International Journal of Pharmaceutics, 77 (1991) 41–46

ADONIS 037851739100361 8

IJP 02535

Kinetic determination of carbocysteine in syrup based on its reaction with 1-fluoro-2,4-dinitrobenzene monitored with a fluoride-selective electrode

J.G. Pentari, C.E. Efstathiou and M.A. Koupparis

Department of Chemistry, University of Athens, Athens (Greece)

(Received 1 March 1991)
(Modified version received 2 May 1991)
(Accepted 20 May 1991)

Key words: Kinetic determination; Carbocysteine; Fluoride-selective electrode; 1-Fluoro-2,4-dinitrobenzene; Micellar catalysis

Summary

A kinetic-potentiometric method is described for the determination of carbocysteine in coloured pharmaceutical preparations based on monitoring its reaction with 1-fluoro-2,4-dinitrobenzene, catalysed by cetyltrimethylammonium bromide micelles, using a fluoride-selective electrode. By measuring the initial slopes $(\Delta E/\Delta t)_0$, kinetic parameters (reaction order and rate constants) were obtained and kinetic determination in the range 7–70 μM was performed. Micellar catalysis was found to enhance the reaction rate 288-fold. The reactions of the related compounds L-cysteine and L-cystine, and the interfering preservative compounds methyl- and propylparabens, were also studied. The proposed method did not suffer any interference from excipients or cloudy and coloured solutions and was evaluated by assaying carbocysteine in commercial formulations and performing recovery experiments. The results showed good agreement with those obtained using methods currently in use.

Introduction

Carbocysteine (*S*-carboxymethyl-L-cysteine, S-CMC), one of the several derivatives of cysteine, is used in the treatment of disorders of the respiratory tract associated with excessive mucus (Martindale, 1982). It has been proven that S-CMC shows a selective activity in reducing bronchial secretion viscosity in laboratory animals (Huyen et al., 1966; Quevauviller et al., 1967). This activity was also confirmed by several clinical trials. S-CMC is administered orally in the form of tablets, capsules and syrups (for adults and children) under several proprietary names (Martindale, 1982).

Raw material of S-CMC can be assayed using the non-aqueous potentiometric titrimetric method of the French Pharmacopoeia (Pharmacopeie Française, 1987). As yet, there is no official method for the determination of S-CMC in its formulations. Only one unofficial HPLC method for the determination of this drug in pharmaceutical preparations, in combination with theo-

Correspondence: C.E. Efstathiou or M.A. Koupparis, Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece.

phylline, was found in the literature (Caccialanza and Gandini, 1981). For the determination of S-CMC in biological fluids a GLC method using pre-column acylation in two steps has also been developed (Dolfini and Testa, 1979). These chromatographic methods are not very suitable for application in routine assays of S-CMC formulations, since they require prior separation of the drug from the turbid and usually coloured formulations, tedious sample treatment before measurement (acylation in GLC), and relatively expensive instrumentation. For these reasons, a simple, fast and inexpensive method for routine analysis is highly desirable.

In this paper, a semi-automated kinetic potentiometric method for the determination of S-CMC in pharmaceutical preparations is described. It is based on the monitoring of the rate of the reaction of S-CMC with 1-fluoro-2,4-dinitrobenzene (FDNB) using a fluoride-selective electrode. Since this reaction is relatively slow, micellar catalysis by cetyltrimethylammonium bromide (CTAB) is used for acceleration.

FDNB undergoes aromatic nucleophilic substitution by primary and secondary amino, phenolic, thiolic, and hydrazino groups, liberating fluoride ions which can be selectively monitored using a fluoride-selective electrode. These reactions have been used to develop kinetic methods for the determination of amino acids (Athanasiou-Malaki and Koupparis, 1987), primary and secondary amines (Athanasiou et al., 1989), hydrazine, hydrazides and azides (Athanasiou-Malaki and Koupparis, 1989a) and phenols (Archontaki et al., 1989). Several applications of these methods in the determination of drugs in formulations have been described. FDNB reactions were found to be catalysed by cationic surfactants, such as CTAB, and this micellar catalysis has been used to decrease the analysis time of equilibrium spectrophotometric (Connors and Wong, 1979) or kinetic potentiometric (Archontaki et al., 1989; Athanasiou-Malaki and Koupparis, 1989b) methods.

The theoretical aspects and several applications of ion-selective electrodes in kinetic methods of analysis have been previously reported (Efstathiou et al., 1985). The combination of ion-selective electrodes with kinetic methods of analysis results in the development of relatively fast, simple, sufficiently precise, and selective methods, with the great advantage of use in turbid and highly coloured samples.

Experimental

Reagents

All solutions were prepared in de-ionised water from analytical reagent grade materials; S-CMC was obtained from Mohes (Spain), and kindly donated by Elpen S.A. Pharmaceutical Co. (Greece). Its purity was confirmed using the French Pharmacopoeia method (Pharmacopeie Française, 1987). The analysed pharmaceutical formulations were obtained from commercial sources.

S-CMC standard solutions A 0.10 M stock solution was prepared by dispersing 1.7921 g of pure substance in a few millilitres of water, adding dropwise 1 M NaOH until the solution became clear and diluting to 100.0 ml with water. This solution was stable in the refrigerator for at least 1 month. Working standard solutions in the range of 7–70 μM were prepared daily from the stock solution. Each standard also contained borate buffer pH 9.0 at a concentration of 0.050 M.

FDNB working solution, 5.0% w/v (0.27 M) in acetone This was prepared by dissolving 1.25 g of fluoro-2,4-dinitrobenzene (Sigma) in 25.0 ml of acetone. This solution was stored in a sealed amber glass vial in the refrigerator when not in use. Under these conditions, it was stable for at least 1 month. This reagent is vesicant and should be handled carefully.

Standard fluoride solution A 0.10 M NaF solution stored in a polyethylene bottle was used.

0.050 M borate buffer, pH 9.0 Borate buffer was used for the final dilution of the pharmaceutical preparations prior to the measurement step.

Working mixed buffer solution A 0.050 M borate buffer, pH 9.0 also containing 30 μM NaF and 2.0 mM CTAB (Merck) was prepared daily. This buffer solution, containing the CTAB micelles, was used in all assays.

Apparatus

The system for potentiometric rate measurements consisted of a combination fluoride electrode (Orion model 96-09) and a digital electrometer (Orion 801 pH pIon meter) interfaced to a microcomputer (Amstrad CPC 6128). The interface between the electrometer and the microcomputer, and the control program (KINMOD) have been described elsewhere (Archontaki et al., 1989). A simple potentiometric recorder can also be used instead of this automated system.

All measurements were carried out in a thermostated ($25 \pm 0.2\,^{\circ}C$), double-wall reaction cell with continuous magnetic stirring. The electrode was stored in 1 mM NaF solution when not in use.

Procedures

Sample preparation For drug assay in liquid preparations (syrups), 5.0 ml aliquots were properly diluted in water so that the drug concentration of the final solutions lay within the range of the calibration curve (7–70 μM). If the preparation contains the interfering preservatives methyl- and propylparabens, the dilution of the sample must be adjusted so that their final concentration is less than 1 μM. The final sample solutions must also contain 0.050 M borate buffer pH 9.0. Care should be taken do drain all of the viscous syrup from the pipette. Solid preparations (tablets and capsules) can also be analysed by preparing appropriate sample solutions.

Measurement procedure 10.0 ml of a working standard or sample solution of the drug and 5.0 ml of the working mixed buffer pH 9.0 (containing the CTAB micelles) were manually pipetted into the thermostated reaction cell. The stirrer was started and after the potential had been stabilised (about 30 s as shown from the electrometer readings), the reaction was initiated by the rapid injection of 100 μl of FDNB working solution and, at the same time, the microcomputer was commanded to collect the data (potential readings vs time, *E-t* curve). The reaction was followed for about 1.5–2 min and its course was shown on the monitor of the microcomputer, the data being stored on a disc. Then the cell was emptied and washed twice with the working mixed borate buffer solution, ready to proceed to the next sample. A blank (water) was included for each calibration graph.

From the recalled data the initial slope $(\Delta E/\Delta t)_0$ (mV s^{-1}) was calculated by least-squares linear regression, by visual choice of the linear part of the reaction curve as shown on the monitor (correlation coefficient being an indication). If a recorder is used to monitor the course of the reaction, the initial slope is calculated graphically. Calibration graphs of $(\Delta E/\Delta t)_0$ vs concentration were constructed using the standard solutions of the drug.

The slope of the electrode response (S), required for the kinetic study, was periodically determined by successive additions of micro-quantities of 0.10 M NaF standard stock solution in 10.0 ml of water mixed with 5.0 ml of 0.050 M borate buffer solution pH 9.0.

Results and Discussion

Kinetic study of the reaction of carbocysteine with FDNB

The reaction of the primary amino group of S-CMC with FDNB is a nucleophilic aromatic substitution, with the formation of an intermediate complex. A dinitrophenyl derivative is finally formed with the liberation of fluoride ion:

$$R-NH_2 + (NO_2)_2C_6H_3F \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (NO_2)_2C_6H_3(F)NH_2R \xrightarrow{k_2} (NO_2)_2C_6H_3NHR + H^+ + F^- \quad (1)$$

From the initial slopes $(\Delta E/\Delta t)_0$ of the *E-t* curves, it is easy to calculate the kinetic parameters of the overall reaction using the equation (Athanasiou-Malaki and Koupparis, 1987):

$$(\Delta E/\Delta t)_0 = S(1/[F^-]_0)k^{st}_{exp}C_{0,S\text{-}CMC}[FDNB]_0 \quad (2)$$

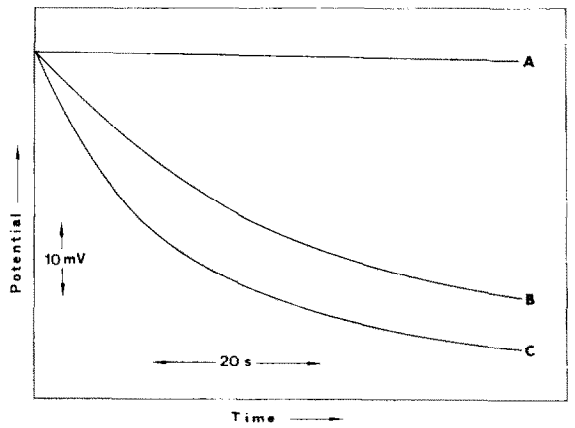

Fig. 1. Effect of micellar catalysis of cetyltrimethyl ammonium bromide on the carbocysteine-FDNB reaction at pH 9 and 25 °C. [FDNB], 1.78 mM; [F^-], 10 μM; [S-CMC], 33 μM; [CTAB]: (A) 0.00, (B) 0.67, (C) 1.00 mM.

where S is the slope of the E vs ln C response curve of the fluoride electrode (ideally being equal to -24.8 mV $(\ln C)^{-1}$ and not affected by the presence of micelles), $[F^-]_0$ is the initial fluoride concentration (10 μM), added in the mixed buffer for obtaining reproducible initial potential readings, $C_{0,S\text{-}CMC}$ is the initial analytical concentration of S-CMC, $[FDNB]_0$ is the initial FDNB concentration (1.78 mM) and k^{st}_{exp} is the experimental stoichiometric overall rate constant of the second-order reaction varying with pH. No correction for the hydrolysis of FDNB was attempted.

From experiments carried out with various concentrations of S-CMC and at a constant concentration of FDNB, the reaction order with respect to S-CMC can be obtained.

The reaction was found to be accelerated favourably by the cationic micelles of CTAB and this micellar catalysis was exploited for shortening the time of the measurement step. The effect of the micellar catalysis of CTAB on the reaction of S-CMC with FDNB is shown in Fig. 1. As shown, micellar catalysis appears even with a subcritical micellar concentration of CTAB (CMC being equal to about 1 mM).

The pH effect on the S-CMC-FDNB reaction was also studied in the range pH 7–9. Since the reactive amino group of S-CMC is expected to have a pK_a comparable to that of cysteine (being equal to 10.3), it is protonated at lower pH values and the reaction rate increases with pH. Higher pH values were not examined since hydroxide ions at higher concentrations interfere with the fluoride electrode (Athanasiou-Malaki and Koupparis, 1987).

TABLE 1

Kinetic parameters of the reactions of the studied compounds with FDNB [a]

Compound	k^{st}_{exp} (M^{-1} s^{-1}) (±SD)		Increase (-fold)
	Uncatalysed	Micellar catalysed	
Carbocysteine	0.0128 ± 0.0005	3.69 ± 0.07	288
Cysteine	0.755 ± 0.001	17.2 ± 0.2	23
Cystine	0.0414 ± 0.0003	6.1 ± 0.2	147
Methylparaben	–	3.94 ± 0.09	–
Propylparaben	–	4.9 ± 0.1	–

[a] Conditions: pH 9.0, 25 °C; [FDNB], 1.78 mM; [CTAB], 0.67 mM.

The rate constants found for the uncatalysed and catalysed reactions of S-CMC with FDNB reaction are listed in Table 1. As shown, a 288-fold increase in the reaction rate was achieved by micellar catalysis at pH 9.0 and 0.67 mM CTAB. Since some other substances, which coexist with S-CMC in crude commercial materials (L-cystine, at less than 0.5% according to the French Pharmacopoeia) or in commercial formulations (methyl- and propylparabens used as preservatives in syrups), also react with FDNB, their reactions, as well as that of the parent compound, L-cysteine, were examined kinetically for comparison. All the kinetic data obtained are shown in Table 1. From the three related compounds, cysteine shows the highest rate for the uncatalysed reaction, probably due to the presence of the very reactive thiolic group in addition to the amino group. The catalysis by the cationic micelles of CTAB is most intense for S-CMC (288-fold), followed by L-cystine (147-fold). This behaviour must be attributed to the attraction of the negatively charged and lipophilic parts of the molecules by the positively charged micelles.

All the reactions were found to be first order

TABLE 2

Analytical characteristics of the determination of cysteine derivatives in aqueous solutions using the kinetic potentiometric micellar-catalysed method

Compound	Linear range tested (μM)	Sensitivity (mV s^{-1} μM^{-1}) ($\pm$SD)	r
Carbocysteine	7–70	0.0163 $\pm$ 0.003	0.9994
Cysteine	5–50	0.076 $\pm$ 0.001	0.9998
Cystine	5–30	0.0268 $\pm$ 0.0007	0.9993

with respect to the reacting compound and the FDNB.

Kinetic determination of carbocysteine in preparations

As concluded from Eqn 2, the initial slope of the reaction curve is proportional to the S-CMC concentration, since the reaction is first order with respect to the analyte. For routine determinations a 0.67 mM concentration of CTAB was used as a compromise between short measurement times with good precision, and high blank readings (due to CTAB-catalysed hydrolysis of FDNB at higher concentrations of CTAB). The borate buffer of pH 9.0 was chosen as a compromise between high sensitivity and low measurable limit (pH also affects the FDNB hydrolysis by base catalysis and reaction with hydroxide).

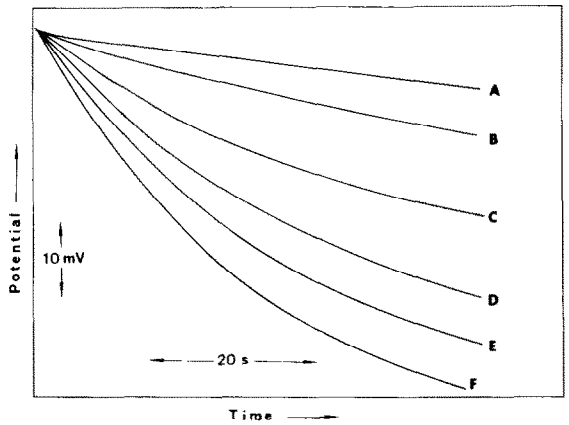

Fig. 2. Typical reaction curves of the S-CMC-FDNB micellar-catalysed reaction for the calibration curve at pH 9.0 and 25.0 °C. (A) Blank, (B) 7.0, (C) 30.0, (D) 50.0, (E) 70.0, and (F) 100 μM.

Typical reaction curves used for the calibration graph of S-CMC are shown in Fig. 2. Data pertinent to the calibration curves (linear concentration range, sensitivity (slope of the curve), and correlation coefficient) of the three related compounds are summarised in Table 2. The precision (RSD) of the measurements was found to be

TABLE 3

Comparison of results obtained by the proposed kinetic-potentiometric method and those currently in use for the determination of carbocysteine in syrups

Formulation	Content (mg/5 ml)			% Relative difference (KP–Ref)
	Nominal	Proposed method [a] (KP)	References (Ref)	
Mucothiol				
for adults [b]	250	259 $\pm$ 5	250 [c]	+3.6
paediatric [d]	100	98.6 $\pm$ 0.9	105 [c]	−6.1
Grossenel [e]	250	261 $\pm$ 4	269 [f]	−3.0
Ectofus [g]	250	272 $\pm$ 3	261 [c]	+4.2
			mean	4.2

[a] Average of three determinations ($\pm$SD).
[b] Lot 8821678, Remek, S.A., Athens, Greece.
[c] Total nitrogen determination of Kjeldahl method (United States Pharmacopeia, 1980).
[d] Lot 8801361, Remek, S.A., Athens, Greece.
[e] Lot 8806, Anfarm Hellas, S.A., Athens, Greece.
[f] Precipitation of carbocysteine at pH 2.9, centrifugation of the precipitate and titration with standard solution of NaOH.
[g] Lot 09A/1249, Coup, Athens, Greece.

1.6% (obtained by five measurements of a standard of intermediate concentration).

The proposed method was applied to the determination of S-CMC in commercial pharmaceutical formulations (coloured syrups). The results obtained were compared to data acquired with methods currently in use and were found to be in good agreement (Table 3). The accuracy of the proposed method was further checked by means of recovery experiments with a syrup sample. The average recovery found was 102.4% (range 98.4–104.5%).

The interference of various common excipients, used in drug formulations, was also studied. Sodium chloride, carboxypolymethylene (carbopol 4000), magnesium stearate, talc, lactose, galactose, sugar, mannitol and glycerin do not interfere with the reaction of amino acids with FDNB.

Pharmaceutical syrups usually contain as preservatives methyl, ethyl- or propylparabens (*p*-hydroxybenzoates), in a total concentration of 0.15% w/v. These compounds possessing a reactive phenolic group also undergo a micellar catalysed reaction with FDNB. Their reaction rates are comparable to that of S-CMC (Table 1). Since their concentration in syrups is much lower in comparison with that of S-CMC (about 13-fold lower in paediatric and 30-fold lower in adults' syrup), this interference can be eliminated by a simple dilution as described in the procedure. It was found that at concentrations lower than 1 μM, parabens do not contribute appreciably to the overall reaction rate, thus causing no positive analytical errors.

Conclusions

The above described method is simple, selective and rapid enough for the determination of carbocysteine in turbid and coloured samples. The proposed low-cost system can be used for routine assays of this compound and its use can be extended to the routine determination of other medicinal substances having amino, phenolic, thiolic and hydrazino groups, ensuring rapid and accurate results.

References

Archontaki, H.A., Koupparis, M.A. and Efstathiou, C.E., Semi-automated kinetic determination of phenolic compounds using a fluoride-selective electrode and based on their micellar-catalysed reaction with 1-fluoro-2,4-dinitrobenzene. *Analyst*, 114 (1989) 591–596.

Athanasiou-Malaki, E. and Koupparis, M.A., Kinetic-potentiometric determination of amino acids based on monitoring their reaction with dinitrofluorobenzene using a fluoride-selective electrode. *Analyst*, 112 (1987) 757–761.

Athanasiou-Malaki, E. and Koupparis, M.A., Kinetic study of the determination of hydrazines, isoniazid and sodium azide by monitoring their reactions with 1-fluoro-2,4-dinitrobenzene, by means of a fluoride-selective electrode. *Talanta*, 36 (1989a) 431–436.

Athanasiou-Malaki, E. and Koupparis, M.A., Kinetic-potentiometric study and analytical applications of micellar-catalysed reactions of 1-fluoro-2,4-dinitrobenzene with amino compounds. *Anal. Chim. Acta*, 219 (1989b) 295–307.

Athanasiou-Malaki, E., Koupparis, M.A. and Hadjiioannou, T.P., Kinetic determination of primary and secondary amines using a fluoride-selective electrode and based on their reaction with 1-fluoro-2,4-dinitrobenzene. *Anal. Chem.*, 61 (1989) 1358–1363.

Caccialanza, G. and Gandini, C., Dosaggio mediante HPLC di *S*-carbossimetil-L-cisteina e teofillina associate in forme farmaceutiche. *Il Farmaco-Ed. Pr.*, 36 (1981) 396–402.

Connors, K.A. and Wong, M.P., Micellar catalysis of an analytical reaction: Spectrophotometric determination of amino acids and peptides after cetrimonium bromide-catalysed reaction with 1-fluoro-2,4-dinitrobenzene. *J. Pharm. Sci.*, 68 (1979) 1470–1471.

Dolphini, E. and Testa, R., Gas liquid chromatographic determination of *S*-carboxymethylcysteine in human urine. *Il Farmaco-Ed. Pr.*, 34 (1979) 114–120.

Efstathiou, C.E., Koupparis, M.A. and Hadjiioannou, T.P., Application of ion-selective electrodes in reaction kinetics and kinetic analysis. *Ion-Sel. Electrode Rev.*, 7 (1985) 203–259.

Huyen, V.N., Garcet, S. and Lakah, L., Hypersécrétion experimentale du mucus bronchique chez le rat: Application à l'étude d'un agent dit mucolytique: la *S*-carboxy-methyl cisteine. *Seanc. Soc. Belge Biol.*, 160 (1966) 1849–1851.

Martindale, The extra pharmacopoeia, 28th Ed., The Pharmaceutical Press, London, 1982, p. 49.

Pharmacopeie Francaise, Xeme Edn., 4e Suppl., La commission Nationale de la Pharmacopeé, Juillet 1987.

Quevauviller, A., Huyen, V.N., Garcet, S. and Lakah, L., Activité des mucolytiques sur la retention experimental de mucus intrabronchique chez le rat. *Therapie*, 22 (1967) 485–493.

United States Pharmacopeia XXI and *National Formulary XVI*, United States Pharmacopoeial Convention, 1980, p. 1207.