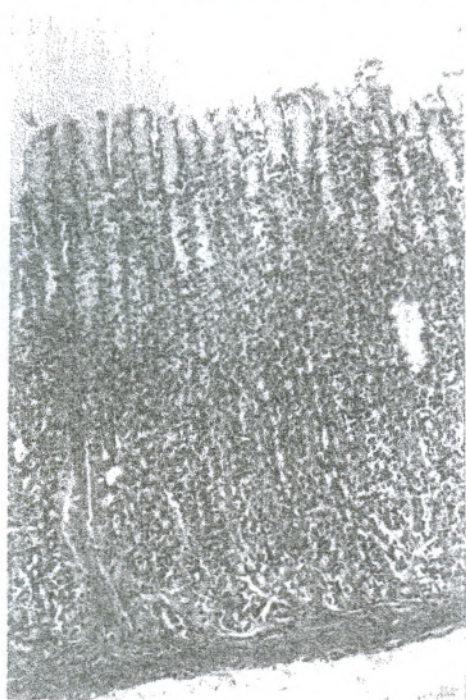


Fig. 10A: Section in the stomach fundus of the control rat.



Fig. 10B: Section in the stomach fundus of the control rat treated with piroxicam- β -cyclodextrin.

comparison, piroxicam gelatin dispersion showed better suppression of ulcer formation in rats than piroxicam- β -cyclodextrin complex.

We could finally conclude that, dispersing piroxicam with natural polymers influences its solubility and dissolution. Gelatin of the tested polymers showed some desirable effects, it increased the solubility and dissolution of the drug. As well as the drug in its dispersion with gelatin offered good protection of the gastric mucosa against the ulcerogenic effect of the drug.

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