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ORIGINAL ARTICLE

The effect of β-cyclodextrin on tenoxicam photostability, studied by a new liquid chromatography method; the dependence on drug dimerisation

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Abstract Tenoxicam (TXM) is an effective anti-inflammatory and analgesic drug, which presents fast photochemical decomposition. In this work in an attempt to investigate the potential β-CD photostabilizing effect on TXM, the photodegradation rate of β-CD complexed drug was monitored under simulated solar irradiation from Xenon arc lamp. The photodegradation was studied at pH 7.5. A new stability indicating Liquid Chromatography method, for TXM in the presence of β-CD was used. According to the obtained results, in the case of free molecules increasing the concentration the photostability is enhanced. The effect of complexation with CDs on the photodegradation rate seems to vary depending on TXM initial concentration. At low TXM concentrations photodecomposition is retarded upon CD complexation, while at high concentrations the process is accelerated. Molecular dimerisation was studied by ^{1}H(1D) NMR and 2D NOESY experiments. 2D ROESY spectra of complexed molecule were evaluated in order to confirm the complexation. TXM dimers could be considered as a critical parameter affecting oxicams photostability, in combination with the already described ESIPT phenomenon.

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Introduction

Tenoxicam (TXM, Scheme 1) is an effective anti-inflammatory and analgesic drug of the group of oxicams, which presents the problem of poor water solubility and fast photochemical decomposition [1, 2]. Inclusion of molecules into the cyclodextrin cavity has been successfully used to improve physico-chemical and pharmaceutical properties of drugs [3–5] photostability being one of them [6–8]. However, it has been also reported that complexation of piroxicam with β-CD enhanced photodecomposition by promoting the ESIPT phenomenon [9, 10].

The present work aims to investigate the photolytic behavior of TXM in CD complexes, under simulated solar irradiation from Xenon arc lamp, according to ICH guidelines. A new approach interpreting the CD effect on TXM photolysis rate is described. For this purpose drug: β-CD complexes were prepared and characterized with the aid of NMR Spectroscopy. The study was performed at pH 7.5, taking into account the fact that the vast majority of formulations intended for intravenous administration are in alkaline media. Initially the free TXM photodegradation was studied and compared to that of the corresponding β-CD complexes. As it has been reported in our previous works, the presence of CDs in sample solutions, influences the spectroscopic data of drugs [11–13], making the accurate quantitation difficult. In addition, the presence of

the photodegradation products makes the drug analysis more complicated. A Liquid Chromatography (LC) method, specific for the determination of TXM in the presence of both photodegradation products and β-CD was necessary for our study. Therefore a new method was developed and validated.

Experimental

Materials

TXM was obtained from Sigma-Aldrich Co, (St Louis, Missouri, USA) and stored under light protection. Reagents used were purchased from Labscan (Ireland) and were of Analytical grade. Water for HPLC use, was deionised and filtered by a Milli – Q Plus water purifying system, Millipore (Bedford; MA, USA). All other solvents were of HPLC grade. The mobile phases were vacuum filtered and degassed through a 0.45 μm Millipore membrane. β-CD was purchased from Sigma Chemicals Co. Deuterated solvents were obtained from Merck KgaA (Darmstadt, Germany).

Methods

Preparation of the complexes

A NaH_2PO_4 solution (2×10^{-2} M) was used as a diluent for TXM initial sample. The TXM concentration of the solution prepared was 30.8×10^{-6} M. For the preparation of drug-CD complexes, the appropriate amounts of β-CD solution were added to the TXM solution in order to obtain 1:1, 1:5 and 1:10 drug-cyclodextrin molar ratios. The mixtures were stirred in a constant temperature water bath at 35 ± 0.1°C for 72 h, until equilibrium was reached. Completion of complexation was evidenced by NMR spectroscopy.

Liquid Chromatography method

The samples were analyzed by a high performance liquid chromatograph, Alliance 2690 Separation Module, Waters Co, (Milford, MA, USA) equipped with a PDA detector. Millennium 32 was used as software facility. The LC column was Xterra RP8 3.5 μm 4.6 × 150 mm from Waters. The mobile phase consisted of 2×10^{-2} M NaH_2PO_4 buffer pH = 2 and methanol (55:45, v/v), the flow rate was regulated at 1 ml/min, the detector wavelength was set at 254 nm and the constant temperature oven at 25°C.

NMR spectroscopy

^{1}H-NMR spectra were recorded on a Bruker DRX-Avance 400 MHz spectrometer. The 2D-NOESY spectrum of TXM (mixing time 1 s) and the 2D-ROESY spectrum of the TXM:β-CD complex (spin lock time 250 ms at transmitter attenuation 23.4 dB) were acquired with 2048 points in the t_2 dimension, and 64 transients were co-added for each of 256 t_1. Additionally, ROESY spectra of the TXM:β-CD complex were recorded using different spin-lock mixing times (ranging between 200 and 500 ms) to ensure the validity of the linear approximation for the ROE cross-peaks. The probe temperature was regulated at 298 K. Chemical shifts were related to external standard DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid) peak at 0 ppm. For the complex formation study, the appropriate amounts of TXM and cyclodextrin were dissolved in D_2O, the pD was adjusted using NaOD 10% and the samples were shaken in a constant temperature bath at 35°C, for 72 h. For the self-titration NMR experiments solutions at the concentration range of 145.2×10^{-6} M–7.26×10^{-3} M were prepared at D_2O, using NaOD 10% for the pD adjustment. The 2D ROESY spectrum of the complex was recorded for a 6.71×10^{-3} M TXM solution at pD = 7.5. The molar ratio of TXM β-CD was 1:2.5.

Photostability studies

The photodegradation experiments were performed with a Suntest CPS Accelerated Exposure Xenon Burner Machine, Atlas Material Testing Technology LLC (Chicago, Illinois, USA), which simulates the solar irradiation in wavelength distribution. Irradiation intensity was 765 $Js^{-1}m^{-2}$. Photodegradation experiments were performed side by side with a validated quinine chemical actinometric system. Samples were stirred throughout the exposure time. At predetermined time intervals 200 μl aliquots of the irradiated solution were withdrawn from the cuvette and immediately diluted as appropriate with mobile phase. The resulting solutions were analyzed by the newly developed LC method. Samples wrapped in aluminum foil were used as dark controls. No concentration changes were observed due to possible thermal reactions. The coexistence of hydrolytic reactions was also excluded by testing the thermal stability of the samples at 50°C for 6 h.

Results

Liquid Chromatography method-validation

System suitability

The relative standard deviation (RSD) of replicate injections was not more than 0.5%. The tailing factor of the peak corresponding to TXM was around 1.0. The resolution between TXM and its previously eluted component was 3.34. The column efficiency determined for TXM peak was about 2000 theoretical plates. The peak purity for the drug substance was found to be better than 0.9991.

Linearity

The assay showed good linearity within the range 0.01–200 ppm (10 concentration levels). The equation of the least square line was 35304x + 706.3. The R^2 value was 1.000. The sum of residuals was zero. The linearity test was repeated with β-CD complexes. The equation of the least square line was 35479x + 8316.8 and the R^2 value was equal to 0.9999. Comparison of the results obtained with the free and complexed molecule, by regression analysis, led to the conclusion that, there is no evidence for systematic differences between the two sets of results.

Limit of detection/quantitation

The limit of detection (signal to noise ratio 3:1) was 0.01 ppm and the limit of quantitation (signal to noise ratio 10:1) was 0.05 ppm.

Precision

The precision of the method was examined by a series of nine repeated assays at the target concentration of 10 ppm. SD = ± 0.0685 and RSD = 0.67%. A second series of nine samples of TXM β-CD was carried out. The target concentration of TXM was 10 ppm and its molar ratio with β-CD was 1:5. SD = ± 0.09 and RSD = 0.88%. Since a difference between the variance values of the two groups was observed, a two-tailed *F*-test was applied revealing that s_1 and s_2 do not present statistically significant difference. The RSD of different samples, containing a standard concentration of 10 ppm TXM and increased concentrations of β-CD (up to the ratio of 1:20) was less than 0.94%.

Accuracy

Recovery experiments were conducted in order to confirm the accuracy of the method. For this purpose TXM samples of known concentration were prepared using a totally photolysed solution, as diluent. The mean recovery was 99.86%. In order to verify that the method is suitable for the assay of TXM in the presence of cyclodextrins as well, samples containing known amounts of the analyte and CD in a totally photolysed TXM solution, were spiked with increasing amounts of TXM. The average recovery of TXM was 99.29%.

NMR spectroscopy study

Self association phenomena

Dimerisation phenomena for oxicams, have already been reported [14, 15]. Self-titration experiments of the free drug in alkaline environment revealed linear dependence of proton chemical shifts upon TXM concentration, which is a strong evidence of dimerisation phenomena (shielding effect). The equation describing the linear chemical shift displacement of TXM H-5′ is provided as an example: y = – 0.0076x + 7.2061 (R^2 = 0.999).

Furthermore, the addition of KCl in TXM solutions resulted in downfield displacement of the signals. This could be considered as a result of shifting the equilibrium to monomers.

In order to collect more information about the structural properties of dimers 2D-NOESY and ROESY experiments were also conducted. Following evaluation of the recorded spectra intermolecular cross

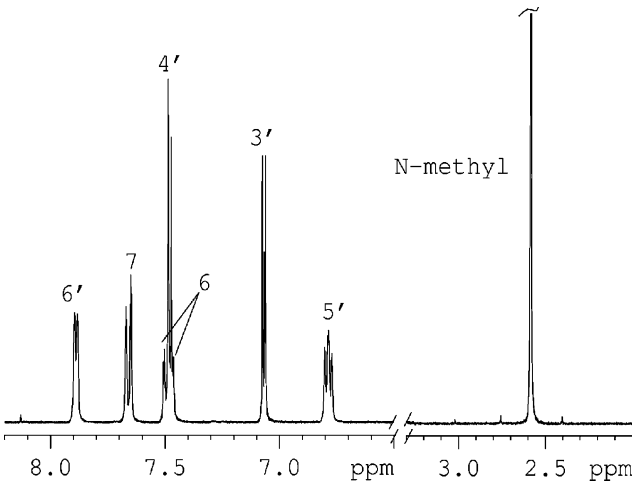


Fig. 1 ^{1}H NMR spectrum of tenoxicam in pD 7.5 solution

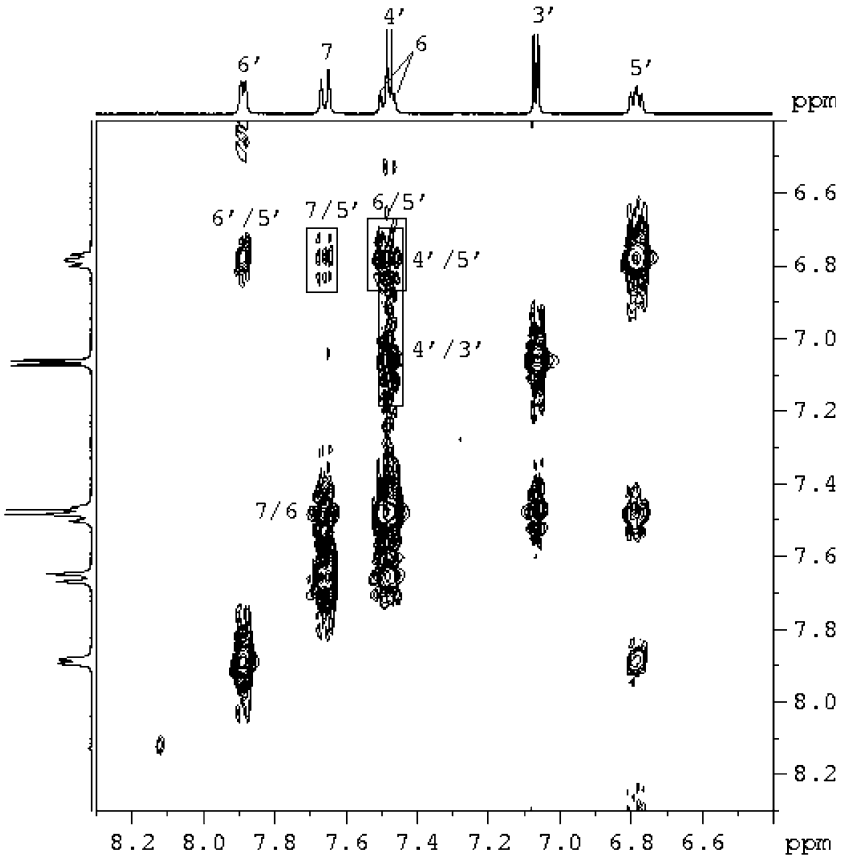


Fig. 2 Partial two-dimensional NOESY spectrum of tenoxicam in pD 7.5 solution

peaks were also detected between the protons of the aromatic and bicyclic part of TXM. Cross peaks were noted between H-5′ of the TXM aromatic ring and the overlapping signals of H-6, and H-4′ protons, which indicate the possible interaction of H-5′ with the H-6. Additionally H-5′ showed cross peaks with H-7 revealing intermolecular interactions, which provide also strong evidence for the TXM dimers. All the aforemen-

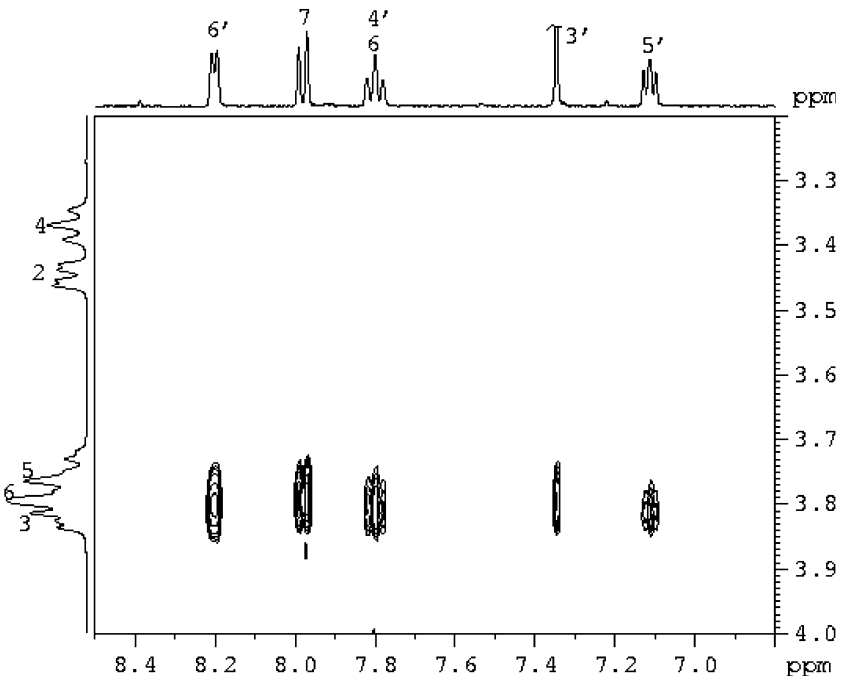


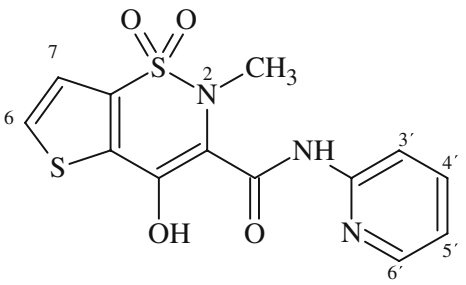
Fig. 3 Partial two-dimensional ROESY spectrum of tenoxicam in pD 7.5 in the presence of β-cyclodextrin with 1:2.5 molar ratio

tioned long-range interactions illustrate also the head to tail orientation of the TXM molecules. (Figs. 1, 2)

Complex characterization

2D-ROESY experiments conducted with TXM-CD complexes (Fig. 3) showed that TXM protons had long-range interactions with the cyclodextrin internal cavity protons H-3, H-5, confirming complexation. In particular cross peaks between H-5′ and H-7 of TXM with H-5 and H-3 of CD respectively are clearly demonstrating the TXM interaction with CD cavity (Sch. 1).

Photodegrdation profiles of free tenoxicam and its CD complexes



Scheme 1 Chemical structure of tenoxicam

Free TXM

The photolysis rate constant of TXM depends on the initial concentration. The reaction follows zero order kinetics at high concentrations (over 15.41×10^{-5} M) and first order kinetics at low concentrations (up to 71.13×10^{-6} M). At intermediate concentrations zero order kinetics change to first order, after a certain time of irradiation (Fig. 4). During the photolytic process a turning point from zero order to first order kinetics is detected. The experiments were performed in triplicate and the relative standard deviation for the rate constants was not more than 0.4%. In all cases an increase of calculated *k* values was observed with higher initial concentration of TXM solutions.

TXM:β-CD complexes

The degradation rate constants of the 1:1 complexes were calculated and compared to those of the free drug. At high tenoxicam concentrations, where zero order kinetics is followed, the complexation with *β*-CD caused a 40.29% increase of the photolytic rate constant. The determined *k* values for the free drug and the complexed were 0.926×10^{-2} and 1.551×10^{-2} min^{-1} respectively. On the contrary, *β*-CD complexation resulted at a 6.2% decrease of rate constant at low tenoxicam concentrations (first order) region. It is noteworthy that increasing the molar ratio of *β*-CD, the photoprotective effect was enhanced as well. At drug:CD molar ratio 1:5 a further decrease of 1.1% was observed for the *k* value, when at 1:10 molar ratio the corresponding increase was 12%.

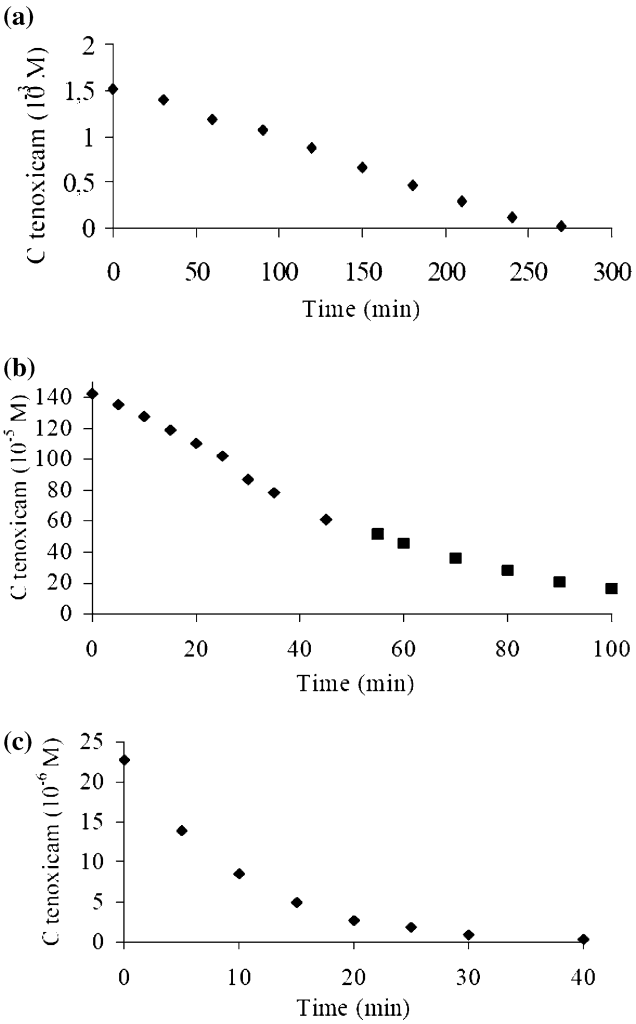


Fig. 4 Tenoxicam photodegradation in aqueous solutions under Xenon lamp irradiation. Tenoxicam concentration: (**a**) 1.51×10^{-3} M (zero order region), (**b**) 14.23×10^{-5} M (region demonstrating the existence of both kinetics) and (**c**) 22.78×10^{-6} M (first order region)

Discussion

In this work the dimerisation phenomena of TXM were evidenced by NMR spectroscopy. Taking into account the already reported influence of molecular

aggregation on to photolytic behavior [16], the probable contribution of dimerisation phenomena to the reaction rate provides a rational approach to the dependence of TXM photostability on drug concentration. Therefore, the varying effect of β-CD on TXM photodecomposition could be attributed to the same phenomenon. On this basis, the enhanced photostability of concentrated TXM solutions could be assigned to dimers formation, enabling different relaxation pathways than these of the monomers.

The accelerating effect of β-CD on photochemical decomposition, at high TXM concentrations, could be considered as a consequence of the dimers dissociation. It is well known that at concentrations lower than 10^{-5} M the equilibrium is shifted towards monomers. Consequently, at the levels where the stabilizing effect of CD is observed, monomers prevail. On the contrary at the concentration of 10^{-3} M, which was the highest one studied, the dimers dominate in the solution. It is noteworthy to mention that at the concentration levels where CDs exhibit photostabilizing effect, the increase of CD molar ratio gives rise to remarkable enhancement of the photoprotective effect. This observation could be considered as supporting evidence to the above. In conclusion, it can be suggested that, molecular dimerisation phenomena is a critical factor affecting oxicams photostability, in combination to the already described ones.

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