

## EFFECT OF MICROSPHERONIZATION ON THE RELEASE AND ULCEROGENIC ACTIVITY OF SULINDAC

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السونداك هو أحد العقاقير الغير كورتيكوستيرويدية التي تستخدم في علاج الإلتهابات والآلام الروماتيزمية ومن أعراضه الجانبية إحداث تهيج وتقرح ونزف في الجهاز الهضمي خاصة المعدة. وقد تم تحضير حويصلات دقيقة للعقار باستخدام بيوتيرات خلات السيليلولوز كمادة مغلفة وذلك بطريقة مستحلب - تبخير المذيب. وقد تم دراسة تأثير حجم الحويصلات والأس الهيدروجيني على معدل انطلاق العقار الذي ثبت أنه يتبع نظام الانتشار. وقد وجد أن معدل الإنطلاق يزداد كلما صغر حجم الحويصلات أو زاد الأس الهيدروجيني ، وقد تم دراسة التأثير التقرحي للعقار المحوصل والعقار الحر في أمعدة الأرانب - وقد لوحظ أن إستخدام العقار المحوصل ليس له أى تأثير جانبي من ناحية التقرح أو النزف في أمعدة الأرانب.

*Sulindac; (SUL) NSAIDS has irritation and ulcerogenic activity on the human stomach. Sulindac microspheres were prepared adopting the solvent evaporation method, using cellulose acetate butyrate as coating polymer with 1-2 drug: polymer ratio. The influence of microsphere particle size and pH of the dissolution medium on the release profile of the drug was investigated. In all cases the drug release mechanism followed Higuchi diffusion model with sustained release profile of sulindac from SUL-CAB microspheres. The smaller particle size gave significantly higher release rate of the drug. The release rate was slower in acidic medium (pH 1.2) than that at pH 7.4. The effect of microspherization on the ulcerogenic activity of the drug in the stomach of rabbits was studied. It was observed that the ulcerogenic activity of the drug was disappeared and the mucosal surfaces showed free of hemorrhage and inflammations when the drug is used as microspheres.*

### INTRODUCTION

Microencapsulation or microsphere preparation has, in recent years, been used to modify or retard drug release in pharmaceutical formulations. Several other aspects in which microencapsulation has been employed include: improving of drug stability,<sup>1</sup> reduction of gastric irritation,<sup>2</sup> taste masking,<sup>3</sup> controlled release<sup>4</sup> and formulation of biologically active materials.<sup>5-8</sup>

Anti-inflammatory drugs are those used to inhibit action of an agent that induces inflammation. One group of these drugs is the nonsteroidal anti-inflammatory drugs (NSAIDS). The acidic NSAIDS have many deleterious side effects on the gastrointestinal tract (GIT) such as irritation, ulceration and hemorrhage.<sup>9-11</sup>

Microencapsulation for oral use has been employed to sustain the drug release and to

reduce or eliminate GIT irritation.<sup>12-14</sup> In addition multiparticulate delivery systems spread out in a more uniform pattern in the GIT, this results in a more reproducible drug absorption and reduces local irritation when compared to single unit dosage forms such as non-disintegrating, polymeric matrix tablets. Unwanted intestinal retention of the polymeric material, which may occur with matrix tablets on chronic dosing, can also be avoided.<sup>15</sup>

Many NSAIDS, acetyl salicylic acid,<sup>16</sup> flufenamic,<sup>17</sup> Ibuprofen,<sup>19</sup> ketoprofen and indomethacin were formulated as microcapsules.

As a microencapsulation technique, emulsion solvent evaporation has been utilized in many reports to prepare sustained release microspheres and microcapsules made of various types of coating materials.<sup>12-14</sup>

Sulindac [5-fluoro-2-methyl-1-(4-methyl

sulphonylbenzylidene) indne-3-yl] acetic acid is a potent (NSAIDS). It is a prodrug and its sulphide metabolites is an inhibitor of cyclo-oxygenase. It is used in musculoskeletal and joint disorders such as osteoarthritis and rheumatoid arthritis.<sup>18</sup>

Reports on microencapsulation of sulindac were scarce. In addition its ulcerogenic activity has not been justified.

These considerations led to the objective of this study to prepare and evaluate oral microparticulate delivery system of this anti-inflammatory drug adopting the emulsion-solvent evaporation technique (O/W). This technique can be used for the entrapment of water-insoluble drugs within water-insoluble polymers. The effect of pH as well as the particle size, of the microspheres, on the release characteristics was investigated. Moreover the ulcerogenic activity of the drug and its microspheres were elaborated in rabbits.

## EXPERIMENTAL

### Materials

- Sulindac (SUL) confirmed to the USP standard.
- Cellulose acetate butyrate (CAB) was obtained from FMC Co. New Wart, DE, USA (Lot. Co.: 5A 823).
- Polyvinyl alcohol PVA 14000 (BDH).
- Polysorbate (Atlas Chemical Co., USA).
- All other materials or solvents were of analytical grade.

### Methods

#### Preparation of SUL microspheres

Microspheres of SUL were prepared adopting the emulsion solvent evaporation technique (O/W).<sup>19,22</sup> The drug and coating material were dissolved in 20 ml. dichloromethane (DCM) and the aqueous phase was prepared by dissolving the surfactant and PVA in 250 ml. of dist. water. The organic phase was emulsified into the aqueous phase by stirring at 1200 rpm using mechanical stirrer (MLW, Medingen, GDR). The mixture was stirred continuously at room temperature until all solvent had been evaporated. The microspheres

were collected by filtration, washed with water and dried at 35°C in a vacuum oven. Microspheres were prepared with core to wall ratio 1:2. Preliminary experiments were carried out to determine the optimum conditions necessary for the preparation of microspheres by this method.

#### Determination of drug content

A 50 mg samples of microspheres were dissolved in DCM. The solution was filtered off using (0.45 µm) millipore filter, the SUL content of the microspheres was determined spectrophotometrically at 285 nm after appropriate dilution.

#### Scanning electron microscope

Scanning electron microscope (JSM-5400 LV, JEOL JAPAN) was used to determine the shape and surface characteristics of the microspheres.

#### Determination of microspheres yield

The yield of the microspheres was determined by the following equation:

$$\% \text{ yield} = \frac{\text{total weight of recovered microspheres}}{\text{wt of drug and polymer used}} \times 100$$

#### Dissolution studies

The dissolution profiles of SUL from the prepared microspheres were determined using USP XX1 paddle apparatus. An amount of the microspheres equivalent to 16.67 mg of the drug was introduced into 200 ml of the dissolution medium of different pH values [1.2, 5.5 and 7.4] using 0.1 HCl and phosphate buffer B.P. at 37 ± 0.5°C and stirred at 50 rpm. Samples were withdrawn at specified time intervals and replaced by the same sample volume of the dissolution medium at 37°C. The drug concentration was determined spectrophotometrically at λ 285 nm. (Shimadzu, Japan). The mean of three determinations was reported.

#### Ulcerogenic activity

The assessment of the ulcerogenic activity of sulindac microspheres was done in two



groups of albino rabbits, three for each (1.5-2 Kg body weight). The rabbits were kept free of diet and water two hours before and after drug administration. An oral dose of the intact drug (8 mg in gelatin capsule) or an amount of microspheres equivalent to 8 mg of the drug were administered two times daily, with 10 ml. water, to each group for 7 days. At the end of the experiment the animals were sacrificed and the stomach was excised and opened along the greater curvature, washed with saline solution and examined for the presence of ulcers and then photographed.<sup>11</sup>

## RESULTS AND DISCUSSION

### Characterization of SUL microspheres

The viscosity of the polymeric solution was regulated to control the size, shape and size distribution of microspheres produced by this method.<sup>20</sup> The microspheres prepared were found to be spherical and free flowing and the sizes could be separated and a more uniform size range of microspheres could readily be obtained.

Fig. 1 illustrates the scanning electron micrograph of SUL microspheres, which are spherical with a slightly rough surface. Very few micropores may be expected to be found on the surface. This may be attributed to the rapid evaporation of DCM. Very few aggregates and very few drug crystals with the absence of polymer flacks were noticed.

The size analysis and drug content of SUL-CAB microspheres are presented in Table 1. Histogram representing the particle size distribution is illustrated in Fig. 2 which showed that about 45% of the batch lie in the range of 1100-900  $\mu\text{m}$  and 29% in the range of 1400-1100  $\mu\text{m}$  i.e about 75% in the range 900-1400  $\mu\text{m}$ .

The drug content of the prepared microspheres was found to be, nearly, uniform for different particle size and it was  $\approx 94.5\%$  (Table 1). This indicates the uniform entrapment of the drug within the microspheres, this is due to insolubility of sulindac ( $< 0.003 \text{ mg/ml}$ )<sup>21</sup> in the aqueous phase during emulsification process which has a significant effect on the percent of drug entrapment.<sup>22,23</sup> Also, the entrapment efficiency is greatly influenced by the type of

organic solvent used. The use of solvents with greater aqueous miscibility e.g., DCM (as in this study) resulted in greater drug entrapment than that expected with organic solvents of low aqueous miscibility e.g., chloroform.<sup>24</sup> In all batches the yield of microspheres is greater than 96%.

### Release studies

The effect of microsphere size on the release profiles of sulindac into phosphate buffer solution (pH 7.4) was illustrated in Fig. 3. It was observed that the highest release rate was attained with microspheres of smaller fraction size (900-500  $\mu\text{m}$ ). This was attributed to the fact that the surface area is inversely related to the fraction size of the microspheres. It was also observed that more than 50% of the loaded drug was liberated after six hours for all ranges of microspheres size.

Fig. 4 shows the effect of pH of the dissolution medium on sulindac release from the prepared microspheres. The obtained data revealed that the release rate of sulindac is highly dependent on the pH of the dissolution media, viz., as the pH is decreased the amount of drug release is significantly decreased. This was attributed to the solubility of sulindac which enhanced by increasing the pH of the dissolution media.<sup>21</sup>

The release of the drug at pH 1.2 is, highly significant, lower than that at pH 5.5 or 7.4. A 3% of microsphere drug content was released at pH 1.2 after 3 hours. These findings would be of great values for controlling the drug release and avoiding the ulcerogenic effect of sulindac in the stomach.

### Drug release kinetics

The release data of SUL from the microspheres were treated according to first order, Higuchi diffusion model mechanisms. Zero order was not applied since the plot of the amount of the drug released versus time don't show a linear plot (Figs. 3 and 4). The data are summarized in tables 2 and 3 which depict that the release kinetics is diffusion controlled, as plots of the amount of drug released versus square root of time were found to be linear (Figs. 5 and 6). Figs. 7 and 8 depict the

**Table 1:** Particle size distribution, mean diameter and drug content of sulindac microspheres prepared at 1:2 drug to polymer ratio.

Particle size range ( $\mu\text{m}$ )	Mean diameter ( $\mu\text{m}$ )	% Frequency	Drug content % $\pm$ (SD)
> 1400	-	4.3	94.72 $\pm$ (1.87)
1400-1100	1250	28.9	94.85 $\pm$ (1.67)
1100-900	1000	45.6	94.37 $\pm$ (1.72)
900-500	700	10.2	95.06 $\pm$ (1.91)
500-300	400	5.7	95.63 $\pm$ (1.62)
300-100	200	3.8	95.17 $\pm$ (1.34)
< 100	-	1.5	96.24 $\pm$ (1.22)

**Table 2:** Effect of pH on the release Kinetics of sulindac from its microspheres (Particle size 1100-900  $\mu\text{m}$ ).

pH of the dissolution medium	First order		Higuchi diffusion model		Spherical matrix model	
	r	K hr <sup>-1</sup> x 10 <sup>-2</sup>	r	D (cm <sup>2</sup> hr)	r	K x 10 <sup>-2</sup>
1.2	0.825	0.2132	0.967	1.3950	0.960	0.0541
5.5	0.994	1.1576	0.986	24.4458	0.994	1.1577
7.4	0.983	7.00180	0.984	28.8694	0.997	1.9147

**Table 3:** Effect of microsphere size on the release kinetic of sulindac from its microspheres (pH 7.4).

Microsphere size $\mu\text{m}$	First order		Higuchi diffusion model		Spherical matrix model	
	r	K hr <sup>-1</sup> x 10 <sup>-2</sup>	r	D (cm <sup>2</sup> hr)	r	K x 10 <sup>-2</sup>
900-500	0.998	10.0361	0.996	32.4214	0.998	3.1120
1100-900	0.983	7.0018	0.984	28.8694	0.997	1.9147
1400-1100	0.996	4.814	0.997	22.7483	0.990	1.1588

r: Correlation coefficient.

K: Specific release rate constant.

D: Diffusion coefficient.

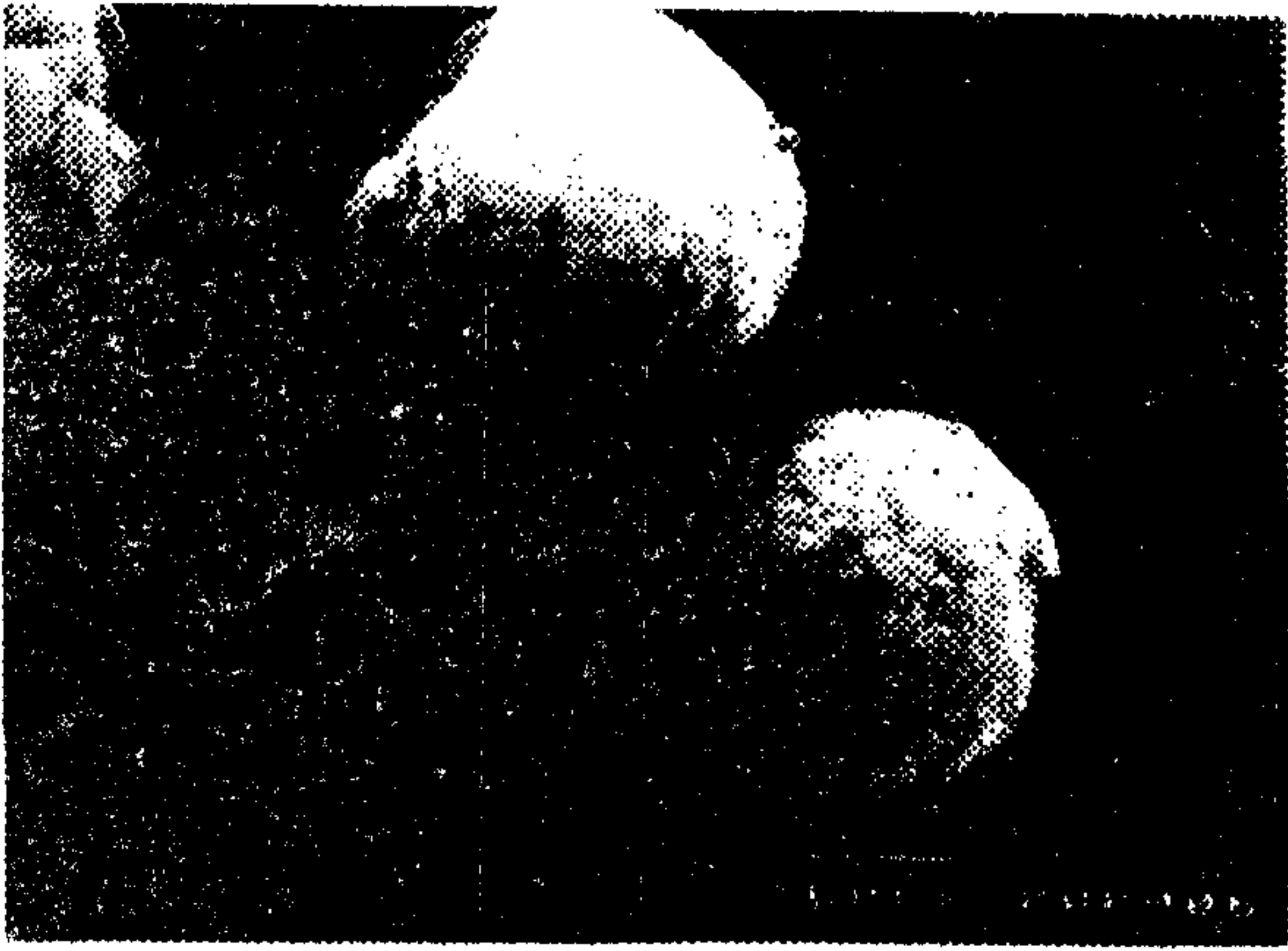


Fig. 1: Scanning electron micrograph of sulindac microspheres prepared with CAB at 2:1 polymer to drug ratio.

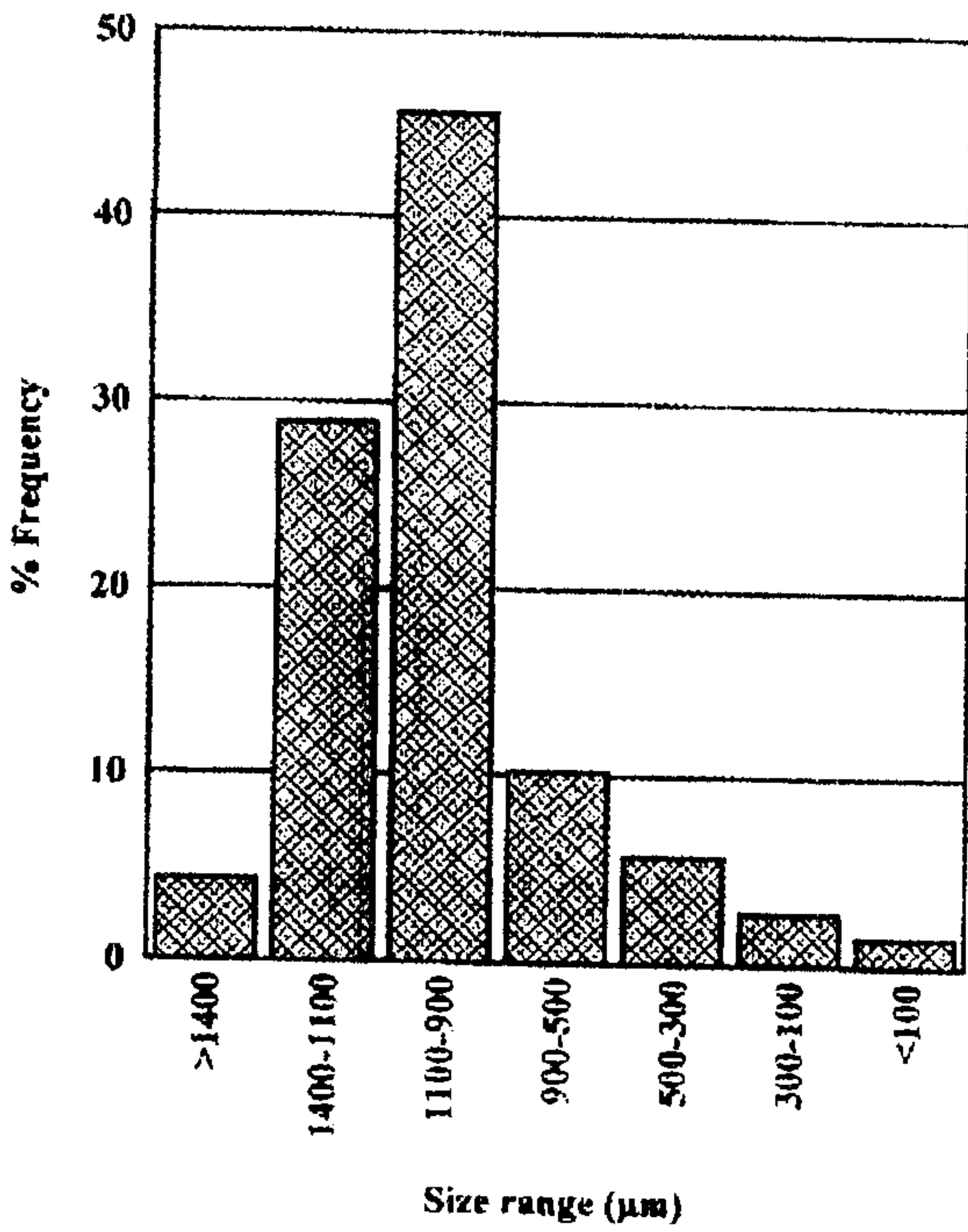


Fig. 2: Particle size distribution of sulindac microspheres prepared with CAB at 2:1 polymer to drug ratio.

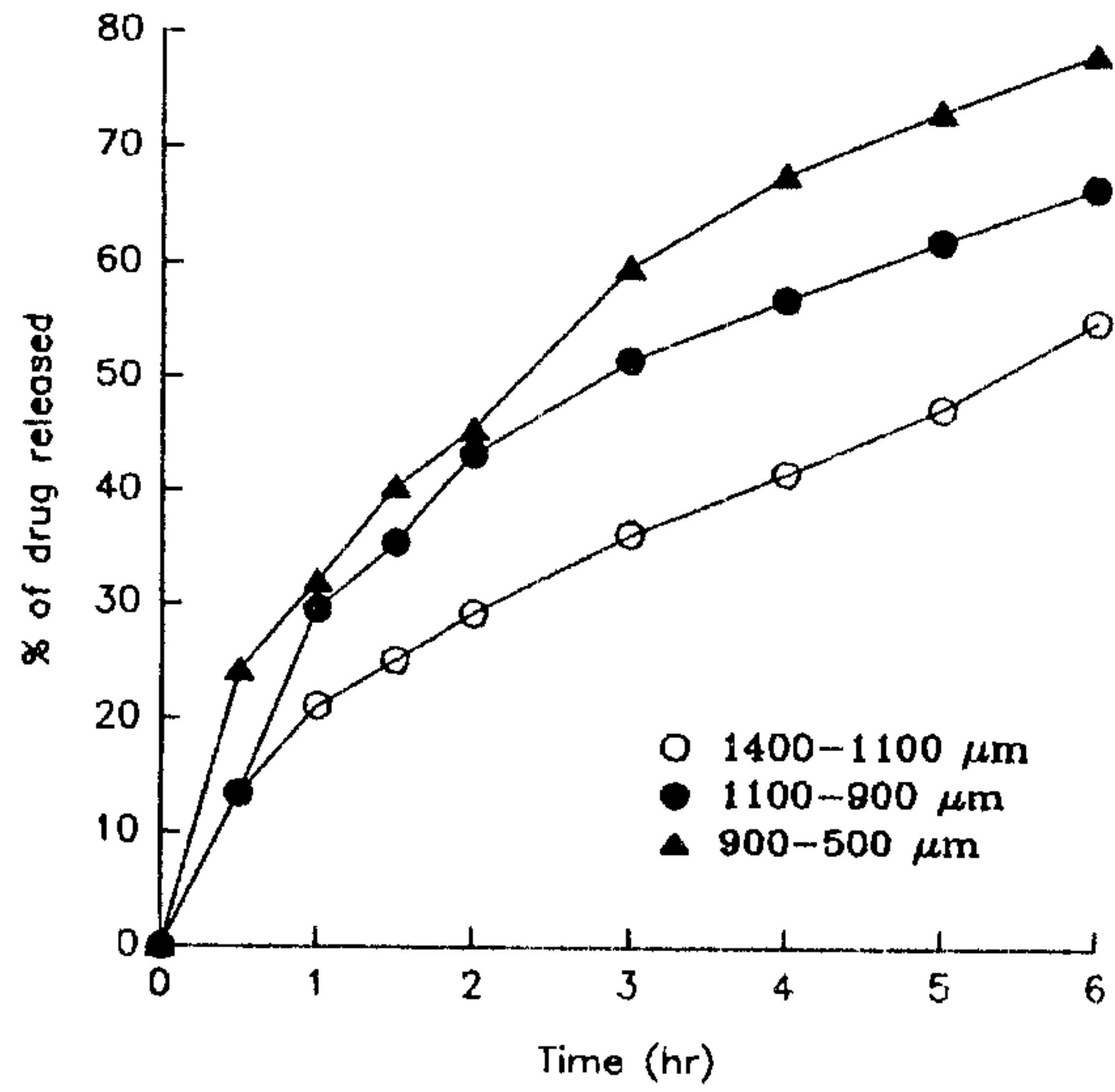


Fig. 3: Effect of microsphere size on the release of sulindac from CAB microspheres prepared at 2:1 polymer to drug ratio into phosphate buffer (pH 7.4).

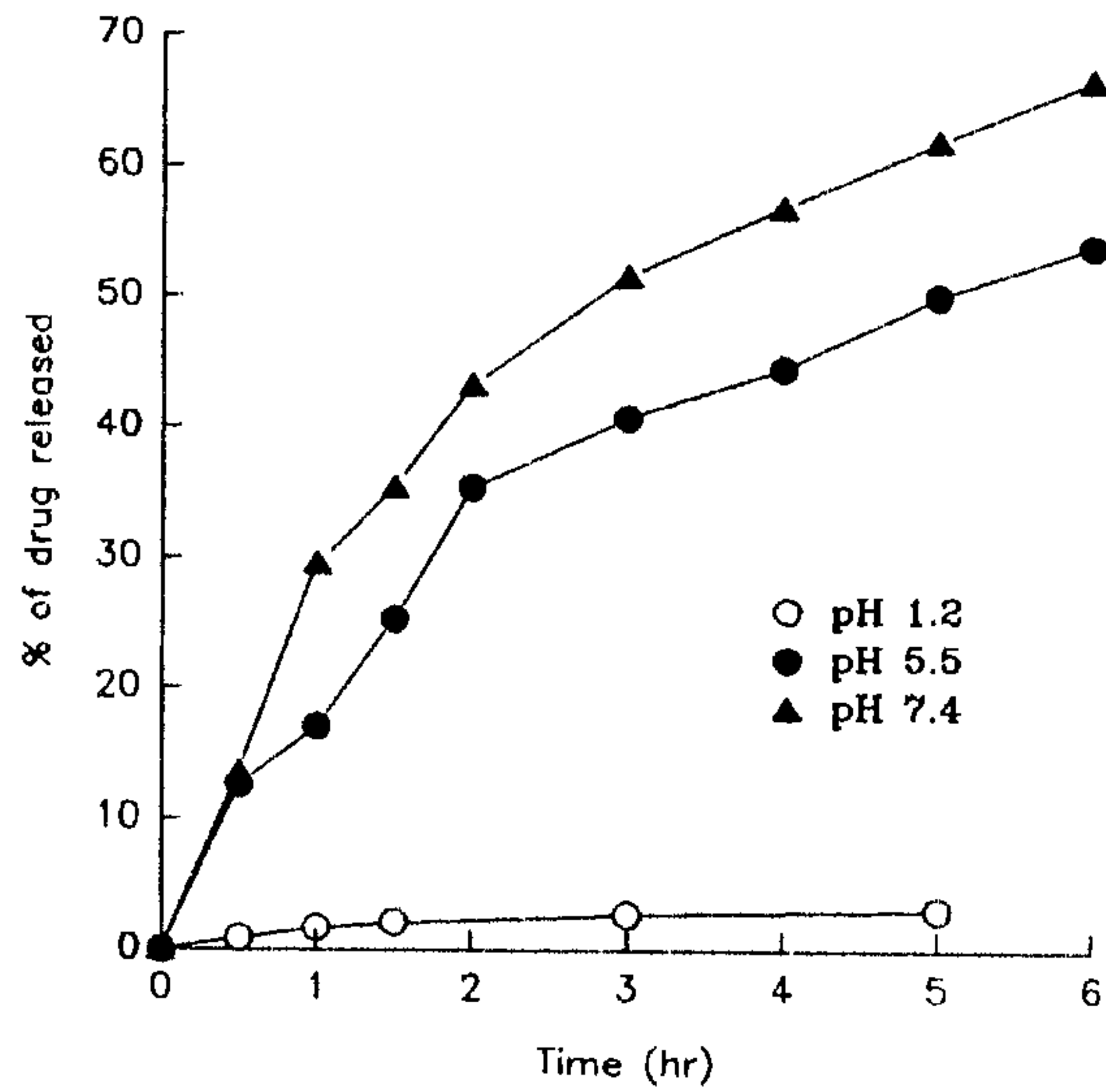
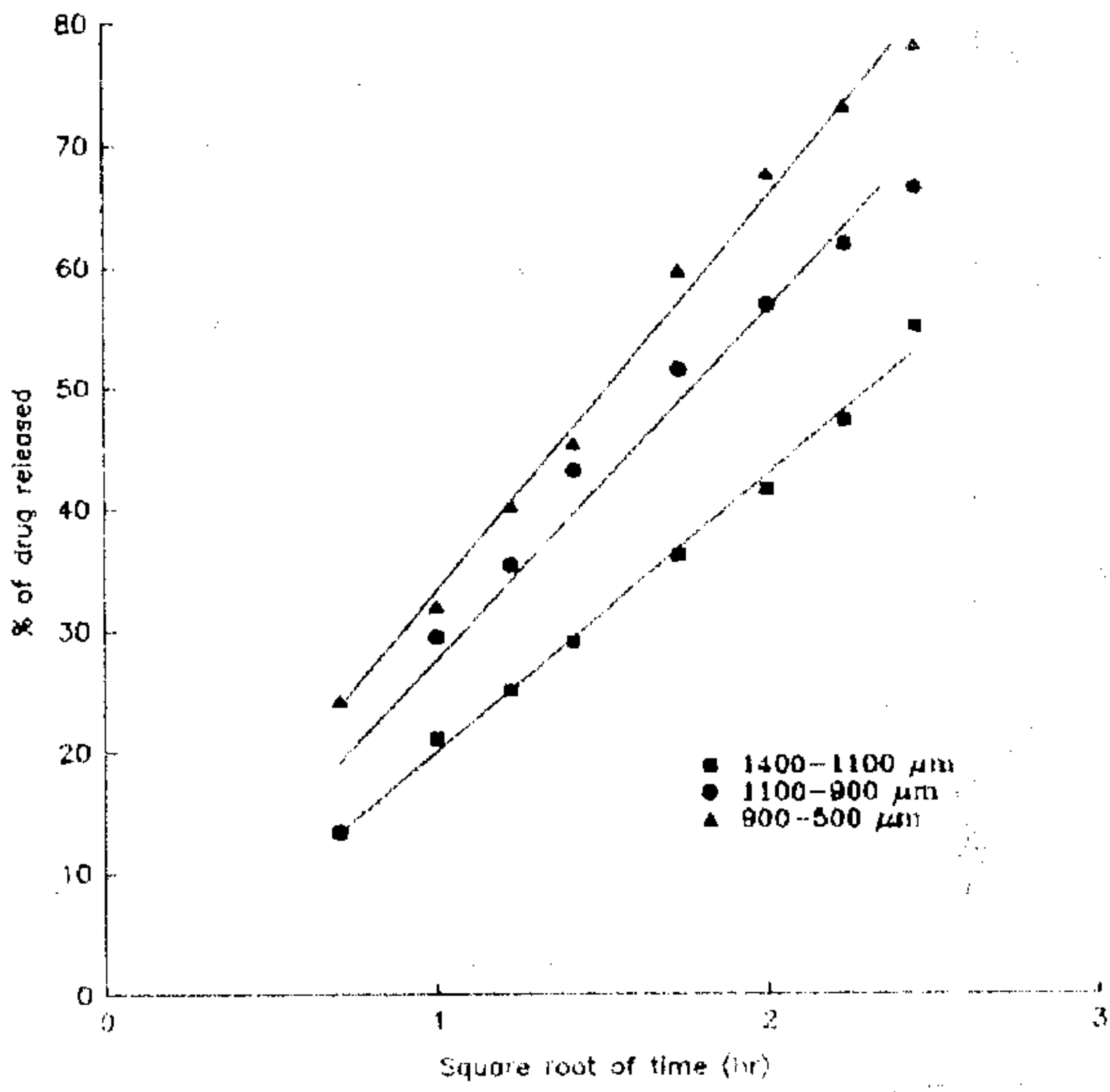
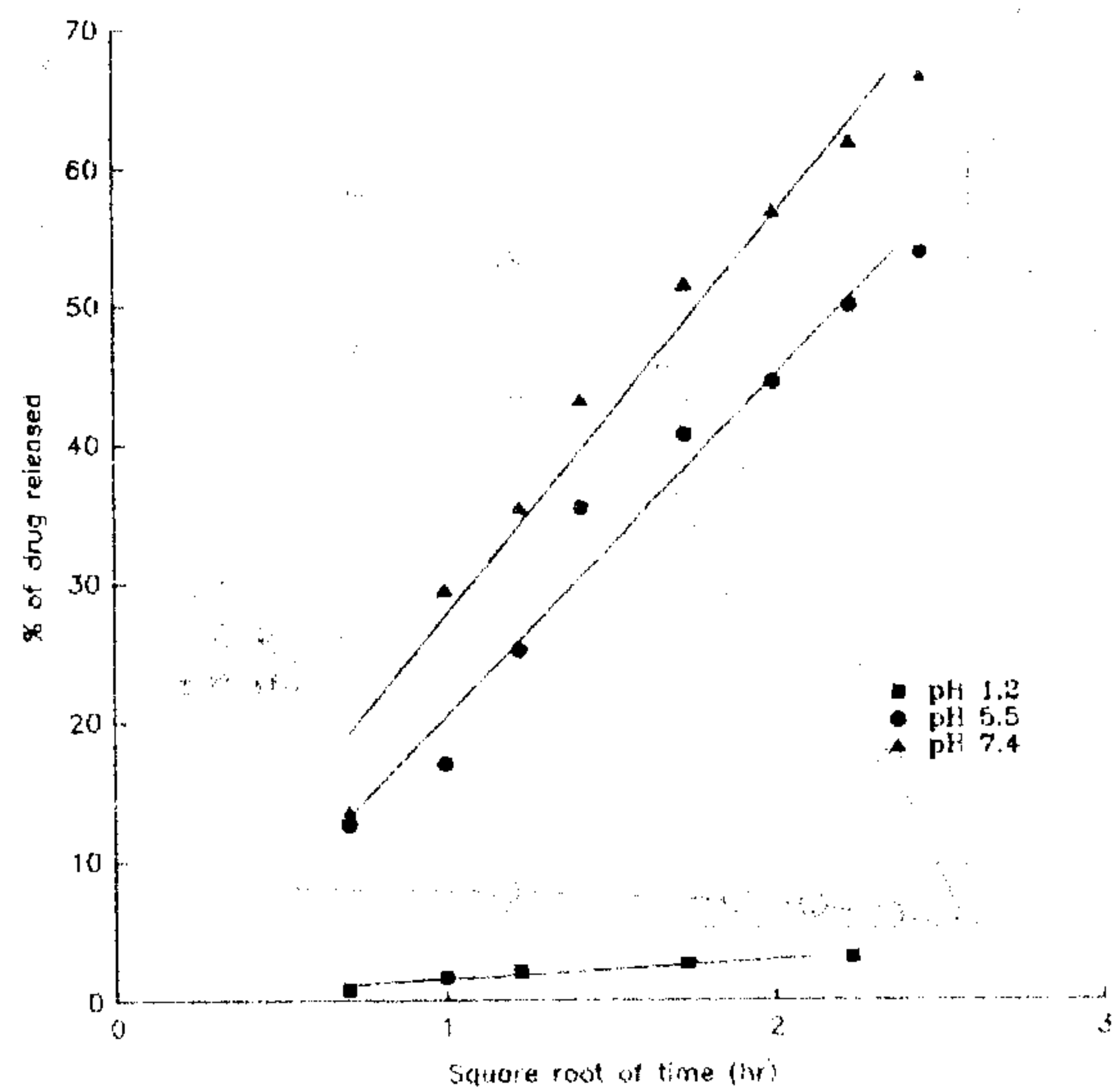


Fig. 4: Effect of pH of the dissolution medium on the release of sulindac from CAB microspheres prepared at 2:1 polymer to drug ratio (P.S. 1100-900  $\mu\text{m}$ ).

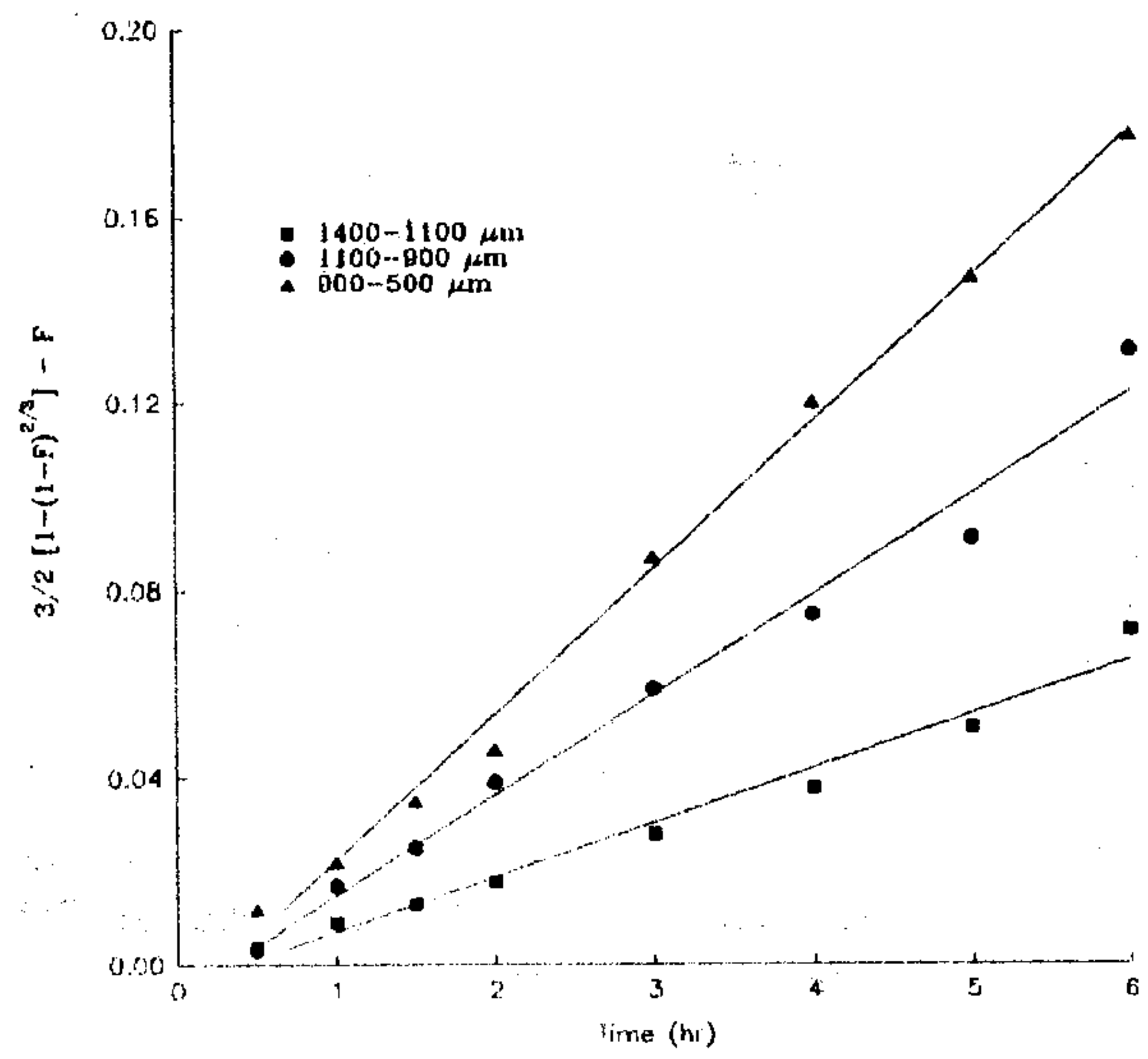




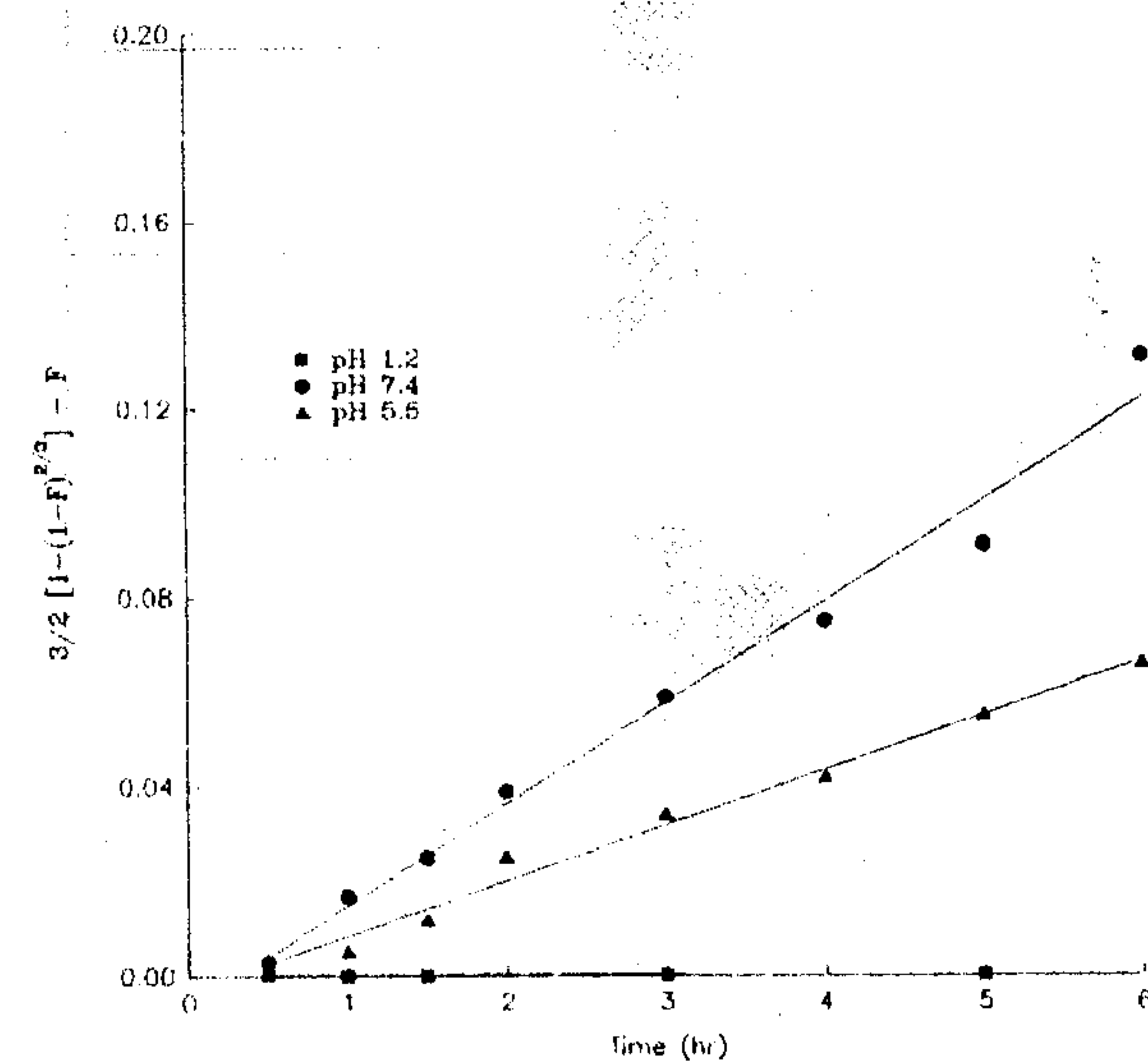
**Fig. 5:** Higuchi plot showing effect of microsphere particle size on sulindac release into phosphate buffer (pH= 7.4).



**Fig. 6:** Higuchi plot showing the effect of the pH of the dissolution medium on the release of sulindac from CAB microspheres (P.S. 1100-900 μm) prepared at 2:1 polymer to drug ratio.



**Fig. 7:** Plot of the release data according to the spherical matrix model showing the effect of the microspheres particle size on sulindac release from microspheres prepared with CAB at 2:1 polymer to drug ratio (at pH 7.4).



**Fig. 8:** Plot of the release data according to the spherical matrix model showing the effect of pH on sulindac release from microspheres (P.S. 1100-900 μm) prepared with CAB at 2:1 polymer to drug ratio.



linearization of the data when spherical matrix model<sup>25</sup> is adopted by applying the following equation:  $3/2[1-(1-F)^{2/3}]-F = K.t$ , where F is the fraction of drug released, K is the diffusion rate at time t.

The correlation co-efficient values obtained by plotting,  $3/2[1-(1-F)^{2/3}]-F$  versus time (hours), revealed linear regression and this confirming that the mechanism for drug release is mainly diffusion controlled.

#### Ulcerogenic activity

The ulcerogenic activity of the coated and uncoated drug was studied in rabbits. The results obtained were interesting and encouraging. The gastric mucosa of the animals administered free drug showed marked ulcerations and massive hemorrhage with complete disappearance of mucosal surfaces in the ulcer region of the fundus and pylorus (Plate I & III). In addition there were also numerous pin point ulcers on the lesser and greater curvatures of the stomach.

Coating of sulindac with cellulose acetate butyrate polymer, on the other hand, induced no ulceration on the stomach of rabbits and the mucosal surfaces appeared free of hemorrhage

and inflammations (plate II & IV). Thus, The microspherization of the drug with cellulose acetate butyrate subdivided the drug in the stomach into very fine particles, each coated with polymer. This may decrease the points of contact between the drug and the stomach. Accordingly, less or no ulcerogenic activity may happen. A similar argument was given by Meshali *et al.*<sup>11</sup> working on the coating of mefenamic acid with Eudragit.

#### Conclusion

Microspheres of sulindac were successfully prepared, using CAB as coating polymer at 1:2 core : coat ratio, adopting the emulsion-solvent evaporation method. The release kinetics were in favor of diffusion controlled mechanism and the release rates were significantly influenced by microsphere size and the pH of the dissolution medium. The significant lower release rate in acidic pH led to study the effect of microspherization on the ulcerogenic activity of sulindac in the stomach. The in-vivo investigation revealed the absence of ulcerogenic activity of sulindac when coated with CAB, in the form of microspheres.



Plate I: Stomach of a rabbit given the intact drug (gross appearance).



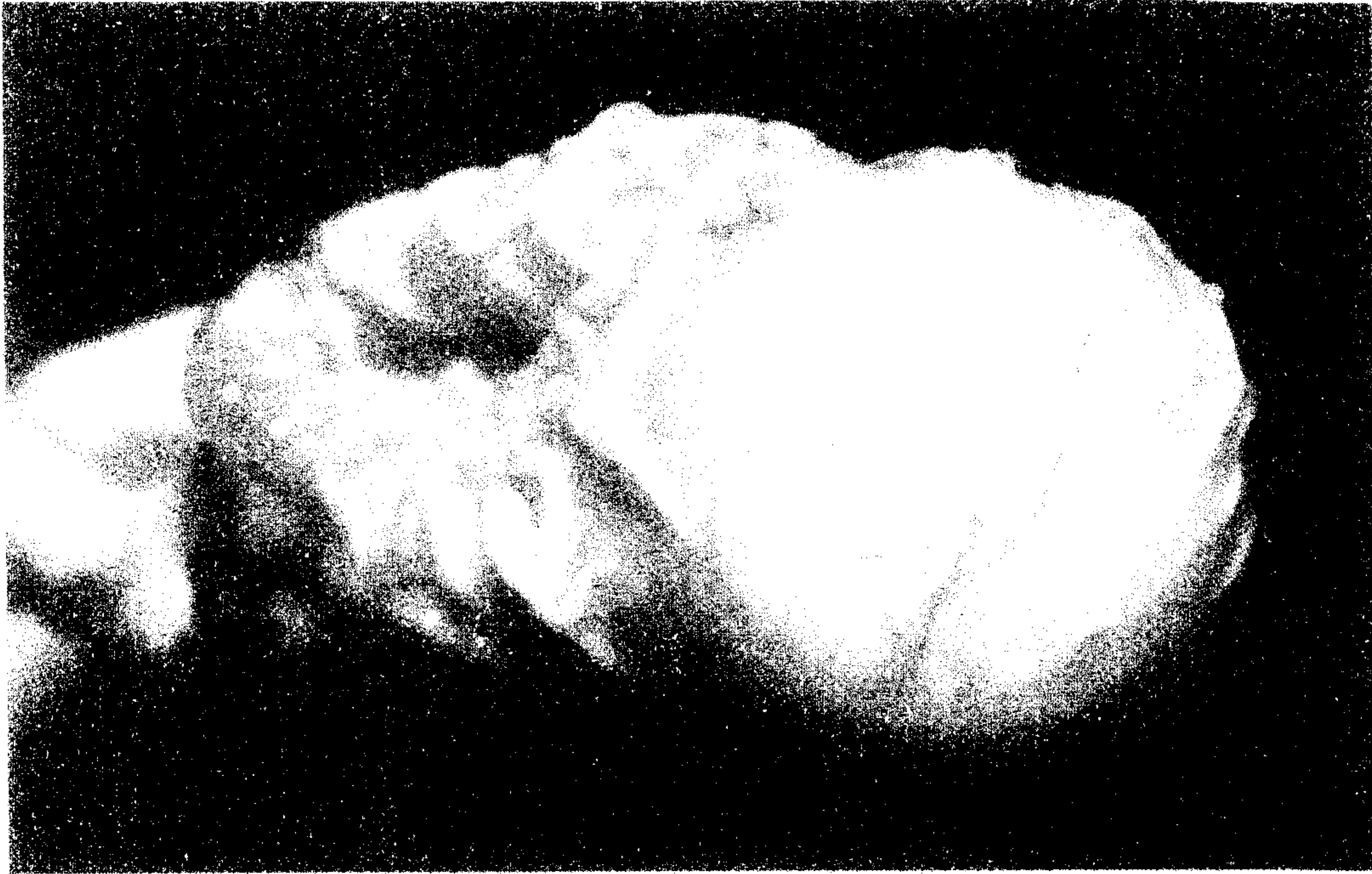


Plate II: Stomach of a rabbit given the drug coated with cellulose acetate butyrate (gross appearance).

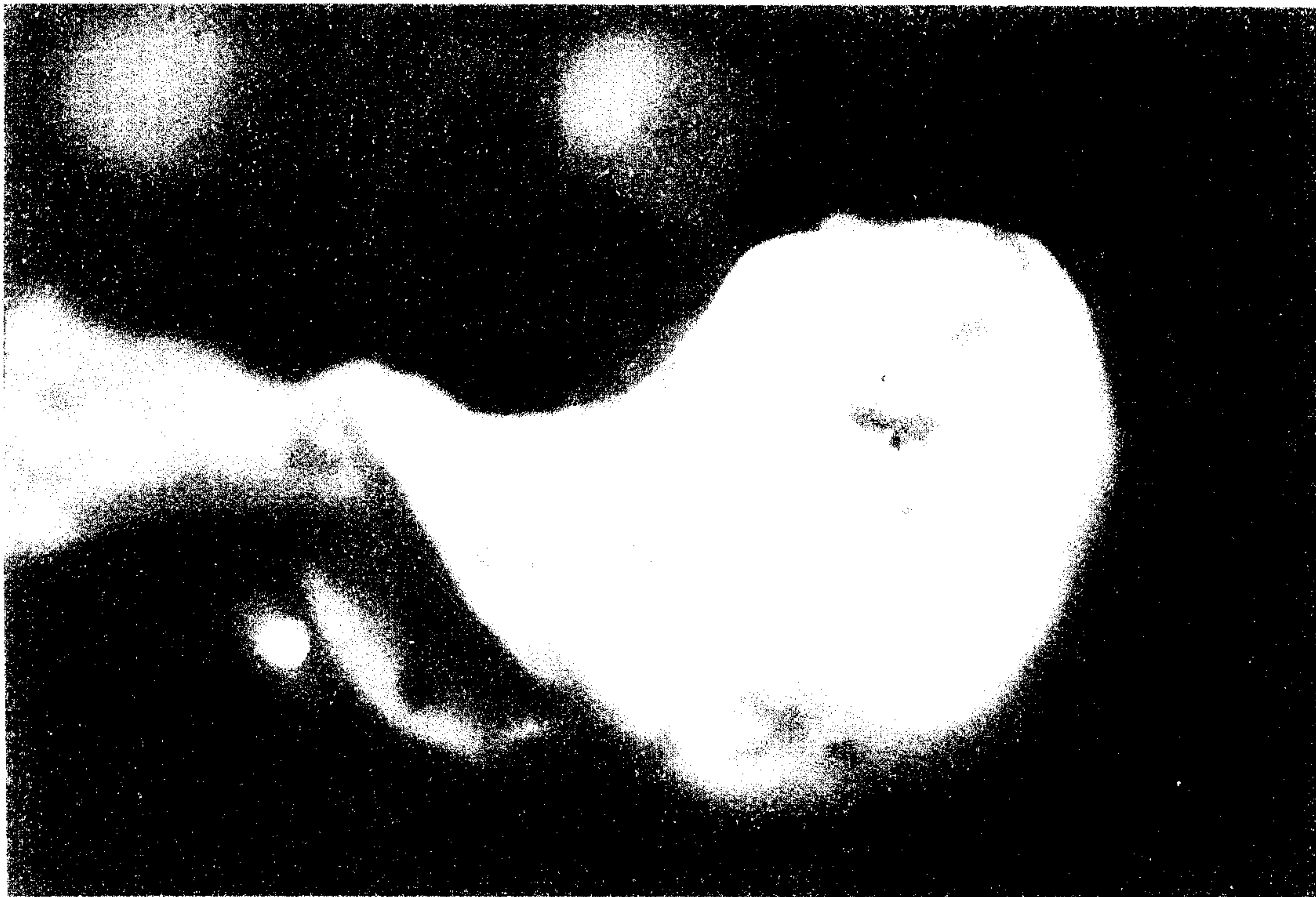


Plate III: Stomach of a rabbit given the intact drug (gross appearance).





Plate IV: Stomach of a rabbit given the drug coated with cellulose acetate butyrate (gross appearance).

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