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FORMULATION AND EVALUATION OF A MICROEMULSION SYSTEM CONTAINING CLOTRIMAZOLE

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ABSTRACT

The objective of this work was to improve the solubility and enhance the antimicrobial activity of a poorly water soluble drug Clotrimazole (CLT) via formulating it as microemulsion systems. Microemulsions with varying mass ratios (Km) of surfactant and co-surfactants (Km 0.5/1, 1/1, 2/1) were prepared using Isopropylpalmitate (IPP) as oil phase, poly sorbate 80 (Tween 80) as surfactant, n-butanol as co-surfactant and distilled water as a aqueous phase. The area of o/w microemulsion region in pseudo-ternary phase diagram was increased with increasing ratio of Tween 80 to n-butanol. The solubility of CLT in microemulsion reached the maximum at Km 1/1 mixture of Tween 80 and n-butanol, the solubility value of CLT increased to 4100-fold, respectively to that of water. The release rate of CLT from microemulsion systems and also the antimicrobial activity reached the maximum with 0.5/1 Km.

1. INTRODUCTION

Microemulsions are clear, thermodynamically stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a co-surfactant[1]. A co-surfactant may be a short chain alcohol, amine, or other weakly amphiphilic molecules[2]. The microemulsion structure can be oil-in-water (o/w), water-in-oil (w/o), or bicontinuous, i.e. effectively continuous in both water and oil. Beside the obvious advantages of microemulsions, including physical stability and ease of preparation, these systems may offer additional benefits for transdermal use. These include an increased drug release rate; due to smaller particle size compared to emulsions, and improved handling due to the transparency of the formulation[3].

Microemulsions and related systems represent pharmaceutically versatile formulations for various applications, including drug delivery to and through the skin[4]. These systems increase the transdermal delivery of a compound by different mechanisms. First, a large amount of drug can be included in the formulation due to the high solubilization power. Second, an increase in the transdermal flux can be expected in that the thermodynamic activity of the drug in the microemulsion can be modified to favor partitioning into the stratum corneum. Third, the surfactants in the microemulsion may reduce the diffusion barrier of the stratum corneum[5].

Superficial fungus infections caused by dermatophytes or yeast are common dermatological problems. The humid atmosphere, warm temperature, dust, socioeconomic standards and abuse of antibiotics may contribute to the high incidence of this type of infection[6].

In 1970, imidazole antifungal compounds were made available, of these; Clotrimazole (CLT) enjoys a broad range of activity against almost all dermatophytes and Candida of clinical interest[7,8].

The objective of this study was to investigate the solubilization of CLT in pharmaceutically accepted non-ionic surfactant systems. In the first part of this study, we constructed the pseudo-ternary phase diagrams of water/IPP/Tween 80 and n-butanol at Km 0.5/1, 1/1, 2/1 in order to determine the effect of alcohol concentration on formulation of microemulsions. In the second part of our study, we determined the effect of microemulsion components alone or in combination on the solubility of CLT which is slightly soluble in IPP then the release rate of CLT from different systems was determined via rabbit abdominal skin. Finally we studied the antimicrobial activity of the systems.

2. MATERIALS AND METHODS

2.1. Materials:

Clotrimazole (CLT) was supplied by Arab drug Company (Cairo-ARE). Isopropyl palmitate (IPP) MerkSharp&Dohme (International, Germany). Methanol, n-butanol (Sigma Chemical CO.St.Louis, MO, USA). Tween 80 (EL-Nasr Pharmaceutical Chemical Co. Cairo-ARE). Triple distilled water was used through the study.

2.2. Preparation of pseudo-ternary phase diagram:

Surfactant was mixed with co-surfactant in fixed weight ratio (0.5/1, 1/1 and 2/1). Aliquots of each surfactant- co surfactant mixture (S_{mix}) were then mixed with oil and finally titrated with aqueous phase (distilled water). Mixtures were gently shaken or mixed by vortexing and kept at ambient temperature (25℃) to attain equilibrium using the method of Abbofazeli and Lawrence[9]. The equilibrated samples were assessed visually and determined as being clear and transparent microemulsions, or crude emulsions or gels.

The physical states were represented on a pseudo-ternary phase diagram with one axis representing water, one representing oil and the third representing the S mix. The influence of mass ratio of surfactant to co-surfactant on the area of o/w microemulsion region was investigated on the pseudo-ternary phase diagram.

2-3. Preparation of microemulsion containing Clotrimazole:

Once the microemulsion region was identified, (CLT) varying from 50 to100 mg was weighted and

added to 2.5 ml of oil S_{mix} mixture with varying Km ratios of surfactant to co-surfactant in fixed ratios of total S mix and oil content (4/1). The oil –S mix was then added to water in ratio 50/50 (W/W) and o/w microemulsion containing (CLT) was produced by vortexing the resultant mixture at ambient temperature.

2.4. Physical stability:

Microemulsions were stored at 4°C and room temperature. Their physical stability was measured by periodic inspection over 3 months for the presence of macroscopic phase separation as shown by cloudiness or the formation of two distinct layers.

2.5. Solubility of Clotrimazole:

The solubility of Clotrimazole was determined in each component of microemulsion systems such as surfactant, co-surfactant and oil, and also in the oil-surfactant mixture, oil-co-surfactant mixture and oil-S_{mix} mixture, as well as in dispersed solutions of each mixture after addition of water as an aqueous phase on each mixture, respectively. An excess amount of (CLT) was add to 5 ml of each dissolution medium and the mixture was stirred for 24 hr at 25 °C (preliminary test proved that this time is enough for equilibrium). Triplicate samples were centrifuged at 4000 rpm for 10 min (Eppendurf centrifuge. Germany) to remove the excess amount of drug undissolved. Then, aliquots of supernatant were taken and the content of (CLT) was quantified by spectrophotometrically at 260 nm after dilution with methanol of analytical grade.

2.6. *In vitro* permeation experiments:

The microemulsion systems containing 0.5/1, 1/1, 2/1 Km ratios were tested for permeation using abdominal rabbit skin (full thickness of abdominal hairless skin). The hair was removed by a hair clipper, and the skin was examined visually and those demonstrated any abnormalities in their surface were discarded. The skin specimens were kept frozen at (-20°C) until used. The specimens were thawed by immersion in water at 37 ± 1°C in a water bath for 4 min, and then soaked in normal saline overnight to ensure complete hydration. Specimens were cut into area of approximately 4cm², each one was fixed at one end of the diffusion cell and the other end was covered with a rubber cap. The dermal side of the skin was facing the receptor solution which was composed of 100 ml of 4 % (v/v) ethanol phosphate buffer 7.6 at 37 ± 1 °C to limit osmosis[10]. Agitation was affected by magnetic stirring at 70 rpm. Each sample equivalent to 10 mg (CLT) was applied on the skin. At each time interval, 1 ml from medium was withdrawn and replaced by an equal volume of the fresh dissolution medium, kept the experiment was kept at 37 ±1°C. The amount of CLT released at different time intervals was determined spectrophotometrically at 260 nm. Each experiment was performed at least three times and the mean was calculated in each case.

2.7. *In vitro* antimycotic study:

Agar-cup diffusion method was adapted, and *Candida pseudo tropicalis* was used. The test was carried out as follows: 15 ml of nutrient media seeded with 24 hrs subculture *Candida pseudo tropicalis* was distributed in each Petri-dish (10 cm-diameter). On solidification, 8 mm holes were made and filled with 0.5 ml microemulsion. In each plate, 2 holes for the medicated microemulsion and another two for the placebo. The-dishes were left for two hours, and then incubated at 37°C for 48 hrs. The inhibition zone was measured by taking the mean of 4 readings. Each two was taken from one hole.

3. RESULTS AND DISCUSSION

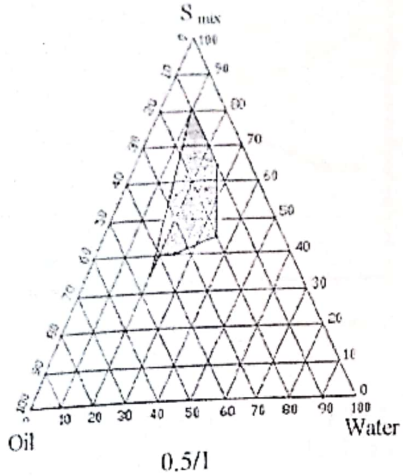
3.1. Phase studies:

For preparation of microemulsion systems, neither the addition of Tween 80 alone nor n- butanol alone can efficiently promote the mutual solubilization of water and IPP. By adequately mixing water, IPP, and suitable amphiphilic compounds, such as Tween 80 plus n-butanol, it is possible to obtain a clear, stable, single phase isotropic system over a wide range of composition.

As shown in Fig. 1, the area of a microemulsion and isotropic regions increased with increasing the ratio of surfactant to co-surfactant. It indicates that the maximum proportions of oil incorporated in microemulsions increased significantly with increasing the ratio of surfactant to co-surfactant. Moreover, the maximum amount of water to be solubilized in the microemulsion without phase conversion or separation has been shown to increase as the mass ratio of surfactant /co-surfactant increased[11] The phase study reveals that the addition of Tween 80 / n-butanol mixture in mass ratio of 2/1 gave the greater and transparent microemulsion. A similar result was obtained from mineral oil/ water solution using Brij 96 as surfactant and glycerin, ethylene glycol and propylene glycol as co-surfactants[12]. Also Zong-GaoGao et al.[12] found that increasing Km of Cremophor EL and Transcutol in microemulsion containing Captex 355 as oil and water as aqueous phase leaded to increase in the region of microemulsion in phase diagram. From a formulation viewpoint, the increased oil content in microemulsions may provide a greater opportunity for the solubilization of poorly water-soluble drugs[13].

3.2. Physical stability:

Physical stability of microemulsion was investigated at 4°C and room temperature: there is no significant change for 3 months in the system i.e. no color change nor macroscopic phase separation which can be indicated by cloudiness and the formation of two distinct layers.



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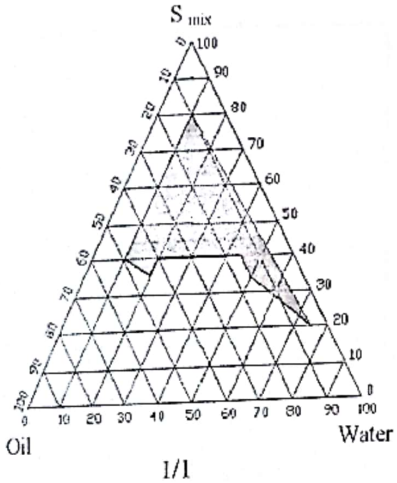

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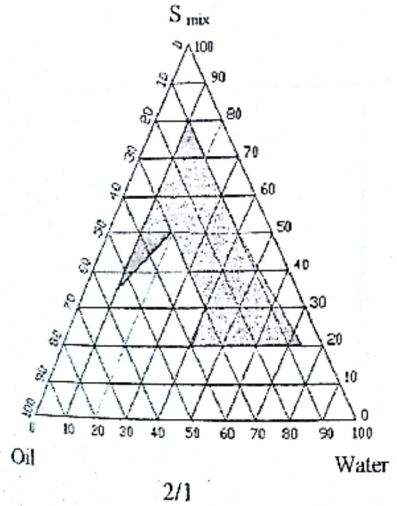

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Fig (1): Effect of Km (mass ratio) of surfactant/ co-surfactant on quaternary system containing Tween 80-n-butanol- IPP and water

3.3. Solubilization of Clotrimazole:

The solubility of (CLT) was found to be 11.79 mg/ml in IPP, less than 0.01 mg/ml in water and very soluble in n-butanol. The solubility of (CLT) in oil-surfactant mixture increases with increasing the content of Tween 80 (Fig. 2). Also the solubility of (CLT) in oil- co-surfactant mixture increased with increasing the content of n-butanol (Fig. 3). The solubility of (CLT) in oil - S_{mix} mixture with varying mass ratio (Km) increased with increasing the surfactant content which indicates that the solubility of (CLT) is greatly affected by the surfactant concentration (Fig. 4). After adding the aqueous phase, the solubility of (CLT) in oil-surfactant-water system increased with increasing Tween 80 content (Fig. 5). The solubility of (CLT) in oil-co-surfactant-water system was also increased with increasing n-butanol content (Fig. 6). The solubility of CLT in system containing all components of microemulsions (Fig. 7) was increased markedly compared with that of systems without surfactant (Fig. 6) or co-surfactant (Fig. 5), and it reached maximum (41mg / ml) at 1/1 S mix of surfactant to co-surfactant[13]. The maximum solubilization power is thought to be achieved by the formation of transparent microemulsion with small droplets. At a ratio greater than 1/1 the mixtures also formed microemulsions, but the lower n-butanol content in the microemulsion system decreased the solubilizing capacity of the microemulsion. A similar result was obtained with Indranil Nandi et al.[14] who found that the solubility of indomethacin and progesteron was increased by 500 and 3300-fold ,respectively.

3.4. *In vitro* permeability:

System composed of IPP/Tween 80/ n-butanol and water gave o/w microemulsion which acts as a reservoir of (CLT) in dispersed phase. After 4 hr only 43.2 %, 42.6 % and 22, 8% of (CLT) was released from microemulsion systems containing S mix 0.5/1, 1/1, and 2/1 respectively (Fig. 8). The higher percentage of (CLT) released from systems prepared by S mix 0.5/1 and 1/1 may be ascribed to the higher percentage of alcohol content which reached approximately 60% and 50% for Km 0.5/1 and Km1/1 compared to 33% for Km2/1. This result is in agreement with that obtained by Thacharodi and Panduranga Roa[15] who found that an increase in alcohol content leads to an increase in permeation from microemulsions due to it act as penetration enhancer.

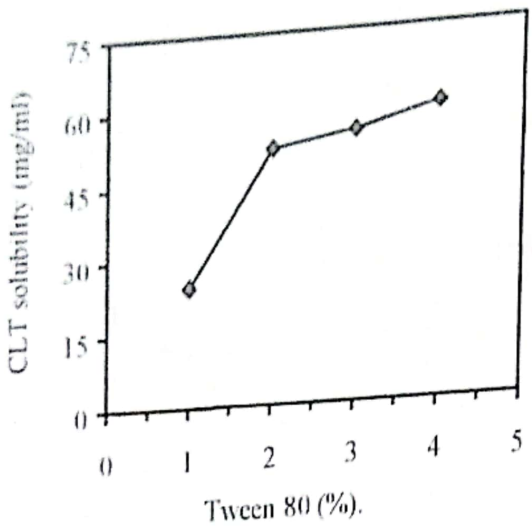

Fig. (2): Effect of Tween 80 concentration on CLT solubility in Tween 80-IPP mixture.

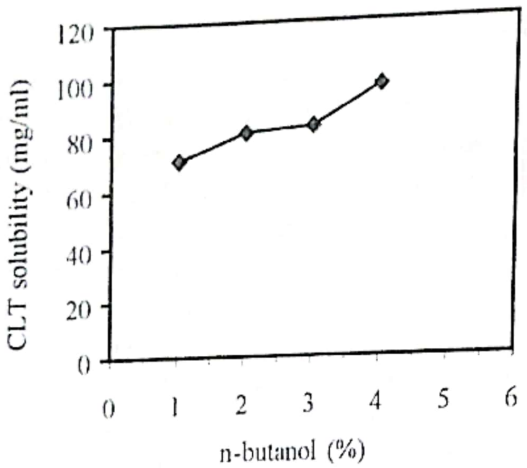

Fig. (3): Effect of n-butanol concentration on CLT solubility in n-butanol-IPP mixture.

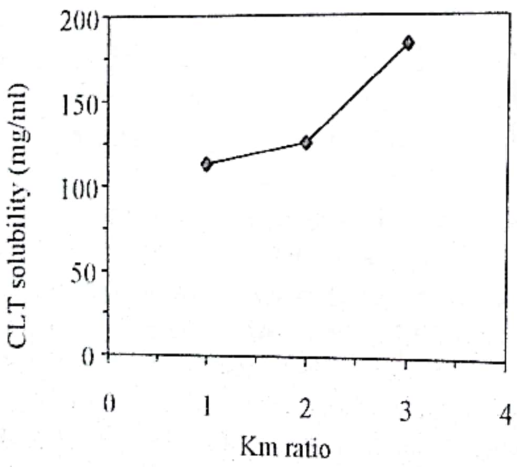

Fig. (4): Effect of Km ratio of Tween 80 and n-butanol on solubility of CLT in a mixture of Tween 80-n-butanol and IPP.

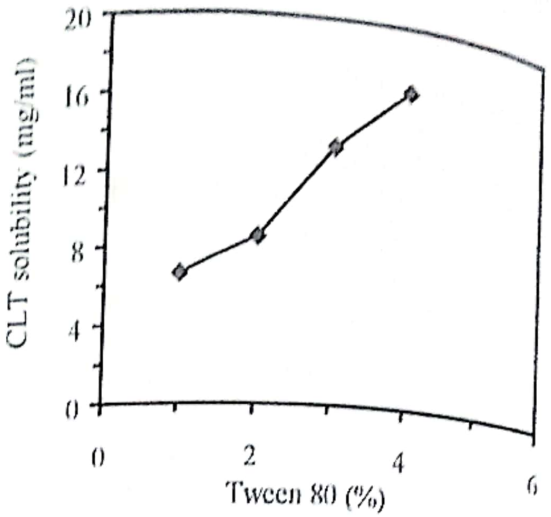

Fig. (5): Effect of Tween 80 concentration on solubility of CLT in a mixture of Tween 80 -IPP and water.

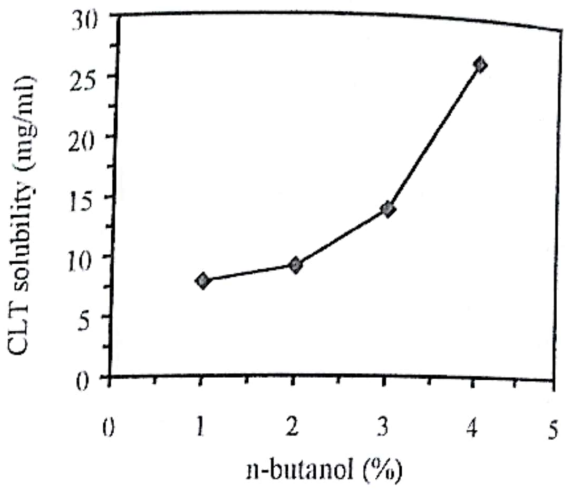

Fig. (6): Effect of n-butanol concentration on CLT solubility in a mixture of n-butanol-IPP and water.

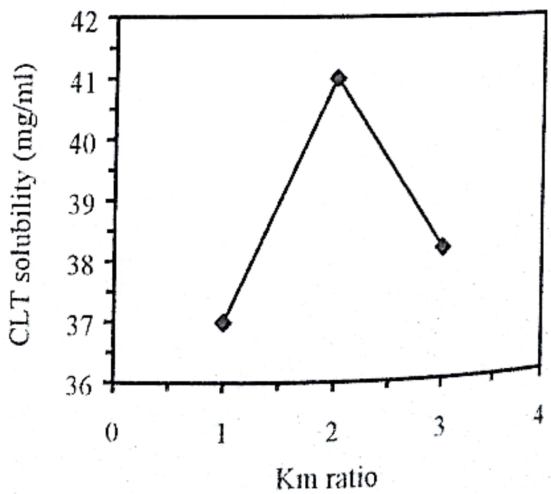

Fig. (7): Effect of Km of Tween 80 and n-butanol on solubility of CLT in microemulsion systems.

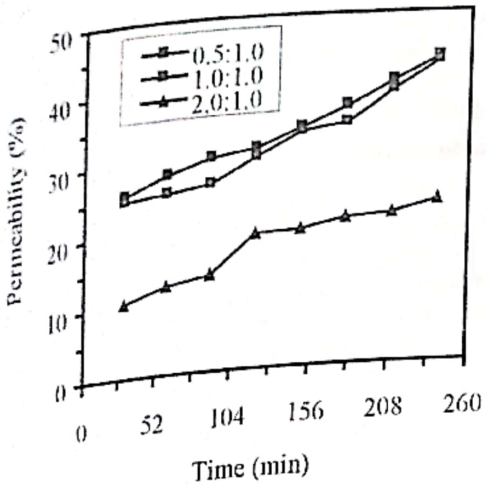

Fig. (8): Permeability of CLT through an isolated abdominal rabbit skin as a function of Km ratio.

3.5. *In vitro* antimycotic activity:

In-vitro antimycotic activity of CLT against *Candida pseudo tropicalis* was performed using 1% CLT solution in methanol, microemulsion system with out drug and microemulsion containing CLT. The area of inhibition zone corresponding to CLT solution in methanol was examined and found to be 6 cm and compared to those of drug solution in different microemulsion systems (0.5/1, 1/1, 2/1). As shown in table 1 a dramatic antimicrobial effect depend on the formulation used was occurred . It is clear that all microemulsion formulations are more effective against *Candida pseudo tropicalis* than CLT solution form. The increased biological activity of microemulsion formulations could be due to the presence of surfactant and co-surfactant which increase the fluidity and causes disruption of the microbial membrane. This action leads to a leaky cell membrane and increase the permeability towards the drug[16]. The above mentioned results show that, microemulsion formulations can be used for topical application of CLT and of limited use for oral and ocular preparations because of the irritant effect of n-butanol and toxicity after oral administration for long period[17,18].

Table (1): *In vitro* antimycotic activity of 1% CLT in different microemulsion systems using agar-cup method and *Candida pseudo tropicalis* as test organism:

Mass ratio (Km)	Inhibition zone (mm)	
	Systems containing CLT	Placebo
0.5/1	37.5	10.0
1/1	29.4	8.0
2/1	24.6	7.0

CONCLUSIONS

- The reported results showed that Oil-in-water microemulsion prepared with isopropyl palmitate ,Tween 80, n-butanol and water could solubilize CLT up to 40mg/ml, which is an 4100- fold increase compared with the solubility of CLT in water.
- The antimycotic activity of microemulsion system was related to the % of co-surfactant, where at higher % of co-surfactant (Km 0.5/1) the largest inhibition zone was obtained 37.5cm, this result was parallel with the permeation of drug through a rabbit skin.

REFERENCES

1) Lawrence M.J., Rees G.D., **Adv. Drug Deliv.**, 6, 45(1), 89 (2000).
2) Paul B.K. and Moulik S.P., **J. Disper. Sci. Tech.**,18, 301 (1997).
3) Scherlund M., Malmsten M., Holmqvist P. and Brodin A., **Int. J. Pharm. Sci.**, 194, 103 (2000).
4) Constantinides P.P., **Pharm. Res.**, 12,1561(1991).
5) Delgado-Charro M.B., Iglesias-Vilas G., Blanco-Mendez J., Lopez-Quitela M.A., Marty J.P. and Guy R.H., **Euro. J. Pharm. and Biopharm.**, 43, 37 (1997).
6) Amin N.A., Hinhawy D.S. and Sorour F.A., **Zagazig Univ. Medical Journal**, l6, 183 (1983).
7) Holt R.J., Ed. By D.C.E.Speller, John Wiley and Sons Chichester, New York, Brisban, Toronto, 113 (1980).
8) Martindale, the Extra Pharmacopoeia 28th Ed., the Pharmaceutical Press, London, 721 (1982).
9) Aboofazeli R., M. Lawrence J., **Int. J. Pharm.**, 93,161 (1993).
10) Gasco M. R., Carlotti M.E. and Trotta M., **Int. J. Cosmo. Sci.**, 10, 263 (1988)
11) Trott M., Cavalli R., Ugagio E. and Gasco M.R., **Int. J. Pharm.**,143, 67 (1996)
12) Kale N.J. and Allen L.V., **Int. J. Pharm.**, 57, 87 (1989).
13) GaoGao Z., Gonchoi H., Jongshin H., Mipark K., Jeong Lim S., Hwang K.J. and Kim C.K., **Int. J. Pharm. Sci.**, 161, 75 (1998).
14) Nandi I., Bari M. and Joshi H., **AAPS Pharm. Sci.Tech.**, 4(1), Article 10 (2003).
15) Thacharodi D. and Rao K.P., **Int. J. Pharm.**, 96, 33 (1998).
16) Attwood D. and Florence A.T., Surfactant Systems, Chapman and Hall, London (1983).
17) Twakabayashi, Horiuchi M., Sakagushi M., Onda H. and Iljima M., **Acta Pathol. Jpn.**, 34, 471 (1984).
18) Nelson B.K., Brightwell W.S., Khan A.J, Burg R. and Goad P.T., **Fundam. Appl. Toxicol.**, 12, 469 (1989).

صياغة وتقييم عقار الكلوتريمازول في المستحلبات الدقيقة

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الهدف من الدراسة هو تحسين الذوبانية وزيادة النشاط المضاد للميكروبات لعقار الكلوتريمازول وذلك عن طريق استخدام أنظمه مستحلبات دقيقه. وقد تم تحضير مستحلبات دقيقه بنسب مختلفة من مواد ذات نشاط سطحى ومواد مساعده باستخدام الايزوبروبيل بالميتات كوسط زيتي والتوين كمادة ذات نشاط سطحي و ن-بيوتانول كماده مساعده وذلك مع الماء. وقد أوضحت النتائج أن زيادة نسبة التوين-٨٠ إلى نسبة ن- بيوتانول يصحبها زيادة في ذوبا نية العقار في الماء وقد وصلت ذوبا نية العقار إلى قمتها عند ١/١ توين: ن-بيوتانول. حيت بلغت الزيادة في ذوبانية العقار ٤١٠٠ مره مقارنة بالماء فقط. كذلك ثبت أن سرعة انطلاق العقار من أنظمة المستحلبات الدقيقة ونشاطه المضاد للميكروبات كان أفضل في الصياغة المحتوية على نسبة ١/٠,٥ توين: ن-بيوتانول.

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