International Journal of Pharmaceutics, 11 (1982) 329–336
Elsevier Biomedical Press

Factors affecting captopril stability in aqueous solution

Peter Timmins **, Ivan M. Jackson and Yu-chang John Wang *

*Squibb Institute for Medical Research, International Development Laboratory, E.R. Squibb and Sons Ltd., Reeds Lane, Moreton, Wirral, Merseyside, L46 1QW (U.K.) and * Squibb Institute for Medical Research, New Brunswick, NJ 08903 (U.S.A.)*

(Received June 4th, 1981)
(Modified version received January 26th, 1982)
(Accepted February 11th, 1982)

Summary

The oxidative and hydrolytic degradation products of captopril, formed under acid and mildly alkaline conditions, have been isolated and identified. The oxidative rate of degradation of captopril over the pH range 2–5.6, in McIlvaine's citrate-phosphate buffer, has been studied at 50°C, the rate showing pH-dependency with maximum stability below pH 4.0. The effect of additives such as anti-oxidants, chelating agents and trace metal contaminants, is also presented. The rate of hydrolysis was studied in hydrochloric acid solutions and extrapolated to the pH range of 2–4. The oxidation reaction was the predominant route of degradation over this pH range.

Introduction

Captopril (3-mercapto-2-methylpropionyl-L-proline; I) is the first orally-active inhibitor of angiotensin-converting enzyme available to medical practitioners and as such represents a completely new therapeutic approach in the treatment of hypertension. As liquid dosage forms of the product (for example, injection, syrups) would be required, its solution stability should be characterized, including modes of degradation in solution and variation of degradation rate with pH.

Like all thiols, captopril would be subject to some degree of oxidative degradation

* Present address: Ortho Pharmaceutical Corp., Raritan, NJ 08869, U.S.A.
** To whom correspondence should be addressed.

0378-5173/82/0000-0000/$02.75

(Cappozi and Modena, 1974). Contaminant metal ions in solution formulations may promote oxidative degradation so knowledge of the extent of this catalysis and the degree of protection offered by chelating agents and/or anti-oxidants is necessary in developing stable formulations.

As the captopril molecule also includes an amide function, degradation via hydrolysis is possible. Assessment of the relative contributions of oxidative and hydrolytic degradation routes in aqueous solution is required, so appropriate measures may be taken to ensure product stability.

This paper describes the preformulation studies carried out to supply some of the basic information required to produce stable aqueous based captopril formulations.

Materials and methods

All chemicals and solvents were 'Analar' grade except propyl gallate, which was laboratory grade reagent, and methanol, which was HPLC grade. Water was distilled from an all-glass still. Captopril, captopril disulphide and 3-mercapto-2-methyl propanoic acid were obtained from the Squibb Institute for Medical Research and were of not less than 99% purity.

Mass spectra were run on an AEI MOS Spectrometer and IR spectra were recorded in potassium bromide on a Pye Unicam SP 1000 spectrophotometer. Thin-layer chromatography (TLC) was carried out on Anachem 20 × 20 cm silica gel GF254 plates having a 0.25 mm-thick layer. The following solvent systems were employed: benzene–glacial acetic acid–methanol (80:20:5 v/v/v; Solvent A), dichloromethane–ethyl acetate–glacial acetic acid (50:50:10 v/v/v; Solvent B) and 95% ethanol–water (70:30 v/v; Solvent C).

The HPLC assay for captopril was similar to that described by Perlman and Kirschbaum (1981). A 20 × 0.5 cm octadecylsilane reversed-phase column (5 μm Shandon Hyperspheres ODS or Altex Ultrasil ODS) was employed and the eluent was a mixture of methanol–water–orthophosphoric acid (380:420:0.4 v/v/v for Hyperspheres and 220:180:0.4 v/v/v for Ultrasil). The flow rate was 1 ml · min^{-1} for the Hyperspheres column and 2 ml · min^{-1} for the Ultrasil column. The eluate was monitored at 220 nm by means of a variable wavelength UV detector.

Samples for analysis were prepared by diluting the solution being examined with HPLC solvent to give a captopril concentration of 0.5 mg · ml^{-1} and 20 μl was injected onto the column via a rotary valve loop injector. Peak areas were used for quantitation.

Isolation and identification of degradation products

Ten ml of captopril solution, 10 mg · ml^{-1}, previously adjusted to pH 2.0 or pH 8.5 with 1.0 N hydrochloric acid or 1.0 N sodium hydroxide, were sealed in 60 ml clear glass (USP Type I) vials. The large headspace ensured adequate oxidation for oxidative degradation to occur according to calculations based on reaction stoichiometry. The sealed vials were stored in a thermostat-controlled oven at 90°C for 10 weeks before being removed, cooled and acidified with 1.0 N hydrochloric acid. The acid solutions were extracted with a 3:1 mixture of chloroform–methanol.

The organic solvent was evaporated to dryness to provide residues corresponding to the organic solvent-soluble products of captopril degradation at pH 2.0 and 8.5.

The residues were examined by TLC (Solvents A and B) and degradation products were isolated for spectroscopy using preparative TLC and Solvent A.

Effect of pH on rate of oxidation

Eight ml of captopril solution, 5 mg · ml^{-1}, prepared in McIlvaine buffers (pH range 2.1–5.6) of constant ionic strength ($\mu = 0.5$) (Elving et al., 1956) were filled (8 ml) into 14 ml glass vials. The sealed vials were stored in a thermostat-controlled oven at 50°C. To ensure oxygen was always in excess the vials were opened daily and air was bubbled through the solutions. The vials were resealed and returned to the oven. Samples were removed at intervals and analyzed for captopril by HPLC.

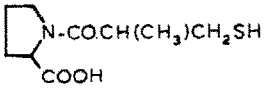
Effect of additives

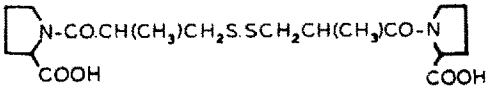
Solutions of captopril containing 5 mg · ml^{-1} were prepared as for the rate profile but at the fixed pH of 4.0 (citric acid-sodium citrate buffer). The following additives were included in different solutions: 0.1% w/v disodium edetate; 0.05% w/v propyl gallate; 0.1% sodium metabisulphite; 5 ppm copper(II) (as copper sulphate); and 5 ppm iron(III) (as ferric chloride). A control solution with no additive was also employed.

The solutions (8 ml) were packed into 60 ml vials sealed and stored at 50°C. Samples were taken at intervals and analyzed for captopril by HPLC.

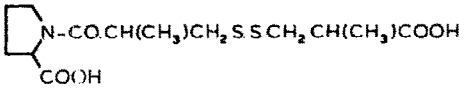
Kinetic studies for hydrolysis

Captopril (5 mg · ml^{-1}) and disodium edetate (0.1 mg · ml^{-1}) were dissolved in hydrochloric acid of various normalities. The solutions were stored in well-filled, sealed glass vials at 90°C. These conditions were chosen to reduce oxygen content of the solutions and remove potentially catalytic metal ions thus limiting oxidative degradation of captopril during the hydrolysis study. Samples were removed at intervals and analyzed for captopril by HPLC.

I 

II 

III $HOOC.CH(CH_3)CH_2S.S.CH_2CH(CH_3)COOH$

IV 

V $HSCH_2CH(CH_3)COOH$

Results and discussion

Identification of decomposition products

When visualized with iodine vapour thin-layer chromatograms developed in Solvents A and B showed 3 spots for solutions degraded at pH 2.0 and 4 spots for solutions degraded at pH 8.5. The uppermost spot in both fractions (TLC solvent A, R_f 0.68) occurred only in very small amounts and was identified as 3-mercapto-2-methylpropanoic acid (V) by comparison with an authentic sample on TLC.

Compounds chromatographing at R_f 0.48 and 0.31 in TLC Solvent A were identified as unchanged captopril (I) and captopril disulphide (II), respectively, by comparison with authentic samples on TLC.

A compound at R_f 0.62 in TLC Solvent A was isolated in sufficient quantity for IR and mass spectrometry. The IR spectrum demonstrated the compound to have a carboxylic acid function (band at 1700 cm^{-1}) but no thiol function (no band at 2500 cm^{-1}). The latter was confirmed in that the compound did not give a yellow colour on TLC plates when sprayed with modified Ellman's reagent (5,5'-dithio-bis-2-nitrobenzoic acid, 0.2% w/v in methanol–ammonia solution, 95:5 v/v). The mass spectrum of this compound showed the molecular ion at m/e 238, corresponding to a molecular formula of $C_8H_{14}O_4S_2$. The absence of an M^+-34 peak confirmed the absence of thiol functions in the molecule, as this peak is always seen in the mass spectra of thiols. This compound was thus identified as 3,3'-dithio-bis-2-methylpropanoic acid (III), an oxidation product of 3-mercapto-2-methylpropanoic acid.

The additional compound occurring in the solution at pH 8.5 was compared on TLC with a partially-hydrolyzed sample of captopril disulphide. The major decomposition product in the latter solution co-chromatographed with the unknown compound from the pH 8.5 solution. It is suggested that this compound may be IV, derived by partial hydrolysis of captopril disulphide. The compound was not obtained in sufficient quantity for structural elucidation by spectroscopic techniques.

Proline, derived from hydrolysis of captopril and its disulphide, is insoluble in the extraction solvent used and could only be detected by TLC in freeze-dried samples of the reaction mixtures. It was recognizable on TLC plates developed in Solvent C by its R_f value and the yellow colour it gave with ninhydrin spray reagent (0.5% ninhydrin in acetone). Visual evaluation of the relative intensities of the fluorescence-quenching spots derived from each fraction on TLC plates indicated that captopril disulphide was the major degradation product under all conditions.

Effect of pH on rate of oxidation

The recoveries of captopril at various time intervals could be fitted to an apparent first-order plot. The regression lines were calculated and the rate constants obtained at various pH values are given in Table 1.

Above pH 4 the rate of captopril degradation increases with increasing pH. The following reaction sequence has been proposed for the uncatalyzed oxidation of thiols (Wallace and Schiesheim, 1962).

$$RSH + B \rightleftharpoons RS^- + BH^+ \qquad (1)$$

TABLE 1

OXIDATION RATE CONSTANT FOR CAPTOPRIL IN SOLUTIONS AT VARIOUS pH VALUES AT 50°C

pH	Rate constant ($days^{-1}$)$\times 10^3$
2.13	8.38
2.59	9.01
2.89	8.22
3.13	8.31
3.53	9.92
3.88	9.13
4.23	12.94
4.67	19.43
5.16	28.93
5.63	42.03

$$RS^- + O_2 \rightarrow RS^{\cdot} + O_2^- \quad (2)$$

$$RS^- + O_2^- \rightarrow RS^{\cdot} + O_2^{2-} \quad (3)$$

$$2\,RS^- \rightarrow RSSR \quad (4)$$

$$O_2^{2-} + H_2O \rightarrow 2\,OH^- + O_2 \quad (5)$$

B is a base; e.g. in aqueous solution hydroxyl ion is present and Eq. 1 becomes

$$RSH + OH^- \rightleftharpoons RS^- + H_2O \quad (6)$$

This would be the case in the less acid solutions where there is sufficient hydroxyl ion for the reaction in Eq. 6 to occur. In addition, as the pH rises the thiol group will begin to ionize (pK_a 9.8, unpublished data) and provides thiolate anion for reaction 2.

Below pH 4 the degradation is pH-independent. According to the Henderson-Hasselbach equation the thiol would be virtually unionized at this pH and furthermore hydroxyl ion concentration is very low at this pH. A different mechanism is therefore required to explain degradation in acidic solution.

The thiol can react with water, although it is not a good nucleophile, to yield thiolate anion and thus Eq. 1 becomes:

$$RSH + H_2O \rightleftharpoons RS^- + H_3O^+$$

This type of solvent-catalyzed initiation of the reaction sequence leading to thiol oxidation was previously described by Wallace and Schiesheim (1962).

Effect of additives

The effect of the various additives is illustrated graphically in Fig. 1. All reactions fitting an apparent first-order plot. Addition of copper and iron at 5 ppm markedly increased the degradation rate ($K_{Cu} = 3.32 \times 10^{-1}$, $K_{Fe} = 9.24 \times 10^{-2}$, $K_{uncat} = 7.51 \times 10^{-3}$ days^{-1}). The catalytic effect of metal ions on thiol oxidation in alkaline and neutral solution has been studied by Cullis et al. (1968) and they found that a wide range of transition metals increased the rate of oxidation, with copper and iron being the most effective catalysts. Copper and iron are the most likely contaminants yielded by formulation additives, containers, closures or manufacturing equipment.

The following reaction sequence was given by Cullis et al. in alkaline media:

$$2\,RS^- + 2\,M^{(n+1)+} \rightarrow 2\,RS^{\cdot} + 2\,M^{n+} \quad (7)$$

$$2\,RS^{\cdot} \rightarrow RSSR \quad (8)$$

$$2\,M^{n+} + O_2 \rightarrow 2\,M^{(n+1)+} + O_2^{2-} \quad (9)$$

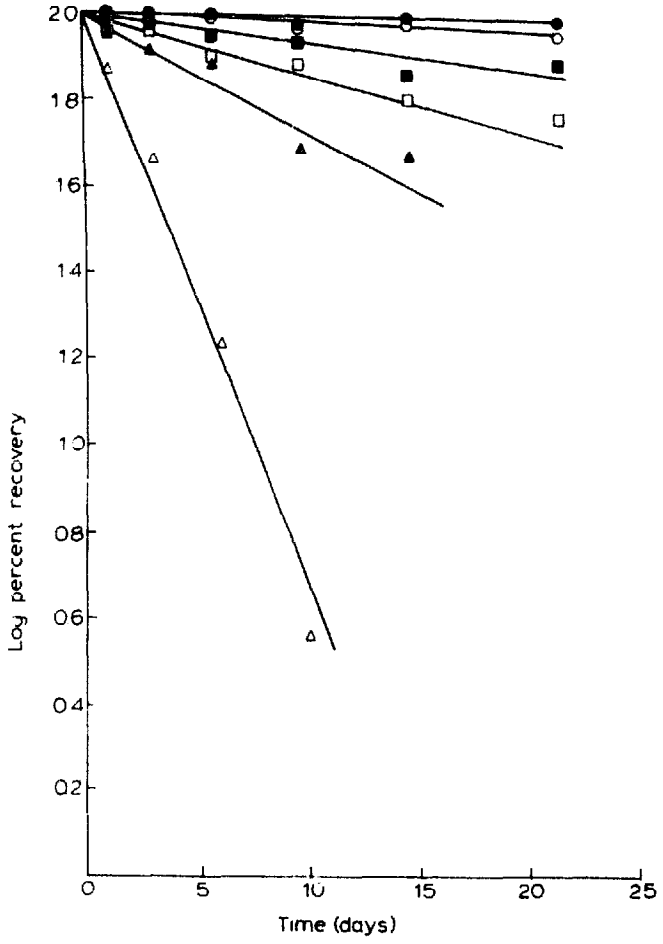


Fig. 1. Degradation of captopril in solution (pH 4 citrate buffer) at 50°C in the presence of various additives. Key: ●———●, 0.1% w/v disodium edetate added; ○———○, no additive; ■———■, 0.05% w/v propyl gallate; □———□, 0.1% w/v sodium metabisulphite; ▲———▲, 5 ppm iron (III); △———△, 5 ppm copper (II).

$$O_2^{2-} + H_2O \rightarrow 2\,OH^- + \tfrac{1}{2}O_2 \qquad (10)$$

(where $M^{(n+1)+}$ is highest oxidation state, e.g. Cu^{2+}, Fe^{3+}). In the case where there is virtually no thiolate anion present the following is suggested:

$$RSH + M^{(n+1)+} \rightarrow RS^{\cdot} + H^+ + M^{n+} \qquad (11)$$

An improvement in stability could be made by adding sodium edetate, which chelates the catalytic metal ions, although Hanaki and Kamide (1978) reported increases in oxidation rate of thiols on adding disodium edetate. Propyl gallate, an anti-oxidant that breaks free radical chain reactions, unexpectedly increased the rate of oxidation. As this study was carried out in the absence of disodium edetate, it is suggested that the increase in rate seen is due to the introduction of catalytic metal ions along with the propyl gallate (laboratory grade, no level of heavy metal impurities given).

The reducing agent, sodium metabisulphite, markedly increased the reaction rate although as Analar grade material it contained low levels of heavy metal impurities. Although the low levels of impurities present could contribute to the increased rate seen, it may be, as sulphite-type anti-oxidants themselves decompose by free radical mechanisms (Schroeter, 1963), the free radicals generated by metabisulphite decomposition may initiate the rapid free radical-mediated decomposition of captopril. As captopril disulphide was the only degradation product detected by TLC and HPLC in freeze-dried solutions containing bisulphite, no evidence for direct attack of metabisulphite on captopril was obtained.

Rate of hydrolysis

The disappearance of captopril by hydrolysis followed first-order kinetics for at least one half-life. In all hydrolysis rate experiments, less than 5% of captopril was found to degrade via oxidation to disulphides (II, III or IV). Rate constants calculated by linear regression are compiled in Table 2. Because of the high temperature employed, pH values were estimated from the extrapolated values of mean molar activity coefficients of HCl (Harned and Owen, 1958). From the Arrhenius equation, the heat of activation for captopril hydrolysis in 0.5 N hydrochloric acid solution was calculated to be 21.4 kcal/mol.

The hydrolytic rate constant was calculated to be 4.4×10^{-7} h^{-1} in 0.5 N HCl solution at 50°C. By assuming that hydrolytic rates are in direct proportion to the activity of hydronium ion, the hydrolytic rate constant would be approximately 10^{-5} h^{-1} at pH 2 and 10^{-7} h^{-1} at pH 4. By comparing these values to $30–50 \times 10^{-5}$ h^{-1} for the oxidation rate constant at pH range of 2–4 it is clear that degradation route in solutions of pH greater than 2 is predominantly oxidation.

With the degradation products and pathways thus identified, the pH region of maximum stability established and the effect of some formulation additives and metal contaminants known, the work has defined some of the steps required to be taken in designing aqueous formulations of acceptable stability.

TABLE 2

APPARENT RATE CONSTANTS (k in $h^{-1} \times 10^3$) OF CAPTOPRIL HYDROLYSIS

Temp. (°C)	Concentration of HCl solution			
	0.5 N	0.2 N	0.1 N	0.05 N
90	17.5(0.459) [a]	8.78(0.845)	6.32(1.13)	3.42(1.409)
80	8.21(0.453)	4.41(0.839)	1.79(1.125)	
70	3.40(0.447)	3.20(0.833)	0.88(1.114)	

[a] Estimated pHs for the solutions are shown in parenthesis.

References

Capozzi, G. and Modena, G., The oxidation of thiols. In Patai, S. (Ed.), The Chemistry of the Thiol Group, Vol. 2, John Wiley, London, 1974, pp. 785–840.

Cullis, C.F., Hopton, J.D., Swan, C.J. and Trimm, D.L., Oxidation of thiols in gas–liquid systems. II. Reaction in presence of added metal catalysts. J. Appl. Chem., 18 (1968) 335–339.

Elving, P.J., Markovitz, J.M. and Rosenthal, I., Preparation of buffer systems of constant ionic strength. Anal. Chem., 28 (1956) 1179–1180.

Hanaki, A. and Kamide, H., Acceleration of copper catalysed auto-oxidation of cysteine by ethylenediamine tetraacetic acid and related polyaminopolycarboxylic acids. Chem. Pharm. Bull. 26 (1978) 325–327.

Harnad, H.S. and Owen, B.B., The Physical Chemistry of Electrolyte Solutions, Reinhold Publishing, New York, 1958, p. 751.

Perlman, S. and Kirshbaum, J., High performance liquid chromatography analyses of the antihypertensive drug captopril. J. Chromatogr., 206 (1981), 311–317.

Schroeter, L.C., Kinetics of air oxidation of sulphurous acid salts. J. Pharm. Sci., 52 (1963) 559–563.

Wallace, T.J. and Schriesheim, A., Solvent effects in the base-catalysed oxidation of mercaptans with molecular oxygen. J. Org. Chem., 27 (1962) 1514–16.