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BIOAVAILABILITY STUDIES OF THEOPHYLLINE MICROCAPSULES

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ABSTRACT

The release of theophylline from microcapsules prepared by using different concentrations of ethylene-vinyl acetate (EVA) copolymer as a coacervation-inducing agent was investigated in simulated gastric and intestinal fluids. Also the bioavailability of theophylline from its microcapsules following oral administration in volunteers using the saliva drug concentration was investigated.

The results pointed out that the higher the concentration of EVA compolymer used, the more sustainment of theophylline from microcapsules. The investigation pointed for a good correlation between the in-vitro release rate characteristics and the in-vivo salivary bioavailability parameters of theophylline microcapsules. The prepared theophylline microcapsules were proven to achieve excellent sustainment pattern and could be used to avoid sub-/or/super therapeutic levels of conventional theophylline products.

INTRODUCTION

Controlled-release formulations function as effective carriers for prolonging drug release for sustained biological action. These drug products are designed as that the rate of systemic drug absorption is limited by the rate of drug release via the drug delivery system. The objective of controling the release rate from the delivery system is to achieve a stable serum concentration.

Theophylline has been popularly used as a bronchodilator for the treatment of chronic asthma and obstructive lung disease in both children and adults[1]. The narrow therapeutic range of serum theophylline concentration (10-20 ug/ml) often resulted in poor drug response or many adverse effects[2,3]. The desirability of maintaining theophylline plasma levels within this narrow range had led to the development of several sustained-release formulations. Therefore, many sustained-release dosage forms are now available commer-

cially, and extensive researches[4-8] on their bioavailability and dissolution characteristics have been reported. El-Yazigi and Sawchuk[9], found a good correlation between the bioavailability parameters and the dissolution characteristics of theophylline from sustained-release dosage forms. Brockmeir[10] compared *in-vitro* dissolution rates with *in-vivo* dissolution rates in humans. Ritschet et al[11] reported correlation between bioavailability parameters in the beagle dog and *in-vitro* dissolution data. Good correlation between theophylline concentration in blood and saliva has been reported[12,13].

Lena et al[14] and Sharma et al[15] suggested that saliva can be used in control of compliance only in children and adults, after administration of sustained release preparations of theophylline. However, Kelly et al[16] confirmed that, saliva can be used in pharmacokinetic assessment of elimination phase of theophylline. Investigations in adults[17] and in children[18] have confirmed the use of saliva for therapeutic monitoring of theophylline instead of plasma, due to high correlation of theophylline concentration in saliva and plasma and low inter and intraindividual difference in the P/S ratio. Moreover, Danhof and Breimer[19] concluded that saliva can be considered of practical value in investigation of pharmacokinetic of elimination of theophylline, as well as, its absorption.

Theophylline microcapsules were prepared from ethylcellulose using ethylene-vinyl acetate (EVA) copolymer as a coacervation-inducing agent[20], or prepared in the form of a drug-resin complex and coated with paraffin or encapsulated with ethylcellulose[21] for sustainment.

The usefulness of EVA copolymer as a drug delivery system for pilocarpine[22], progesterone[23], fluorideion[24], macromolecules such as protein[25,26] and hydrocortisone[27] was reported.

The purpose of our research was to prepare a sustained-release product of theophylline in the form of microcapsules by using various concentrations of ethylene vinyl acetate (EVA) copolymer as coacervation-inducing agent. Also the aim was to investigate the possibility of measuring salivary instead of plasma levels of theophylline after oral administration of sustained-release attempt. In addition, it was an assess the relation-ship between the *in-vitro* release rate and *in-vivo* human saliva concentration of theophylline from sustained-release microcapsules.

EXPERIMENTAL

Materials :

Theophylline supplied by CID Company, Assiut, Egypt. Ethylcellulose (BDH Chemical Ltd. Poole England).

Ethylene-vinyl acetate copolymer (Mitsui Polychemical, Tokyo, Japan).

Disodium hydrogen phosphate (Prolabo, France).

Sodium dihydrogen phosphate (El-Nasr Co., for Pharmaceutical Chem., Abu-Zaabal, Egypt.)

ENOAB theophylline enzyme immunoassay kit No 101 (USA).

Equipment :

Spectrophotometer-UV-150-20 Shimadzu, Japan.

Thermostatically controlled shaker unitronic 320-OR (Seleca).

pH-meter (UqN Tacussel Electronique. Solea, Australia).

Standard sieves of 63 um, 90 um, and 100 um (England).

Methods :

Preparation of Theophylline Microcapsules.

Theophylline microcapsules were prepared by phase-separation method, using EVA copolymer as a coacervation-inducing agent, with the composition in Table 1.

Table 1 : Percent Concentrations of Ethylene-Vinyl Acetate(EVA) Copolymer in Sustained-Release Theophylline Microcapsule Formulations.

Formulations	EVA copolymer conc.(% w/w)
F	0
A	0.43
B	0.83
C	1.5
D	2.5

Three hundred milliliters of cyclohexane containing from 0 to 2.5% of EVA copolymer were separately placed in a 1000-ml three-neck, round bottomed flask equipped with a stirrer, a thermometer, and a reflux condenser. Three grams of each of theophylline and ethylcellulose were added to the stirred cyclohexane-EVA copolymer solution (150 rpm) at room temperature. The system was then heated to 82°C with continuous stirring to give a homogeneous suspension. Then, system was allowed to cool to 24°C and was then stirred for another 10 min. During the cooling process, a phase separation occurred. The microcapsules were separated from the solution by decantation, rinsed with n-hexane, and dried at 40°C in a vacuum drier for 24 hr. Theophylline microcapsules were used for further studies.

Release Studies :

Release of theophylline from microcapsules in simulated gastric fluid and simulated intestinal fluid were determined using 150 mg of theophylline microcapsules (equivalent to 25 mg of theophylline). These amounts were dispersed in 200 ml of the medium at 37°C + 0.5°C in the shaker water bath. The rate of stirring was 50 rpm. Five milliliters of each sample was removed at predetermined intervals and 5 ml of each fresh medium was added to the beaker to maintain the original volume. The drug concentration was analysed spectrophotometrically at 273 nm.

Saliva Bioavailability Studies :

Five healthy volunteers, (23-31 years old) were selected for this study. The volunteers were asked not to drink any caffeine containing fluids before and during this study. Also they were asked not to smoke tobacoo by any mean.

Collecting Saliva Samples :

Blank saliva samples were collected few minutes before administering the preparation. After overnight fasting, a single 100 mg dose of sustained-release preparation containing 50 mg theophylline was administered, with 100 ml water. Saliva samples were col-

lected at appropriate intervals up to 8 hr. A small amount (10 mg) of citric acid, a salivary flow stimulator, was put on the tongue and held in the mouth for 1-2 min. then a 2-ml sample of the saliva was collected in a test tube and kept frozen until analysis. Theophylline concentration was assayed in utilizing a homogeneous enzyme immunoassay technique using ENDAB theophylline enzyme immunoassay kit for the quantitative determination of theophylline in saliva.

RESULTS AND DISCUSSION

The *in-vitro* release studies of the prepared theophylline microcapsules in USP simulated gastric fluid and simulated intestinal fluid at 37°C was carried out and the results are shown in Table 2 and Figure 1. Results pointed out that, microcapsulation retarded the release of theophylline as a function of ethylene-vinyl acetate copolymer concentration. Moreover, the release of theophylline from microcapsules in simulated gastric fluid indicated that, ethylene-vinyl acetate (EVA) significantly control the release rate of theophylline in the following arrangement for the tested formulae:
F› A› B› C› D. However, formula D showed minimal retardation in release with a release rate (9.3587) as shown in Figure 2, the rate of release of theophylline from ethylcellulose microcapsules was related to the concentration of coacervation inducing agent. The higher the concentration of EVA copolymer used, the slower the release rate of theophylline microcapsules. This might be attributed to the lower porosity and thicker wall of the microcapsules reflected by EVA copolymer[20].

There was no correlation between EVA concentration and release rate constant r=0.6758 [Slope=2.9662, intercept=14.3178]. The effect of EVA concentration was manifested clearly in Table 3 and Figure 2 indicating that the order of decrease in release rate is as follows :
F› A› B› C› D. The kinetic data showed a good correlation coefficients between different concentrations of EVA in theophylline microcapsules and the first-order release rate constants of theophylline.

Also, it was found that, the higher the concentration of EVA copolymer used, is the more sustained the release behaviour of theophylline microcapsules. Moreover, theophylline microcapsules prepared by using a higher concentration of EVA copolymer gave the slower the release rate in the simulated intestinal fluid as shown in Figure 2.

A comparison of the release in simulated gastric fluid with simulated intestinal fluids shows that the release of the drug in pH 1.2 is slightly faster than in pH 7. These results were in agreement with conclusion that, any change in the pH of the release medium affected the release rates of different sustained-release theophylline preparation in opposit directions, resulting in either a decrease or an increase in the release rates[11,29,30].

Pharmacokinetic Studies :

Theophylline saliva levels for mean of five subjects is presented in Fig. 3 for oral and absorption step. The saliva concentration versus time curves of theophylline in humans correspond to a one-compartment

open model as shown in Figure 3 by comparing the absorption of theophylline from microcapsules prepared with EVA and without it. Figure 3 indicates that, the higher the concentration of EVA copolymer used, the more sustained the release behaviour of the theophylline microcapsules. Moreover the theophylline microcapsules prepared by using the highest concentrations of EVA copolymer gave a transient steady-state plateau level of theophylline in the saliva after administration without producing a sharp peak of saliva concentration. Saliva data following oral dosing were fitted to one compartment model with first-order elimination step. Theophylline saliva levels were fitted for both absorption and elimination kinetic model.

Absorption of theophylline following oral administration of theophylline microcapsules in five volunteers were presented in Table 3. From the results it was observed that apparent first-order elimination rate is obtained since the rate of theophylline degradation is reported to be proportional to theophylline saliva levels. Also, it was found that calculated saliva elimination rates were highly correlated with three concentrations only of copolymer EVA 0%, 0.43% and 0.83 (r=0.9971, slope=1.5175 and the intercept=0.3224; while there was a good correlation also between all concentrations of EVA with absorption rates of theophylline after oral administration i.e. r=0.9663; slope=1.3912 and intercept=5.5188. These results indicated that as the concentrations of EVA decreased in the theophylline microcapsules an increase in the absorption rate of the drug was observed, thus, the effect of EVA copolymer was manifested sharply at each concentration and at oral administrations after one dosing step.

From the results it was concluded that there was a good correlation between in-vitro and in-vivo results of release rate constants of theophylline microcapsules containing 0% 0.43% and 0.83% with elimination rate constants after oral administration of the microcapsules i.e. r=0.9756; slope=2.9125 and intercept=16.2054.

On the basis of the results obtained, it can be concluded that, saliva can be reliable to be used in bioavailability assessment of theophylline microcapsules.

Table 2 : In-vitro Release Kinetics of Theophylline Microcapsules in Simulated Gastric Fluid.

Formulation	Diffusion			First-order			
	Correlation coefficient r	K(hr^{-1})	Intercept	Correlation coefficient r	K(hr^{-1})	Intercept	$t_{½}$ (hr)
F	0.9416	0.5728	0.8148	0.9694	16.9448	1.9569	40.9
A	0.9897	0.5238	0.6474	0.9868	14.5533	1.9637	47.6
B	0.9876	0.2611	0.6011	0.9941	7.0467	1.9721	98.3
C	0.9849	0.2839	0.5220	0.9980	7.6761	1.9745	90.3
D	0.9858	0.3561	0.1127	0.9948	9.3587	1.9876	74.0

Table 3 : In-vitro Release Kinetics of Theophylline Microcapsules in Simulated Intestinal Fluid.

Formulation	Diffusion			First-order			
	Correlation coefficient r	$K(hr^{-1})$	Intercept	Correlation coefficient r	$K(hr^{-1})$	Intercept	$t_{½}$ (hr)
F	0.9664	0.5268	0.7641	0.9913	15.4536	1.9598	0.0448
A	0.9860	0.4271	0.6914	0.9803	13.0534	1.9651	0.0531
B	0.9944	0.4345	0.6069	0.9728	11.7296	1.9671	0.0591
C	0.9907	0.3539	0.5908	0.9705	9.4573	1.9698	0.0733
D	0.9683	0.2577	0.5534	0.9733	6.9879	1.9739	0.0992

Table 4 : Pharmacokinetic Parameters of Theophylline Microcapsules after Oral Administration in Man.

Formulation	Absorption				Elimination				
	Correlation coefficient r	$K(hr^{-1})$	t (hr)	Intercept	Correlation coefficient r	$K(hr^{-1})$	t (hr)	Intercept	AUC (ug.hr.ml.)
F	0.9081	1.8813	0.3684	0.7445	0.9734	0.3491	1.9851	1.6393	7.7425
A	0.9659	5.0634	0.1369	0.8671	0.9727	0.9196	0.7536	4.2102	20.2215
B	0.9659	4.4291	0.1565	0.0523	0.9805	1.6107	0.4302	7.1748	24.0981
C	0.9464	2.6749	0.2590	0.9408	0.8797	1.1727	0.5909	5.7702	22.6405
D	0.9655	2.3302	0.2974	0.0654	0.9728	0.3593	1.9288	4.858	24.3100

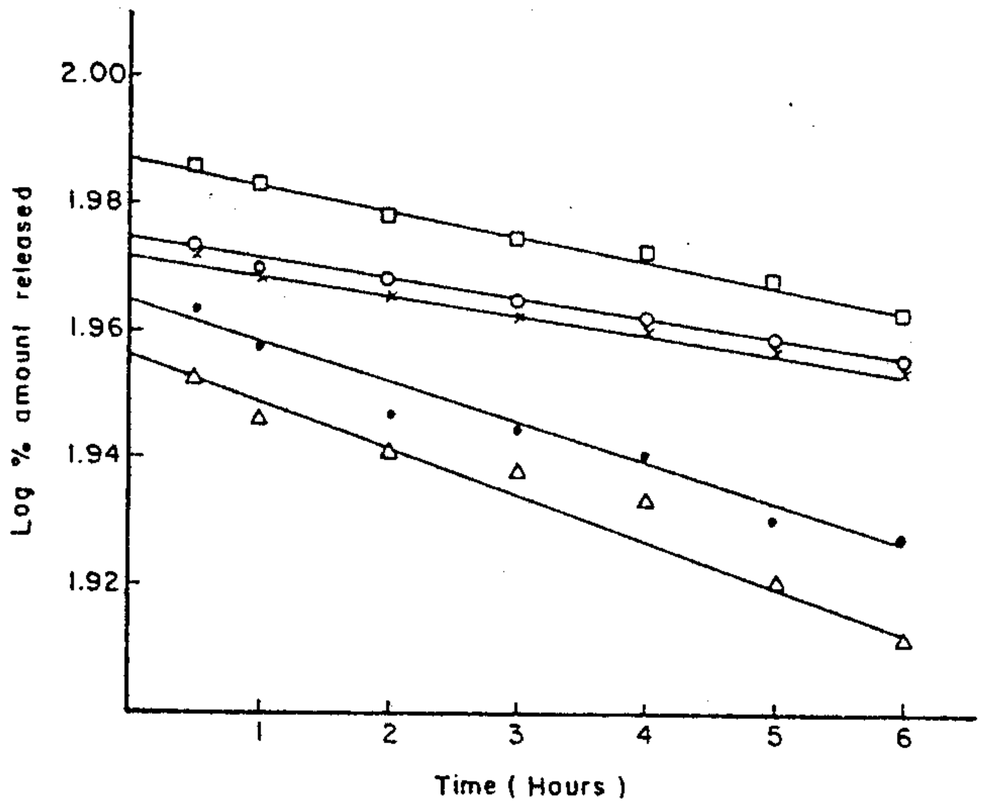

Fig.(1):Effect of ethylene-vinylacetate on the release rate of theophylline from microcapsules in simulated gastric fluid at 37 c°.

□D; ○C; •A; xB; △F

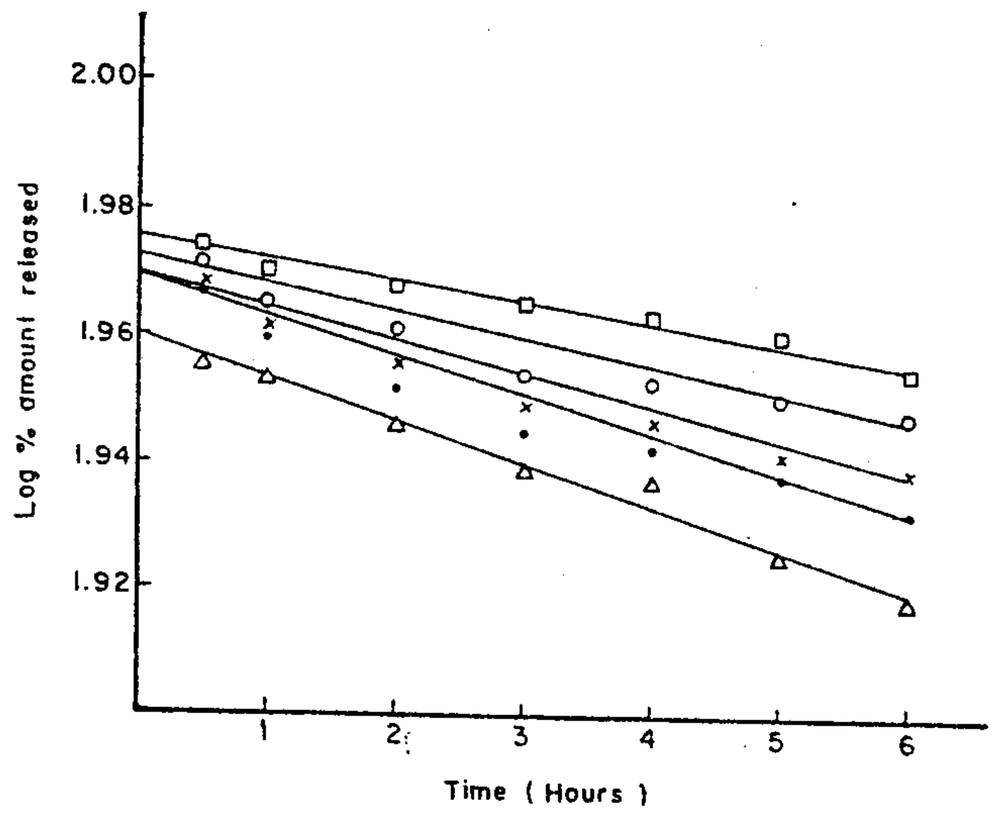

Fig.(2):Effect of ethylene-vinylacetate on the release rate of theophylline from microcapsules in simulated intestinal fluid at 37 c°.

□D; ○C; •A; xB; △F

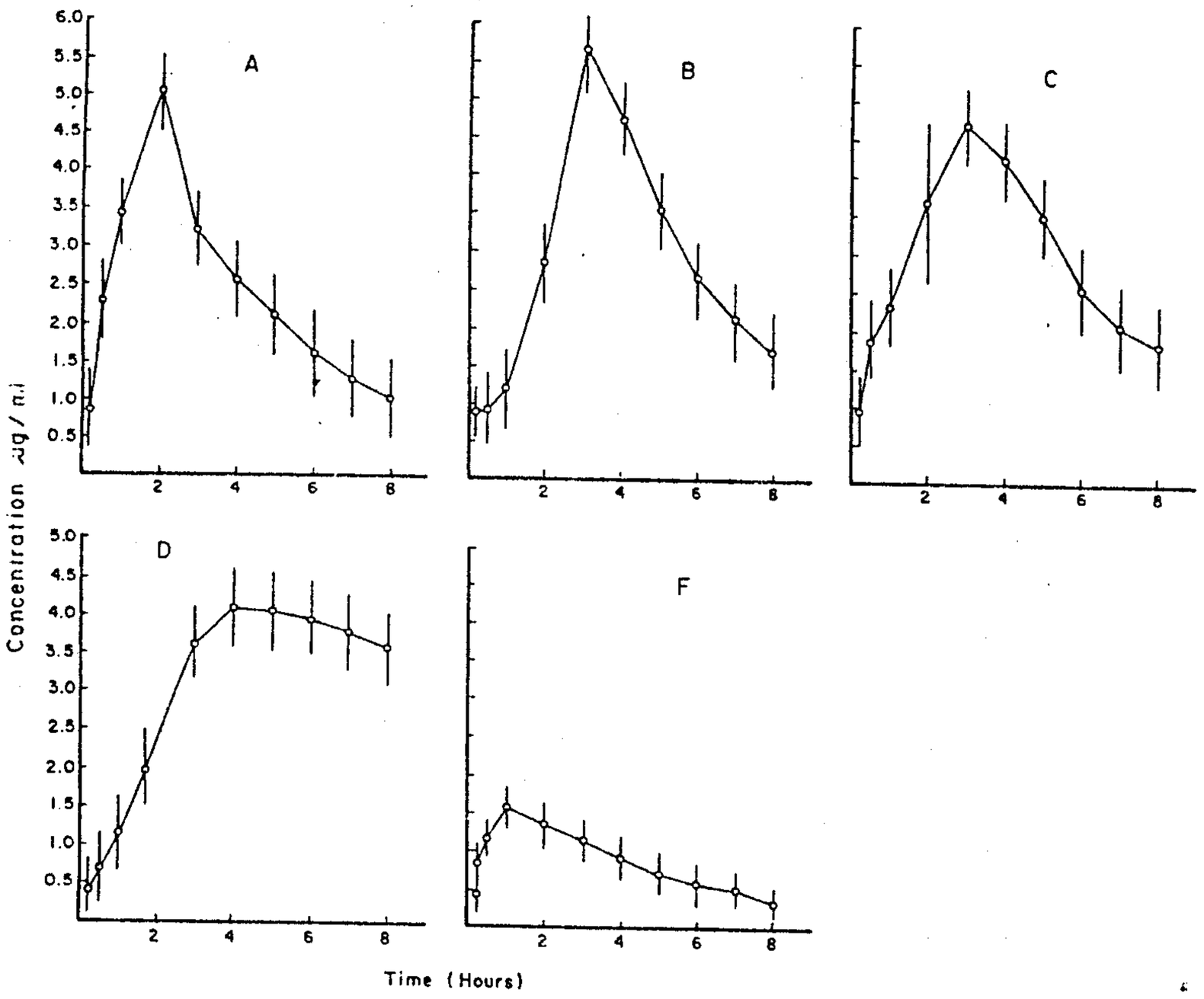

Fig. 3 : Saliva theophylline concentration level after oral administration of theophylline microcapsules.

Concentration of EVA co-polymer A 0.43% ; B 0.83% ; C 1.65% ; D 2.5% ; F 0%.

The bars indicate the standard error of the mean (SEM).

REFERENCES

1-L.Hedeles; M.Weinberger, Pharmacotherapy 3, 2-43 (1983).

2-R.Baptista; D.F.Driscoll, Am. Pharm., N524, 285-293 (1984).

3-R.I.Ogilvie, Clin. Pharmacokinet., 3, 267-293 (1978).

4-M.Weinberger; L.Hendeles; L.wong, and L.Vaughan, J, Pediat., 99, 145-152 (1981).

5-K.J.Simons, F.E.R.Simons, K.D.Plett, C.J.Scerbo, Pharm. Sci., 73, 939-942 (1984).

6-K.J.Simons, E.M.Frith, F.E.R.Simons, J. Pharm. Sci., 71, 505-511 (1982).

7-R.S.Summers; B.Summers, S.Rawnsley, Int. J. Pharm., 30, 83-88 (1986).

8-P.Macheras, M. Koupparis, E.Aposto Lelli., Int. J. Pharm., 31, 73-79 (1987).

9-A.El-Yazigi, R.J.Sawchak, J. Pharm. Sci., 74 161-164 (1986).

10-D.Brochmeir, Arznein-Forsch., 34, 1604-1607 (1984).

11-W.A.Ritschel, H.P.Koch; G.J.Alcorn, Methods. Find. Exptl. Clin. Pharmacol., 6, 609-618 (1984).

12-R.Koysooko, E.F.Ellis, G.Levy. Clin. Pharmacol. Ther., 15, 456-640 (1974).

13-V.P.Shan, S.Riegelman, J. Pharm. Sci., 63, 1283-1285 (1974).

14-S.M.Lena, C.B.S.Wood, P.Hutchins, and P.Turner, Postgrad. Med. J., 56, 85 (1980).

15-J.N.Sharma, A.Blackett, A.Johnston, and N.R.Hathway, Postgrad. Med. J., 57, 153 (1981).

16-H.W.Kelly, W.M.Hadley, S.Murphy, B.G.S.Skipper, Am. J. Dis. Child., 136, 137 (1981).

17-J.H.G.Jonkman, G.H.Koeter, R.Schoenmaker, K.Devries, J.E.Greving, and R.A. de Zwcuw, Eur. J. Clin. Pharmacol., 20, 73 (1981).

18-S.J.Goldsworthy, M.Kemp, and J.D.Warner, J. Royal Soc. Medicine, 74, 215 (1981).

19-M.Donhof, and D.D.Breimer, Clin. Pharmacokinet., 3, 39 (1978).

20-S.Y.Lin, J.C.Yang, J. Am. Pharm. Associ., 76, 219-223 (1987).

21-S.Motycka, C.J.L., Newth and J.G.Nairh, J. Pharm. Sci., 74, 643-646 (1985).

22-M.F.Armaly, and K.R.Rao, Invest., Ophthalmol., 12, 491 (1973).

23-J.Martinez-Manautou, J.Steroid., Biochem., 6, 889 (1975).

24-B.D.Halpern, O.Solomon, L.Kopec, E.Korostoff, and J.L.Ackerman, "Controlled Release Polymeric Formulation" ed. by D.R. and Herris, F.W.m American Chemical Society, Washington D.C., pp. 135-146 (1976).

25-R.Langer and J.Kolkman, Nature (London) 263, 797 (1976).

26-W.D.Rhine, D.S.J.Hsieh, and R.J.Langer. Pharm. Sci., 64, 265 (1980).

27-Fu, J.C., D.L.Moyer. and J.Hugeneier, Biomed. Mater. Res., 12, 249 (1978).

28-E.L.S.Tse, K.H.Valia, D.W.Szeto, T.J.Raimondo, and B.Koplowitz, J. Pharm. Sci., 70, 395 (1981).

29-H.Moller, F.Lagenbucher, Pharm. Ind., 44, 1085 (1982).

30-K.H.Fromming, L.Schwabe, A.H.Staib, et al. Arzneim. Forsch., 33, 446 (1983).

دراسة التوافر الحيوى لحويصلات الثيوفيللين

سهير الشنوانى - سلوى محمود صفوت

قسم الصيدلانيات - كلية الصيدلة - جامعة اسيوط

استهدف هذا البحث تحضير الحويصلات الدقيقة لعقار الثيوفيللين الذى يتصف بمدى مفعول قصير وذلك لزيادة زمن انطلاق وفاعلية العقار وقد استخدمت تركيزات مختلفة من مادة ايثلين فينيل اسيتات فى تحضير هذه الحويصلات الدقيقة وتم دراسة انطلاق العقار من الحويصلات فى الاوساط المشابهة لكل من عصارة المعدة والامعاء وتأثير هذه الاوساط فى انطلاق العقار من الحويصلات وايضا تأثير مادة الحويصلات على معدل امتصاص واخراج العقار فى اللعاب بعد اعطائه للانسان عن طريق الفم .

وقد توصلت النتائج الى ان الحويصلات المحضرة بأستخدام نسبة اعلى من اثيلين فينيل اسيتات تعطى اعلى قدر من زمن الانطلاق فى التحارب المعملية وتعطى ايضا تركيزا للعقار فى اللعاب لفترة زمنية اطول وان هناك علاقة قوية بين معدل انطلاق العقار فى التحارب المعملية والتوافر الحيوى له .

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