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FORMULATION AND EVALUATION OF TOPICAL METRONIDAZOLE

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ABSTRACT

Formulation of metronidazole in the form of topical preparations did not take much attention. Thus, the aim of this work is to formulate metronidazole in the form of gel and ointment. Ten ointment bases were used viz., oleagenous, absorption, emulsion and water soluble bases. The rate of release of the drug from the water soluble polyethylene glycol base was the highest. The effect of some penetration enhancers e.g. dimethylsulfoxide, urea and ethanol on the release rate of the drug from the water soluble and the o/w emulsion bases was studied.

For metronidazole gels, several cellulose derivatives were used viz., methylcellulose, carboxymethylcellulose, sodium carboxymethylcellulose, methocel, Eudragit RL 100 and Eudragit RS 100. The in-vitro release of the drug was maximum from methylcellulose gel followed by Eudragit RL 100. In general the in-vitro release of metronidazole from gel was higher than that from ointment bases.

Preliminary clinical investigations of metronidazole in an ointment and in a gel formulae were performed. These preliminary studies were impressive but further clinical studies are required before recommending metronidazole as an effective topical therapy.

INTRODUCTION

Metronidazole has been used in the treatment of various anaerobic infections, leishmaniasis, mononucleosis, skin disorders, syphilis, topical eosinophilia and vaginitis vincent's infections [1]. Recently trials have been made to formulate metronidazole in the form of cream[1], lotion[1], ointment[2] and gel[3] for topical application for the treatment of some skin diseases. Five ointment bases have been investigated[2], for the *in-vitro* release, stability and the *in-vivo* absorption of the drug through the skin of rabbits[2]. Generally it was found that the formulae tested showed excellent stability independent of drug concentration or type of ointment base. The *in-vitro* data correlated well with the *in-vivo* absorption studies as the release and absorption rate were increased in the following order: polyethylene glycol, w/o emulsion, carbapol gel and oleagenous base[2]. On the other hand metronidazole gel have been developed and used for controlling the offensive odour from fungating tumours and other skin lesions[3].

The aim of this study is to formulate and evaluate metronidazole topical formulations in the form of ointment and gel. Moreover, preliminary clinical studies of the prepared metronidazole topical formulations were assessed on human volunteers complaining either from cutaneous leishmaniasis,schistomiasis or rosacea.

EXPERIMENTAL

Materials:

Metronidazole and Methocel E_{15} kindly supplied by the Nile Co. for Pharm. and Chem. Ind. Cairo, Egypt, PEG 400 and PEG 4000 and N,N dimethylsulfoxide (Prolabo, France), Span 65 (Roth, GFR.), Propylene glycol (Vel-Bios, Leuven, Belgium). Tween 80 (Merck-Schuchardt, Munchen, West Germany), Sodium carboxymethylcellulose (Roth-OHG Karlsruhe, West Germany), Urea (Fluka-West Germany), Sodium lauryl sulphate (Cambrian Chemicals, Beddington, Farm Road, Croydon, England) Carboxymethylcellulose, Sodium hydroxide, Potassium dibasic phosphate and Ethyl alcohol (El-Nasr Pharmaceutical Chemicals Co., Egypt), Methylcellulose (BDH Chemical Ltd. Poole, England). Eudragit RS 100 and Eudragit RL 100 (Rohm Pharma GMBH Darmstadt, West Germany). Methyl parahydroxybenzoate (M.W.Hardy Co. Ltd. London) and Propyl parahydroxybenzoate (BP/USP Naarden International, Holland), White soft paraffin, Beeswax, anhydrous lanolin, hard paraffin, Cetostearyl alcohol and Stearyl alcohol and Triethanolamine. All chemicals were either of analytical or pharmaceutical grade and were used without further purification.

Methodology :

Preparation of Ointments :

The constituents of the ointment bases used are illustrated in Table 1.

Table 1 : Composition of Ointment Bases.

Base Type	Composition	Concentration % w/w
Oleagenous		
a_1	White soft paraffin	100
a_2	White soft paraffin	95
	beeswax[4]	5
Absorption bases		
b_1	White soft paraffin	90
	anhydrous lanolin[5]	10
	Hard paraffin	24
b_2	wool alcohol	6
	white soft paraffin	10
	liquid paraffin[6]	60
Water soluble bases		
c_1	Polyethylene glycol 4000	40
	Polyethylene glycol 400[4]	50
	Polyethylene glycol 4000	47.5
c_2	Polyethylene glycol 400	47.5
	Cetyl alcohol	5.0
Emulsion bases		
(w/o)		
d_1	Emulsifying	30
	white soft paraffin	50
	liquid paraffin	20
	emulsifying wax :	
	Sodium lauryl sulphate	10
	Cetostearyl alcohol	90
	Water[6]	4
d_2	White soft paraffin	64
	Span 65	6
	Distilled water[5]	30
(O/W)		
e_1	Sodium lauryl sulphate	1
	Propylene glycol	12
	Stearyl alcohol	25
	White petrolatum	25
	Purified water[4]	37
e_2	Stearyl alcohol	25
	White petrolatum	25
	Glycerin	12
	Tween 80	5
	Distilled water[5]	33

The fusion method was used to prepare the oleagenous or absorption bases. At the possible low temperature the drug 0.25,0.8% or 1% w/w) was incorporated in the melted base and vigorously. triturated until cold to achieve homogeneity of the drug in the base. To prepare the emulsion bases, the drug alone or with 10% of ethyl alcohol, dimethylsulfoxide and urea as enhancers were dissolved or suspended in the aqueous phase, then warmed and incorporated into the melted oleagenous phase, trituration was continued until complete cooling and a homogeneous ointment was formed. The water soluble bases were prepared according to USP XXI requirements[4]. The drug was dissolved alone or with the above enhancers.

Preparation of Gels :

Metronidazole at different concentrations (0.25, 0.5, 1,2 or 5%) was prepared in different gel bases Table 2. A weighed amount of methylcellulose, carboxymethylcellulose, sodium carboxymethylcellulose or methocel was gently added to the aqueous solution of metronidazole mixed thoroughly using a magnetic stirrer and a bar. The container was left overnight to ensure complete mixing. Eventually in each case a clear viscous-gel is formed. Eudragit RS and RL gels were prepared by addition of PEG 600 as plasticizer and triethanolamine as a neutralizing agent. Gel formulations were prepared one day prior to use and a separate formulation without the drug (as a control) was prepared for each experiment.

Storage of Ointments and Gels:

All ointments and gels were stored in wide mouthed tightly closed plastic containers at room temperature. Preservatives viz., methyl paraben (0.15%) and propyl paraben (0.02%) were added to the stored samples.

In-Vitro Release of Metronidazole from Ointments and Gels:

Two gm from each formulation was accurately weighed and placed on a semipermeable cellophane membrane to occupy 1.5 cm diameter. The loaded membrane with the sample of ointments or gels was stretched over the end of an open ended glass tube (1.5 cm in diameter) which was made tight with rubber band. A volume of 2 ml phosphate buffer of pH 6.8[4] was poured into the tube. The tube was suspended so that the membrane was just below the surface of 100 ml buffer (pH 6.8), at 37 ± 0.5°C contained in 250-ml beaker and was shaken at 25 rpm. at specified time intervals, samples of 5 ml were withdrawn for analysis and were replaced with equal volume of fresh release medium at 37 ± 0.5°C. The amount of metronidazole released was assayed spectrophotometrically at 320 nm using blank similarly treated.

The assay procedure was found to be valid for metronidazole determination. A linear relationship was found between the absorbance and concentration (2-14 ug) of metronidazole (correlation coefficient=0.998). The components of the ointment or gel bases were checked for any interference with the assay procedure and no interference had been detected.

RESULTS AND DISCUSSION

1-IN-VITRO RELEASE OF METRONIDAZOLE FROM DIFFERENT OINTMENT BASES:

The release rates of metronidazole from the tested ointment bases are represented by figures 1 A and 1 B. It is evident from these data that the water soluble bases (c_1 and c_2) exhibit the highest release rate of metronidazole followed in order by the o/w emulsion bases (e_2 and e_1), w/o emulsion base (d_1 and d_2), absorption bases (b_2 and b_1) and lastly the oleagenous bases (a_2 and a_1) Fig.1A and 1B. Figure 1B indicates that the percent of metronidazole released from base c_1 equals 13.3 and 24.8 after 30 and 60 minutes respectively.

The hydrophilic character of polyethylene glycol ointment lead to absorption of water to such extent that eventually the whole ointment would become in solution as observed throughout the experiment. Moreover, the water soluble character and high osmotic pressure of polyethylene glycol bases[7] may lead to the high and rapid release. Comparing the two water soluble bases c_1 and c_2, it was found that the release rate of metronidazole from base c_2 was slower than that from base c_1. This could be explained; that base c_2 seemed to be more viscous than base C_1, due to the presence of cetyl alcohol in the formula. Similar findings were reported by Kaiho et al[8], concerning prednisolone release from ointment bases containing different proportions of long chain fatty alcohol (Cetyl alcohol) where the release was inversly proportional to the vis-

cosity of the base[8]. Figures 1 A and 1 B showed the release of metronidazole from the emulsion bases. It was evident from the results that the release from o/w emulsion bases was higher than that from w/o emulsion bases. From these results it could be concluded that the rate of diffusion of metronidazole was mainly affected by the type of the base. This may be due to the miscibility of the external phase in the o/w emulsion base with the diffusion medium, this result was in good agreement with Attia et al[9] who found that the release of prednisone from o/w emulsion base was higher than that from w/o emulsion base[9].

From figure 1A the release of metronidazole from absorption bases was found to be higher than that from the oleagenous bases, which also showed the lowest release rate of the drug (Fig. 1A). The constituents of the absorption base were found to influence the release rate of the drug. It was observed that base b_2 showed higher release rate than base b_1. This could be attributed to the hydrophilic and emulsifying characters of wool alcohol present in the formulation of absorption base b_2[10]. Also the constituents of the oleagenous base affect the drug release rate (Fig. 1 A). It was observed that base a_2 showed higher release rate than base a_1. This could be attributed to the presence of beeswax in base a_2 which contain self-emulsifying agent which enhance the release of the drug.

2-Influence of Concentration of Metronidazole on the Release from Water Soluble Ointment Bases :

Figure 2: showed the release rate of the drug from the water soluble base (c_1) containing three concentration levels of the drug. It was clearly obvious that the % drug released was slightly increased with increasing the drug concentration in the base. Incorporation of one percent of the drug in the base resulted in the highest release rate of the drug followed in order by bases containing 0.8% drug. It was observed that up to 20 minutes the release rate from the three concentration levels seemed to be not highly different.

3-Effect of Penetration Enhancers on the Release of Metronidazole From Water Soluble and o/w Emulsion Ointment Bases:

In this study the effect of three well known penetration enhancers; dimethylsulfoxide, ethanol and urea at 10% concentration on the diffusion of metronidazole from 1% ointment in polyethylene glycol base (c_1) and in o/w emulsion (e_2) was investigated (Fig.3 and 4). From Figure 3, it was observed that there was nearly no effect of enhancers on the release of metronidazole from the water soluble base. On the other hand, the release of metronidazole was slightly increased on adding 10% N,N-dimethylsulfoxide to the o/w emulsion

formula, followed in order by ethyl alcohol and urea (Fig. 4).

The mathematical evaluation of the *in-vitro* release of the drug was done by using simplified Higuchi equation, zero and first order kinetics[11]. Using these equations the diffusion coefficients, release rate constants and correlation coefficients from different ointment bases were calculated.

The release from suspension of the drug into homogeneous matrix made of soft paraffin and the formation of a saturated layer would account for first order. While, release from multiple bases a_2, b_1, b_2, d_1, d_2 and e_1 may be by diffusion controlled mechanism as evident by correlation coefficient values (0.993-0.997). On the other hand, the release of the drug from water soluble bases in which the drug is completely soluble in the vehicle has to be controlled by the partition mechanism between the preparation and the dissolution medium i.e, follows a zero order and the results obtained confirm this mechanism. The negligible increase in release rate with increase in concentration is another proof, since the confirmation of the diffusion mechanism is the profound increase in release rate by the increase in concentration. In addition, Higuchi publication cited immiscibility of the base in the release medium[12]. So, this mechanism can not be applied to water soluble bases

Metronidazole Gel:

In this study the *in-vitro* procedure was used to investigate the release of metronidazole from different gel formulation. Several bases were used for this study with different concentrations (Table 2).

Table 2 : Composition of Gel Bases.

Gel	Concentrations % w/v
Methylcellulose	5.0
Carboxymethylcellulose	2.5
Sodium carboxymethylcellulose	2 and 2.5
Methocel E15	9.0
Eudragit RS100	10.0
Eudragit RL100	10 and 15

1-Effect of Different Gel Bases on the In-Vitro Release of Metronidazole :

Figure 5 showed the *in-vitro* release of metronidazole from different gel bases. It was observed that the amount of metronidazole released was greater from methylcellulose gel than from the other formulae followed in order by Eudragit RL, Eudragit RS, Methocel, sodium, carboxymethylcellulose and carboxymethylcellulose.

The difference in drug release observed from gel formulae may be attributed to the difference in the viscosity of the polymer. In this respect sodium carboxymethylcellulose 2% showed better release than 2.5% and Eudragit RL in 10% concentration showed better effect than 15% (Fig. 5).

2-Effect of Metronidazole Concentrations on the Release from Methylcellulose and Eudragit RL Gel Bases:

Figures 6 A and 6 B showed the effect of metronidazole concentrations on its release from both methyl-

cellulose 5% and Eudragit RL 10%. The concentrations used were 0.25, 0.5, 2 and 5%. It was observed that the amount drug released from both bases was higher from the formula containing the smallest concentration of metronidazole (0.25%) at the initial release period, but at the end, the release rate seemed to be concentration dependent and an infolding point could be observed after 46 minutes.

Analysis of the data according to different kinetic mechanisms viz., Higuchi, zero and first order kinetics, revealed that the release pattern of metronidazole from the tested bases followed first order mechanism as indicated by the correlation coefficient values (0.980-0.996).

Comparing the release rate of the drug from ointments and that from gels (Fig. 1&5) it could be concluded that the release rate of the drug from gel bases was higher than that from ointment bases at the same concentration level. This finding could be explained by the reported suggestion that the aqueous based gel on starting the experiment mixed with and was readily dissolved by the water that penetrated the membrane, this is in contrast to what has been observed with ointments especially the fatty based ones[13].

The release rates of metronidazole from different ointment and gel bases were redetermined periodically after 2,4 and 7 months. It was worthy to observe that the release rate of the drug from various ointment and gel bases was not affected by aging (Table 3).

Table 3 : Diffusion Coefficient (D) of Metronidazole from Different Ointment and Gel Bases According to Higuchi Diffusion Mechanism Before and After 7 months aging.

Formula	Diffusion coefficient D(cm^2/sec.) Before aging	After aging
1- Ointments		
o/w emulsion base e_2	0.8007	0.800
Water soluble base c_1	2.3080	2.2862
2- Gels		
Methylcellulose 5%	3.6271	3.4651
Eudragit RL_{100} 10%	3.3518	3.1846

Clinical Studies :

Two preparations of metronidazole viz., metronidazole polyethylene glycol ointment and metronidazole methylcellulose gel were subjected to clinical studies. The drug was incorporated at a concentration level of 0.25% and 0.8% in both preparations. The two topical preparations were applied to Egyptian patients residents of the Dermatology Department, Assiut University. The patients were suffering either from cutaneous leisnmaniasis, schistomiasis or rosacea.

The infected patients were followed up for improvements by specialized dermatologists. Preliminary clinical studies showed that the preparations are promising in the treatment of cutaneous leishmaniasis (Fig. 7), schistomiasis and rosacea.

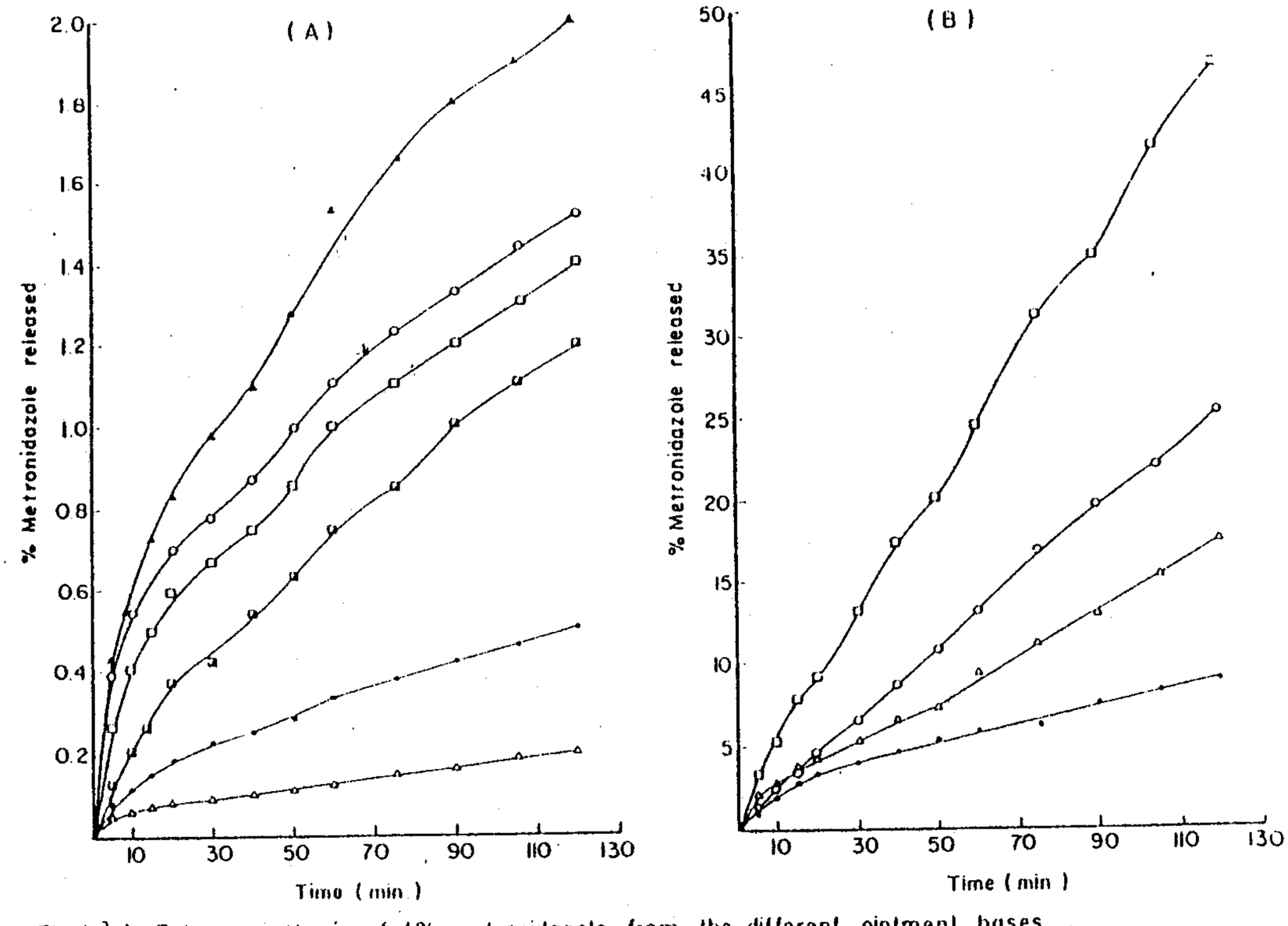

Fig.(1): Release pattern of 1% metronidazole from the different ointment bases

(A) Oleagenous bases △ a_1, • a_2 absorption bases ▫ b_1, ○ b_2, W/O emulsion bases ▲ d_1, ○ d_2

(B) O/W emulsion bases • e_1, △ e_2, water soluble bases ○ c_1, ○ c_2

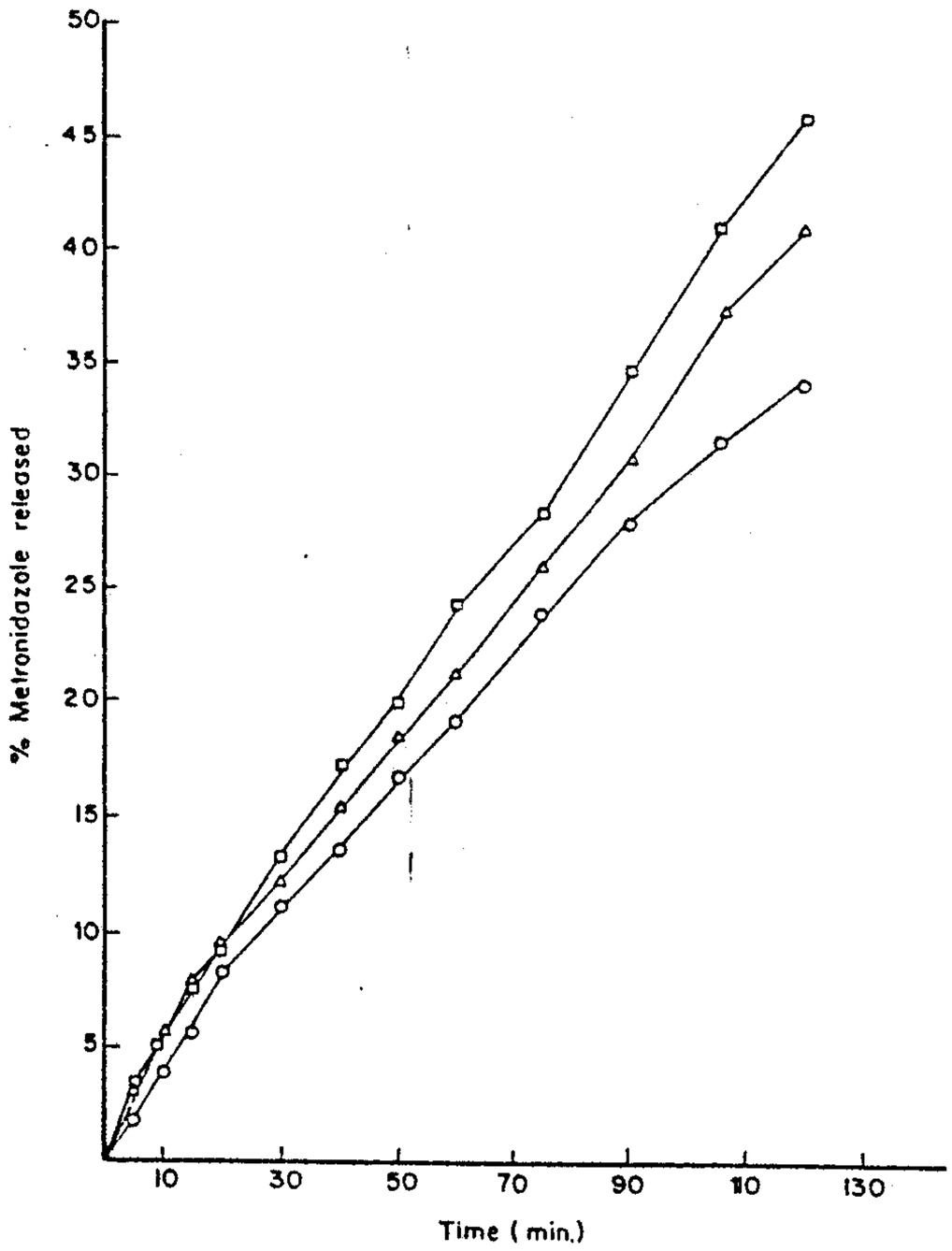

Fig.(2): Effect of concentration of metronidazole on the release from the water soluble ointment base ○ 0.25 %, △ 0.8 %, ▫ 1 %

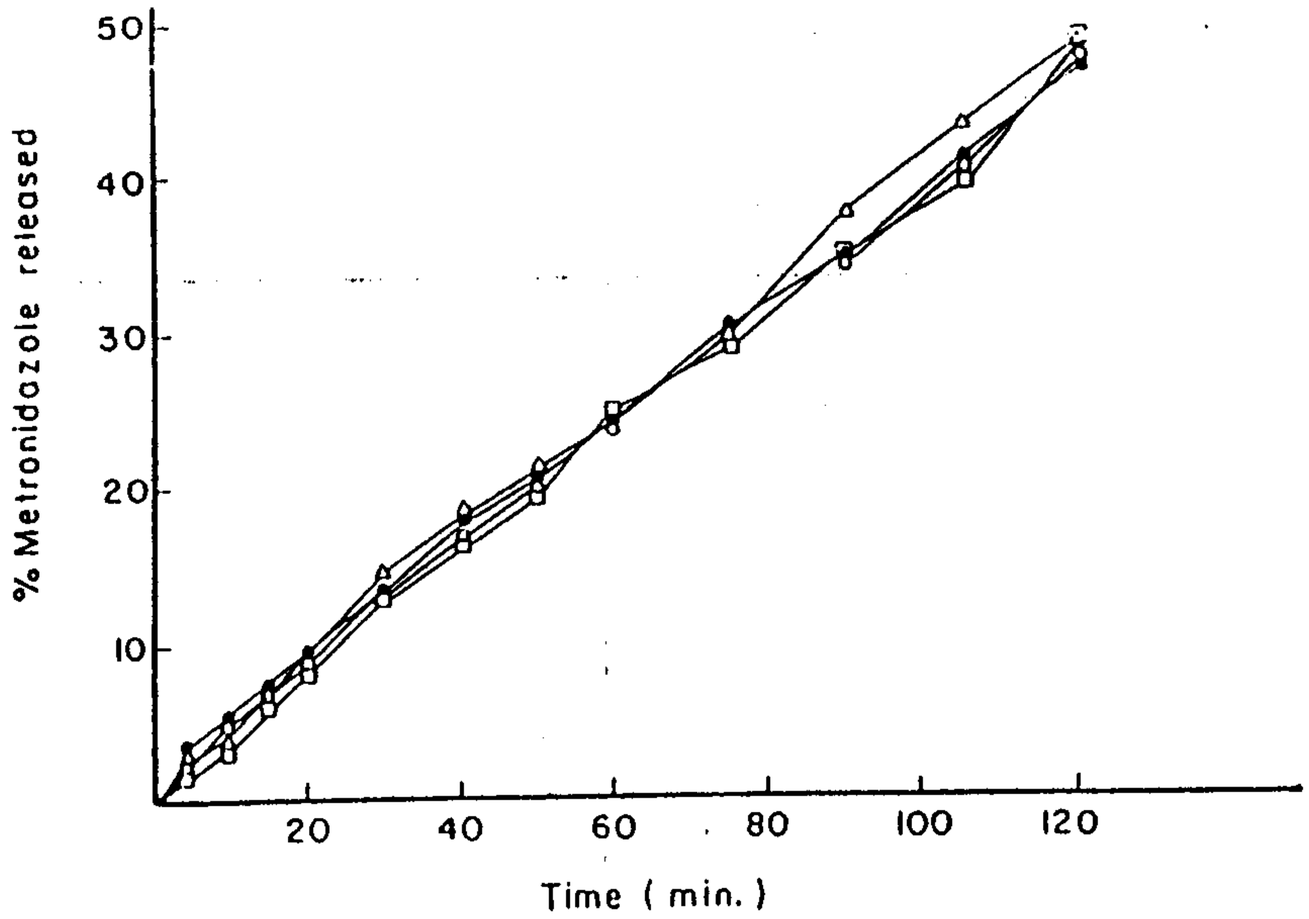

Fig.(3): Effect of penetration enhancers on the release of metronidazole from the water soluble base .
• Control , ∘ Urea , ▵ Alcohol, ▫ N , N dimethylsulfoxide .

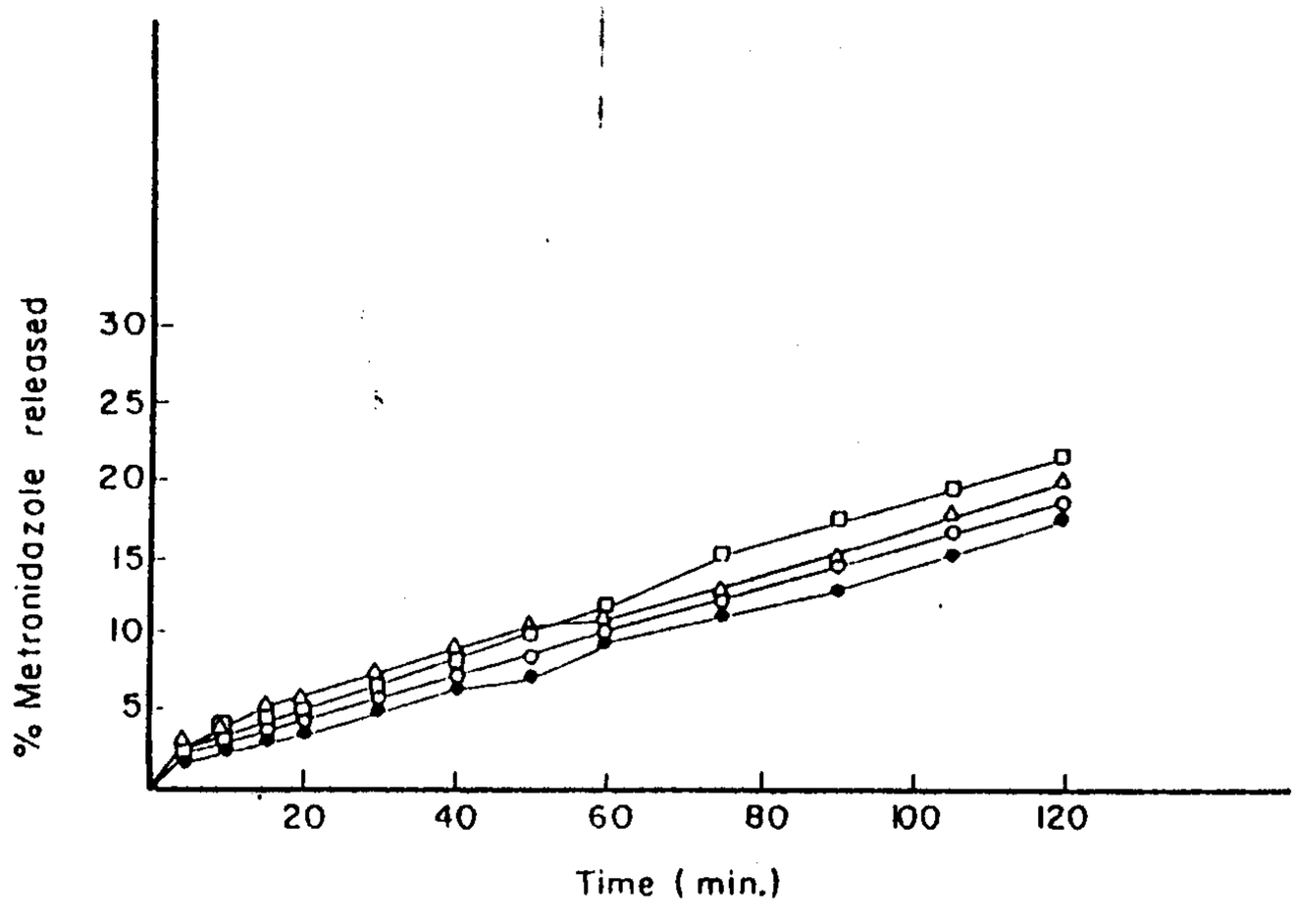

Fig.(4): Effect of penetration enhancers on the release of metronidazole from the emulsion base .
• Control , ∘ Urea , ▵ Alcohol , ▫ N , N dimethylsulfoxide .

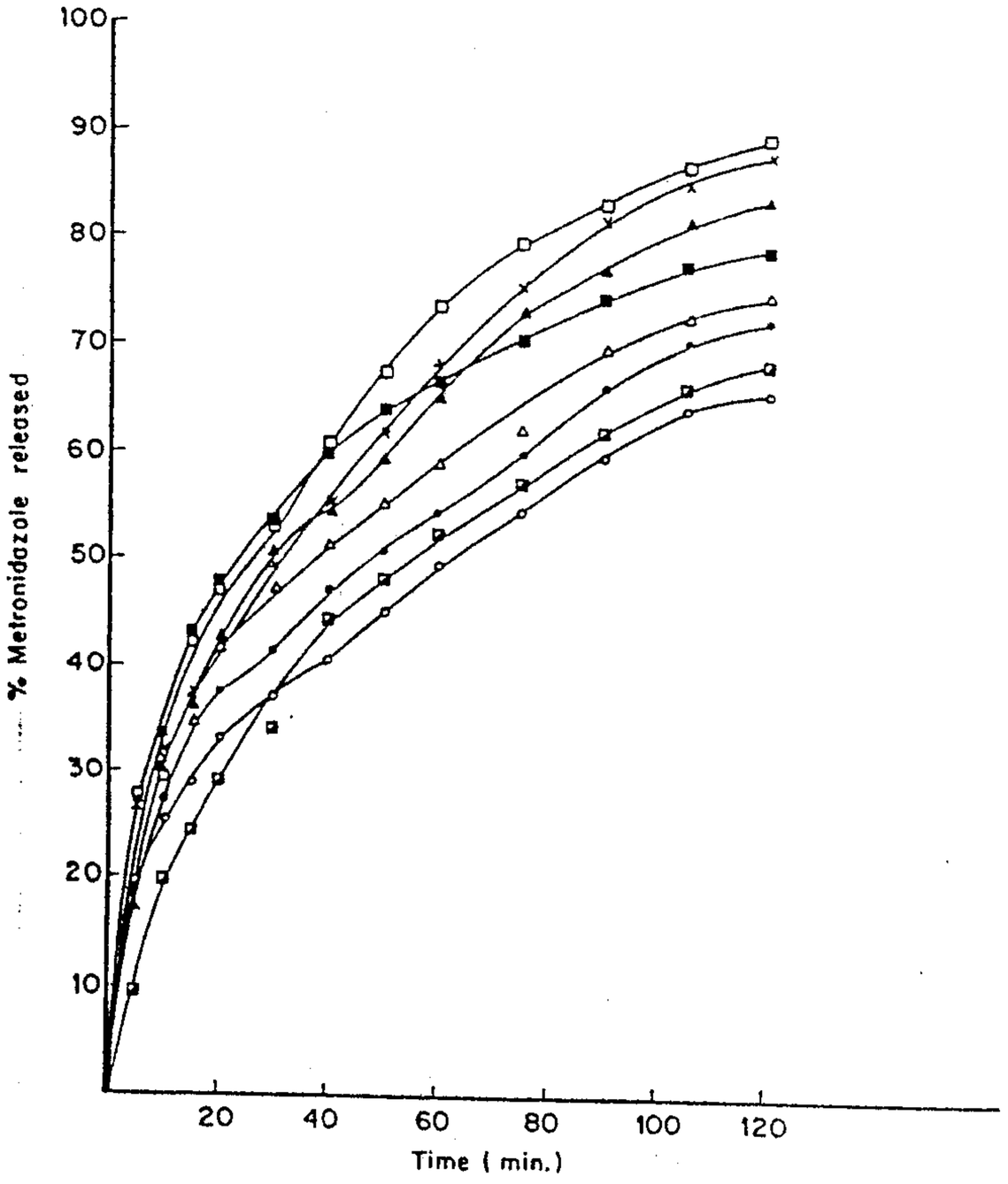

Fig.(5) : Release pattern of 1% metronidazole from the different gel formulations □ Methylcellulose 5% , x Eudragit RL_{100} 10 % , ▲ Eudragit RS_{100} 10% , ■ Methocel 9 % △ Sodium carboxymethylcellulose 2 % , • Sodium carboxymethylcellulose 2.5 %, ▫ Eudragit RL_{100} 15 % and ○ Carboxymethylcellulose 2.5 % .

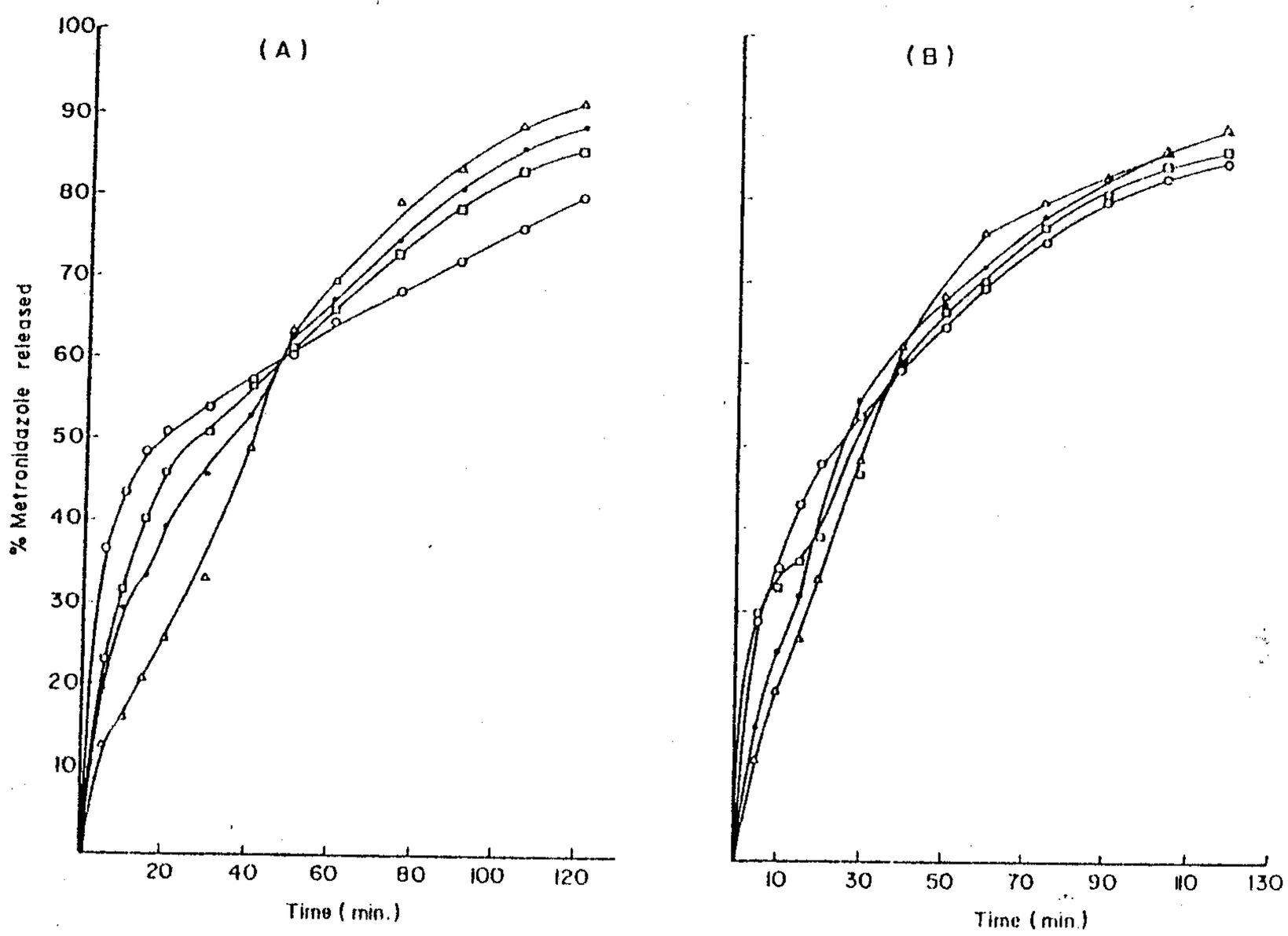

Fig.(6) : Effect of the concentration of metronidazole on the release from 5 % Methylcellulose (A) and 10% Eudragit RL_{100} (B) ○ 0.25 % , □ 0.5 % , • 2 % , △ 5%

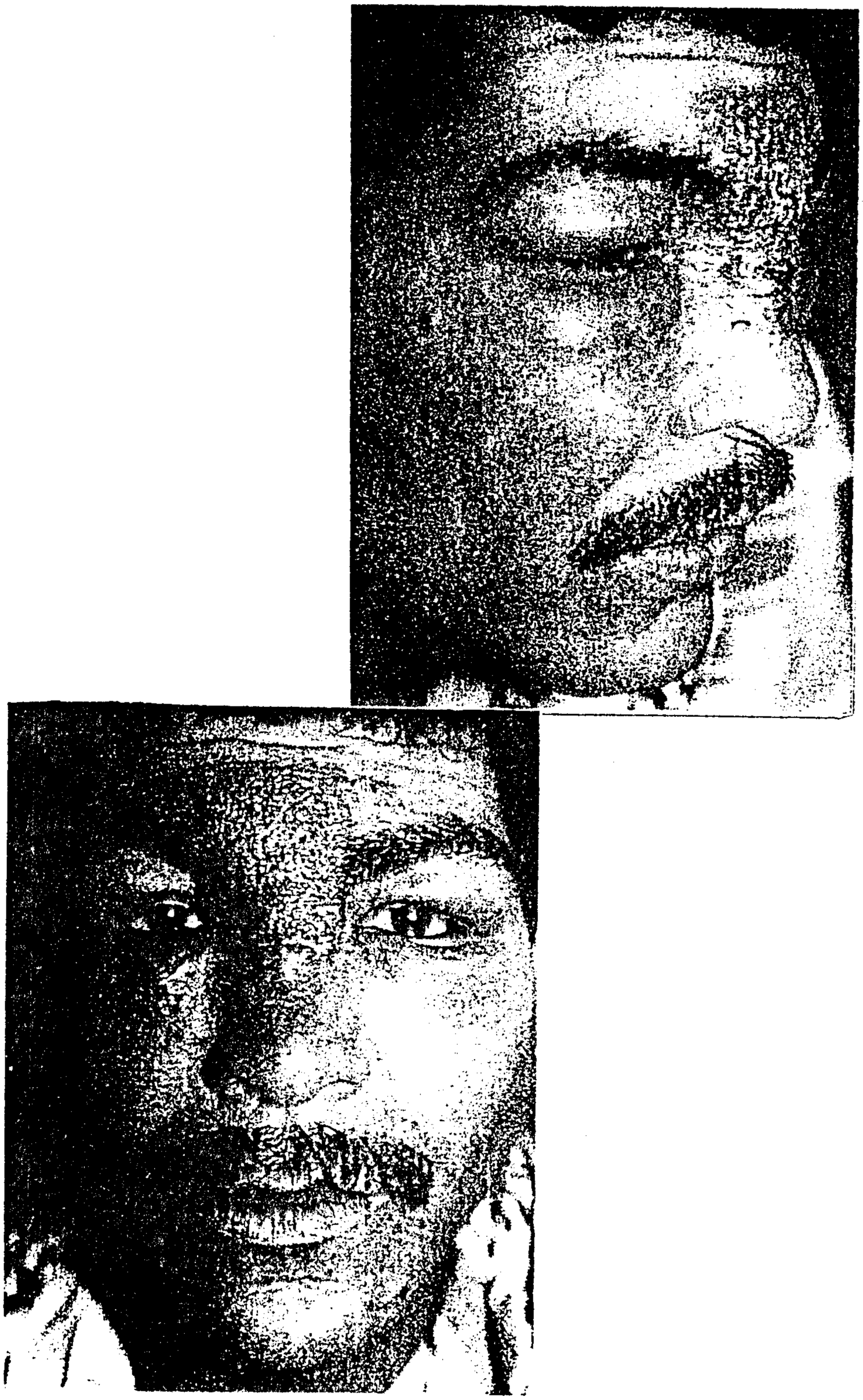

Fig.(7): Photograph of a patient complaining of cutaneous leishmaniasis "A" and "B" before treatment, "C" and "D" after treatment.

(C)

(D)

Cont. Fig.(7)

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صياغة وتقييم مستحضرات المترونيدازول الموضعية

السيد على ابراهيم - سوزان شوقى طوس - تهانى حسن الفحام -
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ان صياغة عقار المترونيدازول فى مستحضرات موضعية لم تحظى بقدر واف من الدراسة . لذلك فقد كان الهدف فى هذه الدراسة صياغة المترونيدازول فى شكل مرهم وايضا فى شكل هلام - ولتحضير مراهم المترونيدازول فقد استخدمت عشرة قواعد لتحضير مرهم المترونيدازول ووجد ان القاعدة القابلة للذوبان فى الماء قد اعطت اعلى معدل لانطلاق العقار . كما ان ثنائى ميثيل السلفوكسيد والبوريا والكحول الايثيلى ليس لها تأثير ملحوظ على معدل انطلاق العقار.

ولتحضير هلام المترونيدازول فقد استخدم بعض مشتقات السليلوز مثال ذلك ميثيل السليليلوز وكربوكس ميثيل السيليلوز وصوديوم كربوكسى ميثيل السيليلوز والمثوسيل والايدراجيت RL ١٠٠ والايدراجيت RS ١٠٠ . وبدراسة معدل انطلاق العقار من هذه القواعد وجد ان الميثيل سيليلوز اعطى اعلى معدل لانطلاق العقار يليه الايدراجيت RL ١٠٠ . وقد اعيد تقييم معدل انطلاق العقار من كل من المراهم والهلام بعد ٢ ، ٤ ، ٧ شهور من تحضيرهم ووجد انه لايحدث اى تغيير فى معدل الانطلاق بالتخزين .

وقد اجريت بعض الدراسات الاكلينيكية على بعض المرضى المصابين بمرض الليشمانيا وبلهارسيا الجلد والطفح الوردى . وقد اظهرت هذه المستحضرات نتائج اولية طيبة لصلاحية المستحضرين فى علاج المصابين لتلك الامراض .

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