Journal of Molecular Liquids 209 (2015) 327–335

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

Oxidative degradation of norfloxacin by a lipophilic oxidant, cetyltrimethylammonium permanganate in water–acetonitrile medium: A kinetic and mechanistic study

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ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form 28 May 2015
Accepted 1 June 2015
Available online xxxx

Keywords:
Norfloxacin
Oxidative metabolism
Cetyltrimethylammonium permanganate
Kinetics
Solvent effect
Activation parameters

ABSTRACT

The present study reports the oxidative metabolism of an established antibacterial drug norfloxacin (NRF) by a lipid compatible lipophilic Mn(VII) oxidant, cetyltrimethylammonium permanganate (CTAP) in acetonitrile–water binary mixture in the presence of acetic acid. The metabolized products are identified as 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, formaldehyde, and ammonia. The kinetics of the reaction is studied in aqueous acetonitrile media in the presence of acetic acid by UV–vis spectroscopic method by monitoring the absorbance of Mn(VII) at 530 nm under pseudo first-order condition. The reaction is found to be first-order with respect to CTAP and fractional order with respect to norfloxacin and acetic acid. Occurrence of Michaelis–Menten type kinetics with respect to norfloxacin confirmed the binding of oxidant and substrate to form a complex before the rate determining step. A suitable ionic mechanism is proposed based on the experimental findings. The proposed reaction mechanism is supported by the effect of solvent polarity and effect of temperature on the reaction rate. High negative entropy of activation ($\Delta S^{\neq} = -259$ to -158 J K^{-1} mol^{-1}) supported the existence of a forced, more ordered and extensively solvated transition state. Further, solvent polarity–reactivity relationship revealed (i) the presence of less polar transition state compared to the reactants, (ii) differential contribution from dipolar aprotic (acetonitrile) and polar protic (water) solvents toward the reaction process through specific and nonspecific solute–solvent interaction and (iii) presence of intramolecular H-bonding in oxidant–substrate complex in acetonitrile rich domain and intermolecular H-bonding between oxidant–substrate complex and water in water rich domain.

1. Introduction

Norfloxacin [1-ethyl-6-fluoro-1, 4-dihydro-4-oxo- 7-(1-piperazinyl)-3-quinolinecarboxylic acid] is a representative member of fluoroquinolone family and widely used as first choice of drug for the treatment of bacterial infections of the urinary, biliary, and respiratory tracts [1,2]. The antibacterial activity of fluoroquinolones arises due to inhibition of growth of DNA gyrase (a critical enzyme to bacterial chromosome replication) of gram-positive and gram-negative bacteria through the formation of a ternary complex between the drug, the enzyme and bound DNA segment [3–7]. Carbonyl and carboxyl moiety of norfloxacin bind with DNA, break the DNA-gyrase complex and are responsible for the antibacterial activity. Fluorine (F) at 6th position and piperazine substituent at 7th position involve in binding interaction with enzyme, giving extra stabilization to the drug–enzyme–DNA ternary complex, and thus responsible for the increase in the effectiveness and potency of the antibacterial [3–7]. Norfloxacin is poorly metabolized in the body. 26–32% of the administered dose remained as such and excreted by renal excretion while only 5–8% of the drug is metabolized mainly to six active metabolites of lesser antimicrobial potency [8]. As studied in human and fungi, the drug is primarily metabolized in the liver by P450 enzymes via N-formylation/acetylation, oxidation and breakdown of the piperazine ring resulting in decreased antimicrobial activity [9–12]. Further, the bioavailability and thus the antibacterial activity of fluoroquinolones drastically decreased by the presence of multivalent metal containing compounds (such as magnesium or aluminum antacid, vitamins or minerals) at gastric pH and is attributed to the formation of metal–chelate complex and alternations in dissolutions [13,14].

In view of potential pharmaceutical importance of norfloxacin and presence of few literatures on the mechanism of oxidation of this drug [15–17], there is a need to understand the mode of interaction with the oxidizing agents and mechanism of oxidative metabolism of this drug in biomimetic medium.

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http://dx.doi.org/10.1016/j.molliq.2015.06.003
0167-7322/

In our efforts in exploring some biomimetic oxidants to oxidize organic substrates in organic solvents, we have reported the oxidation behavior of cetyltrimethylammonium permanganate (CTAP) [18] and dichromate (CTADC) [19] toward various organic substrates. Due to the presence of an amphiphilic long chain quaternary cetyltrimethylammonium (CTA^+) ion, as organic carrier, these oxidants are soluble in organic (lipid) phase via the formation of tight ion pair with the anionic oxidant unit [20]. Further, these oxidants are found to be mild and chemoselective [18,21–25]. Sometimes these oxidants show biomimetic characteristics mainly because of the CTA^+ (containing C_{16} tail and trimethyl ammonium head group) which generates a microheterogeneous environment with different solubilization pockets for the substrates as in the case of micelles, reversed micelles, microemulsions, and vesicles in artificial systems, and proteins and lipid membranes in living systems [25,26].

In the present investigation, we have made an attempt to investigate the oxidative metabolism of norfloxacin by CTAP in aqueous-organic binary solvent mixture in the presence of acetic acid. To achieve the objectives (i) the oxidation products were identified, (ii) kinetics were run by varying [substrate], [acid], and [CTAP] in the reaction process, (iii) a suitable kinetic model, and a plausible mechanism were proposed (iv) solvent isotope effect was analyzed to support the proposed mechanism (v) activation parameters for the reaction were calculated by running the kinetics at various temperature and (vi) solvent effect on the reaction rate was analyzed in media of varied polarities by changing the water–acetonitrile composition and a suitable solvation model was proposed.

2. Experimental

2.1. Materials

Cetyltrimethylammonium permanganate (CTAP) was prepared from the aqueous solution of cetyltrimethylammonium bromide and potassium permanganate as reported earlier [21], and its purity was checked from its melting point and NMR spectral data. Norfloxacin was purchased from Sigma Aldrich, INDIA and was used without further purification. Glacial acetic acid was of Merck grade and used as such. Acetonitrile (Merck, India) was purified by the standard method. D_2O (Sigma-Aldrich) was of NMR grade with 99.8% D. Acetonitrile–water mixed solvents were prepared by carefully mixing the components by volume.

2.2. Kinetic measurements

The oxidation kinetics of norfloxacin by CTAP in acetonitrile–water solvent mixture in the presence of acetic acid was investigated using Shimadzu UV–vis spectrophotometer (UV-1800) fitted with thermostatic cell holders. The temperature in the reaction cell was controlled by circulating water by using a Lauda thermostat with a temperature

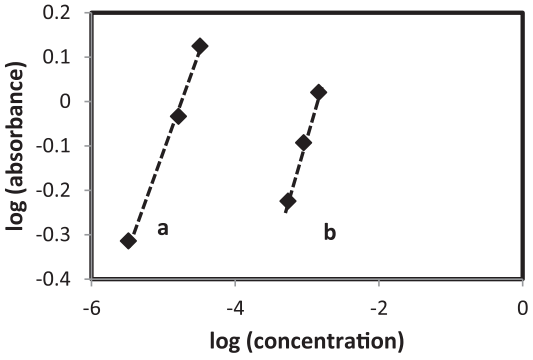

Fig. 1. Limiting logarithmic plot for the calculation of the stoichiometric ratio between norfloxacin and CTAP: (a) log (absorbance) vs. log [norfloxacin] and (b) log (absorbance) vs. log [CTAP].

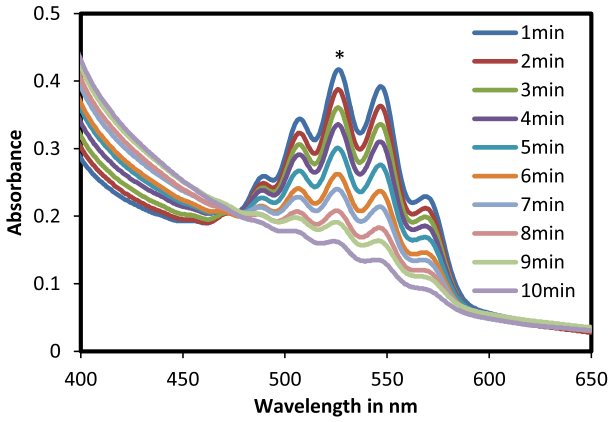

Fig. 2. Successive scan of UV–vis absorption of the reaction mixture consisting of CTAP and norfloxacin in acetonitrile–water mixture in the presence of acetic acid at a time interval of 1 min. (* represents the peak position at which the absorbance is measured for the calculation of k_{obs}).

fluctuation of ±0.05 °C. All the kinetic runs were performed under pseudo-first-order conditions by keeping excess of the norfloxacin (10 times or more) with respect to [CTAP]. The reaction was followed by monitoring the decrease in optical density (OD) of Mn(VII) at an analytical wavelength of 530 nm. The observed rate constants (k_{obs}), were obtained from the linear plots ($R^2 = 0.99$) of log[CTAP] against time for up to 80% completion of the reaction. The effect of the variation of [CTAP], [acid], and [norfloxacin] on the rate constant was investigated by varying the concentration of the desired constituent in the reaction mixture and the values reported are the average of at least triplicate runs and were reproducible within 6% error. The solvent isotope effect ($k(H_2O)/k(D_2O)$) was investigated using mixtures of H_2O/acetonitrile and D_2O/acetonitrile separately in the presence of acetic acid at the mole fraction ratio of H_2O (or D_2O):acetonitrile:acetic acid as 0.71:0.25:0.04.

2.3. Product analysis

The reaction mixture containing the norfloxacin (64 mg, 1 M) and CTAP (80 mg, 1 M) in the acetonitrile–water medium was stirred and

Table 1
Effect of [norfloxacin], [CTAP], and [acetic acid] on the oxidation of norfloxacin by CTAP in acetonitrile–water mixture (mole fraction ratio 0.65:0.3) at 298 K.

$[CTAP] \times 10^4$ M	$[Norfloxacin] \times 10^3$ M	[Acetic acid] M	$10^4 \times k_{obs}\ s^{-1}$
0.11	1.8	1.27	19.6 ± 0.65
0.18	1.8	1.27	20.2 ± 0.68
0.36	1.8	1.27	19.23 ± 0.63
0.73	1.8	1.27	19.64 ± 0.65
1.09	1.8	1.27	18.73 ± 0.6
1.8	1.8	1.27	19 ± 0.6
1.8	0.545	1.27	7.92 ± 0.34
1.8	1.8	1.27	19 ± 0.6
1.8	3.65	1.27	39.62 ± 1.31
1.8	5.45	1.27	53 ± 2.19
1.8	7.3	1.27	58.46 ± 2.2
1.8	9	1.27	60.95 ± 2.35
1.8	10.2	1.27	65.7 ± 2.84
1.8	13.8	1.27	68 ± 3.34
1.8	1.8	0.16	5.5 ± 0.24
1.8	1.8	0.32	7.8 ± 0.31
1.8	1.8	0.63	13.5 ± 0.58
1.8	1.8	0.95	17.31 ± 0.75
1.8	1.8	1.27	19 ± 0.6
1.8	1.8	1.58	19.54 ± 0.71
1.8	1.8	1.9	21.23 ± 0.7