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# EFFECT OF FORMULATION VARIABLES OF MULTIPLE EMULSION ON THE STABILITY AND ANTITREMOR ACTIVITY OF CHLORPHENOXAMINE HYDROCHLORIDE

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في هذه الدراسة أمكن تحضير المستحلبات المتعددة من نوع ماء/زيت/ماء تحتوى على عقار هيدروكلوريد كلورفينوكسى أمين والمسكن والمضاد للرعشة.

وقد أستخدمت صياغات مختلفة لهذه المستحلبات تم تحضيرها فى وجود مواد أضافية فى الوسط المائى الخارجى للمستحلب المتعدد وقد تمت الدراسة على ثبات الصياغات المختلفة للمستحلبات المتعددة بتخزينها عند درجة حرارة ٤°، ٢٥° كذلك تم تقييم أنطلاق العقار من المستحلبات المتعددة المحضرة حديثا بعد التخزين اسبوعيا وبقياس التوصيل الكهربائى.

وأثبتت النتائج أن المستحلبات ثابتة أثناء التخزين لمدة ثلاثة شهور عند درجة حرارة ٢٥ درجة مئوية وأن الصيغة رقم ٣ للمستحلب المتعدد والتى تحتوى على زيت الخروع مع سبان ٦٠ تعطى مفعولا ممتدا كمضاد للرعشة وتقلل النشاط الحركى للفئران أكثر من مفعول العقار فى المحلول المائى.

وقد تم تحليل النتائج أحصائيا وقد أظهرت أن الصيغة المذكورة سابقا للمستحلب المتعدد تعطى مفعولا مسكنا بدرجة أكبر إذا ما قورنت بمفعول العقار فى المحلول المائى.

*W/O/W multiple emulsion formulations of chlorphenoxamine hydrochloride were prepared. The results of dissolution indicate that the release of the drug from w/o/w multiple emulsion was significantly sustained. The incorporation of the additives into the external aqueous phase of w/o/w multiple emulsions based on liquid paraffin increased the release of the drug in the following order: sodium chloride > polyvinyl alcohol > hydroxypropyl methylcellulose > polyvinylpyrrolidone. Stability studies of different emulsion samples were conducted at various temperatures and evaluated by using the conductivity method. The obtained data indicated that the emulsions were stable during the storage for 3 months at 25°C. The formula ($F_{III}$) of the multiple emulsion which contained castor oil with Span 60 possessed a more efficient prolonged antitremor effect and lowered of spontaneous locomotor activity (SLMA) in mice than that of the drug solution. The statistical analysis also revealed that this formula produced a greater sedative effect compared with that of the drug solution.*

## INTRODUCTION

Chlorphenoxamine hydrochloride is an antimuscarinic drug used for its central effects to reduce muscle rigidity and akinesia in certain patients with parkinson's disease[1]. The drug is water soluble and rapidly absorbed at the site of administration. It is given orally in large dose (150-400 mg)[2] that causes gastrointestinal irritation[3]. In order to prolong the release of the drug and to minimize its undesirable side effects, w/o/w multiple emulsion was tried[4-6].

A major problem associated with w/o/w emulsion is creaming, which is probably due to its large size of the multiple drops. Creaming may be reduced by using the thickening agent (e.g.polyvinyl alcohol) in the external aqueous phase[7]. Florence and Whitehill[8,9] have

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investigated the possibility of stabilizing w/o/w systems by forming polymeric gels in the internal or external aqueous phases. Also, the presence of electrolyte appeared to be one of the most important factors in determining the stability and release of materials from the internal droplets of w/o/w multiple emulsions[10].

The objective of the present study was to design a stable w/o/w emulsion of chlorphenoxamine hydrochloride by adding small amounts of thickening agents in the external aqueous phase. Also, the stability as well as drug release from the system would be measured by conductivity method during the time of storage at 25° and 4°C for three months. The antitremor activity and sedative effect of the w/o/w formulations in rats would be compared with aqueous solution of the drug.

## EXPERIMENTAL

### Materials

Chlorphenoxamine hydrochloride (CPX) was a gift supplied by Alfa Wasserman, Bologna(Italy); The non-ionic surfactants used were sorbitan monostearate (Span 60), sorbitan mono oleate (Span 80) and polyoxyethylene sorbitan mono laurate (Tween 20) manufactured by Honeywill Atlas Chemicals Industries, Wilmington (USA). The electrolyte and thickening agents used were sodium chloride; polyvinyl alcohol (PVA) 14.000; polyvinylpyrrolidone (PVP) 44.000, BDH (England); hydroxypropyl methylcellulose (HPMC) Hockest, (Germany). Liquid paraffin and castor oil were used as oily phase of the emulsions.

Dichlorodiphenyltrichloroethane (DDT) was synthesized in the Pharmaceutical Chemistry Department, Faculty of Pharmacy, Assiut University. The experimental animals were albino rats of either sex weighing about 160 -180 gm. An activity cage apparatus (Ot No. 7400 UGO Basile, Biological Research Apparatus, 21025 Camerio-Varese-Italy) was utilized.

### Methods

#### Preparation of w/o/w multiple emulsions

Table 1 shows the composition of the different w/o/w multiple emulsion formulations. Two-step emulsifying procedure at room temperature was used[11]. Primary w/o emulsion for all w/o/w emulsions was prepared by mixing the aqueous phase (50 ml.) with oil containing 2.5% Span (phase volume of the aqueous phase in w/o emulsion was 0.5) at a 7000 rpm for 10 min. Final w/o/w emulsion was prepared by mixing the primary w/o emulsion (50 ml.) at 500 rpm for 2 min. with 1% aqueous solution of Tween 20. One percent w/v of the drug was incorporated in internal aqueous phase of multiple emulsions. One percent w/v of the electrolyte or thickening agents was added in the external aqueous phase of the multiple emulsions.

#### Microscopic observation

The prepared emulsions were observed with an optical microscope (Carl Ziess, Jena, Germany) and photomicrographs were taken.

#### Long-term stability assessment

Stability studies were conducted at temperatures, 4 and 25°C. Physical changes that might occur during storage were followed up by visual observations (as possible creaming, coalescence and phase separation). For more confirmation, changes that might occur on ageing were adopted by measurements of the electrical conductivity of the emulsions using a conductivity meter (KARL-KOLB, D-6072, Dreieich, Germany).

#### Apparent partition coefficient determinations

The partition coefficient of CPX between paraffin oil or castor oil and aqueous phase (pH 1.2) was determined as follows: 25 ml of the appropriate oily phase and 25 ml of the aqueous phase of CPX were kept in contact with gently shaking at 37°C until equilibrium. The aqueous phase was separated and passed through filter paper and measured spectrophotometrically at 259 nm (UV spectrophotometer model Uvidec -320 JASCO) against a blank prepared in an

**Table 1:** Composition of w/o/w multiple emulsion formulations.

| No. of formulae | Oily-phase | Hydrophilic emulsifier (1% w/v) | Lipophilic emulsifier (2.5% w/v) | Additives (1% w/v) |
|---|---|---|---|---|
| $F_I$ | Liquid paraffin | Tween 20 | Span 60 | ---- |
| $F_{II}$ | Castor oil | Tween 20 | Span 60 | ---- |
| $F_{III}$ | Castor oil | Tween 20 | Span 60 | ---- |
| $F_{I+PVA}$ | Liquid paraffin | Tween 20 | Span 60 | PVA |
| $F_{I+HPMC}$ | " | " | " | HPMC |
| $F_{I+PVP}$ | " | " | " | PVP |
| $F_{I+NaCl}$ | " | " | " | NaCl |

* Additive was incorporated into the external aqueous phase of multiple emulsions.
* Chlorphenoxamine hydrochloride (1% w/v) was incorporated into the internal aqueous phase of multiple emulsions.
* Internal phase volume fraction (ratio of the primary emulsion) $\phi = 0.5$.
* External phase volume fraction ratio $\phi = 0.5$.

analogous manner. The apparent partition coefficient was then calculated according to the equation $P_{app} = C_o/C_w$, where $C_o$ and $C_w$ are the equilibrium concentration of CPX in the oily and aqueous phases respectively.

**Release studies**

Release patterns of CPX from w/o/w emulsions were investigated by a dialysis method using glass tubing (20 cm. length) closed at the end with cellophane membrane (Fisher Sci., CO., England) stretched around the end. The w/o/w type multiple emulsions were introduced into the tube (5 ml.) and dialyzed in a volume of 150 ml of water at 37°C. The dialysis solution was agitated at rate of 45 rpm. The drug concentrations were analyzed spectrophotometrically.

**Effect of CPX multiple emulsions on the tremor activity in rats elicited by DDT**

Different groups of rats each consisting of five were utilized in this investigation. For induction of convulsive tremors, rats received an oral dose of an oily solution (in corn oil) of DDT according to the method described by Aldridge *et al.*[12]. DDT was given in a dose of 180 mg/kg. Each rat administered 0.2 ml/100 gm. body weight of the DDT oily solution. Convulsive tremors started 3 hrs. after ingestion of DDT and moderate intensity after 4 hrs.

The protective effects of oral administration of an aqueous solution of the antihistaminic drug (CPX) and four multiple emulsions $F_I$, $F_{II}$ $F_{III}$, $F_{IV}$ against the DDT-induced convulsive tremors were evaluated. In all these treatments, the drug solution or one of the tested formulations was given to the rat and DDT was administered to rats at 1,2,3,4,5,6,7,8 and 9 hours following the oral ingestion of CPX solution or one of its multiple emulsions. The antitremor activity of the drug and its emulsion formulations was evaluated from 4 hrs to 12 hrs following administration of DDT. The CPX aqueous solution and its various multiple emulsions were given to rats in a dose of 9 mg/kg.

**Effect of (CPX) and its multiple emulsion formulations on the spontaneous locomotor activity (SLMA) in mice**

Mice was placed singly in the activity cage and their SLMA was computed for 5 min. intervals before (control value) and after aqueous solution of CPX (or four multiple emulsions) administrations. Following the oral ingestion of the drug or its four emulsion formulations; the SLMA of mice was recorded at 2 hrs intervals over a period of 12 hrs. Moreover, the percentage change in the total score of SLMA of mice (relative to the control or pretreatment

value) was calculated for each mice[13].

Results were calculated statistically using the Student's t-test[14].

## RESULTS AND DISCUSSION

### Evaluation of w/o/w multiple emulsions

### Microscopical examination

Figure 1, shows photomicrographs of freshly prepared w/o/w multiple emulsions $F_I$, $F_{II}$ and $F_{III}$. It is clear from the figures that the formation of very large multiple drops contain vast numbers of internal droplets. This observation is in agreement with Whitehill and Florence[15], who claimed that in photo-micrographs of multiple emulsions, three types could be identified types A, B and C. According to the Figure 1, (a,b and c), type C drops are more predominant in emulsions based on liquid paraffin or castor oil.

($F_I$)

($F_{II}$)

($F_{III}$)

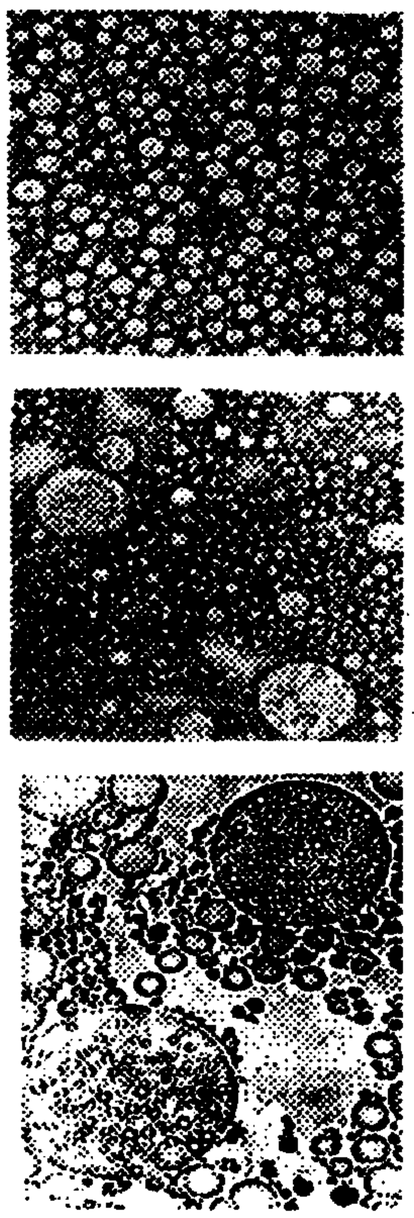

**Fig. 1:** Photomicrograph of w/o/w multiple emulsion freshly prepared.
Figure 1, refere to $F_I$ , $F_{II}$ and $F_{III}$ emulsions respectively in (table 1).

### Long-term stability assessment

All of the w/o/w emulsions were fluid, white and had the same morphological aspects. The physical stability of w/o/w multiple emulsion was examined at 4° and 25°C for 3 months. Phase separation did not occur over during the 3 months of the study at 25°C while partial separation of water was observed after 7 days at 4°C. For more confirmation, the stability of multiple emulsions was determined by using the conductivity method, as shown in (table 2). The electrical conductivity values of freshly prepared emulsions of $F_I$, $F_{II}$ and $F_{III}$ are almost the same ($1.35 \times 10^4$ ohm$^{-1}$). In addition, the data listed in (table 2) indicates that the formula $F_{III}$ is the most stable one for up to 3 months of storage at 25°C.

In solution which contains ionic species, the conductivity is largely dependent on the population of ions. The process of charge neutralization by ion interactions can be observed via conductivity measurements. The decrease in the conductivity of $F_{III}$ after one and second weeks from $1.34 \times 10^4$ ohm$^{-1}$ to $1.10 \times 10^4$ ohm$^{-1}$ was mainly due to the partial formation of the electrically neutral chlorphenoxamine/HCl (2:1) ion triplet[16] and a gradual decline in the concentration of the conductive species chlorphenoxamine/HCl (1:1) ion pair. This resulted in increasing the lipophilic characteristics of the CPX and enhancing the diffusion coefficient of $F_{III}$. This enhancement might depend on the barrier properties of the oil and the type of emulsifiers used in the preparation of w/o/w multiple emulsions.

### Apparent partition coefficient determinations

An accurate analysis of in-vitro drug release from w/o/w emulsion is at first a knowledge of the distribution of the drug in the various phases of the emulsion. The partition ratios of the drug in paraffin oil and castor oil are 93.3 and 22.2 respectively. The drug has a higher affinity for paraffin oil than castor oil. This higher affinity for paraffin oil led to a marked decrease in the release rate of the drug from $F_I$.

**Table 2:** The electrical conductivity of w/o/w multiple emulsion formulations during the storage for three months at 25°C.

| Formulae | Electrical conductivity X $10^{-4}$ $ohm^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Zero time | First week | First month | Second month | Third month |
| $F_I$ | 1.35 | 1.58 | 1.88 | 2.30 | 2.92 |
| $F_{I+HPMC}$ | 1.35 | 1.48 | 1.75 | 2.25 | 2.28 |
| $F_{I+NaCl}$ | 1.34 | 1.22 | 1.20 | 1.65 | 1.79 |
| $F_{II}$ | 1.35 | 1.36 | 1.70 | 2.05 | 2.22 |
| $F_{III}$ | 1.34 | 1.10 | 1.10 | 1.90 | 2.08 |

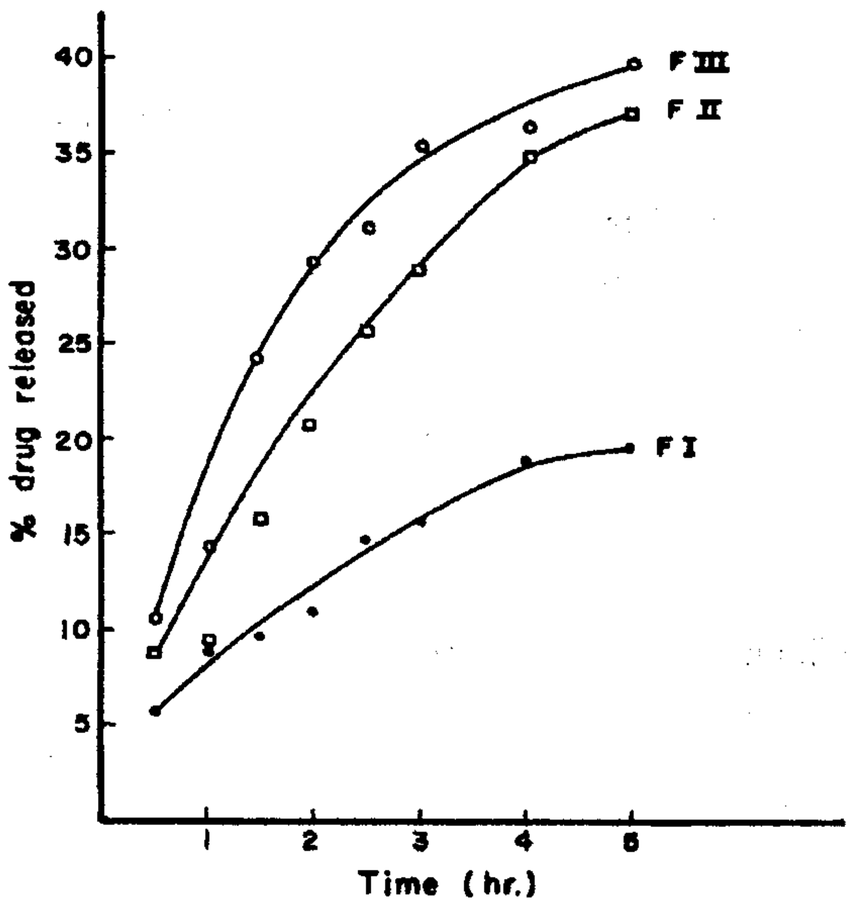


**Fig. 2:** Release profile of chlorphenoxamine hydrochloride from freshly prepared w/o/w multiple emulsions.
(●) $F_I$ : (□) $F_{II}$ and (○) $F_{III}$.

**Release study of the drug**

The release patterns of CPX from different emulsion systems are shown in Figure 2. The release of CPX from $F_{III}$ is higher than that from $F_{II}$. This is attributed to the higher HLB value of Span 60 (4.7) in $F_{III}$ than that of Span 80 (4.3) in $F_{III}$[17]. These results are in agreement with that previously reported by Fincher and Waggoner[18]. Figure 2, revealed that the release of the drug increased in the order of: $F_{III} > F_{II} > F_I$. The above mentioned results point to the existence of a relation between the partitioning properties of the drug in the different emulsion types. When using paraffinic oil, the low viscosity of the membrane w/o/w and the great rate of transfer of the drug from external to the internal phase which resulted in a significant decrease in the release rate of the drug[19]. Also, the stability of the membrane toward rupture and leakage of entrapped materials decreased with decreasing viscosity[19]. Therefore, to enhance the stability and the release rate of the drug, small amounts of electrolyte and thickening agents are added in the external aqueous phase of the multiple emulsions.

**Influence of thickening agents and electrolyte on the release of CPX from w/o/w emulsion based on liquid paraffin**

Figure 3 illustrates the effects of 1% w/v of thickening agents namely: PVA, HPMC, PVP and electrolyte NaCl on the parameters of CPX release from w/o/w emulsions based on liquid paraffin. The incorporation of these additives into the external aqueous phase of w/o/w emulsions enhanced the dissolution rate of the drug in the following order: NaCL > PVA > HPMC > PVP. The presence of 1% w/v NaCl causes an increase in the osmotic pressure in the external phase and transfer of water molecules from internal aqueous phase to the external one. This results in a decrease in the sizes of the internal droplets and consequently decrease in the sizes of oil droplets containing the inner aqueous droplets. Also, it leads to an increase in total surface area for drug release resulted in an increase in the release rate of the drug[20]. The stabilization of the emulsions was achieved by

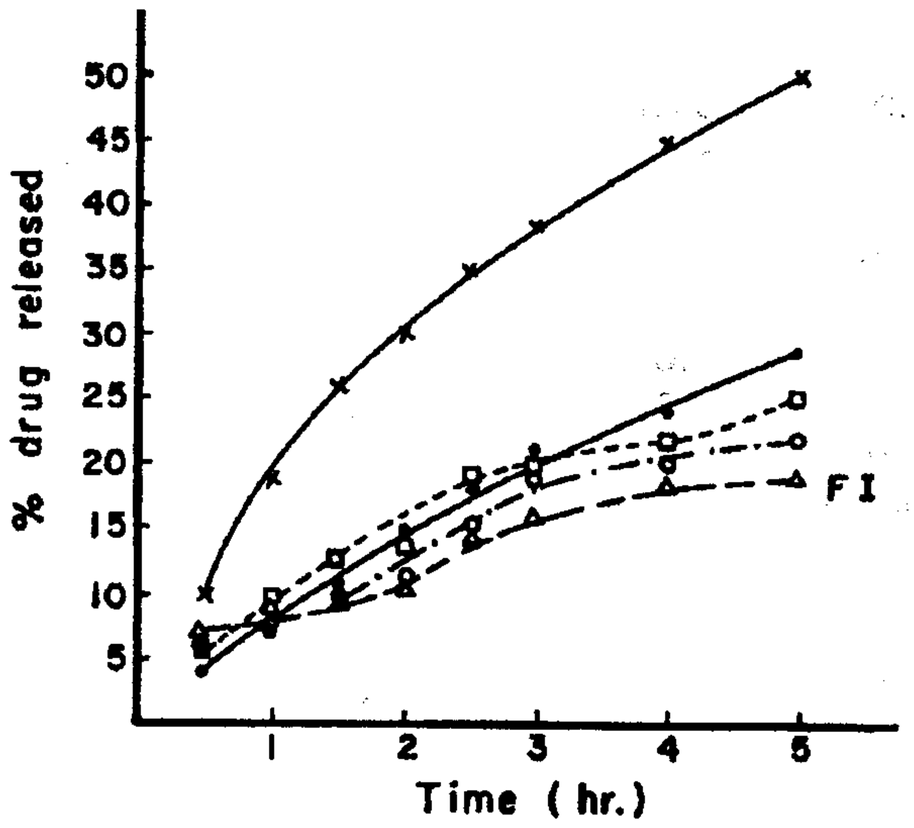


**Fig. 3:** Effect of additives on the release of chlorphenoxamine hydrochloride from freshly prepared w/o/w multiple emulsions. (▲) $F_I$ : (x) $F_{I+NaCl}$ : (●) $F_{I+PVA}$ : (□) $F_{I+HPMC}$ : (○) $F_{I+PVP}$.

the addition of small amounts of NaCl (1% w/v) to the external aqueous phase. This is due to: (a) the presence of electrolyte at this concentration causes a little change in size of multiple drops[21]. (b) also, it causes lower osmotic pressure difference across the oil layer, then passage of water is so slow and the rupture of the oil drops can be avoided[11,22]. (c) the osmolarity may also be adjusted by the presence of the drug in the internal aqueous phase and the small amount of NaCl (1% w/v) in the external one[22]. This approach can lead to equality of pressure on storage which is satisfactory for stabilization of the emulsion.

The incorporation of 1% w/v HPMC (which has a great affinity for water) into the external aqueous phase of emulsions enhanced water uptake from the internal aqueous phase. The action of HPMC on aqueous uptake depends on the amount of HPMC used[23]. There is an increase in the pressure difference due to volume expansion as the polymer swells, the viscous force is also increased. This viscous drag is sufficient to oppose the driving pressure force, thus liquid uptake drops as the viscosity of HPMC increases. The release of the drug was achieved by diffusing the dissolving drug from the polymer gel of external layer. $F_{III}$ showed higher release than all the other formulations except that of $F_{I+NaCl}$.

## Analysis of variance of the In-vitro studies

Analysis of variance[24] of the in-vitro release data from the freshly prepared emulsions (table 3) revealed significant differences between some formulations. The system containing either PVA, HPMC or PVP were of insignificant difference in the release rates of the drug. On the other hand, significant differences were found to exist between emulsion $F_I$ in the presence and absence of NaCl in the release rate of the drug. Therefore, 1 % w/v of NaCl must be included in $F_I$ to avoid the sharp decrease in the release rate of the drug and enhance the stability of multiple emulsions.

## Release kinetic of the drug

The in-vitro release data were kinetically analyzed according to the diffusion controlled release mechanism[25]. The effective diffusion coefficients of the drug from the w/o/w emulsions were calculated according to the equations previously reported by Brodin *et al.*[4].

The diffusion coefficient of CPX has been determined in aqueous solution from w/o/w system both with and without the above mentioned additives (during storage for 3 months at 4°C and 25°C). The data are summarized in (tables 4,5 and figure 4). The diffusion coefficients of the drug from $F_I$ increased by the addition of NaCl, PVA, HPMC or PVP to the external aqueous phase of multiple emulsions. The diffusion coefficients of the drug from emulsion formulations slightly decreased on ageing. This might be due to the presence of sodium chloride ($F_I$) that competes with surfactant for water molecules at the outer o-w interface, which would result in a rigid interfacial layer which would be a more effective mechanical barrier to drug transfer[4,26].

In addition, HPMC acted as a protective colloid which prevented droplets and particles from coalescing or agglomerating, thus

**Table 3:** Effect of formulation on the in-vitro release profile of (CPX), from the freshly prepared multiple emulsion systems.

| Formulae | Amount of drug released (%) after the following time intervals in hours | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 4.0 | 5.0 |
| $F_{I+NaCL}$ | 10.9 | 19.5 | 26.4 | 30.2 | 35.5 | 39.9 | 46.0 | 50.1 |
| $F_{I+PVA}$ | 4.4 | 7.3 | 11.3 | 14.9 | 17.6 | 21.4 | 24.2 | 29.3 |
| $F_{I+PVP}$ | 6.0 | 7.3 | 10.3 | 11.7 | 15.3 | 19.6 | 20.8 | 22.5 |
| $F_{I+HPMC}$ | 6.0 | 10.0 | 13.6 | 14.1 | 19.9 | 20.6 | 22.3 | 25.1 |
| $F_I$ | 6.6 | 8.4 | 9.8 | 11.7 | 15.4 | 15.8 | 18.5 | 19.0 |

Analysis of Variance [24]

| Source of variation | S.S | d.f | S.S/d.f | F | |
|---|---|---|---|---|---|
| | | | | Calculated | Tabulated P= 0.05 |
| Time | 2144.86 | 7 | 306.41 | 25.78 | 2.36 |
| Formuation | 1976.37 | 4 | 494.09 | 41.57* | 2.71 |
| Error | 332.83 | 24 | 11.89 | --- | --- |
| Total | 4454.06 | 39 | --- | --- | --- |

$$T.M.R.T = t_{0.05} \times Q \sqrt{S^2/N} = 6.337$$

| Treatment | Mean % Released | Difference between means | | | |
|---|---|---|---|---|---|
| | | $X_i$-$X_E$ | $X_i$-$X_D$ | $X_i$-$X_C$ | $X_i$-$X_B$ |
| $F_{I+NaCL}$ | $X_A$ = 23.31 | 19.16* | 18.12* | 16.01* | 15.86* |
| $F_{I+PVA}$ | $X_B$ = 16.45 | 3.30 | 2.26 | 0.15 | --- |
| $F_{I+HPMC}$ | $X_C$ = 16.30 | 3.15 | 2.11 | --- | --- |
| $F_{I+PVP}$ | $X_D$ = 14.19 | 1.04 | --- | --- | --- |
| $F_I$ | $X_E$ = 13.15 | --- | --- | --- | --- |

* : Significant difference.
S.S : Sum of squares.
d.f : Degree of freedom.
S.S/d.f : Variance estimate = $S^2$.
T.M.R.T : The Tukey's Multiple Range Test (Q test).

**Table 4:** Diffusion coefficients of chlorphenoxamine hydrochloride from freshly prepared w/o/w multiple emulsion formulations and during the storage for three months at 25°C.

| Formulae | Diffusion coefficients (D x $10^3$) $cm^2/s$ | | | | |
|---|---|---|---|---|---|
| | Freshly prepared | after one week | after two weeks | after one month | after three months |
| $F_I$ | 4.45 | 2.98 | 1.69 | 1.20 | 0.47 |
| $F_{II}$ | 22.50 | 16.13 | 11.27 | 1.46 | 0.93 |
| $F_{III}$ | 30.75 | 16.64 | 13.20 | 7.55 | 3.23 |
| $F_{I+PVA}$ | 15.20 | 9.88 | 8.59 | 6.70 | 3.50 |
| $F_{I+HPMC}$ | 8.73 | 8.75 | 3.26 | 2.68 | 2.30 |
| $F_{I+PVP}$ | 7.94 | 5.52 | 5.13 | 3.13 | 2.57 |
| $F_{I+NaCl}$ | 36.59 | 31.65 | 27.32 | 20.01 | 15.31 |

**Table 5:** Diffusion coefficients of chlorphenoxamine hydrochloride from freshly prepared w/o/w multiple emulsion formulations and during the storage for one month at 4°C.

| Formulae | Diffusion coefficients (D x $10^3$) $cm^2/s$ | | | |
|---|---|---|---|---|
| | Freshly prepared | after one week | after two weeks | after one month |
| $F_I$ | 4.45 | 2.87 | 1.36 | 0.96 |
| $F_{II}$ | 22.50 | 15.37 | 4.91 | 1.19 |
| $F_{III}$ | 30.75 | 19.01 | 9.06 | 1.52 |
| $F_{I+PVA}$ | 15.20 | 7.05 | 5.62 | 1.12 |
| $F_{I+HPMC}$ | 8.73 | 4.35 | 3.08 | 1.14 |
| $F_{I+PVP}$ | 7.94 | 4.90 | 5.55 | 0.96 |
| $F_{I+NaCl}$ | 36.59 | 31.47 | 12.33 | 1.75 |

inhibiting the formation of sediments[27]. The use of these thickening agents in the external aqueous phase provided excellent viscosity stability during long-term storage. The viscous w/o/w system will be stable to creaming and to prevent coalescence of the oil drops themselves, the two major routs of breakdown in multiple emulsions[10,11]. These results indicate that w/o/w systems could be stabilized by addition small amounts of electrolyte or thickening agents.

**Effect of CPX solution and emulsion formulations on the tremor activity in rats**

The antitremor effect of the aqueous CPX solution and their multiple emulsion formulation was recorded over the whole time intervals. There was a progressive decline (except $F_{II}$) in the percentage of rats protected from DDt induced tremors. The data listed in (table 6) revealed that the CPX original solution and their multiple emulsion formulations possess antitremor activity 3 hrs. after DDT oral administration.

Formulae $F_I$, $F_{III}$ and $F_{I+PVA}$ of w/o/w multiple emulsion produced antitremor activity with a peak effect 6 hrs after administration of them, while $F_{II}$ produced a peak effect 8 hrs after its administration and exhibited a longer duration of action (figure 5). The onset of action of the above formulae was in the following

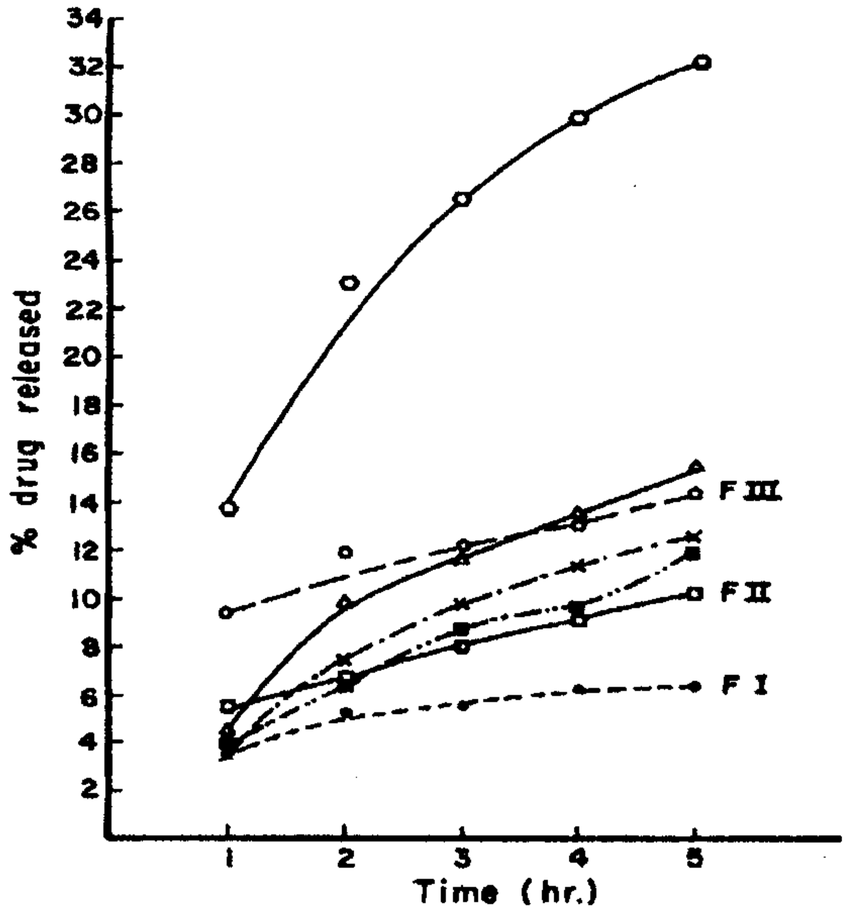


**Fig. 4:** Release profile of chlorphenoxamine hydrochloride from its prepared w/o/w multiple emulsions after storage for three months at 25°C.
(●) $F_I$ : (□) $F_{II}$ : (○) $F_{III}$ : (○) $F_{I+NaCl}$ : (▲) $F_{I+PVA}$ : (■) $F_{I+HPMC}$ : (x) $F_{I+PVP}$.

order: $F_{II} > F_I > F_{I+PVA} > F_{III} >$ CPX aqueous solution. In addition, $F_{II}$ and $F_{III}$ were still acting as antitremor agents until the end of 11 hrs period (table 6 and figure 5). Generally all formulations of the multiple emulsions of CPX produced a more efficient and prolonged antitremor action than CPX original solution.

**Effect of CPX solution and emulsion formulations on the spontaneous locomotor activity (SLMA) of mice**

It is worthy to mention that drugs which depress the (SLMA) of rodents possess a distinct form of sedation behavioral[28,29]. After ingestion of CPX solution a significant diminution of SLMA of mice from 2 to 8 hrs was observed in (figure 6). This decrease in SLMA was time-dependent up to 6 hrs after administration of CPX solution. From the data listed in (table 7) all the formulations showed a significant depression of SLMA of mice. The administration of $F_{III}$ emulsions, was able to bring about a significant fall in SLMA of mice at all the tested time intervals with the peak recorded at 10 hrs following administration of this formula. The peak sedative effects of $F_I$, $F_{II}$ and $F_{I+PVA}$ were attained at 8 hrs, 12 hrs and 6 hrs respectively following their administrations (table 7 and figure 6).

**Table 6:** Effect of chlorphenoxamine hydrochloride multiple emulsion formulations on the convulsive tremors produced by DDT in rats.

| Time after DDT administration (hrs.) | Treatments | | | | |
|---|---|---|---|---|---|
| | Control | $F_I$ | $F_{II}$ | $F_{III}$ | $F_{I+PVA}$ |
| 3 | 60 | 0.00 | 0.00 | 40 | 20 |
| 4 | 100 | 40 | 0.00 | 60 | 40 |
| 5 | 100 | 80 | 80 | 80 | 80 |
| 6 | 80 | 100 | 80 | 100 | 100 |
| 7 | 60 | 80 | 80 | 100 | 60 |
| 8 | 40 | 80 | 100 | 100 | 60 |
| 9 | 20 | 60 | 100 | 80 | 40 |
| 10 | 0.00 | 40 | 100 | 80 | 0.00 |
| 11 | 0.00 | 40 | 100 | 60 | 0.00 |

* Control = aqueous solution of the drug.
* Values represent the percentage of rats protected from the convulsive tremors produced by DDT.
* Groups of 5 rats were used at each of the selected time intervals.

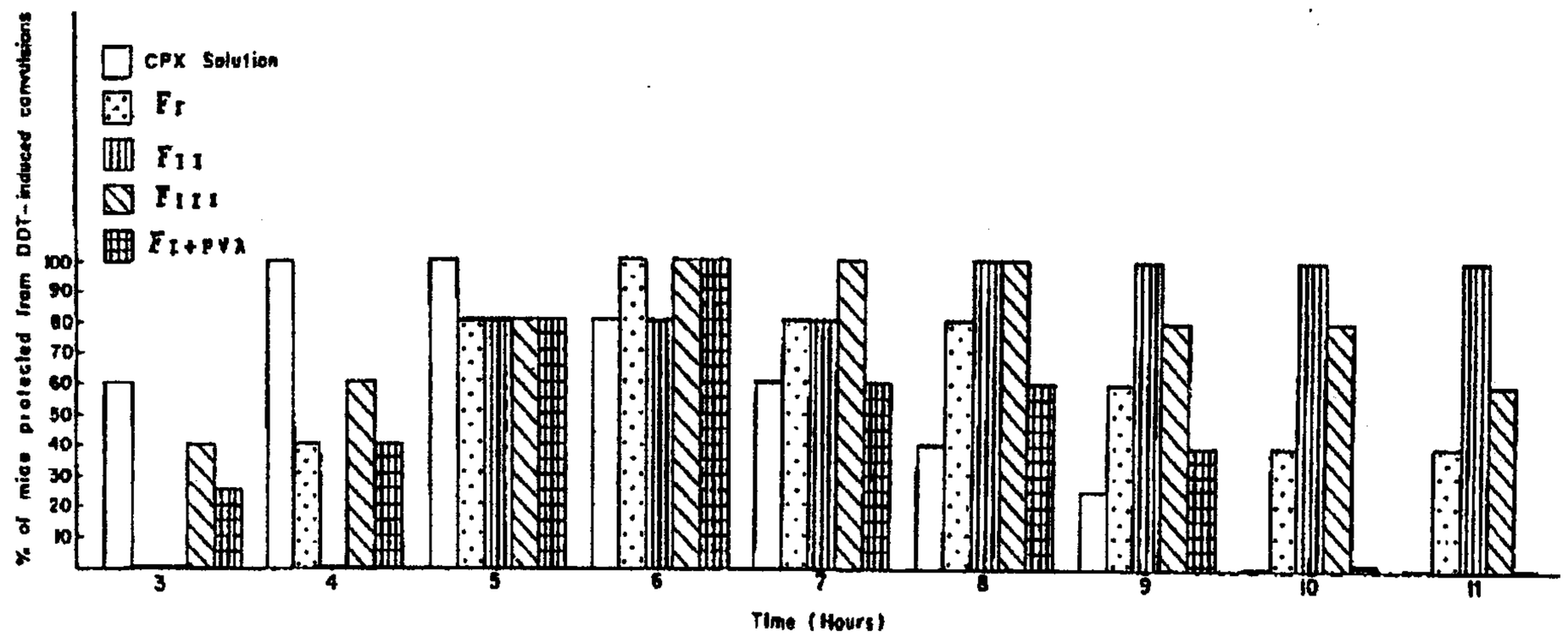


**Fig. 5:** Percentage of protection afforded by aqueous solution of chlorphenoxamine hydrochloride and multiple emulsion formulations ($F_I$ , $F_{II}$ , $F_{III}$ and $F_{I+PVA}$) against DDT - induced convulsive tremors in rats. Values represent the % protection in group of 5 mice.

**Table 7:** Effect of chlorphenoxamine hydrochloride multiple emulsion formulations on the spontaneous locomotor activity (SLMA) of mice.

| Time after drug adminis-tration (hrs.) | Control | $F_I$ | $F_{II}$ | $F_{III}$ | $F_{I+PVA}$ |
|---|---|---|---|---|---|
| 2 | 48.67±3.05 | $22.92^{***}$ ±0.97 | $13.74^{***}$ ±2.28 | $39.35^{x}$ ±4.40 | $37.96^{*}$ ±3.36 |
| 4 | 80.50±5.43 | $43.73^{***}$ ±2.96 | $15.24^{***}$ ±1.43 | $59.72^{*}$ ±4.36 | $72.45^{x}$ ±6.24 |
| 6 | 91.24±4.50 | $75.46^{*}$ ±4.98 | $53.62^{***}$ ±4.23 | $70.53^{*}$ ±5.97 | $68.39^{**}$ ±4.95 |
| 8 | 35.73±2.97 | $86.74^{***}$ ±3.51 | $49.57^{*}$ ±3.54 | $45.92^{*}$ ±2.65 | $28.71^{x}$ ±2.42 |
| 10 | 6.85±2.78 | $34.87^{***}$ ±2.59 | $92.82^{***}$ ±6.95 | $88.39^{***}$ ±6.06 | $8.97^{x}$ ±1.99 |
| 12 | 3.92±2.16 | $26.34^{***}$ ±3.55 | $98.12^{***}$ ±7.96 | $34.75^{***}$ ±2.16 | $10.52^{x}$ ±4.18 |

(x) Insignificant,
Significant difference (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ at 99% C.L.
- Data represent the mean percentage decrease in SLMA values.
- Values are the mean of 5 experiments ± standard error.

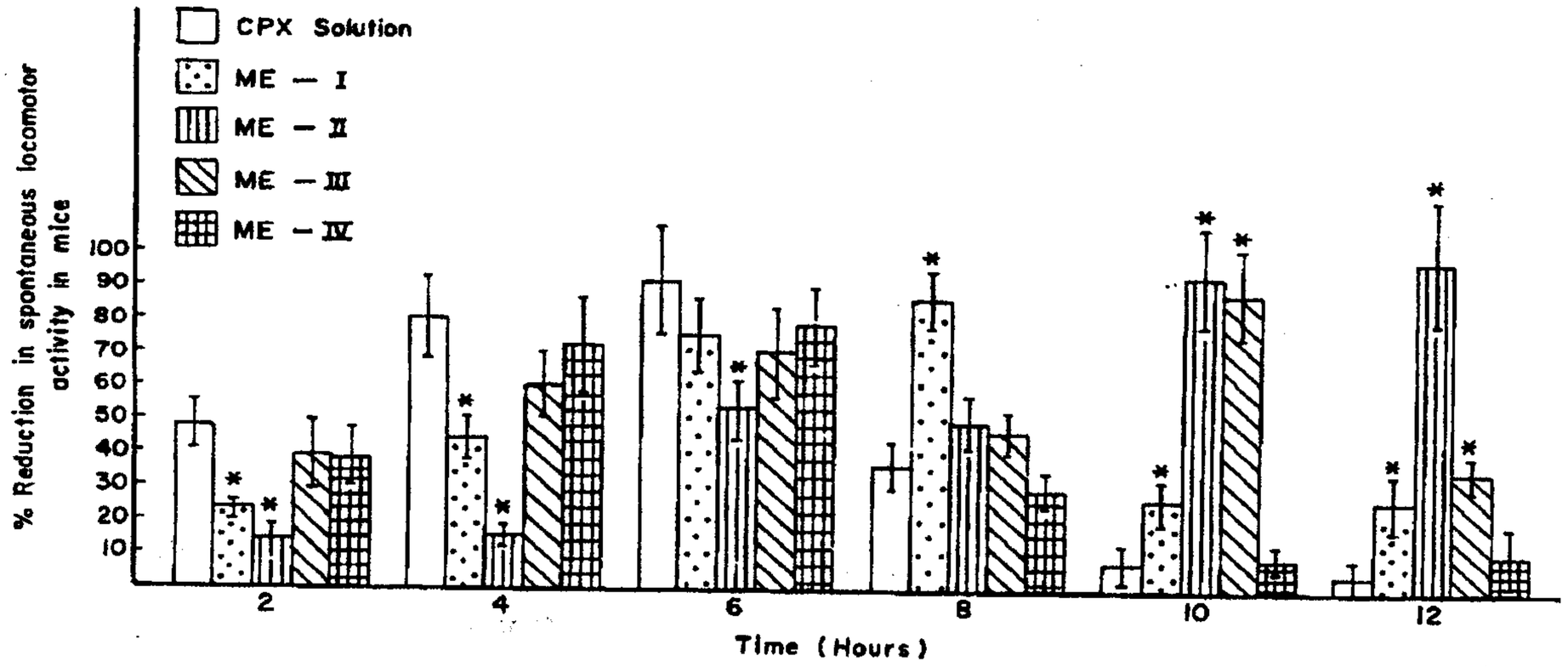


**Fig. 6:** Effect ofaqueous solution of chlorphenoxamine hydrochloride and multiple emulsion formulations ($F_I$, $F_{II}$, $F_{III}$ and $F_{I+PVA}$) on the spontaneous locomotor activity (SLMA) of mice.
Results represent the mean of 5 experiments ± standard deviation.
(*) Statistically different from results obtained in mice treated with chlorphenoxamine hydrochloride solution at $P > 0.05$.

Student's t-test was adopted to demonstrate the differences in sedative effect between CPX solution and their multiple emulsion formulations. The statistical analysis revealed that insignificant differences were observed between CPX solution and $F_{I+PVA}$ at all times except at 2 and 6 hrs. Also, insignificant differences between CPX solution and $F_{III}$ only at 2 hrs, $F_{III}$ elicited a statistically more sedative effect than CPX solution, only at 10 hrs and 12 hrs. There was a significant elevation in the sedative effect of $F_I$ and $F_{II}$ at the tested time (except at 6 hrs for $F_I$ and 8 hrs for $F_{II}$).

**Correlations of the In-vivo and In-vitro studies**

In vivo investigations of CPX from multiple emulsion $F_I$ and $F_{I+PVA}$ were calculated as the percent of protection from convulsive tremor and as the percent decrease in SLMA from 3 hrs to 11 hrs. The amounts of CPX released from multiple emulsions $F_I$ and $F_{I+PVA}$ were calculated from 0 to 300 min. The correlation coefficient of $F_I$ was $r=0.94$ and the regression coefficient was $b=1.8$, while the correlation coefficient of $F_{I+PVA}$ was $r=0.999$ and the regression coefficient was $b=1.15$. From these results, it can be concluded that there was more correlation of $F_{I+PVA}$ than that of the $F_I$. The percent released of the drug from $F_{III}$ in vitro did correlate well with the percent protection from tremor in vivo ($r=0.983$). $F_{III}$ showed a good correlation between the percent released of the drug in vitro and the percent inhibition of SLMA in vivo after oral administration.

From these data, it is concluded that the $F_{III}$ is the most valid correlation and demonstrates that in vitro work will correlate well with in vivo work.

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