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BATCH AND FLOW-INJECTION DETERMINATION OF ETHYLENEDIAMINE IN PHARMACEUTICAL PREPARATIONS

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SUMMARY

The ethylenediamine/pyridine-2-carbaldehyde/copper(I) system is used in a new spectrophotometric method for the determination of ethylenediamine. The batch procedure involves the formation of an orange chelate between the Schiff's base and copper(I) ions at pH 8.5 (borate buffer) and measurement of the absorbance at 475 nm against water after 10—15 min; Beer's law is obeyed over the range 0.5—11.2 $\mu g\ ml^{-1}$ and the molar absorptivity is 6.21×10^3 $l\ mol^{-1}\ cm^{-1}$. Tolerance limits for different amines [36] and other organic compounds [12] are reported. In the optimized flow-injection system, ethylenediamine (1.4—84.6 $\mu g\ ml^{-1}$) is determined at a sample throughput of 55 h^{-1}. The method is sensitive and selective and is satisfactory for the determination of the diamine in aminophylline and pharmaceutical preparations (ethylenediamine contents from 0.031 to 3.23%) with relative errors ranging from −7.4 to +11.1% and relative standard deviations of about 0.65% for both procedures.

Ethylenediamine finds application in the electrochemical industry, as an antioxidant, and as a swelling agent in the manufacture of resins and polymers. It may have to be determined in electroplating baths, in degradation products of resins and polymers, and in factory air. The presence of ethylenediamine in soils and plants can be a measure of contamination by residual pesticides, and its determination in these samples is also of interest. In pharmaceutical use, ethylenediamine is associated with theophylline to give aminophylline, a water-soluble powder with anti-asthmatic properties.

Apart from the numerous titrimetric and chromatographic methods that have been reported for the determination of ethylenediamine, various spectrophotometric procedures have been described. These generally exhibit rather poor sensitivity and selectivity, being based on the formation of copper(II) and nickel(II) ethylenediamine complexes [1] or on the reaction of the diamine with different organic compounds [2—6]. The in-situ synthesis of Schiff's bases has been used to determine amines by titrimetry and spectrophotometry [7]. However, the presence of a suitable metal ion improves both sensitivity and selectivity, providing useful methods for the determination of either organic compound involved in the reaction. Critchfield and Johnson [8] first reported the use of amine/carbonyl compound/

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metal ion systems to determine primary amines spectrophotometrically. Only a few procedures have been described for the evaluation of ethylenediamine in pharmaceutical preparations, e.g., aminophylline [9, 10], tablets [11] and suppositories [7, 12]. A simple, fast spectrophotometric method for determining ethylenediamine is described below. It is based on formation of the copper(I) chelate of the Schiff's base produced by reaction of ethylenediamine with pyridine-2-carbaldehyde. This assay of ethylenediamine is sensitive and selective and is applicable to aminophylline and pharmaceutical preparations by either a batch procedure or flow injection analysis (f.i.a.).

EXPERIMENTAL

Reagents and equipment

Ethylenediamine solutions were prepared from the analytical-grade reagent and standardized against hydrochloric acid (bromophenol blue indicator). The pyridine-2-carbaldehyde used was 99% pure. Copper(I) solutions were prepared from copper(II) nitrate pentahydrate standardized by titration with EDTA, by addition of ascorbic acid. The aldehyde solutions were stable for at least a week, whereas the copper(I) solutions had to be prepared daily. Borate buffer was prepared by adjusting 0.1 mol l^{-1} boric acid to pH 8.5 with sodium hydroxide pellets. Aminophylline, standardized like ethylenediamine, and alkaloids (pharmacopoeial grade) were used. All other chemicals were of analytical grade and distilled water was used throughout.

A Perkin-Elmer Coleman 575 spectrophotometer was used for recording spectra and measuring absorbance at 475 nm. A Hellma QS-1000 18-μl flow cell was used in the flow-injection system. A Metrohm 620 pH meter and a Beckman J2-21 centrifuge were used. The flow manifold was based on a FIAstar (Bifok) with an Eyela MP-3 peristaltic pump and 0.5 mm i.d. teflon tubing. All solutions were degassed in a Selecta ultrasonic bath and samples and standards were injected in triplicate at least. An Apple-II desk computer was used for statistical calculations and for running the optimization program.

Procedures

Dissolution of the samples. For aminophylline and syrup, an appropriate amount was dissolved in water. For injections, the preparation (2.5 ml) was diluted to the mark in a 25-ml volumetric flask with water. Tablets (2—10) were finely powdered; a portion (0.2—0.5 g) was treated with 20—30 ml of distilled water in a ultrasonic bath and centrifuged for 15 min at 15 000 rpm. The solution was transferred to an 100-ml volumetric flask and the precipitate was treated again in the same way. The combined aqueous portions were diluted to the mark. All these working solutions contained between 50 and 150 μg ml^{-1} ethylenediamine.

Batch method for ethylenediamine. The necessary solutions were: (a) 0.1 ml of 99% pyridine-2-carbaldehyde diluted to 50 ml with distilled water; (b) a 500 μg Cu(II) ml^{-1} solution containing 0.4% (w/v) ascorbic acid; (c) a 4.000 g l^{-1} solution of disodium ethylenediaminetetraacetate (EDTA).

For the determination, the sample solution containing up to 110 μg of ethylenediamine was placed in a 10-ml volumetric flask; 2 ml of solution (a), 2.5—5.0 ml of the buffer solution, 0.6 ml of solution (b) and 0.5 ml of solution (c) were added and the mixture was diluted to the mark. After 10—15 min, the absorbance was measured at 475 nm against water.

Flow-injection procedure. The aldehyde stream was 1 ml of pyridine-2-carbaldehyde diluted to 100 ml with the buffer solution. The Cu(I) stream was a 1 + 12 (v/v) dilution of solution (b) of the batch procedure. The manifold is described in Fig. 1. The injected sample (116 μl) contained up to 840 μg of EDA. Peak heights were measured.

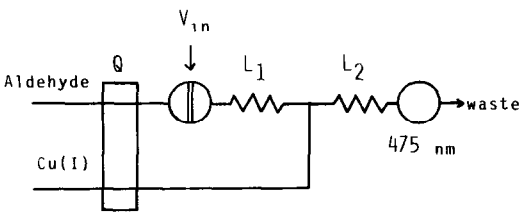

Fig. 1. Flow diagram. Optimum values of Q, V_{in}, L_1 and L_2 are 1.1 ml min^{-1}, 116 μl, 80 cm and 95 cm, respectively.

RESULTS AND DISCUSSION

Study of the ethylenediamine/pyridine-2-carbaldehyde/Cu(I) system

The feasibility of the in situ synthesis of the Schiff's base derived from ethylenediamine and pyridine-2-carbaldehyde was reported previously [13]. In this study, it was found that copper(I) ions offered advantages of sensitivity and stability of the coloured chelate. The maximum absorption wavelength of the system was 475 nm.

The system chosen for the determination of ethylenediamine (en) was optimized. The best pH range was 7.5—11.5 and a borate buffer of pH 8.5 was selected. The optimum molar ratios of the reagents were 1:80 en/pyridine-2-carbaldehyde, 1:12 en/Cu(I) and 1:1 Cu(II)/ascorbic acid. The preferred order of addition was en/aldehyde/buffer/Cu(I). Under these conditions, the chelate attained maximum absorbance within 10—15 min after mixing of the reagents and remained stable for at least 2 h. Attempts were made to increase the sensitivity of the procedure by adding 5—20% (v/v) ethanol, dimethylformamide and dioxane; the maximum wavelength remained 475 nm in all cases and the absorbance was not significantly increased. Heating had little effect on the reaction time, hence room temperature was preferred for convenience.

The chelate was extracted into isoamyl alcohol (λ_{max} = 475 nm; ϵ = 7.6 × 10^3 l mol^{-1} cm^{-1}), isobutyl methyl ketone (partially) and chloroform (λ_{max} = 475 nm; ϵ = 7.5 × 10^3 l mol^{-1} cm^{-1}) in the presence of perchlorate. This offers the possibility of extraction-photometric or atomic-absorption (measuring at the copper line) determinations of ethylenediamine.

The continuous-variations method was applied to ascertain the ethylenediamine/copper molar ratio in the chelate. Two complex species of 2:1 and 1:1 en/metal ion stoichiometric ratios were observed, the latter predominating when Cu(I) was in excess. The electrical charge of the chelate formed under the optimum conditions was studied; the chelate was retained by a cationic resin, thus proving it to be a positively charged species.

Spectrophotometric determination of ethylenediamine

Beer's law was obeyed between 0.5 and 11.2 μg ml^{-1} ethylenediamine, the molar absorptivity, calculated from the calibration graph by least squares, being 6.21 × 10^3 l mol^{-1} cm^{-1}. The Sandell sensitivity was 0.0097 μg cm^{-2}. The relative standard deviation was ±0.61% for eleven samples, each containing 4.6 μg ml^{-1} (P = 0.05). A Ringbom plot showed that the optimum range for accurate determinations was between 1.9 and 9.3 μg ml^{-1} ethylenediamine.

A systematic study of possible interferences from inorganic and organic substances showed that EDTA, up to a final concentration of 200 μg ml^{-1}, not only did not interfere, but also reduced substantially the interfering effects of other species. The presence of EDTA also prevented the precipitation of any Cu(I) excess, thus making centrifugation or filtration unnecessary. Table 1 lists the tolerated levels for some common cations and anions. The organic compounds tested were primary, secondary and tertiary amines, quaternary ammonium salts and alkaloids and other substances accompanying ethylenediamine in the pharmaceutical preparations. The histograms in Fig. 2 show the tolerated levels, expressed as molar ratios, for various organic compounds.

Batch determination of ethylenediamine in pharmaceutical preparations

The method was applied to the evaluation of ethylenediamine in samples of medicines containing aminophylline. Samples containing an exact amount of ethylenediamine were specially prepared from the components of the commercial preparation (see footnotes to Table 2). All the sample solutions were prepared as described under Procedures. A decrease in ethylenediamine content was observed for all prepared solutions stored for some time at room temperature; this is in agreement with the other experimental observations on the stability of aminophylline solutions [14] and of preparations containing ethylenediamine [9, 12]. The fate of the ethylenediamine is not known in most cases. Owing to this, all sample solutions were analyzed within a week after preparation. The calibration equation was $A = 0.0986C$ (μg ml^{-1}) + 0.016, where A is absorbance.

TABLE 1

Tolerance limits[a] for some cations and anions in the determination of 4.6 μg ml^{-1} ethylenediamine

Tolerated amount (μg ml^{-1})		Ion
No EDTA	With EDTA	
10 000	10 000	Alkali metals, SO_4^{2-}, NO_3^-, acetate, As(III), As(V)
750	1000	Ca(II)
500	10 000	Cl^-, Br^-
200	1000	Mn(II)
100	200	Ba(II), Zn(II), Cd(II)
100	1000	Tartrate, oxalate, citrate, PO_4^{3-}, CO_3^{2-}
50	200	I^-
—	200	EDTA
—[b]	100	Bi(III), Al(III), Cr(III), Ag(I), Pb(II), Mg(II), Sr(II)
—[b]	10	Fe(III), Co(II), CN^-

[a]Maximum concentration tested was 10 000 μg ml^{-1}. [b]Turbidity appeared even at low concentrations.

For the determination of ethylediamine in aminophylline, 0.3—0.5 g of aminophylline was dissolved in 50 ml of water and appropriate aliquots were taken; the mean result obtained by the photometric procedure was 14.80 ± 0.10% (n = 3), which agreed well with the mean result obtained by titration with acid (14.57 ± 0.08%). The amounts of ethylenediamine in specially prepared and commercial drugs were evaluated by the standard-additions method as well as from calibration graphs. Details are given in Table 2 which shows good agreement between the two methods of evaluation. Of the commercial samples tested, only the results for tablets are reported. The analysis of commercial syrups and injections gave ethylenediamine contents much below the nominal amounts, possibly because of reaction of ethylenediamine with other ingredients of the preparation on storage at room temperature and exposure to light.

Flow injection method for ethylenediamine in pharmaceutical preparations

Flow injection analysis was used for the determination of ethylenediamine based on the above chemical system. The flow system was optimized and then utilized for the rapid assay of the ethylenediamine content in the pharmaceutical preparations listed in Table 2. In order to simplify the manifold, the buffer was incorporated into the aldehyde reagent. The sample was injected into the aldehyde stream (buffered at pH 8.5) and then mixed with the copper(I) reagent stream (Fig. 1). In numerous preliminary tests, the reagents were mixed following the same order as in the batch procedure, the sample was injected into the copper(I) stream rather than the aldehyde stream, and a 200-μl mixing chamber was used instead of coil L_2 to increase the extent of reaction. Flow rates from 1.0 to 5.0 ml min^{-1} in

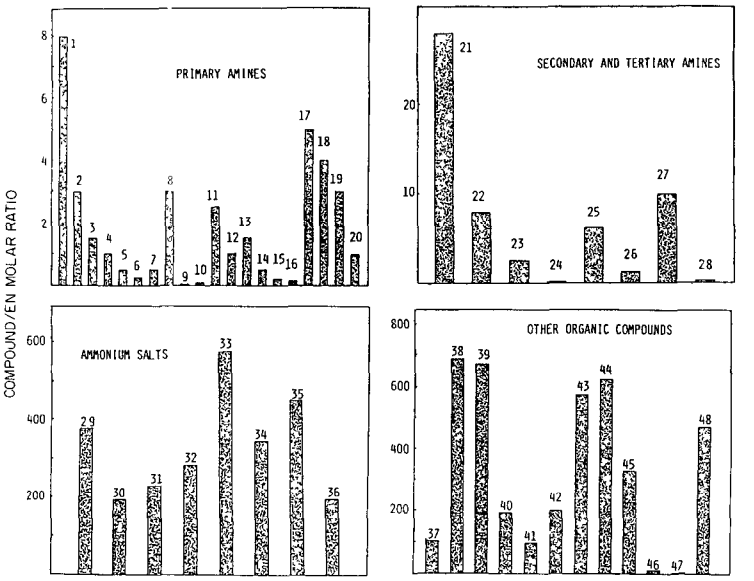

Fig. 2. Interference histograms for some amines and other organic compounds in the determination of 4.6 μg ml^{-1} ethylenediamine: (1) methylamine; (2) ethylamine; (3) n-propylamine; (4) n-butylamine; (5) *p*-phenetidine; (6) benzylamine; (7) *p*-anisidine; (8) 1,3-diaminepropane; (9) 1,4-diaminebutane; (10) benzidine; (11) naphthylamine; (12) hydrazine, (13) aniline; (14) ethanolamine; (15) anthranilic acid; (16) 4-nitroaniline; (17) glycine; (18) alanine; (19) L-serine; (20) hydroxylammonium chloride; (21) diethanolamine; (22) diphenylamine; (23) diethylenetriamine; (24) *N,N*-dimethyl-*p*-phenylendiamine; (25) n-trioctylamine; (26) aminoethylethanolamine; (27) *N,N'*-dimethylaniline; (28) hexamine; (29) trimethylphenylammonium chloride; (30) benzyltri-*N*-butylammonium bromide; (31) tetrabutylammonium chloride; (32) benzyltriethylammonium chloride; (33) triethylammonium chloride; (34) benzyltrimethylammonium chloride; (35) *N*-2-chloroethyl-*N,N*-diethylammonium chloride; (36) *N*-acetyl-*N,N,N*-trimethylammonium bromide; (37) atropine; (38) ephedrine; (39) guaiacol; (40) papaverine; (41) codeine; (42) phenobarbital; (43) caffeine; (44) glucose; (45) fructose; (46) starch; (47) theophylline; (48) mannitol. For ammonium salts and non-amines the ratio shown is the maximum tested.

each stream were tested; the length of coils L_1 (75 cm) and L_2 (85 cm) and the volume of the injected sample (60 μl of 1.8×10^{-3} M ethylenediamine) were kept constant in these tests. These preliminary experiments indicated that a manifold similar to that shown in Fig. 1 would be satisfactory.

In the optimization process, the influence of the buffer solution in the aldehyde stream was examined; tests with three percentages (25, 50 and 100%, v/v) of buffer solutions suggested that the aldehyde reagent should be prepared in 100% buffer solution, i.e., in 0.1 mol l^{-1} borate buffer, pH 8.5. A modified simplex method [15] was used to optimize the interdependent variables, i.e., overall flow rate (Q), volume of injected sample (V_{in}), lengths of the first (L_1) and the second (L_2) coils and the aldehyde (PCA) and Cu(I) concentrations. The maximum value of the absorbance

TABLE 2

Determination of ethylenediamine (en) in pharmaceutical preparations by the batch procedure

Sample	Amount taken	Ethylenediamine content (%)[a]		
		Calibration graph	Standard additions	True value
Tablets[b]	(g/100 ml)			
	0.5270	2.47 ± 0.08 (—2.0)	2.68 (+6.3)	2.52
	0.4900	2.50 ± 0.10 (—0.4	2.52 (+0.4)	2.51
	0.4487	2.44 ± 0.11 (—2.9)	2.38 (—5.2)	2.51
	0.5235	2.60 ± 0.03 (+3.2)	2.47 (—2.0)	2.52
	0.5200	2.28 ± 0.05 (—0.4)	2.12 (—7.4)	2.29
	0.5757	2.26 ± 0.04 (—1.3)	2.19 (—4.4)	2.29
Injections[c]	(ml)			
	0.5	$7.80(\pm0.04)\times10^{-2}$ (—2.13)	8.19×10^{-2} (+2.76)	7.97×10^{-2}
	0.4	$6.92(\pm0.10)\times10^{-2}$ (—4.15)	6.89×10^{-2} (—4.57)	7.22×10^{-2}
	0.3	$8.24(\pm0.06)\times10^{-2}$ (+3.39)	7.91×10^{-2} (—0.75)	7.97×10^{-2}
	0.4	$8.35(\pm0.08)\times10^{-2}$ (+4.47)	8.07×10^{-2} (+1.25)	7.97×10^{-2}
	0.2	$8.31(\pm0.05)\times10^{-2}$ (+4.26)	8.21×10^{-2} (+3.00)	7.97×10^{-2}
Syrups[d]	(g/ml)			
	8.4881/100	$4.00(\pm0.05)\times10^{-2}$ (+11.1)	4.00×10^{-2} (+11.1)	3.63×10^{-2}
		$3.71(\pm0.02)\times10^{-2}$ (+3.05)	3.58×10^{-2} (—1.38)	
	4.1300/25	$3.77(\pm0.02)\times10^{-2}$ (+3.86)	3.79×10^{-2} (+4.40)	3.63×10^{-2}
		$3.66(\pm0.02)\times10^{-2}$ (+0.83)	3.55×10^{-2} (+2.20)	
	5.1093/50	$3.54(\pm0.02)\times10^{-2}$ (—2.48)	3.66×10^{-2} (+0.83)	3.63×10^{-2}
		$3.64(\pm0.04)\times10^{-2}$ (+0.28)	3.63×10^{-2} (0)	
Tablets[e]	(g/100 ml)			
	0.5235	2.71 ± 0.01	2.68	
	0.5311	2.58 ± 0.03	2.78	2.7—3.1
	0.5200	2.85 ± 0.02	2.93	

[a]Results are given as percentage found (average of 3—5 measurements) with standard deviation and, in parentheses, the relative error. [b]Composition: aminophylline, 0.6000 g; ephedrine hydrochloride 0.1045 g; papaverine hydrochloride, 0.1600 g; phenobarbital, 0.1000 g; atropine hydrochloride 0.0010 g; excipients to 2.500 g. Relative standard deviations for the standard additions method were from 1.2 to 12.2%. [c]Composition: aminophylline, 12.4—13.9 mg; guaifenesine, 150.0 mg; chlorphenamine maleate, 1.0 mg; bromhexine chloride, 2.0 mg; lydocaine hydrochloride, 25.0 mg; aqueous solution, 2.50 ml. An aliquot (2.5 ml) of this preparation was diluted to 25 ml. Data are given as weight % in the original preparations. Relative standard deviations for the standard additions method were in the range 0.65—10.69%. [d]Composition: aminophylline, 0.2500 g, ephedrine hydrochloride, 0.1000 g; thyme extract, 3.0000 g, ammonium benzoate, 1.500 g; excipients to 100 0 g. Relative standard deviations for the standard additions method were from 0.18—4.07%. [e]Commercial tablets. The nominal (true) value is based on the calculated content for an aminophylline content of 12—14%.

at 475 nm was attained after 18 experiments, seven of which were used to construct the initial matrix of the simplex. Table 3 shows the optimum values obtained.

The influence of the temperature on the peak height was then studied by heating the second reaction coil at 40, 60 or 80°C (±1°C) in a thermostated bath. The stream was then cooled to room temperature before entering the flow cell. Heating at 80°C resulted in a two-fold increase of sensitivity, but reproducibility was poor. Further, sample throughput decreased because of

TABLE 3

Optimum values of the variables in the flow-injection system

Variable	Q (ml min^{-1})	V_{in} (μl)	L_1 (cm)	L_2 (cm)	PCA[a]	Cu[a]
Value	1.1	116	80	95	5	0,08

[a]Given as molar ratios for the flow method to the batch method.

the longer residence time of the sample in the system, and the manifold became more complicated. Given the choice between sensitivity and reproducibility/simplicity, the optimized system was operated at room temperature.

With the optimized flow-injection system, linear response was obtained between 1.4 and 84.6 μg ml^{-1} ethylenediamine (Fig. 3). The least-squares calibration equation (n = 16) was A = 0.0122 C + 0.043 with r^2 = 0.9998. Precision (r.s.d.) was 0.68% for eleven injections of a 36.2 μg ml^{-1} ethylenediamine solution. A theoretical injection rate of 55 h^{-1} was calculated. Reagent consumption per injection was 8.4 × 10^{-2} mmol of aldehyde and 39 μg of copper(I).

Finally, the flow-injection system was used for the semi-automated determination of ethylenediamine in aminophylline and in pharmaceutical preparations. The results given in Table 4 show good agreement with the true values, confirming the reliability of the new method.

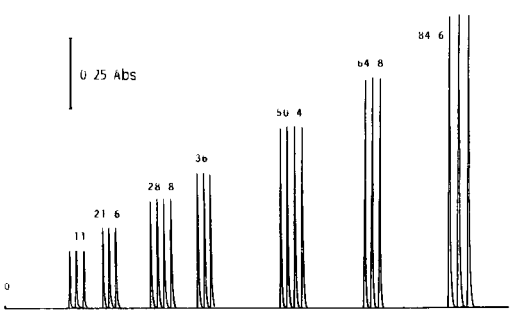

Fig. 3. Peaks obtained by injection of standard solutions of ethylenediamine; the numbers on the peaks are μg ml^{-1} ethylenediamine.

TABLE 4

Flow-injection determination of ethylenediamine in aminophylline and pharmaceutical preparations

Preparation	Solution taken (g/ml)	Ethylenediamine found[a]		True value (%)
		$\mu g\ ml^{-1}$	%	
Aminophylline	0.4644/50	51.4 ± 0.7	13.9 ± 0.5	14.57[b]
	0.3319/50	19.1 ± 0.5	14.4 ± 0.6	
	0.4970/50	56.1 ± 0.8	14.1 ± 0.5	
Tablets	0.4991/100	11.2 ± 0.3	2.79 ± 0.08	2.52
	0.2032/50	45.6 ± 0.8	2.35 ± 0.06	2.36
Injections	—	70.7 ± 0.5	$(7.6 \pm 0.1) \times 10^{-2}$	7.63×10^{-2}
	—	9 4 ± 0.5	$(7.1 \pm 0.1) \times 10^{-2}$	7.02×10^{-2}
Syrup	0.1434/100	15.4 ± 0.8	$(3.3 \pm 0.1) \times 10^{-2}$	3.12×10^{-2}
	0.1128/50	80.2 ± 0.5	$(3.3 \pm 0.1) \times 10^{-2}$	3.12×10^{-2}

[a]With standard deviation for n = 3—6. [b]Calculated from acid-base titration.

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