

PREPARATION AND EVALUATION OF ISONIAZID MICROCAPSULES

S.M.Safwat* and F.A.Omar**

*Pharmaceutics Department, Faculty of Pharmacy, ** Pharmaceutica Medicinal Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

ABSTRACT

Spherical microcapsules of isoniazid (INH) using ethylcellulose and ethylene vinylacetate copolymer (EVA) were prepared by a coacervation-phase separation method. The dissolution characteristics of isoniazid from microcapsules with different concentrations of EVA were almost independent of the content of EVA in phosphate buffer PH 7.4 but dissolution properties of microcapsules in 0.1N HCl and water were found to be affected by the content of EVA and there was a negative correlation between the release rate and the content of EVA in the microcapsules. Kinetic data revealed that the release mechanism from microcapsules followed diffusion controlled and zero order kinetic.

The bioavailability of INH microcapsules was studied in rabbits. The mean maximum serum levels (C_{max}) and time to maximum serum levels (t_{max}) were significantly different for INH microcapsules prepared using ethylcellulose only and those prepared with ethylcellulose and EVA copolymer, compared with INH powder. With regard to the area under the curve (AUC) value, there was no significant difference between INH powder and INH microcapsules.

INTRODUCTION

Nowadays the concept of microencapsulation received increasing attention as a means of formulating pharmaceuticals for controlled release purposes.

Shan-Yang Lin and Juei-Chyi Yand¹ investigated the use of ethylene vinylacetate copolymer in the preparation of microcapsules. Also, Takada et al.² evaluated the

EVA copolymer as a carrier for controlled release rate of a potent anticancer^{3,4}, pilocarpine HCl ; Corticosteroids (prednisolone)⁵; (hydrocortisone)⁶ ; fluoride ion⁷ ; and macromolecules such as proteins⁸. The EVA copolymer can be used to provide controlled release of hydrophilic drugs⁹.

Isoniazid (INH) (pyridine [4]-carboxyhydrazide) is the hydrazide of isonicotinic acid and is the most widely used chemotherapeutic agent for the treatment of pulmonary tuberculosis and extrapulmonary lesions including meningitis and genitourinary diseases^{10,11}. Also, isoniazid has been used in the treatment of lupus vulgaris and leprosy. The release of isoniazid from ethylcellulose microcapsules was previously studied^{12,13} without EVA in the coat. The present investigation was undertaken to determine by means of in vitro experiments the amount of isoniazid released from EC-EVA copolymer microcapsules. The pharmacokinetic parameters of isoniazid from its microcapsules was also depicted.

EXPERIMENTAL

A. Materials:

Isoniazid (Bayer) ; ethylcellulose (Daw. chemical Co., MS.) ; ethylene vinylacetate copolymer (EVA) containing 33% w/w vinylacetate (MSD) Germany ; Tetra methyl ammonium hydroxide (TMAH) ; perchlo-

ric acid ; methyl alcohol (HPLC grade) ; 4-hydroxy benzaldehyde ; dibasic sodium phosphate, dihydrogen sodium phosphate, n-hexane, cyclohexane, hydrochloric acid, methylene chloride ; all chemicals used are of pharmaceutical grade and used as received.

B. Equipment:

-spectrophotometer, UV-150-02, Shimadzu, Japan.

-MLW VEB MLW MEDIZINTECHNIK LEIPZIG centrifuge, Made in GDR Type T51.

-ERWEKA - APPARATEBAU -GmbH. Dissolution Heusenstamm. Fed. Rep. of Germany Type DTD6.

-SP 8810 LC pump (precision isocratic pump) Spectra-Physica. Fed-Rep. Germany. Dupont Instruments U.V.

Spectrophotometer Analytical instrument division.

Wilmington Delaware E.I. dupont de Nemours and Co.

(Inc.). PM 8251 single-pen recorder Made in Holland PHILIPS.

C. Methods:

Preparation of isoniazid microcapsules:

Isoniazid microcapsules were prepared by the phase-separation coacervation technique ; using EVA copolymer and ethylcellulose.

Three hundred milliliters of cyclohexane solution containing the required amount of EVA copolymer were placed in a 500 ml three necked round bottomed flask equipped with a stirrer ; a thermometer and reflux condenser. Isoniazid (3 gm) and ethylcellulose (3 gm) were added to the stirred cyclohexane - EVA copolymer solution at room temperature. The system was then heated to 82° C to form a homogeneous suspension. With continuous stirring, the system was allowed to cool to 24° C, and was then stirred for another 10 minutes. During the cooling process,

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 hours. Isoniazid microcapsules (40-60 mesh ; 250-450 Mm) were used for the subsequent studies.

Dissolution studies:

The dissolution of microcapsules containing the equivalent amount of 50 mg of INH were tested in USP XX paddle dissolution apparatus, at a constant speed of 50 rpm using each of 250 ml 0.1N HCl, water or phosphate buffer PH 7.4 for seven hours, so that sink conditions were maintained during the study¹⁴. The dissolution media were sampled at 0.5, 1, 2, 3, 4, 5, 6, 7 hours. Equal volume of dissolution medium was immediately returned to the dissolution flask after each sampling. The samples were assayed spectrophotometrically for isoniazid content at λ max 267 nm proved to be valid.

Bioavailability studies:

Healthy rabbits weighing (2-2.5 kg) were selected for the study. Isoniazid microcapsules were given orally to each of six rabbit a single dose of (25 mg) of INH after an overnight fast. Food was withheld from 12 hours before to 6 hours after dosing. Serial blood samples were taken from the ear vein all over 24 hours. Sera were immediately separated by centrifugation at 7000 rpm for 15 minutes and stored at -20°C. Before assay, the serum was allowed to reach room temperature, vortexed and centrifuged. INH was assayed by using high performance liquid chromatography (HPLC) method¹⁵.

Each sample was assayed in comparison with calibration curve containing serum from each rabbit before each treatment (time=0) spiked with known amounts of synthesised hydrazone derivative with 4-hydrox-

ybenzaldehyde. The synthesis of the hydrazone derivative with 4-hydroxybenzaldehyde:

Procedure:

To a stirred solution of 4-hydroxybenzaldehyde (0.619, 5 mmole) in 10 ml methanol was added a solution of isonicotinic acid hydrazide (0.675 g; 5 mmole) in 10 ml of water. The mixture was stirred for 30 minutes at room temperature and the precipitated product filtered off and dried. Recrystallization from aqueous ethanol give 1.0 gm of the crystalline product (82 %), m.p. 283-5°C, IR cm^{-1} ; 3260 cm^{-1} (OH), 1650 cm^{-1} (CO-NNH⁻¹); UV; (methanol): λ max 320 nm; 226 nm.

Calibration curve :

An amount of 2.7 mg of the pure crystalline hydrazone (as external standard) was dissolved in 10 ml methanol to give a stock solution 1.1×10^{-3} M. One ml. of this solution was diluted to 10 ml with methanol. Aliquots of the final solution were injected into HPLC and the corresponding peak areas at the specified retention time were recorded.

HPLC assay:

Stock solution (INH - 4 HBA external hydrazone in methanol) 1.1×10^{-4} M; Solvent: MeOH : H₂O : HClO₄ 70% : TMAH (400:1600:1:1). Column: RPC - 18, 25 cm x 4.5 mm; with cartridge precolumn as guard column. Flow rate: 1.5 ml/min. Sensitivity: 0.005 and λ max 320 nm. Chart speed: 0.5 cm/min. Observed retention time: 9.4 min.

The correlation coefficient of the injected volumes in μl and peak Height (mm) was $r = 225.622$ and the intercept $a = 0.588$ according to the equation $Y = a+bc$ and the relation was depicted as pseudo-first order relation.

RESULTS AND DISCUSSION

The average yield of microcapsules from tested microcapsules was $92.8 \pm 1.5\%$ and the percent of isoniazid entrapped in the microcapsules was 90.1 ± 1.2 . Microscopic examination of the encapsulated microcapsules suspended in cyclohexane showed them to be spherical and coated with thin layer of EVA-copolymer and ethylcellulose¹⁶.

In Vitro dissolution studies:

In order to elucidate the mechanism of drug release from EVA-EC microcapsules, dissolution studies were conducted in three different dissolution media, 0.1N HCl, distilled water and phosphate buffer (pH 7.4), respectively.

The dependency of the drug dissolution profile from microcapsules prepared with different ratios of EVA copolymer is illustrated in figures. (1-3) INH microcapsules A, B, C, D and E prepared with 0, 0.25, 0.5, 1 and 2% EVA copolymer dissolved slowly over seven hours in phosphate buffer pH 7.4 especially the microcapsules (E).

The analysis of the kinetic data obtained from the release of INH were summarized in Table 1. From which it is observed that the mechanism of the release of INH from microcapsules following the diffusion model and agreed with both zero-order and square-root of time equations (figer 4).

As shown from the tables that the release rate of INH from microcapsules was approximately constant in phosphate buffer pH 7.4 and was inversely proportional to the increased amount of EVA copolymer in the microcapsules. The correlation between the percent amount of EVA

and the release rate from microcapsules was always negative.

It can be seen from figure. 5, that the release of INH shows maximum decrease in phosphate buffer pH 7.4 and constant release in acidic pH media. The decreased order in the release rate is as follows in acidic media:-Microcapsules A> C> B> E> D, respectively.

The controlled released can be seen in phosphate buffer pH 7.4 where the pka of INH equals 1.85 less than the pka of INH in acidic pH which equals 10.77¹⁷. H. Tomida *et al.*¹⁸ reported that the increased release rate of diclofenac sodium from the gel resulted as the decreasing of pH in alkaline medium.

Bioavailability studies:

Actual drug concentrations in blood plasma samples were determined using HPLC method¹⁵. It was necessary to synthesise a hydrazone derivative to use as an external standard. (calibration curve figure. 6 representing relation between concentration and corresponding peak heights was developed). The correlation coefficient was 0.9997.

The bioavailability and pharmacokinetic parameters were estimated from the plasma concentration of INH after administration of either INH powder INH microcapsules (A) without EVA copolymer and INH microcapsules (E) with 2% w/w EVA copolymer in the coat.

The plasma concentration versus time curves of INH in rabbits corresponded to a one compartment open model, as shown in figure 7.

The absorption of INH from the pure powder was very rapid and high; where as the microencapsulated INH with 2% w/w EVA copolymer produced less and gradual decrease in the absorption and a more sustained profile of the plasma levels of isoniazid.

The mean values of the maximum plasma level (C_{max}) and time to maximum plasma level (t_{max}) were significantly different ($p>0.05$) for microcapsules (A) and microcapsules (E) compared with that of INH powder.

The area under the curve (AUC) was obtained by using the trapezoidal rule. The values of AUC of both INH powder and microcapsules (A) were significantly different ($p>0.05$) and the AUC of microcapsules (E) was highly significant ($p>0.001$) different from microcapsules (A). This may be due to the higher value of the INH plasma concentrations of microcapsules (E). As seen from figure 4 the absorption characteristics of isoniazid after oral administration differ to a certain extent. Also, as observed for the drug, the oral administration of microcapsules resulted in a corresponding variation in the absolute bioavailability.

In conclusion, isoniazid microcapsules prepared using ethylcellulose and ethylene vinylacetate copolymer forming coat give high level of drug bioavailability in comparison to those microcapsules prepared by using ethylcellulose only as the coat.

Table 1:- Kinetic characteristics of INH released from Microcapsules

(a) Q/t									
Microcapsules	phosphate buffer PH 7.4			0.1N HCL			Distilled Water		
	r	slope	intercept	r	slope	intercept	r	slope	intercept
A	0.9982	3.6008	3.2545	0.9984	5.4503	2.5418	0.9981	4.9004	1.9314
B	0.9960	3.0649	2.6293	0.9992	4.7947	1.9165	0.9964	4.6568	2.4444
C	0.9964	3.7059	2.3317	0.9988	5.2291	1.6841	0.9949	5.6679	2.1635
D	0.9957	3.5407	2.9676	0.9987	4.3677	2.2896	0.9941	3.8658	2.1313
E	0.9961	3.4986	2.4258	0.9988	4.3473	1.6831	0.9974	4.1583	1.4594

(b) log Q/log t									
Microcapsules	r	slope	intercept	r	slope	intercept	r	slope	intercept
	A	0.9661	0.4105	0.5154	0.9546	0.4341	0.5402	0.9879	0.4415
B	0.9588	0.4255	0.4311	0.9546	0.4894	0.4814	0.9769	0.4265	0.5468
C	0.9522	0.4674	0.4185	0.9571	0.5066	0.4638	0.9786	0.4511	0.5865
D	0.9649	0.4274	0.4914	0.9652	0.4375	0.5147	0.9865	0.4173	0.5016
E	0.9545	0.4641	0.4016	0.9601	0.4651	0.4444	0.9748	0.4578	0.4403

r: Correlation coefficient.

Q: Amount of drug released at time t in hours.

Table 2:- The Mean plasma levels following administration of INH microcapsules

Time (minutes)	plasma concentration (ug)		
	powder INH	Microcapsules	
		A	E
15	1.15(+0.56)	1.85(+0.37)	1.65(+0.25)
30	2.30(+0.64)	4.00(+0.55)	2.75(+0.33)
45	7.90(+0.35)	9.40(+0.56)	3.65(+0.15)
60	4.3 (+0.46)	2.30(+0.71)	2.55(+0.19)
240	1.85(+0.32)	1.11(+0.66)	1.85(+0.50)
120	1.20(+0.33)	0.65(+0.34)	1.75(+0.45)
180	0.55(+0.76)	0.42(+0.32)	1.35(+0.33)

Table 3:- Pharmacokinetic parameters of INH Microcapsules after Oral Administration in Rabbits.

Formula	* C _{max}	t	AUC	I _{rel}	t ^{1/2}	F ^{**}
	Mg/ml ⁻¹	max, n	Mg, n. ml ⁻¹	min ⁻¹	h	
Pure INH Powder	2.2812±0.65	5.13925 ±0.95	531±0.75	0.0457	1.516	--
INH Microcapsules (A) (EC)	2.7463±0.45	5.7718 ±8.50	433±0.67	0.0214	2.962	81.54
INH Microcapsules (E) (EC/EVA)	2.2919±0.77	17.08865±0.25	473±0.85	0.0142	4.883	89.08

* = Calculated C^{max}F^{**} = Absolute bioavailability from blood level data.

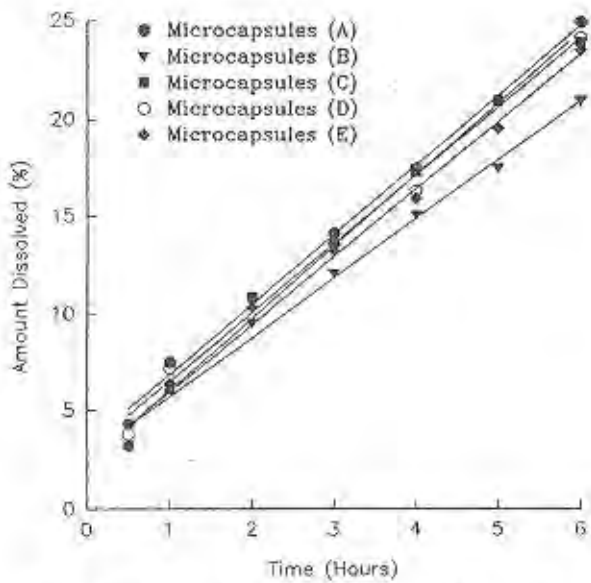


Fig. 1: Dissolution Profiles for Isoniazid Microcapsules in Phosphate Buffer pH 7.4 at 37°C.

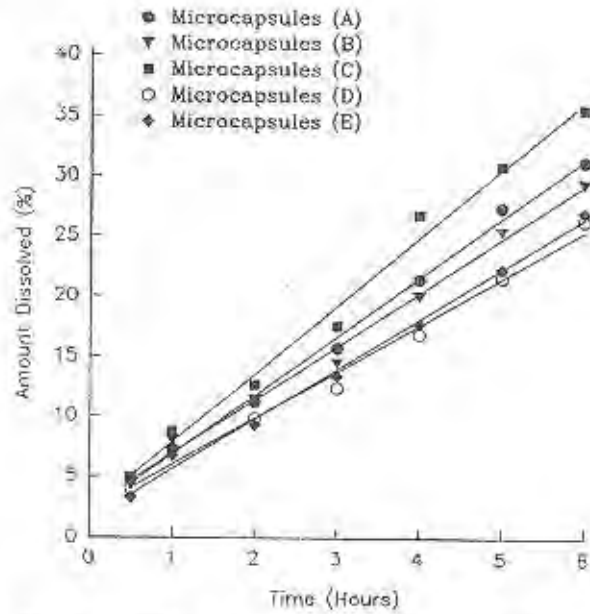


Fig. 3: Dissolution Profiles for Isoniazid Microcapsules in Distilled Water at 37°C.

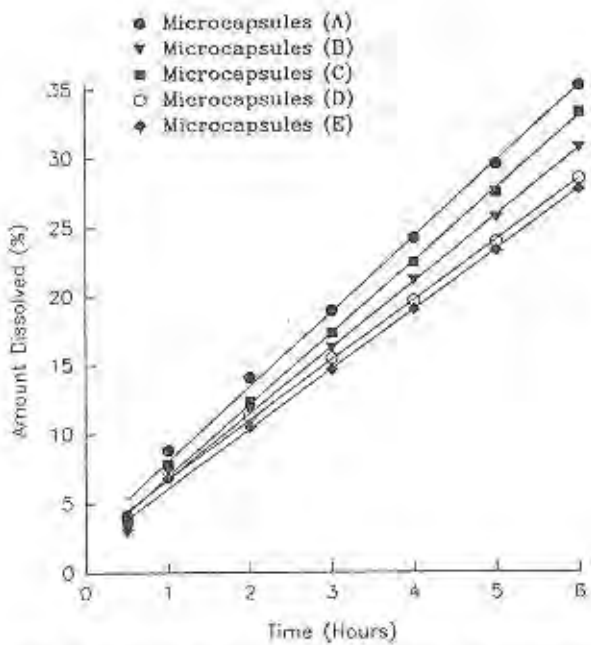


Fig. 2: Dissolution Profiles for Isoniazid Microcapsules in 0.1 N HCl at 37°C.

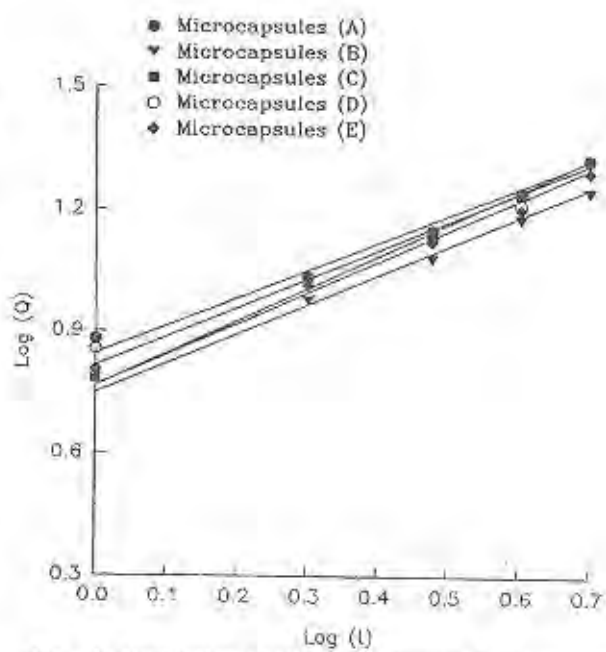


Fig. 4: Dissolution Profiles for Isoniazid Microcapsules in Phosphate Buffer pH 7.4

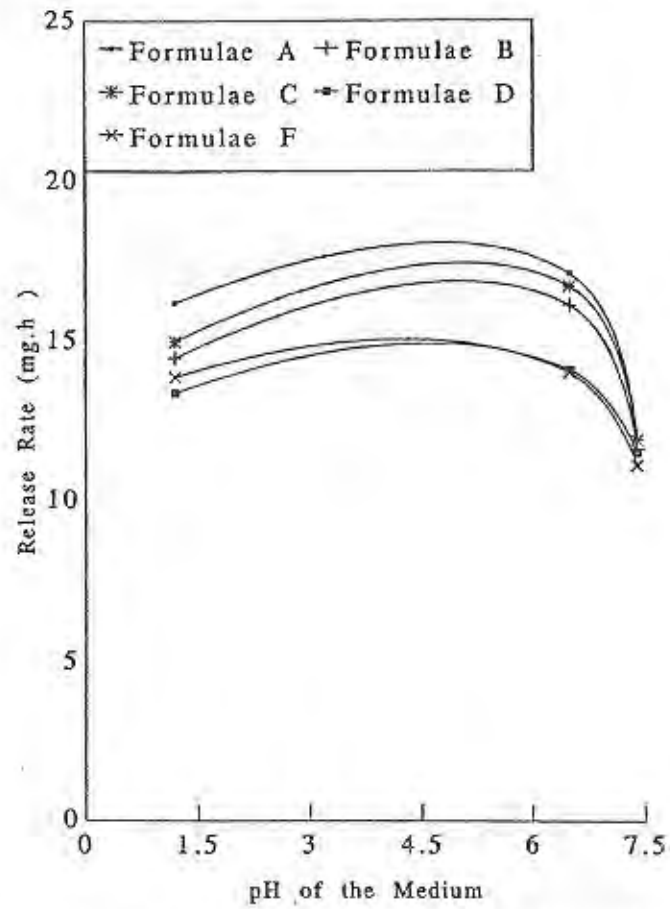


Fig. 5 : Effect of pH on the release Rate of Isoniazid from EVA Copolymer Microcapsules.

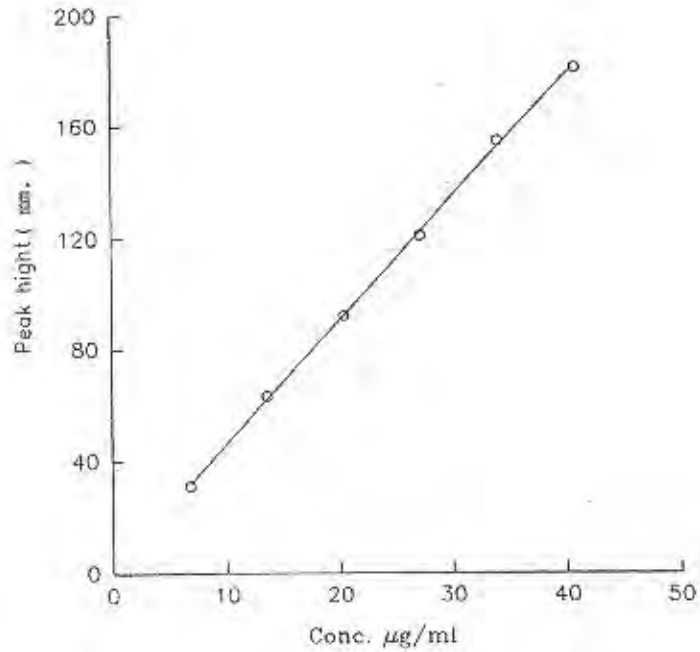


Fig. 6 : Calibration curve of INH-4-hydroxybenzaldehyde hydrazone (external standard)

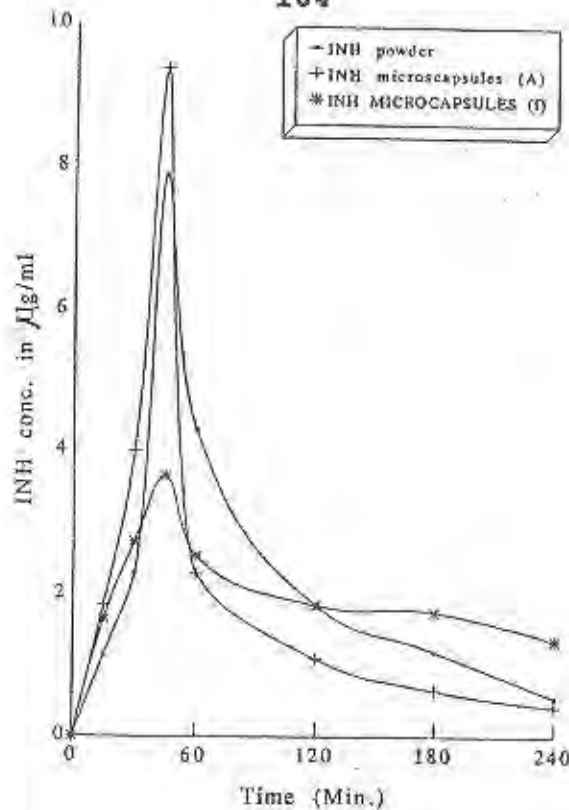


Fig. 7 : Average Blood Level Curves of INH Microcapsules.

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تحضير وتقييم حويصلات الايزونيازيد

سلوى محمود صفوت - فرغلى عبد الحميد عمر

قسم الصيدلانيات - كلية الصيدلة - جامعة اسيوط

قسم الكيمياء الصيدلية - كلية الصيدلة - جامعة اسيوط

فى هذه الدراسة تم تحضير حويصلات تحتوى على ايزونيازيد باستخدام عديد من البلمرات ايثيلين فينيل خلات وايثيل السليلوز. وتم دراسة انطلاق العقار من الحويصلات تحت تأثير استخدام تركيزات مختلفة من عديد البلمرات.

وقد وجد أنه بزيادة نسبة ايثيلين فينيل خلات مع ايثيل السليلوز زاد التحكم بالنقص فى معدل انطلاق العقار فى محلول الفوسفات المنظم عند الأس الهيدروجينى ٧.٤ وعند اعطاء الحويصلات بالفم لمجموعات من الارانب وتقدير كمية العقار فى بلازما الدم لمدة ٢٤ ساعة باستخدام كروماتوجرافيا السوائل تحت الضغط العالى. وجد أن تركيز العقار فى الدم يوجد بدرجة عالية فى الدم عند ابتلاع الحويصلات بدون عديد البلمرات وأن تركيزات العقار فى الدم يقل بصورة واضحة عند ابتلاع الحويصلات المحضرة بعديد البلمرات وعلى ذلك فان استخدام طريقة التحوصل بمخلوط من عديد البلمرات وايثيل السليلوز لعقار الايزونيازيد أدى الى التحكم فى انطلاق العقار بالمقارنة مع استخدام العقار بدون تحوصل مطلقا.