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PREPARATION AND EVALUATION OF PHENYLTOLOXAMINE CITRATE MICROSPHERES

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تم تحضير حويصلات دقيقة للفينيل تولوكزامين بطريقة تبخير الوسط المنتشر (المذيب لكل من العقار والبولمر) للمستحلب. وقد تم استخدام خلات بيوترات السيليلوز كمادة مغلفة بنسبة العقار إلى البولمر (١:٢ ، ١:١ ، ٢:١) وقد تم فصل الاحجام المختلفة للحويصلات بطريقة المناخل وتقييم كل حجم من الحويصلات المأخوذة على حدة لتقدير كمية الدواء وخصائص الاذابة.

وبدراسة الصفات الانطلاقية للحويصلات المحضرة في المحاليل المشابهة للوسط المعدى والمعوى أتضح أن معدل الانطلاق أبطأ فى حالة الحويصلات الاكبر حجما وبزيادة نسبة مادة الغلاف إلى العقار من ٢:١ إلى ١:٢ كما أظهرت النتائج أن الحويصلات المحضرة بهذه الطريقة تعطى تأثيرا طويل المدى كمضاد للهستامين بأستخدام حقن الفورمالين المذاب فى ٥٪ من المحلول الملحى فى الأرانب بالمقارنة بمسحوق الدواء الغير متحوصل.

وقد تمت دراسة قدرة كل من الحويصلات ومسحوق الدواء على فتران التجارب كمسكن للألم بطريقة استخدام السطح الساخن وقد وجد أن الحويصلات تعتبر نموذجا جيدا وأمنا فى تأثيراتها كمضاد للهستامين وكمسكن طويل المدى بالمقارنة لمسحوق الدواء الغير متحوصل.

Microspheres of phenyltoloxamine citrate (PTC) with cellulose acetate butyrate (CAB) were prepared using an emulsion-solvent evaporation technique. Good reproducibility in microspheres preparation was observed. The recovery of drug from different microspheres varied between 87 and 94%. The release rates of the drug from these microsphres were decreased as compared with that from the drug powder. Different release characteristics were obtained by changing the drug-to-polymer ratio and varying the particle size of microspheres. As the polymer to drug ratio increased, the microsphere size distribution shifted to the smaller size and the release of the drug decreased. The release of the drug showed pH-dependence. The release kinetics of the drug from CAB microspheres were studied. The antihistamine and the analgesic activities of PTC alone and PTC microspheres were evaluated in vivo. The antihistamine and analgesic activities of the tested microspheres in vivo were found to be greater and more prolonged than that of the intact drug.

INTRODUCTION

Phenyltoloxamine citrate is an ethanolamine derivatives with the properties and uses of the antihistamines. It is widely used for the treatment of prurities, urticaria, angioedema, nasal sneezing and rhinorrhea associated with the common cold and allergic rhinitis[1]. It can be given by nasal or oral route. PTC is a potential candidate to be formulated in a sustained release dosage form because of:

- its short half-life (1-3 hours)[1],
- frequent dosing (50 mg) three or four times daily[2],
- This medication has been given with milk, water or food to lessen gastric irritation[1].

The use of a microparticulate drug delivery system (CAB microspheres) may be useful for overcoming the problems arising from the oral administration of the drug and ensures an extended release of the drug. Microparticulate delivery system offers the advantage over other sustained release systems, that is the coated particles could be widely distributed throughout

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the gastrointestinal tract. This potentially improves drug absorption and reduces side effects related to localized build up of irritating drugs against the gastrointestinal mucosa[3]. The emulsion-solvent evaporation technique has been described in the literatures, and has been applied to polymer like ethylcellulose and CAB[4-6].

The aim of this investigation was to develop sustained release microspheres of PTC using CAB polymer. Other objectives were to study the morphology, size distribution of the microspheres, the effect of drug-to-polymer ratio on release rate of the drug from microspheres, the effect of different pH's, and the kinetics of drug release from microspheres. In addition, the analgesic and antihistamine activities of PTC microspheres has been carried out on both rats and rabbits.

EXPERIMENTAL

Materials

Phenyltoloxamine citrate was obtained from (CID Co., Egypt). Cellulose acetate butyrate was obtained from (FMC Co., NY, lot No.5A825). Sorbitan monooleate, acetone, n-hexane, ethylacetate and liquid paraffin were of analytical grade.

Methods

1- Microspheres preparation

Microspheres were prepared by an emulsion-solvent evaporation technique. Acetone was used as the polymer solvent and the drug was dispersed in the polymer solution. This dispersion was poured into 100 ml of liquid paraffin containing 2% sorbitan monooleate. Emulsification was performed by using two-blade mechanical stirrer at 750 rpm at room temperature for 5 hrs. The liquid paraffin was decanted and the collected microspheres were washed twice with 100 ml of hexane, thereafter filtered and air dried for 12 hrs. The collected microspheres were sized through standard sieves. The fraction of microspheres remaining on each sieve was collected for further study.

Different ratios of 2:1, 1:1 and 1:2 drug-to-polymer were studied.

2- Recovery of microspheres

The dried microspheres were weighed to determine the total recovery (%) by the following equation:

$$\text{Recovery (\%)} = \frac{\text{Weight of the collected microspheres}}{\text{Total weight of drug and polymer used}} \times 100$$

3- Drug content

To determine the total drug content of the CAB microspheres, an extraction method was performed. Fifty milligrams of microspheres were added to 20 ml of ethylacetate to dissolve the polymer coating and PTC was extracted with 100 ml of simulated intestinal fluid (pH 7.4). The amount of PTC in the aqueous phase was assayed spectrophotometrically at 270 nm. Each determination was performed in triplicate.

4- Scanning electron microscopy

The surface characteristics of the microspheres 1:1 drug-to-polymer ratio were observed with electron scanning microscope (JSM-25 S3,Jeik Co., Japan). Randomly chosen microspheres were coated with gold palladium foil (45 nm) prior to examination.

5- In vitro dissolution studies

The USP paddle apparatus was used for all in vitro release studies. Simulated intestinal fluid (pH 7.4) containing 0.02% tween 80 was used as the release medium. An appropriate amount of microspheres equivalent to 50 mg of PTC was transferred into 500 ml of release medium at 37 $\pm$ 0.5°C and stirred at 50 rpm. Five milliliters samples were taken at appropriate intervals, filtered and replaced by 5 ml of the fresh medium to maintain a constant volume. The amount of the drug in samples was assayed spectrophotometrically at 270 nm. Each determination was performed in triplicate.

When the effect of different pH's on the release of PTC from microspheres was studied, the release medium consisted of simulated

gastric fluid (pH 1.2), simulated intestinal fluid (pH 7.4), citrate buffer (pH 4 and 5), phosphate buffer (pH 6), all containing 0.02% tween 80.

6- Evaluation of analgesic activity

Analgesia was measured by the hot-plate reaction time test exhibited by groups of albino rats (200-220 gm) each comprising 6 animals. Two groups were given saline solution (control group) and PTC alone (2.7 mg/kg) respectively. The other three groups were given microspheres of different ratios (2:1, 1:1 and 1:2 drug-to-polymer) of the same size (515 μm) in dose equivalent to 2.7 mg/kg. Different formulae of the drug were given orally by stomach tube. Rats were placed separately on a 55°C hot plate surface within a hallow cylinder. Licking of paws or jumbing was taken as the end point for the determination of the reaction time[7]. After which the animal was rapidly removed from the hot plate(Rocrel Model-DS 30).

A cut-off reaction time of 30 seconds was used in order to avoid tissue damage. Reaction time latencies were determined during 8 hours periods following each medication.

The analgesia test is considered positive when the average reaction time (seconds) of the treated animals is significantly different from that of the control group.

7- Evaluation of antihistamine activity

PTC acts by competing with histamine for H_1- receptor sites on effector cells. They thereby prevent, but do not reverse, responses mediated by histamine alone[1]. This experiment was performed to determine the antihistamine effects of PTC microspheres. Four adult male rabbits, of average weight about 1.8-2.2 kg, for each group were used. The calculated dose of the drug (equivalent to 1.05 mg/kg) was orally administered. Rabbits in each group were injected with 50 μl formalin in 5% saline[8] , subcutaneously for inducing inflammation after oral administration of the drug or PTC microspheres (515 μm) by 20 min. Post injection by 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr and 8hr, the size of inflammation, degree of redness and edema were determined.

RESULTS AND DISCUSSION

Characterization of microspheres

A scanning electron micrograph of PTC microspheres is shown in (Fig. 1). Spherical microspheres with uniform and smooth surfaces were obtained. Microspheres prepared with different ratios of drug-to-polymer, resulted in products with various particle sizes distribution (Fig. 2). Size distribution measurements showed that more than 85% of the microspheres had diameters ranging from 400 to 1100 μm. In spite of a large degree of overlap in the particle size of the microspheres, there was a clear tendency in particle size increase with increasing PTC concentration (Fig. 2). This was attributed to an increase of the dispersion viscosity caused by the addition of PTC particles which yielded larger emulsified dispersions and consequently, larger solid microspheres[9].

PTC is insoluble in the polymer solution, some drug was lost when this mixture was poured into the mineral oil phase and subsequently emulsified. This resulted in less actual drug content of the microspheres than theoretical values calculated from the drug-to-polymer ratios (Table 1). The recovery yield of the microspheres was found to be related to the final particle diameter and PTC content. Recovery was higher when the microsphere batches were composed of larger

Fig. 1: Scanning Electron Micrograph of Phenyltoloxamine Citrate Microspheres Prepared with Cellulose Acetate Butyrate at 1:1 Drug-to-Polymer Ratio.

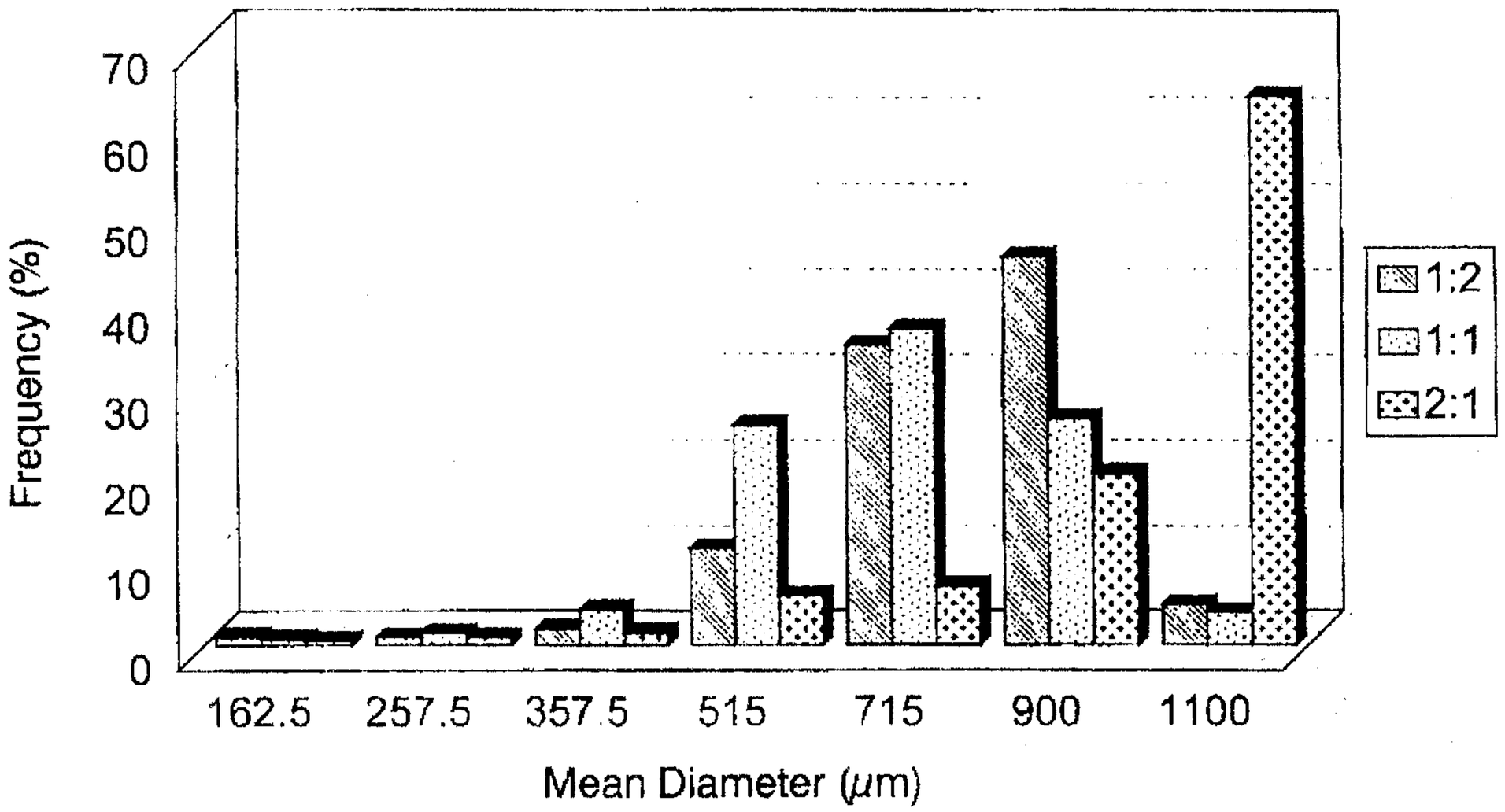

Fig. 2: Size-Frequency Distributions for Different Ratios of Drug to Polymer Microspheres.

Table 1: Recovery and actual drug content of phenyl-toloxamine citrate microspheres at different drug-to-polymer ratios.

Drug-to polymer ratio	Initial weight of drug and polymer (gm)	Microspheres recovered (gm)	Microspheres recovery (%)	Assayed drug content (%)±SD
1:2	5.62	5.28	93.87	87.5 ±2.30
1:1	7.50	7.16	95.47	90.63 ±1.79
2:1	11.25	10.90	96.89	93.75 ±1.50

SD= Standard deviation of triplicate sample determinations.

particles and higher PTC content (Table 1). In addition, PTC content determinations in various particle size ranges (>1000, 900, 715 and 515 μm) of a microspheres batch were performed. No significant variation in the content was observed, indicating that the ratio between the PTC and the CAB remained practically constant in each batch regardless the variation in the particle size distribution of the microspheres.

In vitro release studies

In vitro release of PTC from microspheres with different drug-to-polymer ratios in simulated intestinal fluid of pH 7.4 is shown in (Fig. 3). As expected, drug-to-polymer ratio decrease resulted in greater delays in the release rate due to the thicker coating membrane. This is in agreement with findings by several researchers[10,11]. Fig. 3 showed that the release of PTC from microspheres is greatly retarded in comparison to the intact powder.

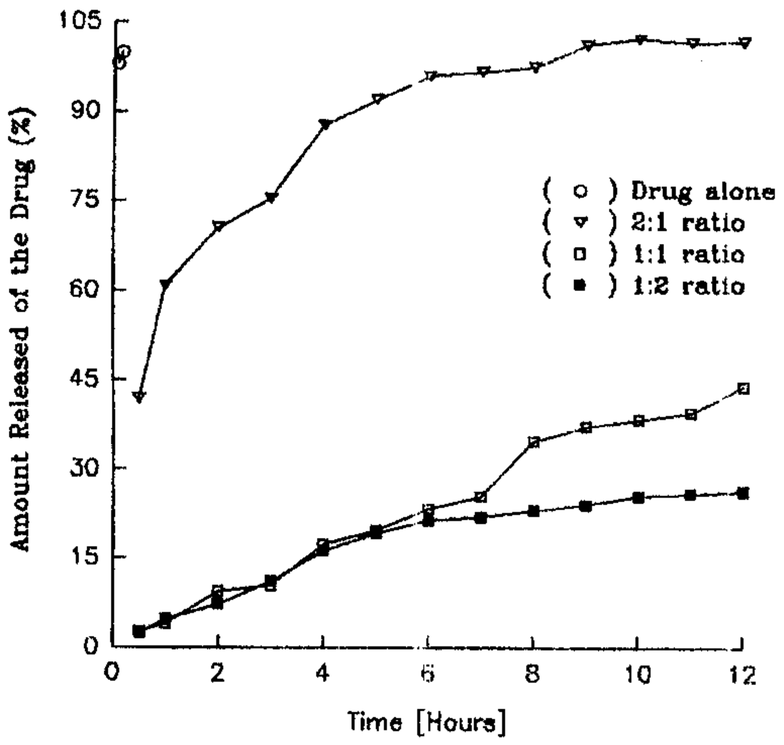

Fig. 3: Effect of Phenyltoloxamine Citrate–Cellulose Acetate Butyrate Ratios on the Release of the Drug from Microspheres (900 μm) at pH 7.4.

Figs. 4-6, showed the release behavior of PTC from four different particle size microspheres prepared. The release rat of PTC from the different sizes of microspheres was decreased in the following manner: 515 μm > 715 μm > 900 μm > more than 1000 μm, irrespective of drug-to-polymer ratio. The drug content in all sizes of microspheres in each ratio is almost the same (Table 1). Therefore, the faster drug release from the smaller size microspheres may be explained by the larger surface area of the smaller size microspheres[12].

The release profile of PTC from microspheres in different pH dissolution media is depicted in (Fig. 7). The drug release from CAB microspheres in simulated gastric fluid was found to be higher than that in simulated intestinal fluid and other different pH's. This can be attributed to the basic nature of the drug (pka = 9.4)[1]. However, the release of PTC from microspheres in simulated gastric fluid (pH 1.2) is still greatly lower when compared with the pure drug (Fig. 7); a characteristic which can overcome the gastric irritation caused by administration of the drug in plain form.

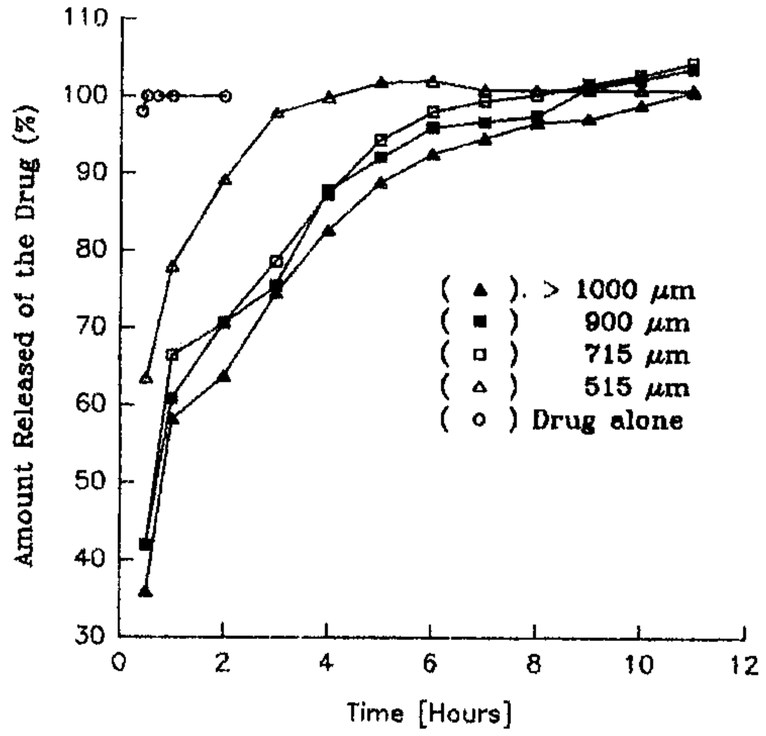

Fig. 4: Effect of Microsphere Mean Size on the Release of Phenyltoloxamine Citrate from CAB microspheres with a 2:1 Drug to Polymer Ratio at pH 7.4.

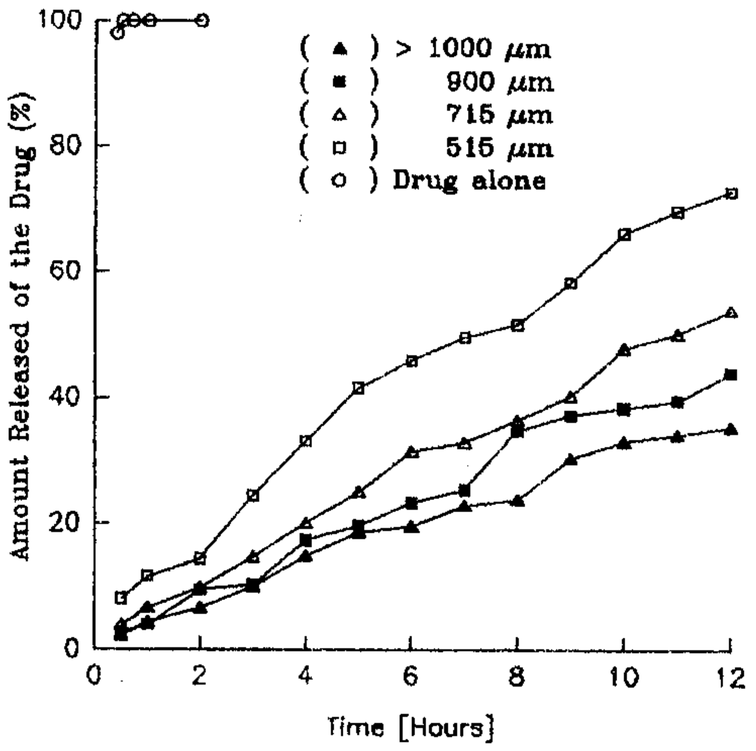

Fig. 5: Effect of Microsphere Mean Size on the Release of Phenyltoloxamine Citrate from CAB Microspheres with a 1:1 Drug to Polymer Ratio at pH 7.4.

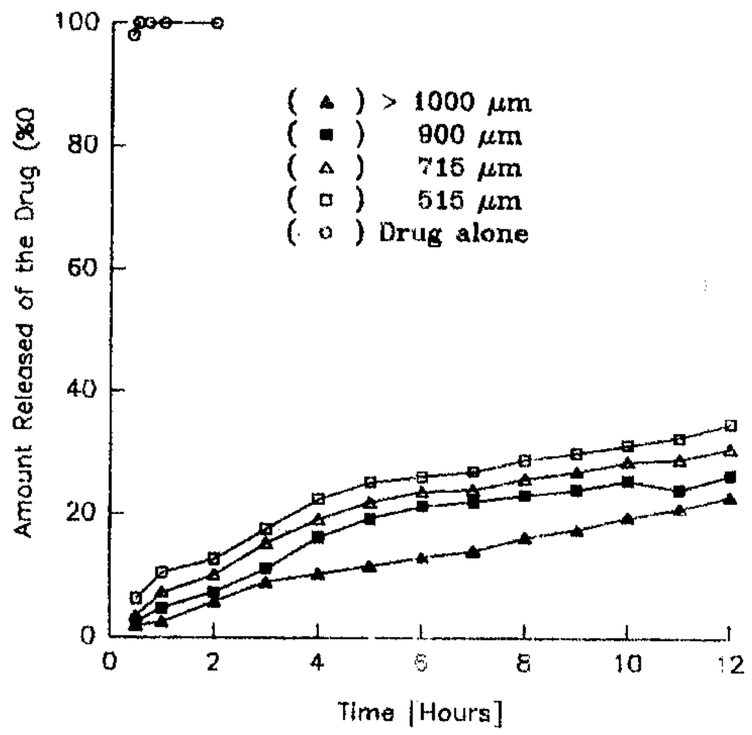

Fig. 6: Effect of Microsphere Mean Size on the Release of Phenyltoloxamine Citrate from CAB Microspheres with a 1:2 Drug to Polymer Ratio at pH 7.4.

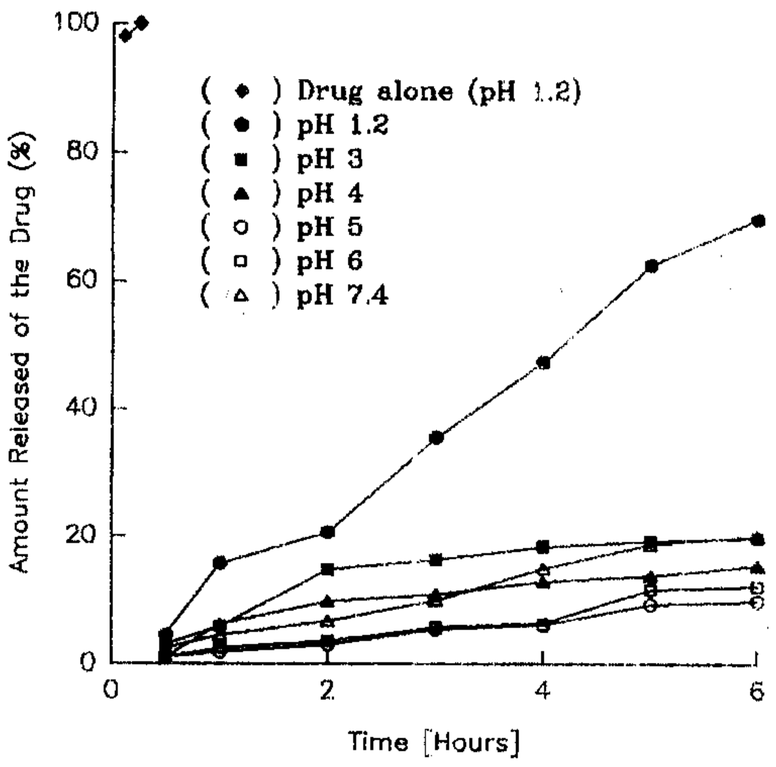

Fig. 7: Effect of the Dissolution Media on the Release of Phenyltoloxamine Citrate from CAB Microspheres (900 μm) with a 1:1 Drug to Polymer Ratio.

Release kinetic studies

In order to obtain meaningful information for the release models, the drug release profiles were fitted to various models (first order, Higuchi square root of time and zero order models). Table 2 summarizes the correlation coefficients for the different release kinetic models for the CAB microspheres formulated with a 2:1, 1:1 and 1:2 drug-to-polymer ratios. Models with higher correlation coefficients were judged to be a more appropriate model for the release data. The release of PTC from CAB microspheres formulated with 1:1 and 2:1 drug-to-polymer ratios was first order release model. For the CAB microspheres with a 1:2 drug-to-polymer ratio, the release models were tested for the entire dissolution test of 12 hours because of the slow drug release. The square-root of time release model was followed by microspheres formulated with a 1:2 drug-to-polymer ratio.

A student's t-test[13] on the $T_{50\%}$ (time to release 50% of the drug) showed that there was a significant difference between the 1:1 and 1:2 drug-to-polymer ratios of the same particle size (515 μm). Microspheres of 1:1 drug-to-polymer ratio had a $T_{50\%}$ of 7.5 hours and met our release profile target, while 1:2 drug-to-polymer ratio had a $T_{50\%}$ more than 12 hours. $T_{50\%}$ of 2:1 drug-to-polymer ratio (size 515 μm) was twenty minutes. A student's t-test showed there was significant decrease in the $T_{50\%}$ when the drug was increased from 1:2 to 2:1 ratio of the CAB microspheres.

Analgesic activity of PTC microspheres

PTC prevents the initiation and transmission of nerve impulses by decreasing the permeability of nerve cell membrane to sodium ions. This action decreases the rate of depolarization of the membrane and prevents the generation of the action potential[1]. The study of the analgesic activity of PTC powder and PTC microspheres revealed that all tested formulations have the ability to protect rats from thermal pain. When PTC microspheres were given in the ratios of 1:2 and 2:1 drug-to-polymer produced marked and significant prolongation in the hot plate latencies at different recording time periods (Table 3). As expected, 2:1 drug-to-polymer ratio gave the highest analgesic activity after 1 hr.

Antihistamine acidity of PTC microspheres

Antihistamines diminish or abolish the main actions of histamine receptor sites on tissue; they do not inactivate histamine or prevent its synthesis or release. Histamine H_1 receptors are responsible for vasodilation, increased capillary

Table 2: Comparisons of correlation coefficients and release rate constants of phenyltoloxamine citrate microspheres from dissolution data fit to various release models.

Drug-to polymer	Particle size (μm)	Zero-order		First-order		Square root	
		K_z	r	K_f	r	K_s	r
2:1	>1000	5.5	0.954	-0.16	-0.993	19.1	0.979
	900	5.6	0.954	-0.19	-0.990	19.8	0.983
	715	5.8	0.941	-0.24	-0.970	20.2	0.961
	515	5.9	0.904	-0.45	-0.910	20.6	0.905
1:1	>1000	1.4	0.993	-0.01	-0.995	5.9	0.985
	900	1.8	0.992	-0.02	-0.995	7.4	0.982
	715	2.1	0.994	-0.03	-0.996	8.8	0.983
	515	2.7	0.990	-0.05	0.993	11.6	0.989
1:2	>1000	0.7	0.992	-0.01	-0.995	3.0	0.998
	900	0.8	0.946	-0.01	-0.966	3.8	0.985
	715	0.9	0.944	-0.02	-0.957	4.0	0.990
	515	0.9	0.956	-0.02	-0.969	4.1	0.990

K = release rate constants, K_z ($mg.hr^{-1}$), K_f (hr^{-1}), K_s ($mg.cm^{-2}.hr^{-½}$). r = correlation coefficients.

Table 3: Effect of different ratios of phenyltoloxamine citrate-cellulose acetate butyrate microspheres on the latencies of pain threshold.

Time (hr.)	Reaction time in seconds after varying time periods				
	Control	Pure drug	2:1	1:1	1:2
0.3	10.9 ±0.92	18.9^{**} ±0.50	15.8^{*} ±0.33	14.8^{*} ±0.47	16.5^{**} ±1.19
1	11.8 ±0.91	25.9^{***} ±1.27	25.0^{***} ±1.63	19.2^{**} ±1.03	23.4^{***} ±0.47
2	10.3 ±0.30	24.1^{***} ±0.81	18.3^{**} ±0.38	21.9^{**} ±0.99	22.8^{***} ±1.11
3	10.0 ±0.47	17.4^{**} ±0.48	13.2^{*} ±0.27	18.6^{**} ±0.65	21.5^{**} ±1.38
4	11.2 ±0.11	15.1^{*} ±0.99	14.3^{*} ±0.52	15.5^{*} ±0.52	21.7^{**} ±1.43
5	11.1 ±0.18	11.8^{x} ±0.76	13.9^{*} ±0.34	14.9^{*} ±0.41	20.4^{**} ±0.90
6	10.9 ±0.19	13.6^{*} ±0.19	13.8^{*} ±0.92	13.6^{*} ±0.35	16.4^{**} ±0.37
8	10.0 ±0.23	13.4^{*} ±0.50	14.3^{*} ±0.80	13.8^{*} ±0.34	14.8^{*} ±0.40

- (^{x}) Insignificant, Significant difference (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$ at 99% C.L.
- Results represent mean ± S.E of 6 observations.

permeability, flare and itch reactions in the skin. Therefore PTC is used for the symptomatic relief of hypersensitivity reactions including urticaria and angioedema[14].

Tables 4, 5 present the antihistamine activity of PTC and its microsphere formulations against inflammation induced by 50 μl formalin in 5% saline. At all tested time intervals the magnitude of the antihistamine activity produced by the formulations of 2:1, 1:1 and 1:2 drug-to-polymer ratios were prolonged and greater than that of its control and pure drug. Formula of 1:2 drug-to-polymer ratio showed a significant decrease in the size of inflammation after 5, 6 and 8 hours than that its control and pure drug. Edema and the degree of redness were vanished after orally administration of PTC microspheres (2:1 and 1:2 ratios) at 5, 6 and 8 hours. This may be due to the presence of the CAB polymer in the microspheres and the vasoconstrictor property of the drug[1], which temporarily reduces the swelling associated with inflammation of the mucosal membrane.

Student's t-test[13] was adopted to demonstrate the differences in analgesic and antihistamine activities between PTC powder and their microspheres (515 μm) formulations (Tables 3 and 4). Microspheres of 1:2 drug-to-polymer ratio elicited a significant ($p \leq 0.05$) prolongation in the analgesic and antihistamine activities than PTC powder and its control. Concerning the antihistamine effect of the drug, the statistical analysis revealed insignificant differences between PTC powder and different ratios of microspheres at 2 and 3 hours (Table 4).

Table 4: Effect of different ratios of phenyltoloxamine citrate - cellulose acetate butyrate microspheres on the size of inflamed area induced by subcutaneous injection of 50 μl formalin in 5% saline in rabbits.

Time (hr.)	Mean diameter of the response (mm)				
	Control	Pure drug	2:1	1:1	1:2
1	5.3 ±0.49	3.2 ±0.40	5.1^{**} ±0.30	5.2^{**} ±0.12	5.5^{**} ±0.30
2	5.0 ±0.37	3.1 ±0.37	4.5^{*} ±0.19	3.4^{X} ±0.18	3.2^{X} ±0.40
3	4.3 ±0.35	4.5 ±0.35	4.0^{X} ±0.30	2.9^{*} ±0.11	3.3^{*} ±0.17
4	4.9 ±0.48	4.6 ±0.48	3.0^{*} ±0.10	2.0^{**} ±0.21	1.9^{**} ±0.36
5	4.9 ±0.40	4.9 ±0.19	2.0^{**} ±0.19	2.0^{**} ±0.30	1.5^{***} ±0.17
6	4.7 ±0.39	4.8 ±0.22	2.0^{**} ±0.17	1.9^{**} ±0.47	1.5^{***} ±0.19
8	4.1 ±0.40	4.3 ±0.17	2.0^{**} ±0.37	1.9^{**} ±0.23	1.4^{***} ±0.45

-(^{X}) Insignificant, Significant difference (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$ at 99% C.L.
- Values are the mean of 5 experiments ± S.E.

Table 5: Effect of different ratios of phenyltoloxamine citrate - cellulose acetate butyrate microspheres on allergic reaction and inflammatory signs induced by subcutaneously injection of 50 μl formalin in 5% saline in rabbits.

Time (hr)		Control	Pure Drug	2:1	1:1	1:2
1	(D.R.) Edema	+++ +++	++ ++	++ ++	+++ ++	++ +++
2	(D.R.) Edema	+++ +++	+ ++	++ ++	+++ ++	++ ++
3	(D.R.) Edema	+++ +++	+ ++	+++ ++	++ +	+ +
4	(D.R.) Edema	+++ +++	++ ++	++ ++	++ +	+ +
5	(D.R.) Edema	+++ +++	+++ +++	+ ++	+ +	+ +
6	(D.R.) Edema	+++ +++	+++ +++	++ ++	- -	- -
8	(D.R.) Edema	+++ +++	+++ +++	++ ++	- -	- -

(D.R) = Degree of redness.

The tabulated t-factor of PTC at (12, 0.05)[15] is equal to 2.18, while the estimated t-factor of the different ratios 2:1, 1:1 and 1:2 in relation to the pure drug is equal to 2.67, 2.41 and 2.71 respectively. These results represent that significant differences were found between the pure drug and the different ratios of PTC microspheres.

In vitro / in vivo correlation

The relationship between the percentage released of the drug from microspheres in vitro and the antihistamine or analgesic activities of PTC microspheres in vivo was assessed. A correlation between the size of inflamed area (antihistamine response) of PTC microspheres (at all ratios) orally administered and the percentage released of PTC from microspheres in vitro was observed as shown in (Fig. 8). The correlation coefficients 0.920, 0.895 and 0.881 were obtained for 1:2, 2:1 and 1:1 drug-to-polymer ratios respectively. This indicates that the percent released of the drug from 1:2 microspheres in vitro did correlate well with the antihistamine activity of the drug in vivo. The second relationship examined was between the latencies of pain threshold (analgesic response) of PTC microspheres orally administered in vivo

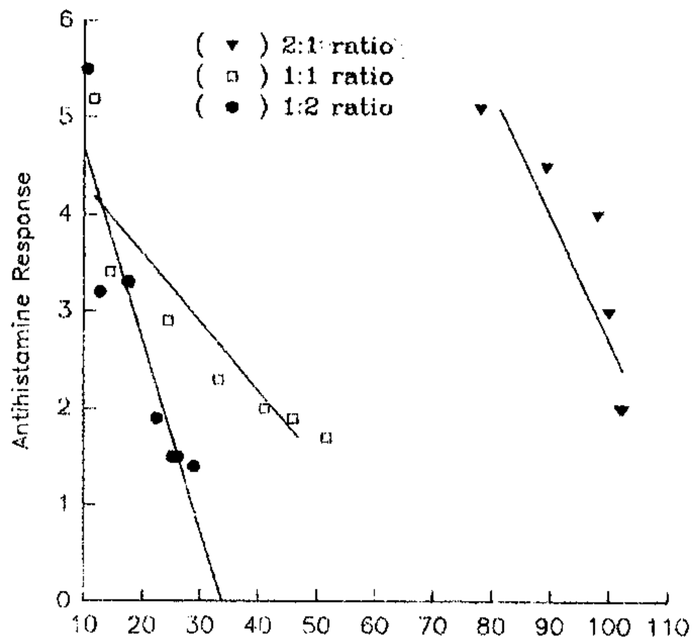

Fig. 8: Relationship Between The Percentage Released of PTC Microspheres from In Vitro and The Size of Inflammation Area (Antihistamine Response) In Vivo at The Same Time.

and in vitro release percentage of PTC microspheres (Fig. 9). Higher correlation coefficients of 0.982, 0.950 and 0.920 were obtained for 2:1, 1:1 and 1:2 drug-to-polymer ratios respectively. Microspheres of different ratios showed a strong correlation between the percent released of the drug in vitro and the analgesic activity of the drug in vivo.

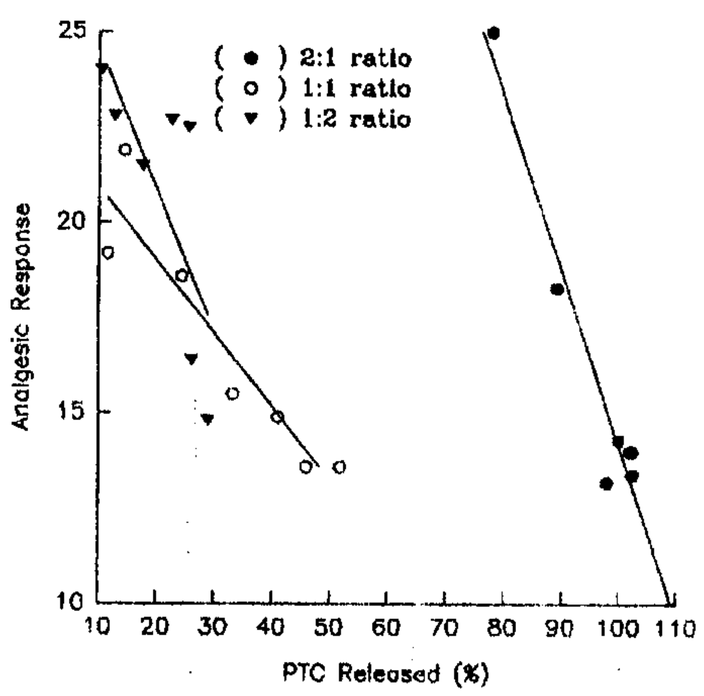

Fig.9: Relationship Between The Percentage Released of PTC Microspheres from In Vitro and The Latencies of Pain Threshold (Analgesic Response) In Vivo at The Same Time.

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