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FORMULATION AND EVALUATION OF COLON-TARGETED TEGAFUR MICROSPHERES

F. A. Mohamed, G. A. El-Gendy and A. S. Ali

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

التيجافور هو أحد العقاقير المضادة للسرطان ويستخدم لعلاج بعض أنواع سرطان الجهاز الهضمي ومنها سرطان المستقيم. تم تحضير حويصلات دقيقة للعقار بإستخدام بيوترات خلات السليلوز وذلك بطريقة المستحلب – تبخير المذيب. وقد تم الحصول على حويصلات دقيقة منتظمة كروية الشكل حرة الإنسياب. وقد زادت نسبة الإنتاج عن ٩٧٪ بينما تم حوصلة أكثر من ٩٥٪ من العقار. وقد تم دراسة تأثير حجم الحويصلات والأس الهيدروجينى للمحلول على معدل إنطلاق العقار. وقد أثبتت الدراسات أن إنطلاق العقار يتبع نظام الإنتشار. وقد أعطت الحويصلات الأصغر حجما معدلات إنطلاق أسرع للعقار. وكذلك تبين أن العقار ينطلق بمعدل أبطأ فى الوسط الحامضى (الأس الهيدروجينى ١,٢) مما يرجح إمكانية توجيه العقار نحو القولون فضلا عن إطالة مدة الإنطلاق.

Tegafur (TGF) is an antineoplastic drug which used for treatment of neoplasm of gastro-intestinal tract including colon cancer. Cellulose acetate butyrate microspheres containing the drug were prepared by using the emulsion-solvent evaporation technique. The microspheres were spherical and free flowing. The yield was greater than 97% and more than 95% of the drug was encapsulated. The effect of microsphere size and pH of the dissolution medium on the drug release was investigated. Sustained release profiles of TGF from TGF-CAB microspheres were observed. In all cases the drug release followed Higuchi diffusion model. Microspheres of smaller size gave a relatively higher dissolution rate of the drug. The drug release was slower in acidic medium (pH 1.2) suggesting that using CAB microspheres would be of value for controlling the drug release and potential colonic-targeting of the drug.

INTRODUCTION

Microencapsulation is now considered one of the most frequently employed methods for production of controlled-release dosage forms.[1] This approach has been applied to a numerous drugs of variable physicochemical properties and belonging to different pharmacological classes including: albutrol sulfate,[2] valproic acid,[3] propranolol,[4] nifedipine,[5] isoniazid,[6] chlorpromazine hydrochloride,[7] theophylline,[8] diclofenac sodium,[9] chlorpheniramine maleate,[10] phenazopyridine hydrochloride,[11] progesterone,[12] glibenclamide,[13] vitamin A,[14] furosemide,[15] erythromycin[16] and propoxyphene hydrochloride.[17] Several other aspects in which microencapsulation has been employed include: improving of drug stability,[18] reduction of gastric irritation,[19] taste masking,[20] controlled release suspension[21] and microencapsulation of biologically active materials.[22-25] Recently microencapsulation or matrix tablets prepared with pH-dependent polymers has been shown to be of potential application for colonic targeting of drugs.[26-28]

Tegafur (TGF) {5-fluro-1-(tetrahydro-2-furyl) uracil} is an antineoplastic agent which appears to act by the release of flurouracil in the body. It has been used in the management of neoplasms of the breast and gastro-intestinal tract including colorectal cancer.[29] On our hand only one publication concerning microencapsulation of TGF using beeswax has been published.[30] The present work was conducted to prepare and evaluate TGF-cellulose acetate butyrate microspheres with the aim of obtaining controlled release formulation of the drug and studying the possibility of its colonic targeting.

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EXPERIMENTAL

Materials

- Tegafur (TGF) B.P. (Wako C. Ltd., Japan).
- Cellulose acetate butyrate (CAB) was obtained from FMC Co., NewWart, DE, USA (Lot. No.: 5 A 825).
- Light mineral oil: Fisher Scientific Co., Fair Lown, N.J., (Lot No.: 84485).
- Sorbitan monooleate (Atlas Chemical Co., USA).
- All other materials or solvents were of analytical grade.

Methods

Preparation of TGF microspheres

Microspheres of TGF were prepared by using the emulsion solvent evaporation technique.[31] A quantity of 3 gm. of CAB was dissolved in 20 ml of acetone and the equal amount of drug was dispersed in the polymer solution. The dispersion was emulsified onto liquid paraffin containing 1% w/v sorbitan monooleate using mechanical stirrer adjusted at 900 rpm. Stirring was continued until complete evaporation of acetone at room temperature. The microspheres were separated by filtration, washed several times with n-hexane and left to dry in vacuum oven at 30°C.

Determination of drug content

A 100 mg samples of microspheres were crushed in a glass mortar and extracted with 50 ml of distilled water with the aid of sonication for 10 min. The solution was filtered off using membrane filter (0.45 μm Millipore). The extraction process was repeated for further four times and the combined extract was adjusted to 500 ml. The drug concentration was determined spectrophotometrically after appropriate dilution.

Scanning electron microscope

Scanning electron microscope (JSM - 25 S3, Jeik Co. Japan) was used to determine the shape and surface characteristics of the microspheres.

Determination of the yield of the microspheres

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

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

Dissolution studies

The dissolution profiles of TGF from the prepared microspheres were determined using USPXXI paddle apparatus. An amount of the microspheres equivalent to 100 mg of the drug was introduced into 500 ml of the dissolution medium of different pH values [1.2, 4.8, 5.5 and 7.4] using 0.1 N HCl and phosphate buffer B.P. The temperature of the dissolution medium was adjusted at 37±0.5°C and stirred at 50 rpm. A 5 ml samples were withdrawn at specified time intervals and replaced by the same volume of the dissolution medium at 37°C. The drug concentration was determined spectrophotometrically at 271 nm. The means of three determinations were reported.

RESULTS AND DISCUSSION

Characterization of TGF microspheres

Spherical and free flowing microspheres (flowability was determined by cone method) of TGF-CAB were prepared by utilizing the emulsion-solvent evaporation technique. Figure 1 shows the scanning electron micrograph of TGF-CAB microspheres. The microspheres were spherical and exhibited slightly rough surface. Very few micropores may be expected to be found on the surface. This could be attributed to the rapid evaporation of the organic solvent. No aggregates, drug crystals or polymer flacks were observed.

The particle size distribution and drug content of TGF-CAB microspheres are presented in Table 1. Histogram representing the particle size distribution is shown in Figure 2. It is obvious that using a stirring rate of 900 rpm resulted in microspheres of narrow range of variation in particle size. More than 40% of the batch lie in the range of 300-200 μm. Preliminary trials indicated that stirring rate had

a significant influence on the particle size distribution of the prepared microspheres. Using stirring rate higher than 900 rpm resulted in a smaller microspheres while slower rate gave larger irregular microspheres.

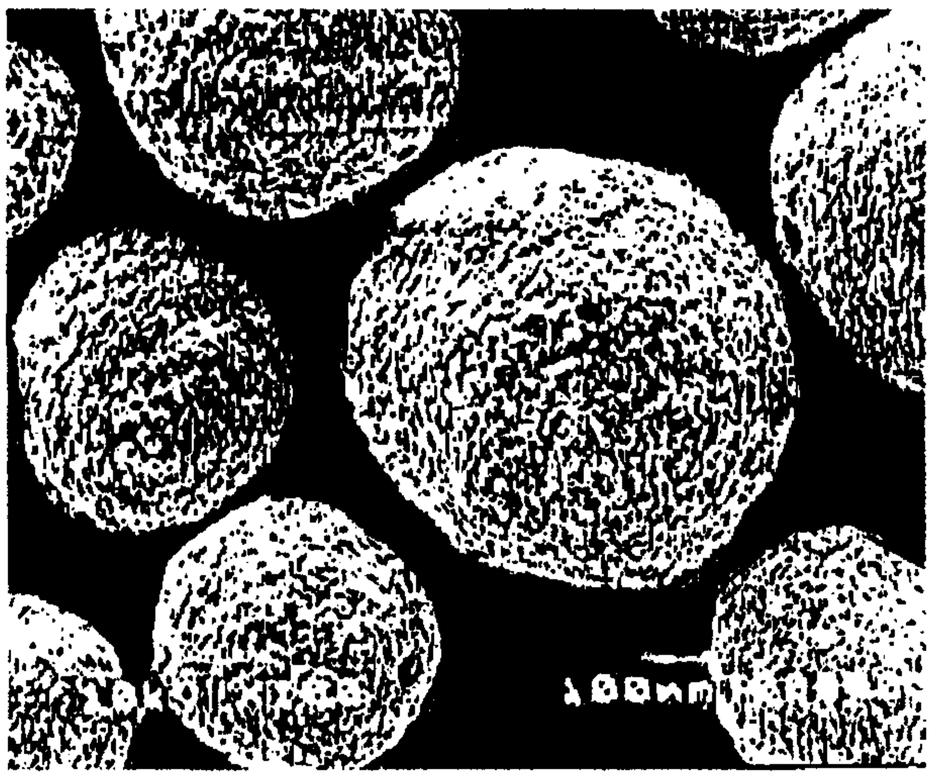

Fig. 1: Scanning electron micrograph of tegafur microspheres prepared with CAB at 1:1 polymer to drug ratio.

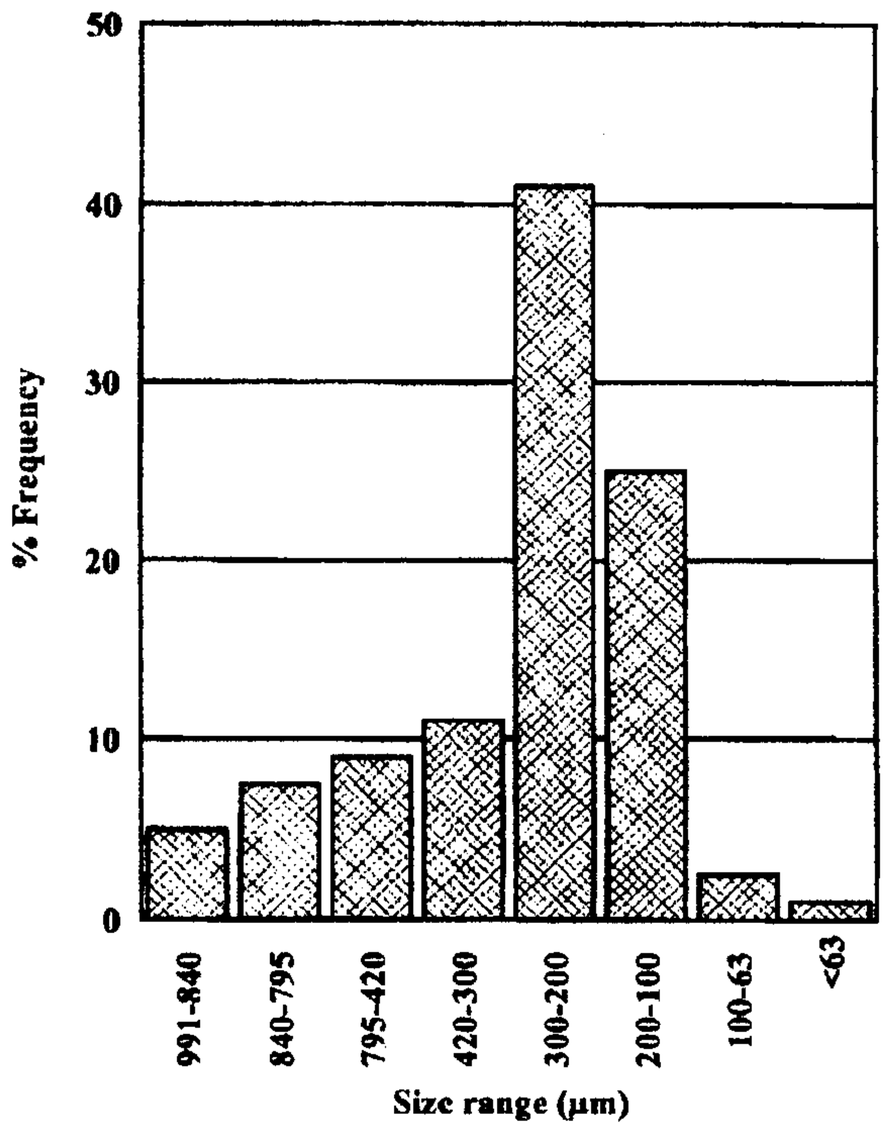

Fig. 2: Particle size distribution of tegafur microspheres prepared with CAB at 1:1 polymer to drug ratio at 900 rpm.

The drug content was higher than 95% for microspheres of different particle size (Table 1) indicating the uniform entrapment of the drug within the microspheres. In all patches, the yield of microspheres was greater than 97%.

Dissolution studies

Figure 3 shows the dissolution profiles of TGF from microspheres of different ranges of particle size (fractionation was carried out by sieve analysis) into pH 7.4 dissolution medium. The highest release rate was attained with microspheres of smaller fraction size (300-200 μm). This finding was attributed to the fact that the surface area is inversely related to the fraction size of the microspheres.

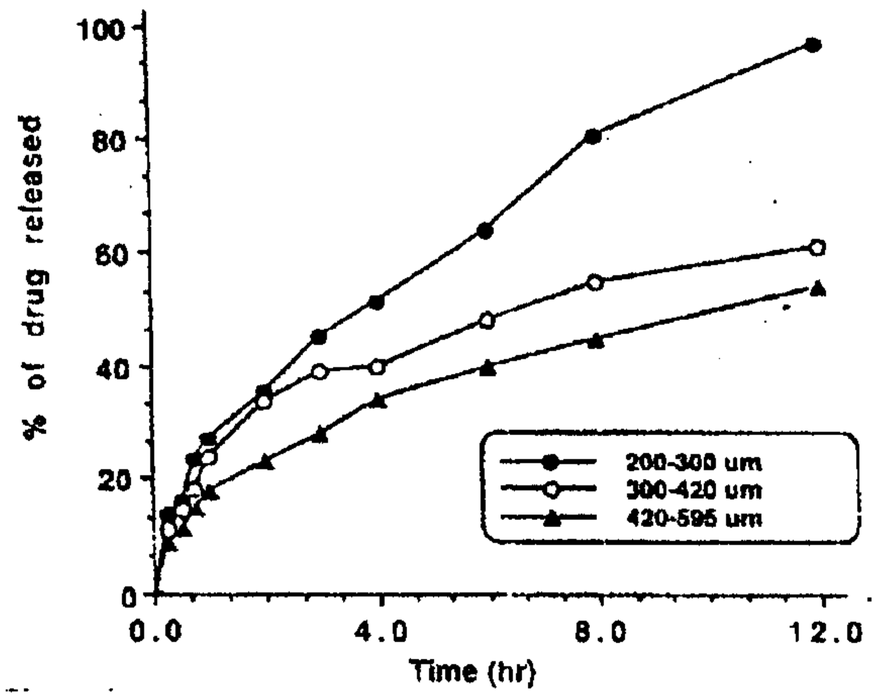

Fig. 3: Effect of microspheres size on the release of tegafur from CAB microspheres prepared at 1:1 polymer to drug ratio into phosphate buffer (pH 7.4).

The effect of pH of the dissolution medium on TGF release from the prepared TGF-CAB microspheres is shown in Figure 4. The obtained results indicated that the release rate of TGF from the microspheres is pH dependent. The release of the drug at pH 1.2 is significantly lower than at other investigated pHs (4.8, 5.5 and 7.4). In view of these results micro-encapsulation of TGF using CAB would be of value for controlling the drug release and potential colonic targeting.

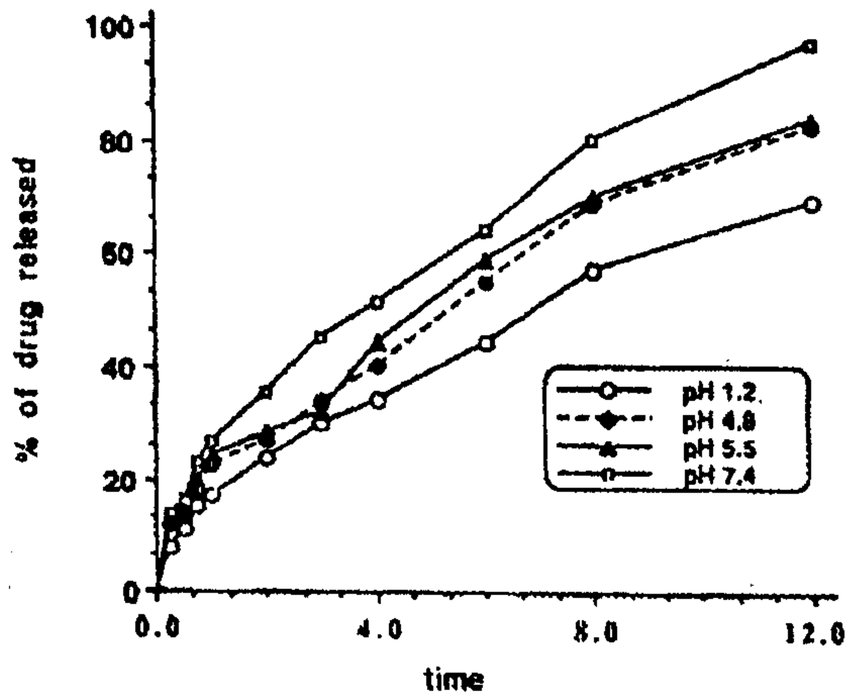

Fig. 4: Effect of pH of the dissolution medium on the releases of tegafur from CAB microspheres prepared at 1:1 polymer to drug ratio (P.S. 200-300 μm).

Drug release kinetics

The release data of TGF from the microspheres were treated according to first order and Higuchi diffusion models.[32] Zero order was not applied since the plot of amount released versus time don't show a straight line (Figs. 3 and 4). The fitting of TGF release data onto the investigated models were determined by least squares linear regression analysis. The results of analysis are presented in Table 2 and Table 3. The obtained data revealed that TGF release from the microspheres of various fraction size follows Higuchi diffusion model regardless of pH of the dissolution medium (Figs. 5 and 6). Further confirmation was attained by applying the following equation described by Baker and Lonsdale[33] for the release of drugs from the spherical matrices.

Table 1: Particle size distribution, mean diameter and drug content of tegafur microspheres prepared with CAB at 1:1 polymer to drug ratio.

Particle size range (μm)	Mean diameter (μm)	% frequency	Drug content % $\pm$ (SD)
991-840	915.5	5.2	95.60 (2.30)
840-795	717.5	7.3	96.05 (1.71)
795-420	507.5	8.9	97.53 (1.48)
420-300	360	10.5	98.36 (1.68)
300-200	250	40.9	97.23 (2.01)
200-100	150	25.0	97.40 (1.87)
100-63	81.5	2.0	98.15 (1.19)
<63	---	0.20	---

Table 2: Effect of microsphere size on the release kinetics of tegafur from TGF-CAB microspheres at pH 7.4.

Microsphere size (μm)	First order		Higuchi diffusion model		Spherical matrix model	
	r	K hr^{-1}	r	D (cm^2/sec) x 10^{-4}	r	K x 10^{-3}
300-200	0.915	0.157	0.993	11.15	0.963	31.11
420-300	0.868	0.132	0.984	4.13	0.986	7.57
595-420	0.881	0.140	0.998	3.33	0.999	5.52

Table 3: Effect of pH on the release kinetics of tegafur from TGF-CAB microspheres of particle size 300-200 μm.

pH of the dissolution medium	First order		Higuchi diffusion model		Spherical matrix model	
	r	K hr^{-1}	r	D (cm^2/sec) x 10^{-4}	r	K x 10^{-3}
1.2	0.906	0.168	0.996	5.97	0.985	10.29
4.8	0.926	0.161	0.991	8.31	0.981	16.72
5.5	0.873	0.147	0.990	8.37	0.984	17.90
7.4	0.915	0.157	0.993	11.15	0.963	31.11

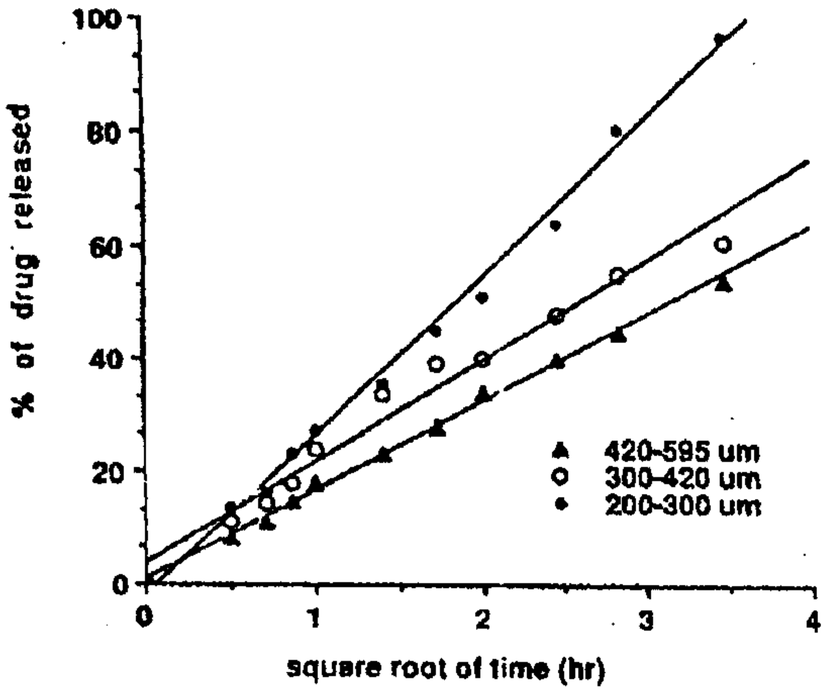

Fig. 5: Higuchi plot showing effect of microsphere particle size on tegafur release into phosphate buffer (pH= 7.4).

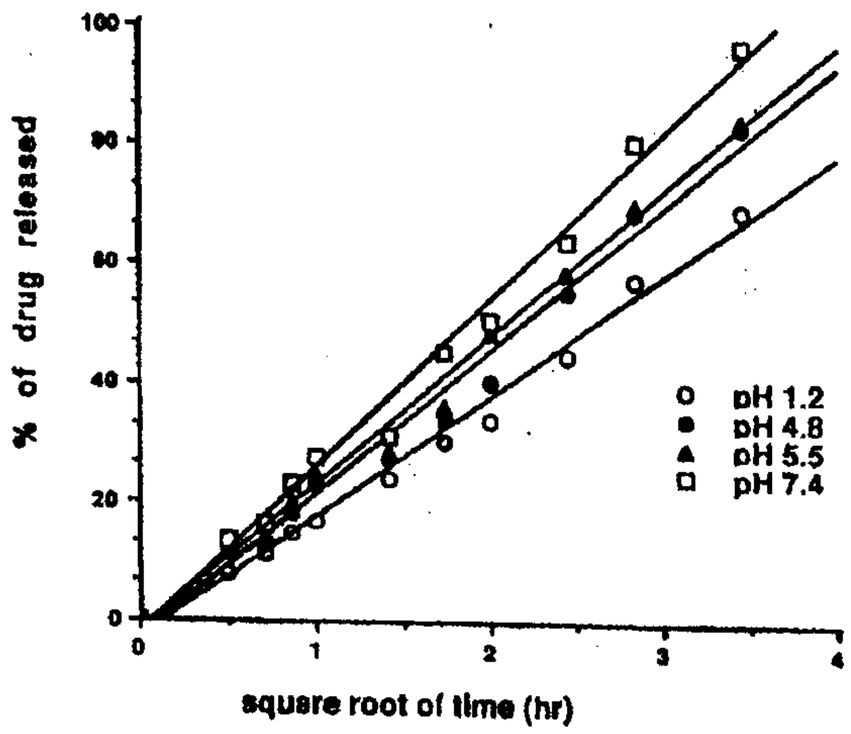

Fig. 6: Higuchi plot showing the effect of the pH of the dissolution medium on release of tegafur from CAB microspheres (P.S. 300-200 μm) prepared at 1:1 polymer to drug ratio.

$$3/2\,[1 - (1-F)^{2/3}] - F = K.\,t$$

where F is the fraction of drug released, K is the diffusion rate and t is the time. The correlation coefficient values obtained by linear regression applying the above equation are shown in Tables 2 and 3. Plotting of data according to spherical matrix model is shown in Figures 7 and 8. The obtained results confirmed that the mechanism for drug release is mainly diffusion controlled.

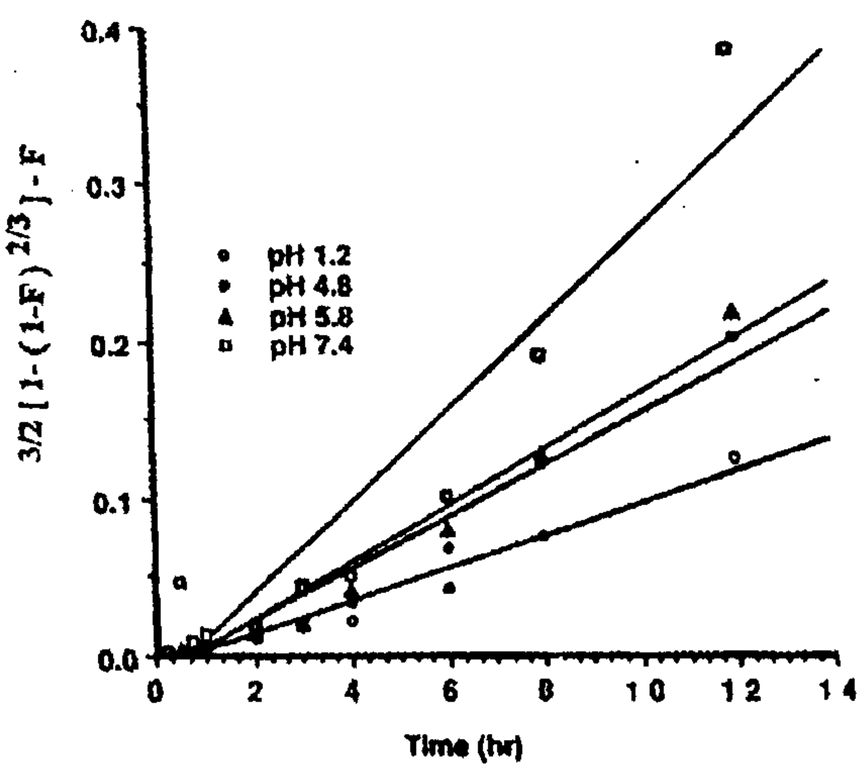

Fig. 7: Plot of the release data according to the spherical matrix model showing the effect of pH on tegafur release from microspheres (P.S. 300-200) prepared with CAB at 1:1 polymer to drug ratio.

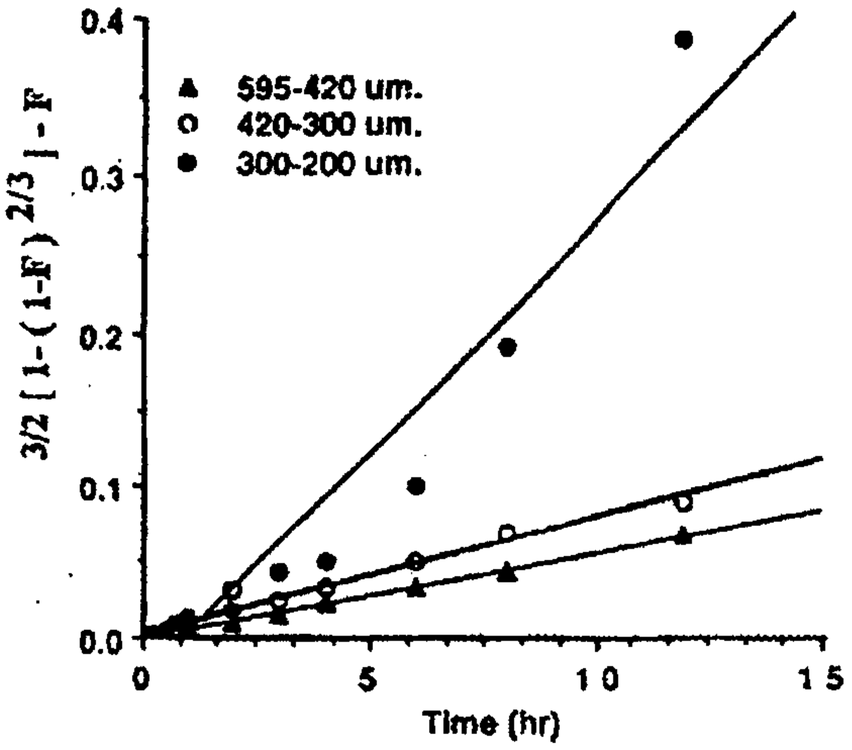

Fig. 8: Plot of the release data according to the spherical matrix model showing the effect of the microsphere particle size on tegafur release from microspheres prepared with CAB at 1:1 polymer to drug ratio (at pH 7.4).

Conclusion

Microspheres of TGF were successfully prepared using CAB as a coating polymer at 1:1 core:coat ratio by adopting the emulsion solvent evaporation technique. The drug release was inversely related to microsphere size. The drug release kinetic followed the diffusion controlled mechanism. Significantly slower release rate was obtained at pH 1.2 dissolution medium suggesting the possibility for colonic targeting of this antineoplastic drug. *In-vivo* investigation was already started and will be the subject of next publication elsewhere.

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