DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 13(9-11), 1973-1987 (1987)

RELEASE CHARACTERISTICS AND BIOAVAILABILITY OF NORFLOXACIN FROM SUPPOSITORY BASES.

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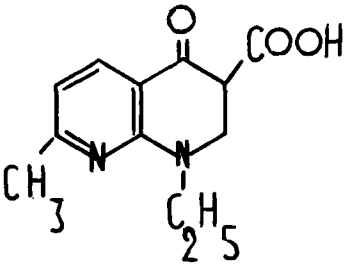
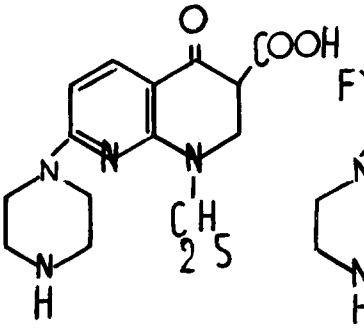
The in vitro release of norfloxacin (a new broad spectrum antimicrobial agent whose pharmacokinetics are characterised by varied gastrointestinal absorption and irregular plasma level) from different suppository bases was studied in order to obtain suitable formulation with good release and satisfactory drug concentrations in plasma. The bioavailability of suppositories containing 200 mg drug made from the bases that showed the best in vitro release was investigated. The release rate was in the order of PEG 400, 1540, 4000 mixture in ratio of 20: 33: 47 respectively > Witepsol H15 > Witepsol W 35 > Witepsol E 75. Addition of Tween 20, Tween 80 and Myrj 45 to the Witepsol members greatly increased the release rate especially at surfactant concentration of 5×10^{-5} mol.g.$^{-1}$ of the drug. The amount of the increase was found to be dependent on the partition coefficient of the used bases, their melting range and the HLB of the added surfactants. The breaking load test revealed that the PEG mixture base has the greater elasticity and the longer softening interval. The bioavailability results of norfloxacin after rectal administration in albino rabbits was found to be in agreement with the in vitro data. The results showed that the drug was regularly absorbed and provided AUC ranging between 3.25 and 7.05 µg hr m^{-1} which are satisfactory enough to give its antimicrobial activity.

1. INTRODUCTION

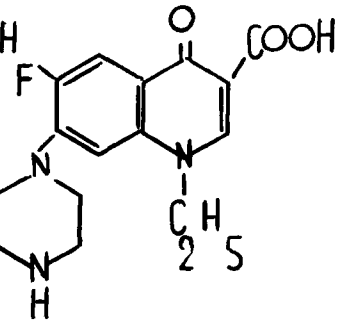
Norfloxacin is an antibacterial oxoquinoline carboxylic acid structurally related to nalidixic acid and oxolinic acid. The compound has a braod spectrum of activity against pathogenic bacteria (1). Like pipemidic acid it contains a piperazinyl ring which confers antipseudomonas activity, but in addition it has a 6-flurine atom which contributes to its greater potency (2).



Nalidixic acid (Naphthridine derivative)



Pipemidic acid (Pyridonpyrimidine derivative)



Norfloxacin (Quinoline derivative)

Norfloxacin was found to be four to five times more effective than gentamicin or Carbenicillin (3). Its oral administration was found to be five times more active than pipemidic or nalidixic acid in treatment of experimental urinary tract infection (4).

The pharmacokinetics of norfloxacin are characterised by varying gastrointestinal absorption which is greatly affected by food intake (5,6). The drug has not been administered intravenously to human so that the exact determination of the degree of gastrointestinal absorption have not been possible (7).

Previous studies on administration of norfloxacin (8-10) have given little or no informations regarding the routes of administration and no informations about the rectal use of this new promising drug.

The purpose of this study was to investigate the in vitro release and the in vivo rectal absorption of norfloxacin from different suppository bases in the hope that suitable formulation of good release and satisfactory drug-plasma concentration could be obtained. The results have been explained on the bases of the partition coefficient and the melting range of the used bases, and as function of breaking load and elasticity of the formed suppositories.

2. EXPERIMENTAL

2.1. Materials:

Norfloxacin: Merck Sharp and Dohme Research Laboratories. West Point, PA, USA.

Witepsols H 15, W 35 and E 75: Dynamit Noble, West Germany.

Polyethylene glycols 400, 1540, 6000 Prolabo, France.

Tweens 20, 80 & Myrj 45: Atlas Chem. Ind. USA.

Cellophane membrane; Spectroper; M.W. cutt of 12000-14000: Fisher. Sci. Co., USA.

All reagents were analytical or reagent grade purity.

2.2. Methods

Construction of the standard curve of norfloxacin:

A known amount of the drug was dissolved in the least amount of NaOH 1N and then diluted with distilled water. The maximum absorbance was determined using Unicam SP 1800 spectrophotometr and was found to be 284 nm. Serial dilutions of

norfloxacin were made to obtain concentrations of 2, 4, 6, 8, 10, 15, 20 & 30 µg/ml and the UV absorbance of the concentrations was measured. The drug was found to obey Beer's law in all concentrations.

2.2.1. Drug surfactant coprecipitates

Tweens 20, 80 and Myrj 45 were individually mixed with the specified amounts of norfloxacin to produce concentrations of 0.5, 3 and 5 x 10^{-5} mol. $g.^{-1}$ of the drug. The mixture was then dissolved in chloroform. The solvent was evaporated and the solid mass was pulverized, sieved and the fraction 45-63 µm was kept in a desicator until used.

2.2.2. Preparations of norfloxacin suppositories

All suppositories were prepared by the fusion method (11) using a metal mold (ERBO, Prazisions - Formenbau GmH, Mod. 128 B1 Kauale). Drug displacement (12) in the used bases was first determined, and the amount of norfloxacin required was calculated.

2.2.3. Content uniformity

Thirty suppositories were randomly selected from each base, 10 of which were assayed individually. A preweighed suppository was melted and dissolved in 15 ml of phosphate buffer 7.5±0.1 (prepared by dissolving 6.89 g of Na_2HPO_4 in 250 ml of NaOH (0.2 mol/L) and 400 ml of water were then added, adjusting the resulting solution with NaOH 0.2 mol/L to a pH 7.5±0.1, and diluting with water to 1000 ml. It is a simulated intestinal fluid USP without pancreatin (13).

The absorbance was measured on the spectrophotometer at 284nm. Blank suppositories were tested, and it was found that the

suppository bases had no effect on the UV absorbance at 284 nm.

2.2.4. Breaking or hardness test

This was designed to measure the brittleness and fragility of the suppositories. It was determined using Erweka Hardness Tester (SBT, West Germany).

2.2.5. Release of norfloxacin from suppository bases:

Two different methods were used:

Method A: The USP rotating basket dissolution apparatus. Each suppository was placed in the wire basket which was lined inside with filter paper and lowered into a flask containing 600 ml of phosphate buffer pH 7.5. The filter paper was used as a barrier for the diffusion of the suppository base (12). The basket was rotated at 100 rpm at 37±0.1°C.

Five milliliters samples were withdrawn at time intervals and assayed for their drug contents. Five milliliters of fresh phosphate buffer was added to the dissolution medium to compansate for sampling.

Method B: Adopted by Krowczynsky (14). One suppository was placed onto a glass tube (15x3.5 cm) with cellophane membrane firmly tied at its end. The tube was vertically suspended in 250 ml beaker containing 30 ml of the phosphate buffer. The temperature was maintained at 37±0.1°C in a thermostatically controlled water bath. One ml samples were withdrawn at time intervals, then compensated with equal amounts of the fresh buffer solution after each withdrawal. The drug content in each sample was assayed as before.

2.2.6. Partition coefficient measurements:

The partition coefficient measurements of the drug between the used bases and the release medium was determined by mixing the drug with the base and buffer in a bottle. The bottle was then rotated in a thermostatically controlled water bath for a certain time at 37±0.2°C and tested as previously reported (15).

2.2.7. In vivo study:

Twelve healthy white New Zealand male rabbits (2.5-3 kg) were randomly divided into 4 groups of 3 animals each.

The animals were fasted 18 h prior to the experiment but had free access to water. To stimulate the rabbit for rectal evacuation, a glass tube (0.5x3 cm) slightly lubricated with liquid paraffin was inserted in the rectum. Several minutes latter, the glass tube was removed & the rabbits usually defecated. The suppository was then inserted in the rectum. The anal end was pinched with a Clip for one hour to prevent expulsion of the suppository.

The in vivo tested suppository bases were PEG and each individual Witepsol base containing Tween 20 in concentration of $5x10^{-5}$ $mol.g.^{-1}$.

2.2.8. Assay of norfloxacin in plasma:

A modification of the method adopted by Enadi et al. (16) was followed. Heparinized blood samples were taken at zero, 1,2,4,6 & 8 hours. The samples were centrifuged immediately for 10 minutes at 4000 rpm. Blood plasma was collected and stored at -15°C in dark until ready for assay. Reverse phase octadecyl C_{18} column was used for the extraction of norfloxacin from the biological matrix. The column was prepared

from a 25 ml analytical burrite in which 5 g octadecyl C_{18} (7-10 µm fraction) was introduced. After washing the column with 4 ml of acetonitrile and 10 ml of water, 0.5 ml of plasma and 0.5 ml of water were poured into the column. The column was then washed with 10 ml of water and 2 ml of acetonitrile. The elution of norfloxacin retained on the lypophylic phase of the column was performed with two aliquots of 0.5 ml of a mixture of methanol and phosphoric acid (90-10 V/V). The eluate was collected and dried at 50°C. The dried samples were recovered with 100 µl of the mobile phase and 20 µl were injected into the liquid chromatograph. A selectable wavelength detector was used to monitor the eluate at 284 nm. The mobile phase consisted of a mixture of acetonitrile and 0.05M phosphate buffer pH 2.5 (80:20 v/v). Under these conditions norfloxacin shows a retention time of 241 sec.

2.2.9. Calibration curve:

A 100 mg/L solution of norfloxacin was prepared by dissolving the compound in a small volume of NaOH 1N and diluted to full volume with distilled water (stock solution). Norfloxacin free plasma samples were prepared from the stock solution to obtain concentrations of 0.5, 1, 2, 3, 4 and 5 µg/ml (Fig.3)

3. RESULTS AND DISCUSSION

The drug uniformity test has shown that the drug content in the prepared suppositories was in the range of 98.6-101% which are acceptable limits of the B.P 1980a.

Fig. 1 shows the breaking load of the different suppository bases versus temperature. The two steep curves of the Witepsols W 35 and E 75 containing Tween 20 indicate brittle bases with lower elasticity and smaller softening intervals.

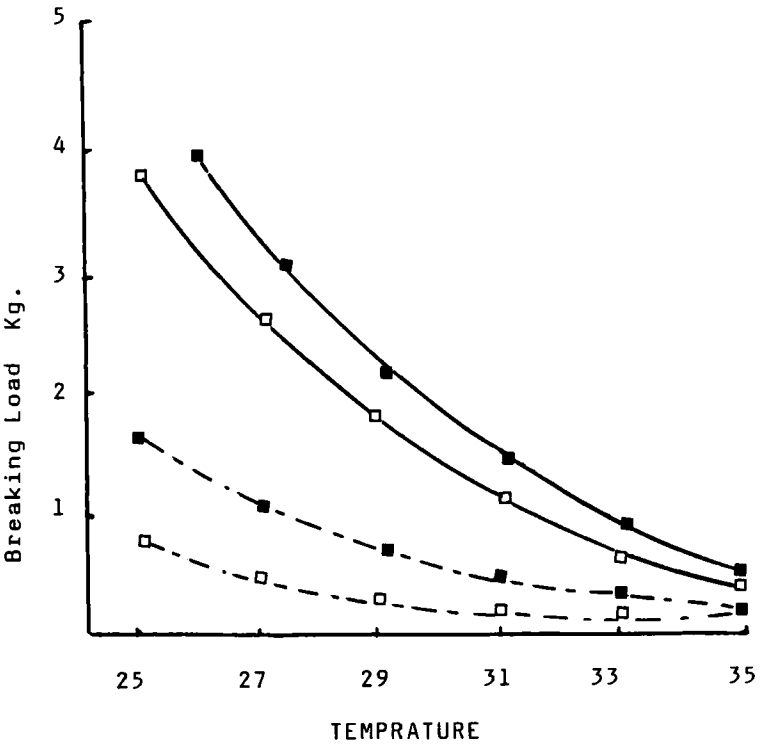


Fig. 1 : Breaking loads of the different suppositories as a function to temperature .

Key : Polyethylene glycol, witepsol H_{15}, witepsol W_{35}, witepsol E_{75}

On the other hand the two flat curves of the PEG and Witepsol H 15 base contained the same drug-surfactant ratio indicate a greater elasticity of the two bases.

The release rates of norfloxacin from the different bases are shown in Fig. 2. The figure represents the results obtained from Krowczynsky method. The USP dissolution apparatus method did not give reproducible results due to the erosion of the water soluble bases. The release of the drug

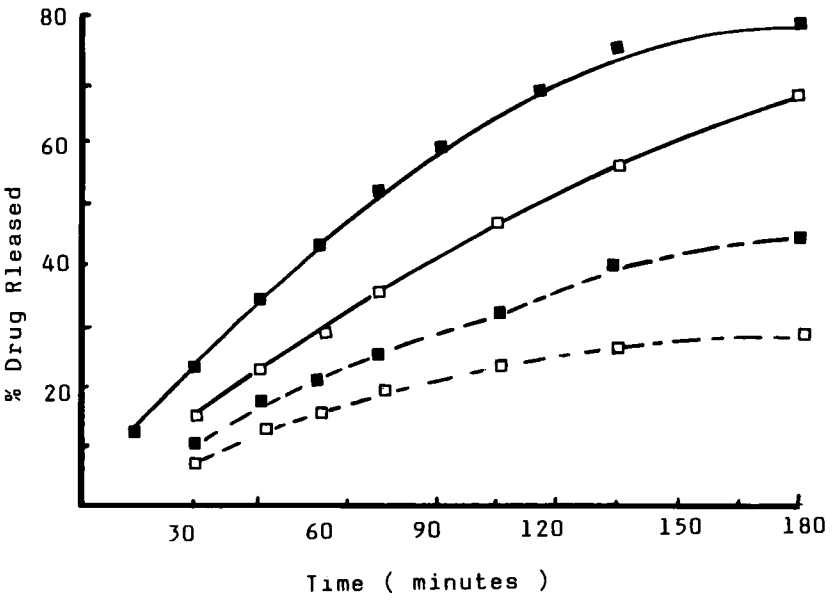


Fig. 2 : Rlease of Norfloxacin from different suppository bases.

Key : as in Fig. 1.

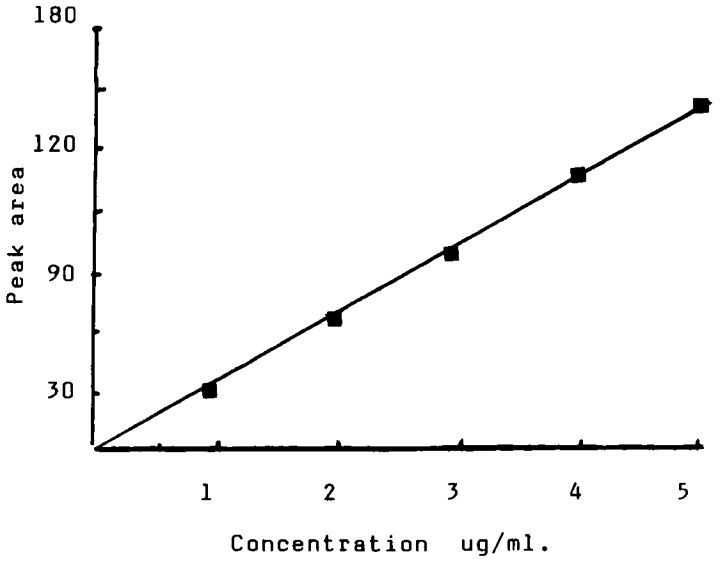


Fig. 3 : Plasma calibration curve of Norfloxacin.

Table 1: Partition coeffecient of norfloxacin between the different bases and the release medium .

Suppository bases	C_{sb}	C_w	Partition coefficient C_{sp}/C_w
Witepsol H15	1645.5	289.7	5.68
Witepsol W53	1798.6	152.5	11.79
Witepsol E75	1943.8	92.8	20.95

C_{sb}: Concentration of norfloxacin after equilebrium µg/10g base

C_w : Concentration of norfloxacin after equilebrium µg/10ml buffer solution

from the hydrophobic bases was found to decrease from 6-9% in the Krowczynsky method compared to their release in the USP dissolution apparatus method. This reflects the irregularity of the leaching of the drug in the former case and also the hindering of drug passage through the quasicrystalline bound water membrane net work in the later case. The release of norfloxacin from the tested bases was in the following order PEG > Witepsol H 15 > Witepsol W 35 > Witepsol E 75. which was in agreement with the partition coefficient results of these bases in Table 1. The higher release of the drug from PEG could be attributed to the great hydrophilic property and the solubilizing effect of this base [17-19]. The increased partition coefficient values of the oleagenous bases in the order of Witepsol E 75 > Witepsol W 35 > Witepsol H 15 indicating more affinity to the rather hydrophobic norfloxacin. The order of release from the Witepsol members depended mainly on the comparatively low

melting ranges of them. Accordingly the release from Witepsol H 15 (33-35°C) was greater than that from Witepsol W 35 (35-37°C) and Witepsol E 75(37-39°C). These results were in agreement to those obtained by Thomas and McCormack (20) and parallel to those obtained for benzocaine and adiphenine release from different bases (17,18). The effect of surfactant on the release of norfloxacin from the Witepsol members is shown in Table 2. It is clear that in all cases the surfactant concentration $5x10^{-5}$ mol.g.$^{-1}$ of the drug shows the higher release data. The release rate was found to vary with the nature and amounts of the surfactant. Myrj 45 was observed to produce the least amount of drug release. Tween 20 showed the best release data while Tween 80 was intermediate between the two. Tween 20 is characterised by its relatively higher HLB (16.7) which could enhance the wetting of the fatty bases with the release medium rather than Myrj 45 HLB (11.1) and Tween 80 HLB (15.0). The increase in the HLB values means an increase in the hydrophilic properties of the Witepsole bases, consequently decrease their affinity to the hydrophobic drug. Another explaination is that, Tween 20 and 80 are characterised by their ester-ether linkage which is superior in enhancing drug release rather than Myrj 45 which has only an ester linkage. The in vivo results in Fig. 4 shows the mean plasma concentrations of norfloxacin from the different bases. The maximum drug concentration (C_{max}) varies in the same order of the in vitro results in Fig. 2. After C_{max} had been reached the plasma wash out curves decreased along a faster slope related to the rapid distribution phase of the drug-plasma concentrations to provide AUC values of 3.25, 4.59, 6.73 and 7.05 all in μg.hr.ml^{-1}

Table 2: Effect of surfactants on percent release of norfloxcin from suppository bases.

Time (minutes)	Tween 20			Tween 80			Myrj 45			Tween 20			Tween 80			Myrj 45			Tween 20			Tween 80			Myrj 45		
Surfactant Conc. mol.g^{-1}x10^{5}	0.5	3	5	0.5	3	5	0.5	3	5	0.5	3	5	0.5	3	5	0.5	3	5	0.5	3	5	0.5	3	5	0.5	3	5
15	10.2	12.3	16.1	9.0	10.1	12.0	8.0	8.1	8.0	7.-	9.9	10.3	6.0	7.2	9.3	5.1	5.6	5.2	5.6	8.5	10.1	4.0	4.9	7.0	5.3	5.2	6.1
30	19.1	21.1	26.6	18.8	19.3	22.3	17.1	17.9	18.1	14.3	18.0	19,9	11.2	12.3	14.1	11.2	11.3	12.0	10.0	13.1	18.1	9.0	10.1	12.1	10.3	10.7	11.0
45	26.2	30.1	35.9	26.0	27.2	29.9	24.1	24.1	26.0	20.2	24,9	28.8	18.1	14.1	22.3	17.1	17.8	19.2	16.3	19.1	25.3	16.0	17.0	20.0	12.1	12.9	14.3
60	34.3	37.6	45.3	33.1	36.7	39.8	31.2	31.3	34.0	26.3	30.0	37.9	22.3	24.3	28.4	22.0	99.1	24.0	19.2	99.9	30.0	20.0	21.1	26.0	18.1	18.9	20.0
90	46.1	52.1	60.0	45.3	49.1	52.7	42.1	43.3	48.0	37.1	40.1	49.9	32.0	34.2	40.0	32.1	32.7	33.3	26.2	30.0	40.1	25.0	24.1	34.1	22.7	22.9	26.3
120	56.2	63.1	72.4	56.8	60.6	64.8	53.2	55.9	60.4	45.2	50.1	60.0	40.1	45.2	50.6	40.1	41.8	43.0	31.0	35.0	50.0	30.1	34.0	44.1	28.3	29.1	31.0
150	65.2	72.2	81.3	65.1	69.3	74.8	62.3	68.0	70.8	51.2	56.4	68.6	46.1	50.2	58.9	42.3	44.2	50.9	36.1	34.1	57.0	32.0	36.0	50.0	32.9	34.4	36.3
180	74.3	76.2	86.4	72.3	78.0	83.1	70.8	73.1	74.3	56.3	60.3	70.2	51.2	56.3	62.7	45.3	51.9	53.8	37.9	40.0	60.0	35.0	40.1	55.4	33.1	36.4	40.0

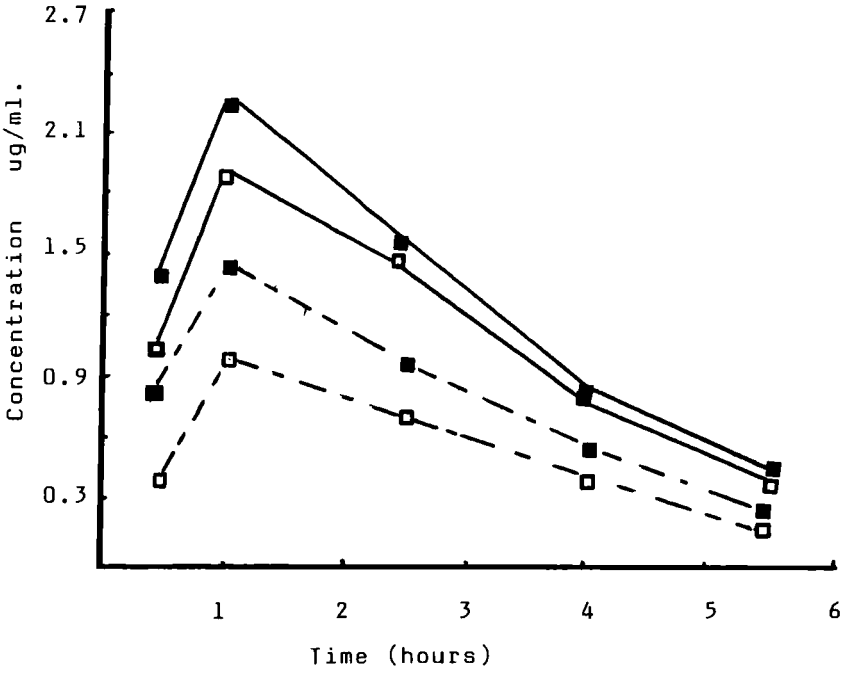


Fig. 4 : Plasma concentration of Norfloxacin in relation to time after adminestration of four types of suppositories.

Key : as in Fig. 1.

for the Witepsols E 75, W 35, H 15 and PEG bases respectively. The pharmacokinetic results agree with those obtained by Abico et al. (21) on powdered drug samples using a microbiological assay.

It was therefore believed that norfloxacin could be regularly distributed into tissues after administring the drug from any of the tested suppository formulations.

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