Bull. Pharm. Sci., Assiut University
Vol. 12, Part 1, pp 136 - 151

CORRELATION BETWEEN GASTRIC IRRITANCY AND BIOAVAILABILITY OF INDOMETHACIN MICROSPHERES.

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ABSTRACT

Indomethacin microspheres were prepared by an emulsionsolvent evaporation method. Ethylcellulose was used as the matrix material at 1:1 drug/polymer ratio. The prepared microspheres were tested for drug content, dissolution characteristics, antiinflammatory effect in rats and for their possible gastric effect in rabbits. Acontrolled release pattern of the medicament was found to be existed from the prepared microspheres. The prepared microspheres gave an antiinflammatory effect in rats as that produced by the intact medicament. Histological examination of rabbit's stomach proved the superiority of the tested microspheres over the intact drug.

INTRODUCTION

The aim in designing a sustained-release dosage form is to obtain rapidly a desirable blood concentration of the drug, to maintain such a concentration at a roughly constant level for a suitable period of time, to reduce the frequency of drug administration, and to reduce the incidence and intensity of side effects[1]. Many pharmaceutical means for the preparation of sustained release dosage forms have been investigated, and it was reported that microspheres is useful method[2]. Microcapsules are either of the film or matrix type. In the matrix type

microcapsules the drug is homogeneously dispersed in a matrix formed by the polymer chains[3]. This type of microcapsules is better described as microspheres. The method of preparation is the determinant factor of the microcapsules type.

Indomethacin is a nonsteroidal anti-inflammatory agent. The drug exhibits analgesic and anti-pyretic activity[4]. The drug is rapidly and almost completely absorbed from the gastrointestinal tract in healthy adults. Clinical trials of indomethacin as an anti-inflammatory agent have demonstrated that the medicament releives pain, reduce pain and tenderness of the joints, increase grip strength and decrease the duration of morning stiffness. It is also very effective in the treatment of acute gout, although it is noturicosuric[5-7]. There were many reports of adverse reactions to indomethacin, included gastrointestinal ulceration and bleeding[8]. Wilson[9] reported that indomethacin adhesion to the mucosal surface is unlikely to be the mechanism responsible for its side effects. These side effects resulted in sloughing of epithelium, slight reddening, localized areas of reddening, pin-point area of bleeding haemorrhagic lesions, larger area of bleeding profuse bleeding, breakdown of epithelium, perforating ulcers and intestinal adhesions[10,11]. The ulcerogenic properties of indomethacin in rats were found to be dose dependent and followed 36-60 hrs after its oral administration[12]. Nakamura et al[13] followed the gastrointestinal mucosal damage of indomethacin in rats 15 hours after its oral administration. The effect was reported to be dose dependent. Dearden and Nicholson[10] examined the stomach lining for its possible damage 4.5 hours after oral administration of indomethacin. Knoll et al[14] studied the gastrointestinal changes two weeks after indomethacin administration(3.5 mg/kg). Muller et al[15].tested the mucosal lesions in healthy volunteers after 5 and 10 days treatment with indomethacin 50mg three times daily.

The ulcers were prepyloric in some patients and had an appearance falsely suggestive of malignancy in the others[16]. Mouth ulcers developed in some patients with dentures upon receiving indomethacin for rheumatoid arthritis[17]. Also, rectal bleeding occurred after several months of treatment with indomethacin 150 mg daily as suppositories[18]. Reduction of dose or discontinuation of drug led to complete healing in all reported cases.

Solid dispersions of 10% indomethacin in PEG 6000 were more gastro-irritant than 10% physical mixes in PEG 6000 or lactose[19]. The larger size fraction of indomethacin dispersion was more gastro-toxic than the finer ones[20]. A microsphere form of indomethacin without adverse effect on the stomach was reported using soyabean oil[21]. An encapsulated hydrophilic beads of indomethacin with more durable serum drug level was reported by Suryakusuma and Jun[22]. However, no significant difference in availability between a regular preparations of indomethacin(25mg three times daily) and a sustained dosage form (75 mg twice daily) was also reported[23]. Hilton and Summers[24] studied the oral absorption of indomethacin in rats. They reported that encapsulation of drug does not offer any advantage over convention dosage forms. On the other hand, Laakso et al[25] found that the bioavailability of indomethacin sustained release tablets in humans was less than that of commercial capsules.

In the present study, we prepared indomethacin microspheres and investigated their dissolution characteristics, gastro-toxicity and anti-inflammatory properties.

EXPERIMENTAL

Microspheres Preparation :

Ethylcellulose (N 100, Hercules, Inc. Wilmington) solution in ethyl acetate was prepared at 10% concentration. Indomethacin (B.P. grade) was dissolved in the polymeric solution. The obtained solution was added while stirring to an aqueous medium containing 2% emulsifier. Stirring was continued for about three hours. The formed microspheres, after ethyl acetate evaporation were separated by decantation and filteration. The microspheres were then dried in an oven at 50°C for about 12 hours and separated into sieve fractions. Procedures for the preparation of microspheres are shown in Figure 1.

Determination of Drug Content :

100-mg samples representing the different sieve fractions were crushed in a mortar. Simulated intestinal fluid was added to the mortar content. The content was filtered, to remove coat fragments, and quantitatively transferred to 100-ml measuring flask. The flask was completed to volume by simulated intestinal fluid. The drug concentration was measured spectrophotometrically at 319 nm.

Dissolution Studies :

Indomethacin dissolution studies were performed using the USP paddle method, under sink conditions (1000 ml of dissolution media) at 37°C. The speed of rotation was 100 rpm. The dissolution medium was either the USP artificial gastric fluid (pH 1.2) or the pH 7.2 phosphate buffer. The media were used without enzymes but containing 0.02% w/v polysorbate 80 added to overcome poor wettability of indomethacin powders and the microspheres[26]. Samples (5 ml) were withdrawn at intervals and assayed spectrophotometrically at 319 nm. using fresh medium as a blank.

Gastric-Irritancy Studies :

Adult male healthy rabbits weighing about 1.8-2.2 kg. were used. The animals were kept under control for one week before study. They were divided into three groups of four rabbits. One group received indomethacin

microspheres (equivalent to 25 mg), the second group received the intact drug (25 mg) while, the third one served as the control. The calculated amount of drug was filled into hard gelatin capsules. Each rabbit received one capsule daily for 15 consecutive days. The rabbits in the control group received an empty capsules. The rabbits were sacrificed 5 hours after swallowing the lost capsules. The stomachs were removed, small sections of the lesser and greater curvatures were obtained and dehydrated. The preparations were stained with haematoxylin and eosin and examined by the light microscope. Photomicrographs were taken for the different preparations.

Anti-inflammatory Effect

The anti-inflammatory effect of indomethacin based on its ability to inhibit the increase in local vascular permeability, induced by intradermally injected prostaglandin was investigated[27].

Male rats of about 150 gm body weight were used throughout. Two microspheres size fractions and intact drug were tested. The drug was administered orally at a dose of 50 mg/kg[28]. The abdominal fur was clipped 24 hr before testing. The rats were anaesthetized with intraperitoneal secobarbital injection before the prostaglandin intradermal injection. Potamine blue 6Bx (100 mg/kg) was injected intravenously 30 minutes before the prostaglandin injections. A series of intradermal injections was made into the clipped abdominal skin. Each of the prostaglandin injections were contained in 0.1 ml of tyrode solution and adjusted to pH 7.4. Forty five minutes after the intradermal injections the rats were killed and blueing was examined from the underside of the abdominal skin. The degree of vascular permeability was estimated by measuring the mean diameter of each blue reaction site.

RESULTS AND DISCUSSION

Indomethacin microspheres were prepared using ethylcellulose at drug-polymer ratio of 1:1. An emulsion-solvent evaporation method was adopted (Fig. 1). An emulsifier was added to facilitate

emulsification and aid the separation of single microspheres rather than aggregates. The type as well as the concentration of emulsifier was found to be a critical factor in the adopted procedures. The adopted technique is simple, inexpensive, rapid and reproducible. The yield was found to be more than 90% under the tested conditions. Sieve analysis revealed that more than 95% of the isolated microspheres were of particle size range 90-1000 u. Drug content was found to be 50±3.0% for the tested size fractions.

In Vitro Dissolution Studies :

The results illustrated in Figures 2 and 3 showed that : The dissolution of indomethacin microspheres is greatly retarded in comparison to the intact powder. The dissolution of indomethacin in simulated intestinal fluid was found to be greater than that in simulated gastric fluid. A result which can be attributed to the acidic nature of the medicament. The dissolution rate of indomethacin microspheres was also affected by the microsphere particle size. In this respect; fractions of coarse particle size showed a slow rate of dissolution in comparison to those of the fine particle size. A result which is mainly attributed to the fact that increasing the particle size is accompanied by decreasing the surface to volume ratio and thus decrease the rate of dissolution.

Analysis of indomethacin dissolution from the prepared microspheres according to first order kinetics and diffusion controlled mechanism[29,30] showed that : The dissolution is best described as diffusion-controlled release dependent ($Q-V_{ss}\sqrt{t}$). Figure 4 showed the linear relationship between the percentage dissolved from microspheres of intermediate size fractions in both media and the square root of time. The results are in agreement with those obtained by Lai et al[31] working on the release of indomethacin from polymeric matrices. On the other

hand, first order kinetics was reported to be a better description of indomethacin release either from ethylcellulose-polyethyene glycol microspheres prepared by solvent evaporation technique[32] or from an oral sustained release gelatin matrix[33].

Histological Examination :

Histological examination of the control stomach wall showed that the wall is regular and linned with epithelial cells. The epithelial cells, are tall columnar and contain an oval nuclei. The fundic glands are distributed around the epithelial cells. The mucous neck cell are observed very near to the epithelial cells. They are cuboidal with flat nuclei. The oxyntic cells are large rounded with rounded nuclei and the cytoplasmic is acidophilic. The peptic cells are cuboidal with rounded nuclei and basophilic cytoplasmic (Figures A-B)ones.

Histological examination of the treated stomach showed normal epithelium. There is huge proliferation in the cells of the fundic glands of the three areas. The effect was more marked when the stomach treated with intact drug (Figures C-D) than that in case of treatment with microspheres (Figures E-F). In treatment with intact drug macroscopic examination revealed the presence of localized areas of reddening. Also, a breakdown in epithelium was more manifested.

Anti-inflammatory Effect :

The study of the ability of prepared micropheres to inhibit the increased vascular permeability induced by intradermally injected prostaglandin revealed that : The prepared microspheres gave a comparable anti-inflammatory effect as that produced by the intact drug. Table 11 illustrates the effect after one hour. The effect in case of microspheres was found to be more prolonged. The found results favour the use of such products of indomethacin. These products give comparable anti-inflammatory effect as the intact drug but less undesirable effect on the gastric mucosal tissues.

Table 1: Sieve Analysis and Drug Content of Indomethacin Microspheres

Fraction Size (μ)	Amount of Microspheres in Each Fraction (% ± SE)	Drug Content of Microspheres (%)
> 1000	1.6 ± 0.27	-
630-1000	27.4 ± 0.92	51.72
400-630	43.5 ± 1.41	49.20
200-400	22.2 ± 0.81	47.24
90-200	3.2 ± 0.54	-
< 200	2.1 ± 0.49	-

Table : Comparison of the Ability of Different Indomethacin Preparations to Reduce the Capillary Permeability When Given Orally to Rats.

Preparation	Mean Diameter of the Response(mm)in Four Rats			
	1	2	3	4
Control	12	10	11	11
Microspheres (200-400 μ)	5	7	5	10
Microspheres. (400-630 μ)	1	3	5	5
Intact Drug	1	1	1	1

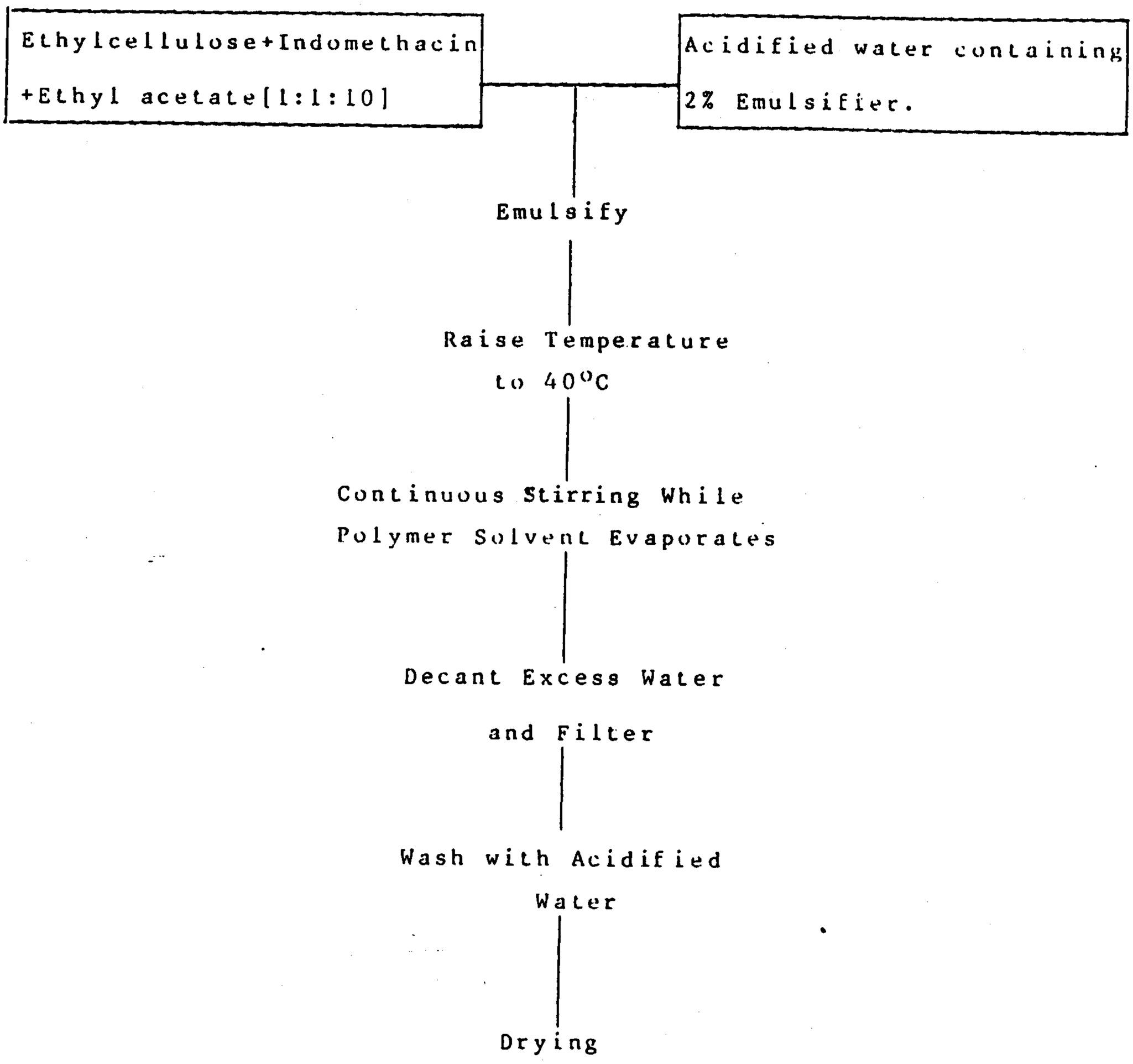


Figure 1: Preparation of Indomethacin Microspheres

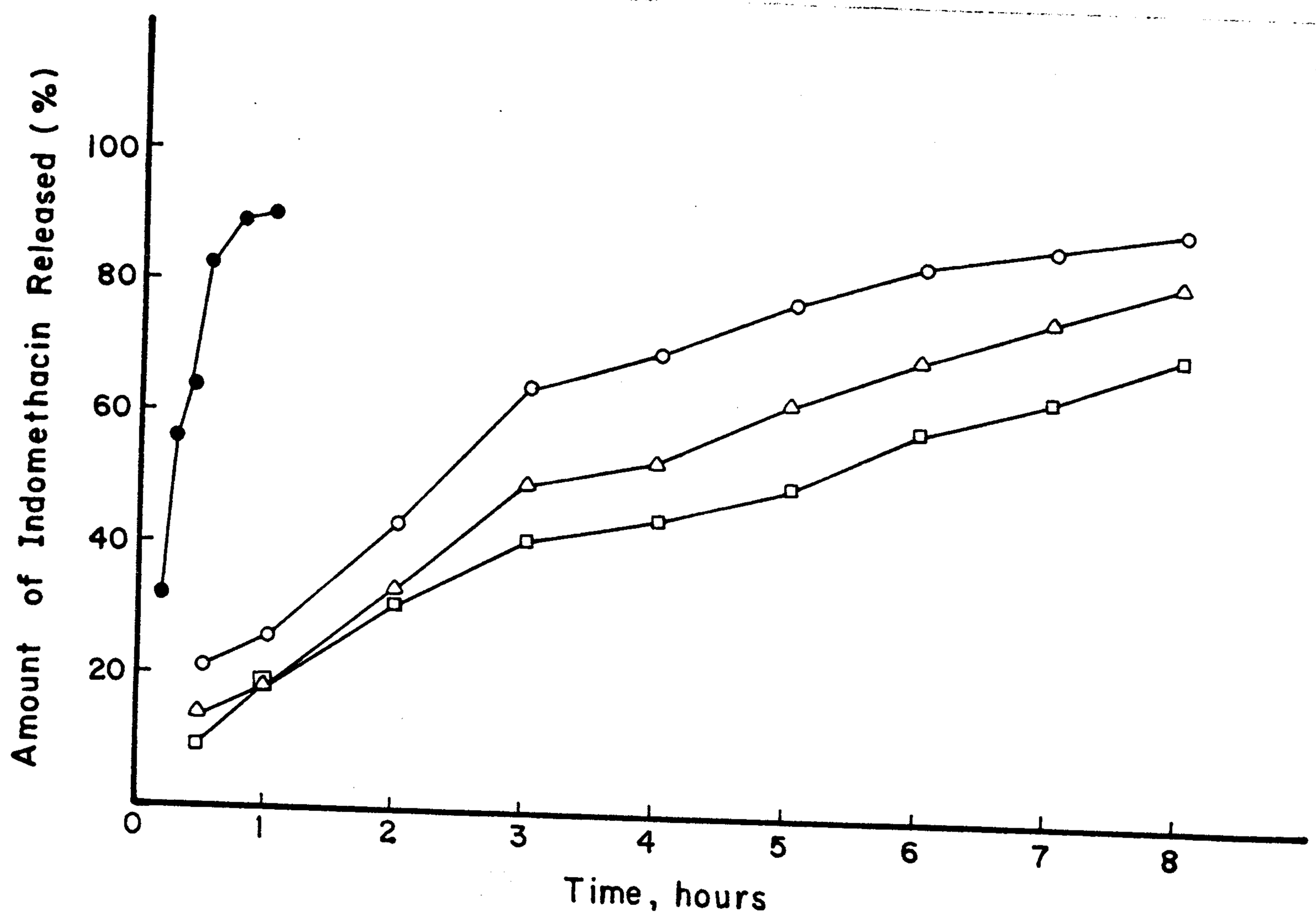


FIGURE 2

In-Vitro Release of Indomethacin from its Microspheres in simulated gastric fluids.

- ● Intact drug
- ○ Microspheres 200 — 400 μ
- △ // 400 — 630 μ
- □ // 630 — 1000 μ

Correlation Between Gastric Irritancy and Bioavailability of Indomethacin Microspheres.

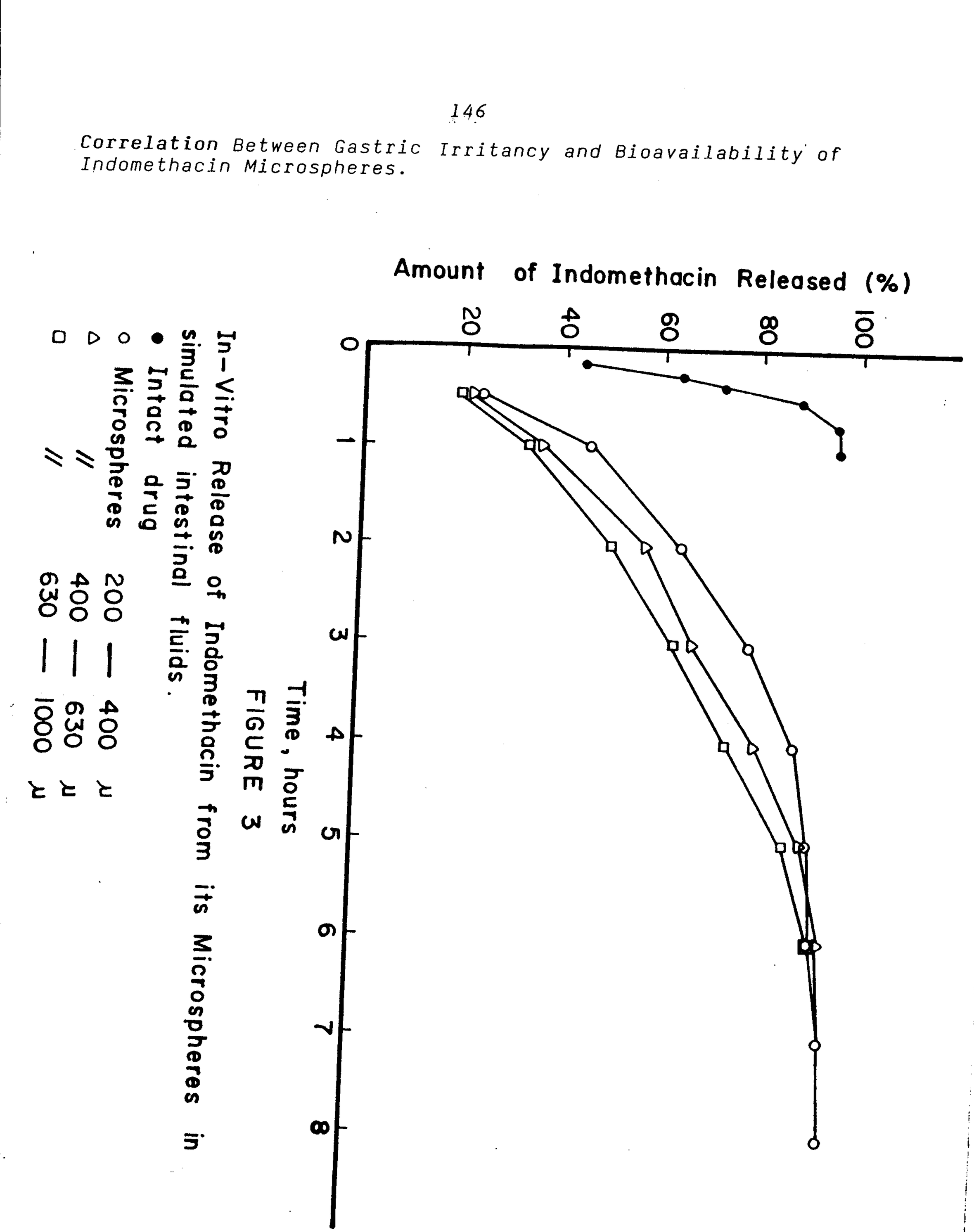


FIGURE 3

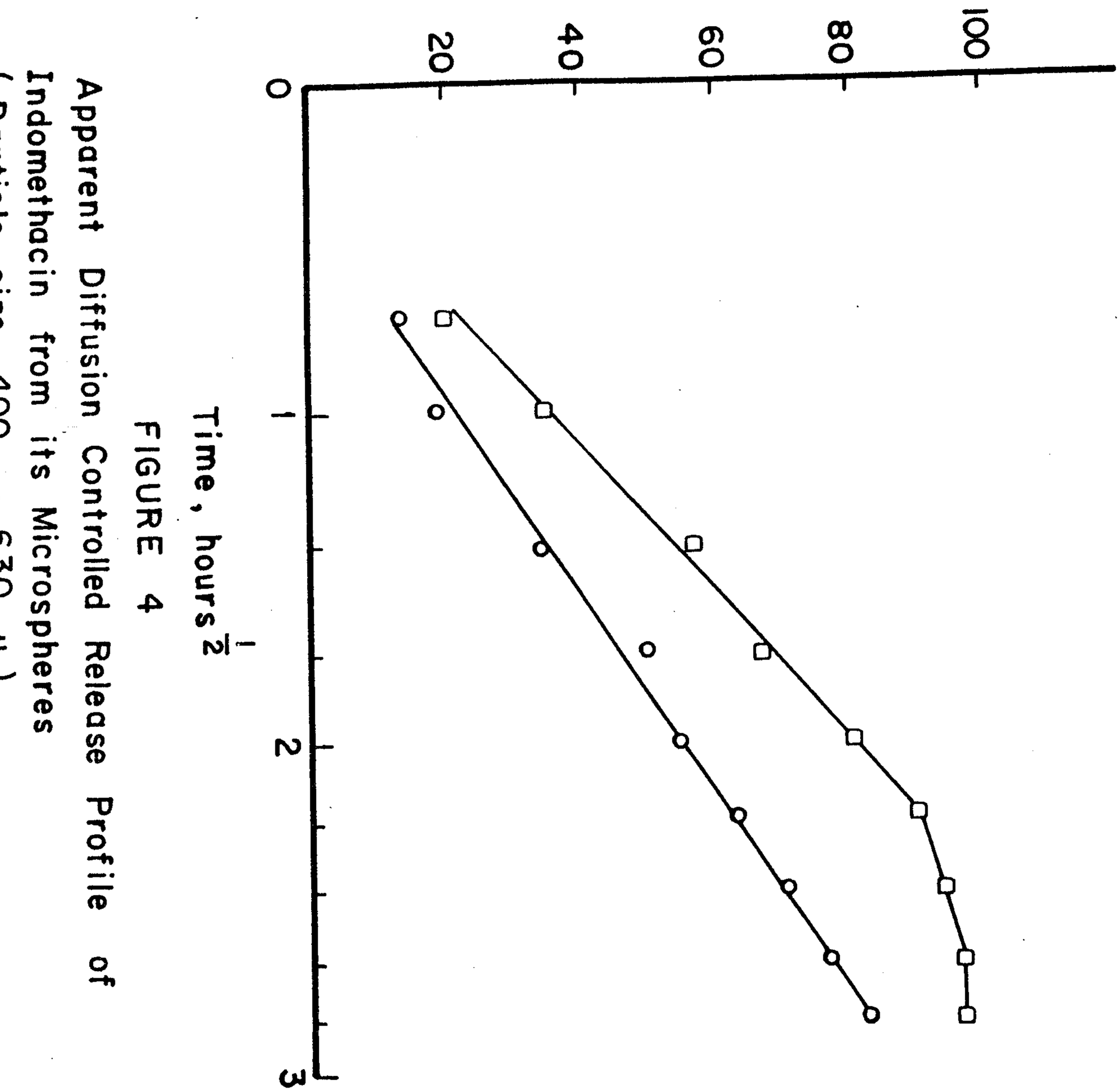


FIGURE 4

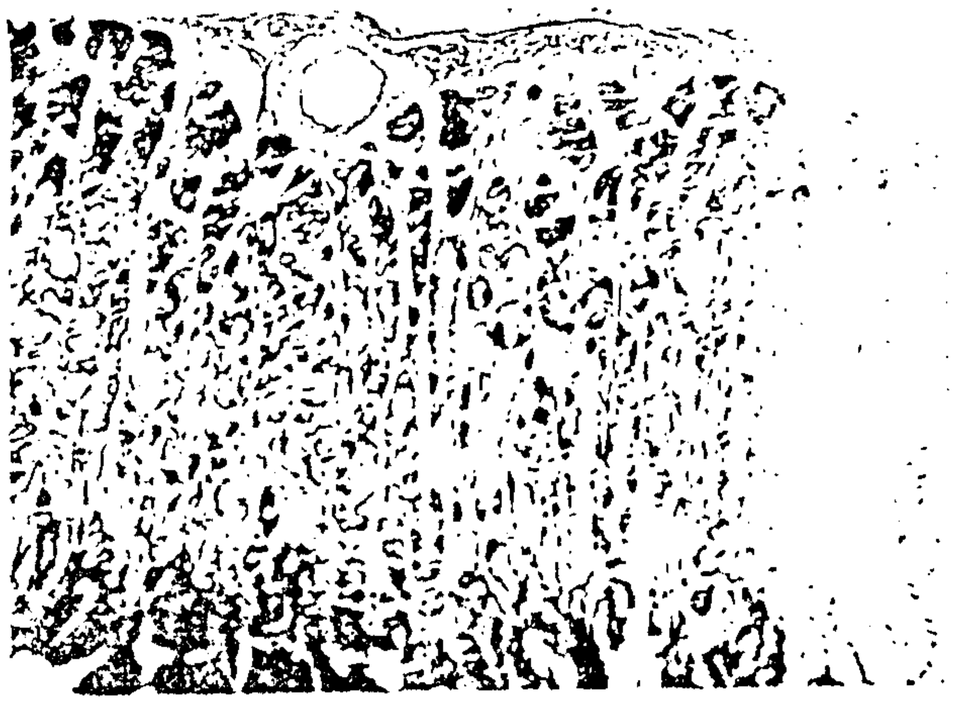
Apparent Diffusion Controlled Release Profile of Indomethacin from its Microspheres (Particle size 400 — 630 μ).
□ In simulated intestinal fluid.
○ In simulated gastric fluid.

Correlation Between Gastric Irritancy and Bioavailability of Indomethacin Microspheres.

(A)

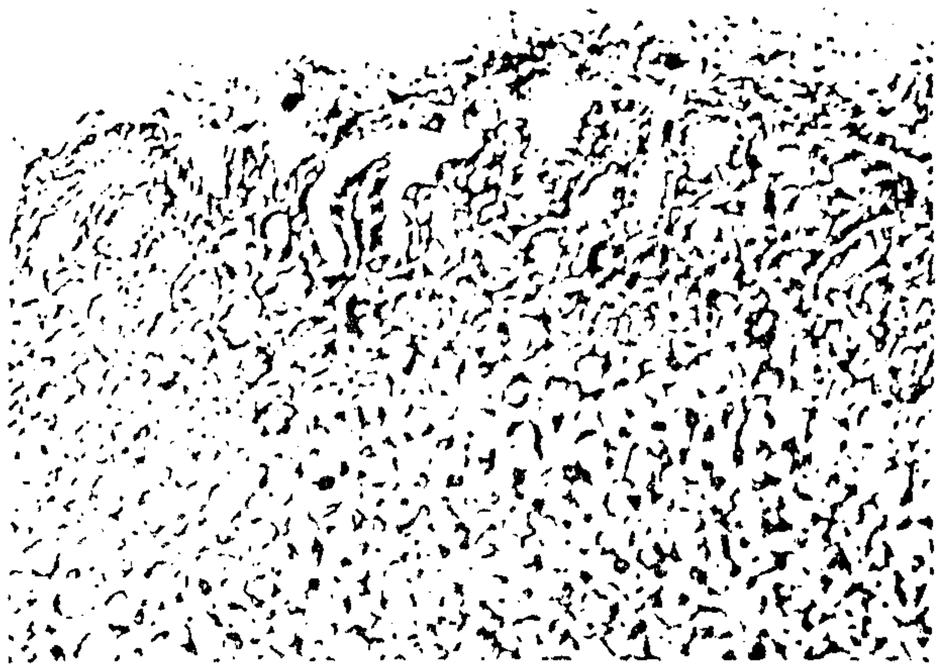
(B)

(C)



(D)

(E)



(F)

Figure 5 : Photomicrogaphs of Gastric Mucosa of Rabbits Receiving Indomethacin ; (A&B) Control , (C&D) Intact Drug , and (E&F) Microspheres.

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العلاقه بين التهابات المعده والتوافر الحيوى للحبيبات الدقيقه للاندوميثازيـــن

سلوى محمود صـــفوت ، حسين عبدالمنعم ، امتثـــــــــــــــال مصطفى

قسم الصيدلانيات ـ كلية الصـــيدلة ـ جامعة أســـــــــــــــيوط

قسم الفسيولوجى ـ كلية الطـــب ـ جامعـــــة أســـــــــــــــيوط

تم تحضير الحبيبات الدقيقه للاندوميثازين بطريقة تبخير الوسط المنتشر (المذيب لكل من العقار والبولمر) للمستحلب . وقد تم استخدام اثَيل السيليلوز كمادة مغلفه بنسبة البولمر الى العقار (١ : ١) . وقد تم فصل الاحجام المختلفه للحبيبات بطريقة المناخل وتقييم كل حجم من الحبيبات المأخوذه على حـــــــده لتقدير كمية الدواء وخصائص الاذابه والتأثير المضاد للالتهابات فى الفـــــئران البيضاء باستخدام حقن البروشتاجلاندين .

وقد أثبتت النتائج أن الطريقه المستخدمه فى تحضير الحبيبات تعطى أكثر من ٩٠ ٪ من الحبيبات التى حجمها من ٩٠ الى ١٠٠٠ ميكرون . وقد تمت دراسة الاذابه باستخدام المحاليل المشابهه للمعده والامعاء عند درجة ٣٧ درجه مئويـة وكما أظهرت النتائج أن الحبيبات كبيرة الحجم تعطى انطلاقا ممتدا أكبر كمـــا أنها تعطى تأثيرا مضادا للالتهابات لفترة أطول بالمقارنه مع الدواء الغـــــير متحوصـــل .

وتمت المقارنه بين أنسجة معده الارانب بعد اعطاء كل من مسحوق الدواء والحبيبات الدقيقه ووجد أن الحبيبات تعتبر نموذجا جيدا وأمنا فى تأثيراتهـا على أنسجة المعـــــده .

received in 15/3/1989 & accepted in 12/9/1989