POLAROGRAPHIC DETERMINATION OF NORFLOXACIN

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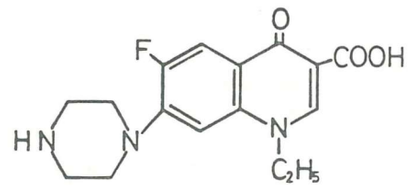
In recent years there is an increasing interest in the determination of biologically active molecules by electrochemical methods. this communication reports on the polarographic behaviour of norfloxacin.

Norfloxacin is a new broad-spectrum antibacterial agent, this substance exhibits greater antibacterial activity againts both gram-positive and gram-negative bacteria than other nalidixic acid analogs.(1)

NORFLOXACIN



The electrochemical study of norfloxacin was carried out in aqueous solution, to stablish its polarographic characteristics and to develop an analytical method for its determination

Solubility and polarographic behaviour of norfloxacin are very affected by the pH solution, in the pH 5-9 range, norfloxacin is an sparingly soluble solid, which shows amphoteric behaviour, being soluble in acid and basic media

EXPERIMENTAL

Apparatus. A Metrohm 506 polarograph an polarographic stand with three electrodes (Metrohm E-A 1029/1 dropping mercury electrode, Metrohm E-A -285

Portugaliæ Electrochimica Acta, 7 (1989) 65-68

platinum counterelectrode and saturated calomel electrode (S.C.E.). A Radiometer potentiometer and a Metrohm AG-9100 combined glass and S.C.E. were also used.

Reagents. Pure norfloxacin was supplied by Liade Laboratories. All other chemicals used were of analytical grade.

Procedure. Suspensions of the pure solid product were used for potentiometric titration using both 0.1000 M HCl and 0.1000 M NaOH reagents. Solutions of norfloxacin at the desired concentrations were prepared using 0.4 M Britton-Robinson buffers. The solutions were placed in the polarographic cell and, after removing oxygen by bubbling nitrogen througt it, the corresponding polarograms were recorded in the -1.2 to -2.0 V. potential range (versus S.C.E.). When the differential pulse polarography technique was used a ΔE of -50 mV was applied. The drop time in D.C.P. and D.P.P. techniques was 2 s/drop.

RESULTS

Potentiometric titration of norfloxacin. Norfloxacin is a sparingly soluble solid which shows amphoteric behaviour, being soluble in acid and basic media and may be titrated with either strong acids or strong bases. Titration curves for the reacction of norfloxacin with 0.1000M NaOH and 0.1000M HCl show two breaks (Figure 1). Its two acid-base ionization constants were calculated by potentiometric titration (glass electrode). Values of $pK_{1a} = 5.50 \pm 0.17$ and $pK_{2a} = 9.67 \pm 0.21$ were obtained. The low solubility ofnorfloxacin in water may be explained by the predominance of an undissociated species instead of with an internally internally ionized molecule (zwitterion) in the pH 5-9 range

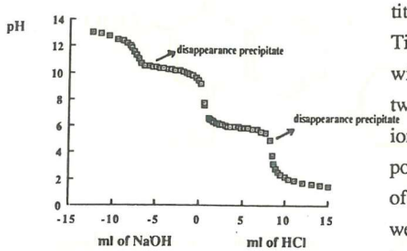
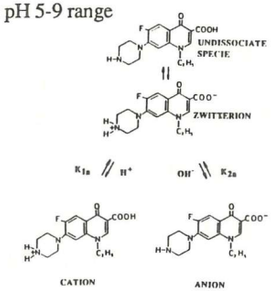
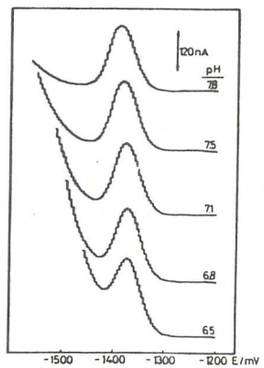


FIGURE 1.-Potentiometric titration



Acid base equilibria between undissociated, zwitterion, anion and cation species of norfloxacin

Polarographic behaviour of norfloxacin. In the polarographic reduction of norfloxacin two waves (DC) or peaks (DP) have been observed. First peak appears in the pH 6.5-10 range; the plot of Ep potential versus pH fits to two straight lines, one dependent (Ep = - 0.31 - 0.12pH) and another independent of pH (Ep = - 1.31 - 0.010pH), with a interception at a pH value of 8.7, close to the pK_{2a} value potentiometrically calculated. The second peak appears in the pH 8.5-10.4 range and its Ep potential remains practically constant at the -1.7V value. Besides, the intensities of the first and second peaks are also very affected by the pH of solution (Figure 2, 3)



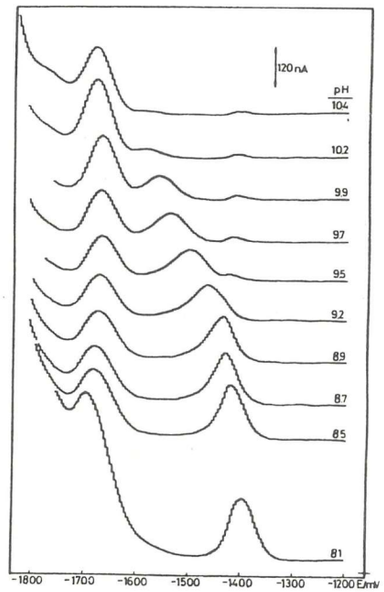


FIGURE 2.-Influence of pH on the polarographic behaviour [Norflox] = 4.80 10^{-5} M

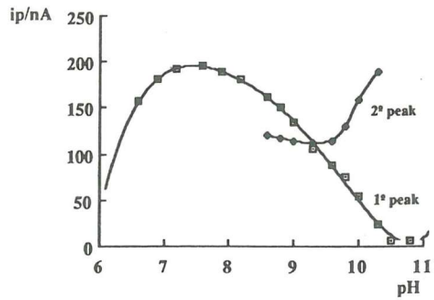


FIGURE 3.-Influence of pH on the peak curent of the first and secon peak [Norflox] = 4.80 10^{-5} M

The analytical possibilities of the first peak are adventageous and have been investigated using as supporting electrolyte a Britton-Robinson buffer (pH=8.0). In this medium norfloxacin yields only a well defined peak at -1.4 V (vs S.C.E.). The influence of the drop time, the pulse amplitude, temperature and concentration of norfloxacin in the polarographic response were studied. The polarographic process is irreversible and there in an adsorption process on the electrode surface.

Analytical applications. The analytical usefulness of polarography for the determination of norfloxacin was studied. Calibration show a good linearity between limiting current (DC) or peak (DP) and norfloxacin concentration in the following ranges:

$il(nA) = 3.8 + 9.7\ 10^5\ C(M)$, (r=0.9998) range $1.05\ 10^{-5}$ M to $3.93\ 10^{-4}$ M

$ip(nA) = -0.35 + 3.9\ 10^6\ C(M)$, (r=0.9998) range $1.94\ 10^{-6}$ M to $2.43\ 10^{-5}$ M

For the norfloxacin concentrations higher than $2.43\ 10^{-5}$ M, and when the DP technique is used, the graphs calibration is not linear. Detection limits of 0.9 µg/ml (DC) and 0.05µg/ml (DP) for both techniques were found. its relative standar deviations were ±1.6 % and ±0.8 % respectively. The polarographic method proposed was applied to direct determination of norfloxacin in pharmaceutical preparations and good results were obtained(Table1)

Table 1

Determination of norfloxacin in Tablets

Technique	norfloxacin mg/tablet	Content according to manufacturer's laboratory
DC	449 ± 25	400 mg/tablet
DP	375 ±21	

REFERENCES

1.-BURNIE, J.; Drugs of Today, 20, 391-5 (1984)

DESIGN AND DEVELOPMENT OF A FLOW THROUGH GLUCOSE REACTOR

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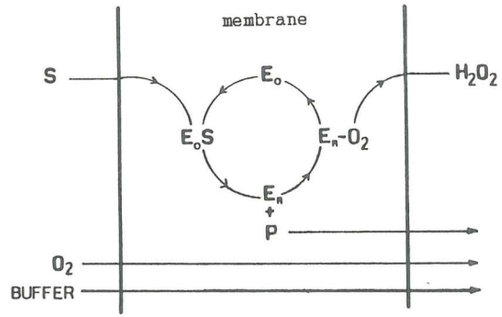
A flow-through mini-reactor containing immobilised glucose oxidase (GOD) was designed, developed and evaluated. The immobilisation of glucose oxidase directly on an electrode surface caused very high losses in activity [1]; immobilisation on a nylon support was much more satisfactory. A mini-reactor was developed, containing a nylon membrane with covalently immobilised GOD, and coupled to the inlet of a wall-jet cell electrochemical detector [2], where the hydrogen peroxide produced by the enzymatic reaction was detected.

The mechanism of reaction of GOD with glucose is of the double displacement type (ping-pong) [3]



where S - substrate; P - product, and E_O and E_R are oxidized and reduced forms of the enzyme respectively.