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FORMULATION AND EVALUATION OF CIPROFLOXACIN HYDROCHLORIDE SUPPOSITORIES

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في هذه الدراسة تم صياغة السبروفلوكساسين هيدروكلوريد بإستخدام قواعد اللبوسات الدهنية المصنعة المختلفة مثل السبوسير إيه إم ، والسبوسير سي إم ، والنوفاتا بي ، والنوفاتا بي دى ، والويتبسول دبليو ٣٥ والقاعدة الطبيعية زبدة الكاكاو.

وفى هذه الدراسة تم تعيين كمية العقار فى اللبوسات بمختلف القواعد وتعيين درجة الصلابة والوقت اللازم لليونة اللبوسات.

وبدراسة انطلاق المضاد الحيوى (السبروفلوكساسين هيدروكلوريد) من هذه القواعد باستخدام الماء المقطر عند درجة حرارة ٣٧°م وبقياسه عند الطول الموجى ٢٧٨ وجد أن أعلى معدل انطلاق كان من قاعدة السبوسير إيه إم ويليها نوفاتا بى دى ، نوفاتا بى ، الويتبسول دبليو ٣٥ ، زبدة الكاكاو ثم السبوسير سى إم على التوالى.

وقد تم دراسة التوافر الحيوى لهذه اللبوسات التى تحتوى على ١٠٠ مجم من العقار فى الأرانب التى يتراوح وزنها من ١,٥-٢ كيلوجرام.

وبإستخدام الكروماتوجرافيا ذات الضغط العالى لتعيين تركيز العقار فى البلازما - فقد وجد أن العقار قد أمتص بصورة جيدة من اللبوسات ويتفاوت هذا الامتصاص تبعا للقاعدة المستخدمة وقد وجد أن هناك توافق بين نتائج انطلاق العقار من هذه القواعد فى الماء عند ٣٧°م وبين نتائج التوافر الحيوى للعقار من مختلف القواعد.

Formulation of the antibiotic, ciprofloxacin hydrochloride suppositories was carried out using different semisynthetic lipophilic bases as suppocire AM, suppocire CM, Novata B, Novata BD, Witepsol W35, and natural lipophilic base as Cocoa butter.

The in-vitro release study of 100 mg ciprofloxacin hydrochloride suppository from these bases was performed in distilled water at 37° using thermostatically controlled mechanical shaker water bath and the drug was measured at 278 nm. The results obtained revealed that suppocire AM gave the highest release rate followed by Novata BD, Novata B, Witepsol W35, Cocoa butter and Suppocire CM respectively.

The bioavailability of ciprofloxacin hydrochloride from suppositories containing 100 mg of the drug after rectal administration in rabbits (1.5-2 kg), was carried out. HPLC procedures were adopted for the determination of drug in plasma. The results obtained revealed that it was in agreement with the in-vitro data. The results showed that the drug was regularly absorbed and provided AUC ranging between 10.3 to 20.23 μ.hr./ml. Other pharmacokinetic parameters as C_{max}, T_{max}, K_a, K_e, $t_{½abs.}$ and $t_{½el}$ were also calculated.

INTRODUCTION

Quinolone derivatives as antibacterials have aroused much interest because of their potency and efficacy.[1-4] The first analogue of this class of synthetic agents used clinically was nalidixic acid for treating urinary tract infections. In recent years, synthesis of the newer 4-quinolones has renewed the interest in this family of compounds. The addition of 6-fluoro and 7-piperazinyl groups to the molecule resulted in enhanced activity against Enterobacteriaceae, as well as the activity against a wide range of gram-negative organisms and gram-positive

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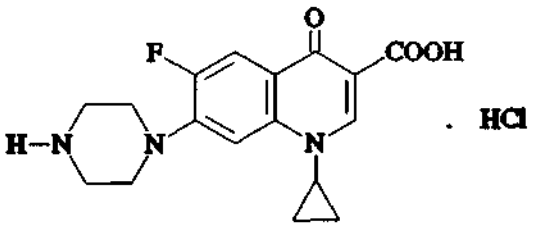
cocci. The increased potency of the new fluoroquinolones has greatly expanded their potential clinical usefulness.[5,6]



The chemical formula of ciprofloxacin hydrochloride.

The primary mechanism of action of fluoroquinolones is the inhibition of DNA gyrase. So, penetration in the bacterial cell is a major determinant of their antibacterial activity.[7] Ciprofloxacin hydrochloride which is a mono fluorinated quinolone, is not considered as hydrophobic since its octanol/water partition coefficient (0.031) is less than one.[8] It has a good tissue and bacterial cell penetration with a wide spectrum of activity, and is effective agaisnt gram-positive and gram-negative bacterial species. The efficacy of ciprofloxacin hydrochloride has led to using it for treatment of various bacterial infection diseases. However ciprofloxacin hydrochloride is considered to be less effective than other fluoroquinolones like pefloxacin and ofloxacin in some interacellular infections. This has been attributed to relatively short interacellualr residence time.[9]

The rectal route is a convenient means of administering drugs to patients who are unwilling or unable to swallow medication, such as unconscious patients, infants, children, and patients who are vomiting. A pediateric suppository may prove useful when children are unable to take an oral antibiotic.[10]

Ciprofloxacin hydrochloride is available in the form of tablets only, thus the aim of this study is formulation of ciprofloxacin hydrochloride in the form of suppositories and investigation of *in-vitro* release and *in-vivo* rectal absorption of ciprofloxacin hydrochloride from different lipophilic suppository bases.

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride was purchased from Sigma Chemical Co. (St. Louis Mo., USA) and was used as received. Cocoa butter (Premium vegetable oils SDN BHD Malaysia). Witepsol W35 (Dynamit Nob., G.F.R.). Suppocire AM, Suppocire CM (Gattefossé-France). Novata BD, Novata B (Henkel KGaA, Germany)

Apparatus used

- Double beam spectrophotometer (150-02) Shimadzu, Ltd., Japan.
- Erweka hardness tester for suppositories, (SBT model), Erweka, G.F.R.
- Erweka softening time tester (mode SSP),. Erweka G.F.R.
- Thermostatically controlled mechanical shaker water bath GFL Germany.
- A centrifuge (Centurion Scientific LTD, UK).
- Knauer HPLC system consisted of a knauer HPLC delivery pump 64, a Knauer spectrophotometric detector, C-R6A chromatopac recording integrator, and a stainless steel C-18 Eurospher 80 column.

Methods

1- Preparation of ciprofloxacin hydrochloride suppositories

The suppositories were prepared by the fusion method using metal mold weighing 1 gm. The base was melted. 100 mg of ciprofloxacin hydrochloride was incorporated in the base. Then the melt was poured into mold, and allowed to cool and congeal into suppositories. The formed suppositories was removed from the mold. Drug displacement values in the used bases were determined and the amount of ciprofloxacin hydrochloride per suppository was calculated.

2- Drug content uniformity of the prepared suppositories

The test was carried out on the suppository batches soon after preparation. Ten suppositories

under test were randomly selected from each batch and assayed individually for drug content. A preweighed suppository was melted and extracted with five portions each of 20 ml of warm distilled water. The aqueous extract was collected throughly into 100 ml volumetric flask. Samples were withdrawn, diluted and measured spectrophotometrically at 278 nm. The amount of the drug was calculated in each suppository. Blank suppositories were tested, and it was found that the suppository bases did not interfere with the spectrophotometric assay of the drug.

3- Breaking or hardness test for the suppositories

The hardness of the prepared suppositories was determined at 25° using Erweka hardness tester (type SBT) and applying its respective method.

4- Determination of softening time for suppositories

The softening time of fatty suppositories was determiend using Erweka softening time tester (Type SSP) the tester was connected to recirculating thermostatically controlled water bath at 37°. The time in minutes required for complete softening of the suppository was recorded as the softening time.

5- *In-vitro* release of ciprofloxacin hydrochloride from the prepared suppository bases

One suppository was placed onto a glass tube with standard cellophane membrane (30/32). The cellophane membrane was soaked in distilled water overnight, withdrawn, rinsed and firmly stretched and tied at the tube end. The tube was vertically suspended in a 250 ml beaker containing 100 ml distilled water. The temperature was maintained at $37\pm0.1°$ in a thermostatically controlled water bath. Five ml samples were withdrawn at specified time intervals, then compensated with equal volume of distilled water after each withdrawal. The drug content in each sample was assayed as mentioned before.

6- *In-vivo* investigations of ciprofloxacin hydrochloride suppository

Twenty four healthy rabbits, weighing 1.5-2 kg, were divided into six groups, each group of four animals. The animals were fasted for food 18 hr prior to the experiment but had free access to water. The rabbits were evacuated before insertion of suppositories. The suppository weighing 1 gm was then inserted in the rectum. The anal end was pinched with a clip for 1 hr. to prevent explusion of the suppository. The *in-vivo* tested suppositories bases were containing 100 mg of ciprofloxacin hydrochloride.

7- Assay of ciprofloxacin hydrochloride in plasma

Heparinized blood samples were taken at ½, 1, 2, 3, 4, 5, and 6 hr. The samples were centrifuged immediately for 15 min. at 4000 rpm. Blood plasma was collected and stored at -15° untill ready for assay. Samples of 200 µl were withdrawn and added to 200 µl of methanol in order to deprotenize the plasma. The samples were centrifuged for 15 min. Twenty five µl of the clear supernatant were injected in HPLC apparatus for analysis. The HPLC system consisted of a Knauer HPLC delivery pump 64, a Knauer varaible wavelength spectrophotometric detector monitored at 278 nm for detecting the drug concentration, C-R 6A Chromatopac recording integrator; a stainless steel (25x0.5 cm.i.d.) C-18 Eurospher 80 (5 µ particles) column, attached to cartridge guard column. The mobile phase was a buffer solution consisted of 1.5 gm potassium dihydrogen phosphate (KH_2PO_4), and 1.3 gm disodium hydrogen phosphate (Na_2HPO_4) dissolved in 450 ml of deionized water and 550 ml of methanol (45:55). In all runs, the flow rate was 1 ml/min.

RESULTS AND DISCUSSION

The results obtained from the drug uniformity content test showed that the drug content in the prepared suppository was in the range of 98% to 100.2%.

Table (1) represent the results of hardness and softening time of different suppository bases. The study of hardness using Erweka hardness tester indicated that the suppository of

Table 1: Softening time and hardness values for the investigated lipophilic suppository bases.

Type of bases	Softening time (min)		Hardness (Kg)	
	non-medicated	medicated	non-medicated	medicated
Suppocire AM	3	2.5	2.6	3.4
Suppocire CM	12.5	15	4.6	5.5
Novata B	6	6	5.8	5.8
Novata BD	7	7	5.6	5.6
Witepsol W35	7.5	8	5.2	5.6
Cocoa butter	5	4.5	4	5.4

Suppocire AM base showed the lowest hardness (3.4 kg), while the other bases showed about the same hardness with little difference. Determination of softening time of the prepared suppositories showed that the Suppocire AM base gave the shortest time of softening (3 min), while the Suppocire CM base showed the longest one (12.5 min).

The *in-vitro* release of ciprofloxacin hydrochloride from the various lipophilic bases are shown in Fig. 1. The release of ciprofloxacin hydrochloride after 4 hours was 99%, 87.5%, 81.9%, 69.1%, 55% and 21.3% from Suppocire AM, Novata BD, Novata B, Witepsol W35, Cocoa butter, and Suppocire CM respectively. The highest quantity of released ciprofloxacin hydrochloride was from Supocire AM after 4 hours.

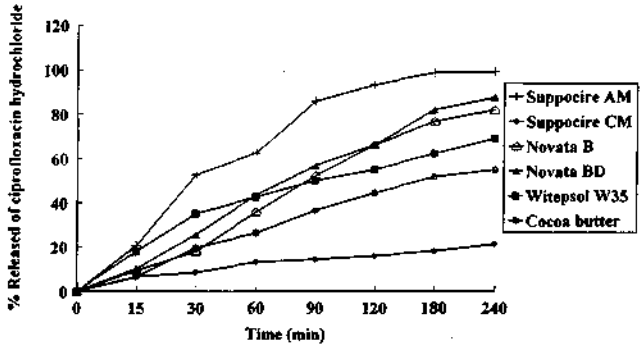


Fig. 1: The release rate of ciprofloxacin hydrochloride from the investigated lipophilic suppository bases.

The different brands of semisynthetic bases showed different release rates. Each brand contains a unique combination of tri-, di- and monoglycerides which have certain characteristics to that base. Cocoa butter being primarily a mixture of fatty acid triglycerides showed lower release rate than other bases. The nature of the base clearly has an effect on the release rate and the amount of the drug released. It may appear that the hydroxyl value of suppository base is of some significance to the release of the drug from suppositories.[11] The synthetic suppository bases are mixtures of fatty acid esters with certain amounts of glycerides. The hydroxyl value of a base is determined by the presence of mono- and diglycerides and therefore represents the availability of free hydroxyl groups. Ciprofloxacin hydrochloride release from bases with lower hydroxyl values (Suppocire AM with hydroxyl value $\leq$ 6 and Novata BD with hydroxyl value $\leq$ 15) was shown to be faster than from those of higher hydroxyl value. These results could be accounted for on the basis of simple partitioning between aqueous and lipid phases.[10]

Consideration of hydroxyl value alone could not account for these release results, however, since Suppocire CM despite having lower hydroxyl value than Novata BD and Novata B, showed lower release rate. Examination of softening time and liquification time showed that Suppocire CM have the longest softening time (12.5 min) and longest liquification time (+60 min),[12] which could

result in decrease of drug release. Also Suppocire AM has the shortest softening time (Table 1), and shortest liquification time (8.7)[12] which explain the faster and complete release of ciprofloxacin hydrochloride from Suppocire AM.

The results obtained by Webster *et al.*,[10] of *in-vitro* release of amoxicillin from lipophilic suppositories was in agreement with these results. The release rate was found to be in the order of Novata BD > Novata 299 > Witepsol W35 > Suppocire A32 > Theobroma respectively. The drug release from bases with lower hydroxyl value (such as Novata bases) was shown to be faster and more complete than from those of higher hydroxyl value and the release also depend on the solidification point of the bases.[10]

Another explanation that might account for the decrease in the release of ciprofloxacin hydrochloride was the complexation of the drug with hydroxyl groups in the bases used.

The release data were analyzed according to zero order, first order, and Higuchi diffusion model (Table 2). The calculated results obtained indicated that the release rate is first order or diffusion Higuchi model, because of the very near correlation coefficient. Successive evidence for the relative validity of diffusional and first-order models was obtained by plotting log amount of drug released per unit surface area vs. log time. In the case of a diffusional process, a straight line with a slope of 0.5 is obtained.[13] The results obtained indicated that it is first-order.

The *in-vitro* methods of evaluating dosage forms provide only indirect evidence of the therapeutic utility of the drug in a given dosage form. It would be more desirable to employ a direct mean of ascertaining the utility of a particular dosage form. In order to do this, *in-vivo* methods are needed which will reflect the true safety and efficacy of the dosage forms. The estimation of the bioavailability of a drug in a given dosage from is direct evidence of the efficiency with which a dosage form performs its intended function.

Plasma ciprofloxacin hydrochloride was analyzed using HPLC. The calibration curve of ciprofloxacin hydrochloride from palsma was linear in the range of 0.8 μg/ml to 5.5 μg/ml, and the correlation coefficient was 0.9996.

Mean plasma level profiles of ciprofloxacin hydrochloride obtained following administration of ciprofloxacin hydrochloride suppository in different investigated bases are graphically represented in Fig. 2. The figure show that, the maximum plasma concentration after one hour, and the maximum drug concentration (C_{max}), varies in the same order as the *in-vitro* results. The peak heights were 4.53, 2.64, 3.57, 3.17, 3.3 and 3.03 for the Suppocire AM, Suppocire CM, Novata BD, Novata B, Witepsol W35 and Cocoa butter bases respectively. Table (3) represents the plasma concentration and its ±SD after rectal administration of suppositories in rabbits.

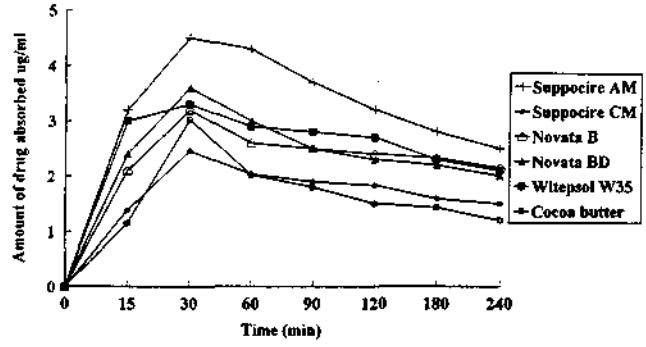


Fig. 2: The mean plasma profile of ciprofloxacin hydrochloride absorbed from the investigated lipophilic bases.

Pharmacokinetic analysis

A one compartment model was adopted to calculate the pharmacokinetic parameters, including rate constants and half-lifes for absorption and elimination, and AUC (Table 3). Both C_{max} and T_{max} were obtained directly from the plasma measurment data. The K_e was estimated from the terminal slope of the plasma concentration time curve after logarithmic transformation and application of linear regression.

The absorption rate constant, Ka, is determined by the method of residuals,[14] using the following equation:

$$A\, e^{-Kat} = A\, e^{-Kt} - C$$

Table 2: Kinetic study of release rate of ciprofloxacin hydrochloride from the investigated lipophilic suppository bases.

Suppository bases	Zero order		First order		Higuchi diffusion	
	r	$Kx10^{-2}$	r	$Kx10^{-3}$	r	K
Suppocire AM	0.852	70.45	0.990	2.11	0.962	15.02
Suppocire CM	0.961	13.97	0.970	7.10	0.992	2.82
Novata B	0.962	82.21	0.996	9.22	0.991	16.59
Novata BD	0.933	70.43	0.990	7.17	0.980	14.54
Witepsol W35	0.932	45.50	0.980	3.94	0.983	9.33
Cocoa butter	0.940	47.12	0.971	3.21	0.982	9.65

r: correlation coefficient.
K: release rate constant.

Table 3: Plasma concentrations of ciprofloxacin hydrochloride after rectal administration of suppositories to rabbits.

Suppository bases	No. of animals	Mean plasma concentration at different time intervals after administration of ciprofloxacin suppositories (μg/ml, mean ± SD)						
		0.5	1	2	3	4	5	6
Suppocire AM	4	3.22 ±0.17	4.53 ±0.14	4.32 ±0.17	3.71 ±0.29	3.21 ±0.22	2.82 ±0.22	2.52 ±0.23
Suppocire CM	4	1.38 ±0.10	2.46 ±0.15	2.04 ±0.16	1.87 ±0.16	1.83 ±0.14	1.59 ±0.17	1.51 ±0.17
Novata BD	4	2.4 ±0.55	3.57 ±0.10	3 ±0.10	2.44 ±0.12	2.25 ±0.11	2.2 ±0.10	2 ±0.13
Novata B	4	2.1 ±0.13	3.17 ±0.11	2.6 ±0.11	2.5 ±0.15	2.4 ±0.12	2.3 ±0.11	2.2 ±0.11
Witepsol W35	4	3 ±0.16	3.3 ±0.16	2.88 ±0.15	2.75 ±0.15	2.67 ±0.13	2.28 ±0.12	2.06 ±0.15
Cocoa-butter	4	1.15 ±0.88	3.03 ±0.83	2.02 ±0.11	1.89 ±0.12	1.52 ±0.18	1.47 ±0.22	1.16 ±0.18

Table 4: Pharmacokinetic parameters of ciprofloxacin hydrochloride from the investigated suppository bases.

Pharmacokinetic parameters	Suppocire AM	Suppocire CM	Novata B	Novata BD	Witepsol W35	Cocoa butter
C_{max} (ug/ml)	4.53±0.14	2.46±0.15	3.17±0.11	3.57±0.10	3.3±0.16	3.03±0.83
T_{max} (hr)	1	1	1	1	1	1
$K_{abs.}$ (hr^{-1})	0.20	0.10	0.11	0.17	0.11	0.10
K_{el} (hr^{-1})	1.04	0.34	0.48	0.51	0.49	0.24
$t_{½\ abs}$ (hr)	3.47	6.79	6.29	4.07	6.38	6.76
$t_{½\ el}$ (hr)	0.67	2.05	1.43	1.35	1.43	2.82
AUC (μg/hr/ml) 0-6 hr.	20.23 ±0.84	10.67 ±1.60	14.35 ±0.63	14.78 ±0.59	15.60 ±0.89	10.30 ±0.73

C_{max} : maximum concentration
T_{max} : time at which maximum concentration obtained
$K_{abs.}$: absroption rate constant
K_{el} : elimination rate constant
$t_{½\ abs}$: half-life of absorption
$t_{½\ el}$: half-life of elimination
AUC : area under the curve

where Ka= absorption rate constant, K= elimination rate constant, C= plasma concentration, t= time in hours. The area under the curve AUC was calculated using the trapezoidal rule.[14]

From Table (4) it is observed that the AUC is directly proportional to the amount absorbed and there is a correlation between the amount of the drug released *in-vitro* and *in-vivo* absorbed amount. The AUC of ciprofloxacin hydrochloride Suppocire AM suppository was the largest area which indicated the highest amount absorbed, while those of the drug with Suppocire CM and Cocoa butter were the lowest.

From Table (4) it is observed that all the lipophilic bases gave the same T_{max} (1 hr), while C_{max} was different according to the type of base investigated. The data show that Suppocire AM gave the highest C_{max} followed by Novat BD, Witepsol W35, Novata B, Cocoa butter and Suppocire CM. It is also observed very slight difference between the C_{max} of Novata B and Witepsol W35 suppository bases. The pharmacokinetic parameters obtained, (Table 4), indicated high absorption rate and high elimination rate constants of Suppocire AM suppository.

The *in-vivo* study revealed that there is a correlation between the *in-vitro* and the *in-vivo* results obtained.

Conclusion

The results obtained from this study revealed that:

1- A good release of ciprofloxacin hydrochloride from the investigated lipophilic suppository bases as Suppocire AM, Novata BD, Novata B and Witepsol W35.
2- Good absorption of the drug from these bases after rectal administration.
3- There is a good correlation between the *in-vitro* and the *in-vivo* results obtained.

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