metric techniques [7]. The advantages of the proposed methods for analytical purposes lie in the rapid determination of LCD in pharmaceutical dosage forms, easy preparation of the sample, fair enough reproducibility, use of inexpensive instrumentation. It does not require separation procedures such as filtration, extraction and expensive grades of solutions.

Experimental

1. Apparatus and reagents

The voltammetric experiments were performed using a Bioanalytical System (BAS 100W) electrochemical analyser. A glassy carbon working electrode (BAS), a Ag/AgCl reference electrode (NaCl 3M, BAS), a platinum wire auxiliary electrode (BAS) and a standard one-compartment three-electrode cell of 10 ml capacity were used in all experiments. Before each experiment the glassy carbon electrode was polished manually with alumina ($\phi = 0.01$ μm), in the presence of bidistilled water on a smooth polishing cloth. Operating conditions for differential pulse voltammetry (DPV) were: pulse amplitude 50 mV; pulse width 50 ms; scan rate 20 mVs^{-1} and for square wave voltammetry (SWV); pulse amplitude 25 mV; frequency, 15 Hz; potential step 4 mV.

LCD and its pharmaceutical formulation were kindly provided by Glaxo Smith-Kline Pharm. Ind. (Istanbul, Turkey). A stock solution of LCD (1×10^{-3} M) was prepared in methanol and kept in the dark in a refrigerator. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution by the selected supporting electrolytes, namely sulphuric acid (0.5 M), phosphate buffer (0.2 M; pH 2.5–11.0) and Britton-Robinson buffer (0.04 M; pH 2.06–11.04) were used.

2. Analysis of tablets

Ten tablets were throughly ground and mixed. An amount of powder equivalent to 1×10^{-3} M of LCD was accurately weighed and transferred into a 25 ml calibrated flask and completed to the volume with methanol. The contents of the flask were sonicated for 10 min to effect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte.

In order to know whether the excipients show any interference with the analysis, known amounts of the pure drug were added to the different preanalysed formulations of LCD and the mixtures were analysed by the proposed methods.

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Preparation and characterization of sparfloxacin-β-cyclodextrin complexes

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Sparfloxacin, (*cis*)-5-amino-1-cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-di-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid, is a fluoroquinolone antibacterial reported to have a low aqueous solubility [1]. It has been reported that the absorption of sparfloxacin after oral administration is a long process and peak concentrations are generally not observed before 3 to 5 hours which leads to interpatient variability [2, 3]. Cyclodextrins by virtue of formation of water soluble inclusion complexes have been known to increase the solubility and dissolution rate of poorly water soluble drugs [4–6].

The present study was an attempt to improve the solubility, dissolution rate and bioavailability of sparfloxacin by forming inclusion complexes with β-cyclodextrin (β-CD). Inclusion complexes of sparfloxacin with β-CD were prepared by kneading and solid dispersion techniques in molar ratio of 1 : 1 and evaluated by differential scanning calorimetry, powder X-ray diffractiometry, fourier transform infrared spectroscopy, phase solubility and dissolution studies.

Solubility of SFLX increased linearly with increasing concentration of β-CD, showing a typical A_L-type phase solubility curve [7], characteristic of complexation with a stoichiometric ratio of 1 : 1. The apparent 1 : 1 stability constant, K_c, was found to be 57.33 M^{-1}.

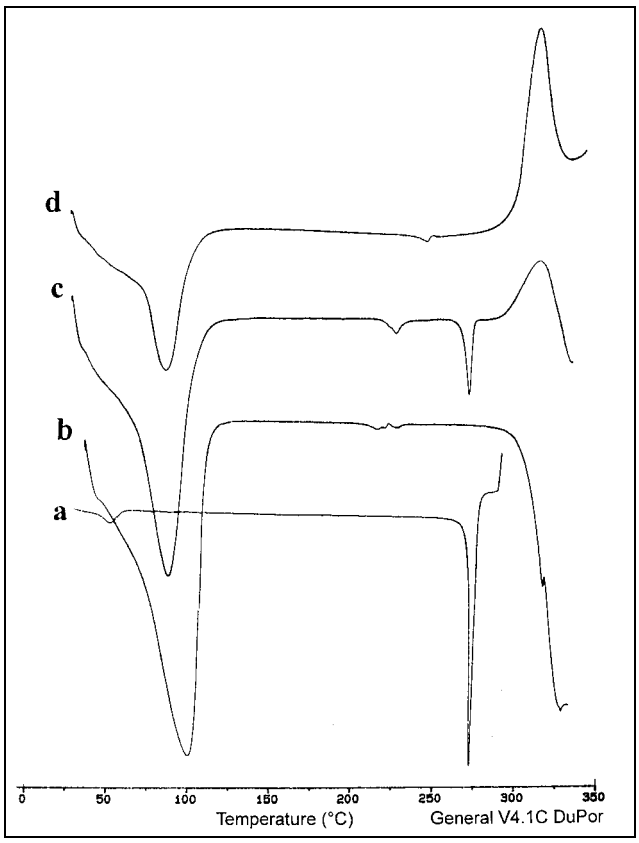

Fig. 1: DSC thermograms of the sparfloxacin-β-cyclodextrin system. a: sparfloxacin, b: β-cyclodextrin, c: 1:1 complex by solid dispersion d: 1:1 complex by kneading

The DSC thermograms for the various samples are shown in Fig. 1. The thermogram of pure SFLX showed a sharp endotherm near 265 °C, which is indicative of its melting temperature, followed by an exotherm which signifies its decomposition. A couple of early endotherms near 50 °C and 100 °C indicates the loss of moisture. The DSC thermogram for β-CD showed two endotherms, the first near 100 °C indicative of loss of moisture and another near 320 °C indicative of fusion. The thermogram for the complex prepared by the solid dispersion technique was found to be a combination of peaks for the two pure substances except for slight reduction in the intensity, indicating a low or no complex formation. Thermogram for complex prepared by kneading method in 1 : 1 ratio exhibited a complete disappearance of the endothermic peaks, indicating complete complexation.

X-ray diffraction patterns for both SFLX and β-CD exhibited intense and sharp peaks indicative of their crystalline nature. The X-ray diffraction pattern for the complex prepared by the solid-dispersion technique exhibited peaks characteristics of both the pure drug and the β-CD, although a few peaks were reduced in intensity indicating slight interaction between the two. In the complex prepared by the kneading technique, the peaks were greatly reduced in intensity with some peaks characteristic of SFLX being totally absent, indicating complex formation.

Since FTIR is a highly sensitive method of analysis, spectra of complexes prepared by both the methods showed some or other change from parent spectra (i.e., pure SFLX and β-CD). Some complex formation could thus be assigned to both the methods of preparation. The peaks characteristic of SFLX were found to be diminished in the complex prepared by the solid dispersion technique. Some new peaks were also observed at 1371 and 1461 cm^{-1}. In the complex prepared by the kneading technique also, the peaks were found to be highly diminished and a major peak of SFLX at 1712 cm^{-1} due to carboxylic acid group C=O stretching was completely missing.

The release profile of the uncomplexed drug and the complexes prepared by both the techniques is shown in Fig. 2. It can be seen that after 5 min only 7% of uncomplexed drug was dissolved and even after 60 min only 47 of drug went into solution whereas in case of the sparfloxacin-β-CD inclusion complex in 1 : 1 ratio prepared by the kneading technique, 90.9% drug was released within 5 min and almost whole of the drug was released within 20 min.

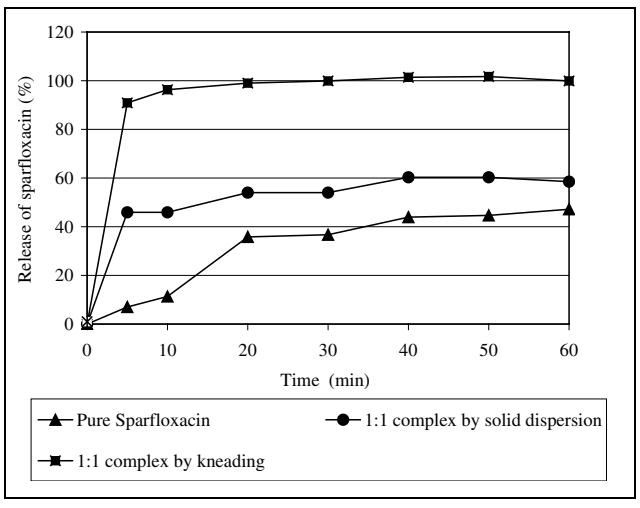

Fig. 2: Dissolution profile of pure sparfloxacin and different drug-β-cyclodextrin complexes

Based on the above results, it can be concluded that sparfloxacin can be included into the cavity of β-CD to form an inclusion complex. The complex prepared by the kneading method in stoichiometric ratio of 1 : 1 :: sparfloxacin : β-CD is one which shows maximum complex formation. The complex exhibits a significantly enhanced dissolution rate of SFLX in water and may be useful in developing new pharmaceutical dosage forms of SFLX.

Experimental

1. Materials

SFLX was obtained as a gift sample from Torrent Laboratories, India, while β-CD was obtained from Cerestar Inc., USA and used as such. All other chemicals and solvents used were of AR grade.

2. Preparation of inclusion complexes

Complexes of the drug with β-CD were prepared in a molar ratio of 1 : 1 by the techniques of solid dispersion and kneading. The quantities of SFLX and β-CD taken were 392.41 mg and 1135 mg, respectively.

3. Evaluation of solid inclusion complexes

The prepared solid inclusion complexes were evaluated by phase solubility studies, differential scanning calorimetry, powder X-ray diffractiometry, fourier transform infrared spectroscopy and dissolution studies.

3.1. Phase solubility studies

Phase solubility studies of the complexes were carried out according to the method described by Higuchi and Connors [7]. An excess amount of the drug was added to the solution of β-CD in water (pH 6.8) at various concentrations (2–8 mMl^{-1}). The flasks were sealed and magnetically stirred for 72 h at 25 ± 1 °C. After equilibrium was attained, the samples were filtered, diluted suitably and the absorbance recorded at 292 nm using Spectronic-21 UV spectrophotometer (Bausch & Lomb).

3.2. Differential Scanning Calorimetry

Differential thermal analysis of the samples was performed using DUPONT model 910 (USA) system in an inert atmosphere of Argon at the rate of 10 °C min^{-1} between 50 °C and 350 °C using a sample size of 1.3 mg.

3.3. Powder X-ray Diffractiometry

X-ray diffraction pattern of the samples was obtained using a high resolution X-ray diffractiometer (RU-200B, Rikagu, Japan) using a Copper filter monochromator and a scan speed of 4 °C min^{-1}, a voltage of 40 KV and a current of 50 mA.

3.4. Fourier-Transform Infra Red Spectroscopy

The FTIR spectra of the samples were recorded on Magna IR 750 Nicolet series II FTIR instrument using the KBr disc technique. Scanning was done from 4000 to 500 cm^{-1}.

3.5. Dissolution studies

Dissolution studies were performed on Paddle type USP dissolution test apparatus (Veego Labs., India) using 900 ml of water (pH 6.8) at 37 ± 1 °C as the dissolution media and a stirring speed of 50 rpm. Samples were withdrawn at 5, 10, 20, 30, 40, 50 and 60 min., filtered, diluted suitably and analyzed spectrophotometrically at 292 nm.

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