Flow injection biamperometric determination of chloramine-T in environmental, pharmaceutical and veterinary samples

M. Catalá Icardo[a], D. Giménez Romero[a], J.V. García Mateo[b], J. Martínez Calatayud[a]

[a] *Departamento de Química Analítica, Universidad de Valencia, Valencia, Spain*
[b] *Departamento de Química. Colegio Universitario C.E.U., Moncada, Valencia, Spain*

* Corresponding author. Tel. and fax: C34-6-386-4062. *E-mail address:* jose.martinez@uv.es (J.M. Calatayud).

Abstract
A flow injection assembly for the determination of chloramine-T is proposed. The sample (213 ml) is inserted into the carrier, de-ionized water flowing at 4.1 ml min^{-1}. This carrier merges with a mixture of potassium iodide and sulphuric acid, and the resulting solution flows to the flow cell through a reactor 66 cm long. The chloramine-T oxidises the iodide to tri-iodide. The resulting iodide/iodine ratio is biamperometrically tested. The calibration graph is linear up to 65 mg ml^{-1} chloramine-T; the limit of detection is 0.5 mg ml^{-1}; the relative standard deviation (RSD) of the calibration slope is 2.8% for a series of eight independent calibrations. The RSD of a series of 74 peaks for 40 mg ml^{-1} chloramine-T) is 0.8%, and the sample throughput 220 h^{-1}. Few foreign substances interfered. The method is applied to pharmaceutical, veterinary and waste water samples.

1. Introduction

Chloramine-T is used in medical and veterinary treatment as a wound disinfectant and general surgical antiseptic. It is also used as general disinfectant (germicide) [1] in cattle ranches; this last use makes it a pollutant in waste waters. It also finds use as an analytical oxidant for inorganic species [2–9] and organic compounds [10–17].

Various methods have been proposed for the determination of chloramine-T; most of them based on titrimetric determinations using iodide [18] or vanadium(II) in acetic acid [19] as titrant, and amplification reactions [20]. Flow methods with spectrophotometric detection [21], gas chromatography [22] and liquid chromatography [23] have also been employed for the determination of chloramine-T in foodstuffs. In spite of its general use, few studies have been devoted to the determination of chloramine-T. In the present work a flow injection (FI)-biamperometric procedure for the determination of chloramine-T in pharmaceutical, veterinary and environmental samples is proposed.

The method is based on the oxidising properties of chloramine-T. The reaction of chloramine-T with an acidic solution of iodide produces a stoichiometric amount of iodine which is detected biamperometrically using two platinum electrodes polarised at 100mV.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical-grade reagents and in purified water ($18M\Omega cm$) using a Sybron/Barnstead Nanopure II water purification system provided with a fiber filter of 0.2 μm pore size. Chloramine-T, potassium iodide and sulphuric acid were from Scharlau, $Al(NO_3)_3 \cdot H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$, KNO_3, $K_2Cr_2O_7$, $NaNO_2$, $Na_2CO_3 \cdot 10H_2O$ and urea (Panreac), $Cd(NO_3)_2 \cdot H_2O$, $Mn(NO_3)_2 \cdot 6H_2O$, $NaNO_3$, $NaCl$, $Zn(NO_3)_2 \cdot 6H_2O$ (Probus), $Cu(NO_3)_2 \cdot 3H_2O$ from u.c.b. and $Na_2S_2O_3 \cdot 5H_2O$ (Merck). Stock solutions of chloramine-T and potassium iodide were made freshly, protected from room light and degassed by sonication for 30 min prior to use.

2.2. Apparatus

The flow manifold used comprised a PTFE coil of 0.5mm i.d., a Rheodyne 5041 injection valve and a Gilson Minipuls 2 peristaltic pump. The flow cell was laboratory-made from two methacrylate blocks [24]; two Pt wires (99.99% pure, from Afora) 4 cm long x 0.5mm i.d. were aligned with each other along the flow axis and used as electrodes. The wire length exposed to the flow solution was about 2 cm. Analytical outputs were obtained by using a potentiostat [25] interfaced to a Pentium 75MHz computer.

2.3. Flow injection assembly

The proposed FI manifold is depicted in Fig. 1.A volume of 213 μl of aqueous chloramine T solution was inserted into a de-ionized water carrier stream flowing at 4.1 ml min^{-1}, and the resulting solution was mixed with an in situ prepared mixture containing 0.2 mol l^{-1} potassium iodide in 1.5 mol l^{-1} sulphuric acid (both at a flow rate of 1.1 ml min^{-1}). The solution was driven to the detector flow cell through a 66 cm long x 0.5mm i.d. PTFE coil to ensure thorough mixing. Finally, the I_2/I_3^- ratio was biamperometrically measured at 100 mV.
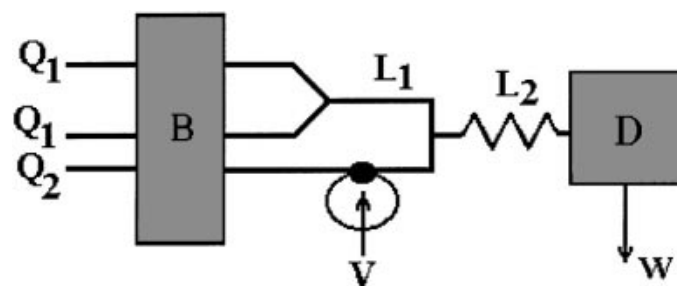
Figure 1. FI-manifold for chloramine-T determination. B: peristaltic pump; V: injection valve; D: potentiostat; W: waste; Q_1: reductant solution (sulfuric acid+iodide); Q_2: carrier solution (di-ionized water); L_1 and L_2, reactors.

2.4. Optimisation of FI empirical variables

The optimisation of chemical, FI and detector variables were performed by a sequential combined methodology. First the chemical, iodide concentration, ionic strength and acidic media were optimised. Then with the selected chemical values, the hydrodynamic variables (FI parameters) were optimised by using a multivariate method (the modified simple method, MSM).

The initial simplex was selected according to Yarbro and Deming [26]. The variable region was standardised by following the modification of Morgan and Deming [27]. The different simplex vertices were obtained with the aid of software based on the method of Nelder and Mead [28] with the target FI variables and the value (in mA) corresponding to each combination of such variables provided by the simplex inputs. The program is written to optimise the height of the output peak. Two consecutive simplexes were performed; the interval for each variable in the second being restricted to the zone that gave the best results in the first. Then some of the higher vertices were selected for a new comparative study to choose the output resulting in the best compromise sensitivity (peak height)-sample throughput (peak–base width)-reproducibility (RSD).

2.5. Procedures for preparation of samples

(a) *Clorina*. (Squibb Industria Farmacéutica, s.a., Barcelona, Spain) and (b) *Halamid* (Fort Dodge Veterinaria, S.A., Girona, Spain). These preparations are available as powders. The manufacturer's stated compositions was 2.5 g of chloramine-T per bag and 99.5% for *Clorina and Halamid*, respectively. An appropriated amount of *Clorina* or *Halamid* was weighed and diluted with de-ionized water to obtain a solution containing chloramine-T of ca. 30 µg ml^{-1}.

(c) *Galenic preparation*. It was prepared according to *The Military Pharmacopoeia* [29].

(d) *Water samples*. Water from irrigation ditches located in Moncada and Burjassot (Valencia), and ground water from Moncada (Valencia) were doped with a known amount of chloramine-T to obtain a solution of ca. 30 µg ml^{-1}chloramine-T. The content of chloramine-T in the resulting solutions was determined. Water from irrigation ditches yielded a blank signal and thus the amount of chloramine-T was calculated by standard addition. For determining chloramine-T in waste water from a rabbit farm located in Navarrés (Valencia), a chloramine-T solution (3 g of *Halamid* per litre) was employed as a disinfectant solution of rabbit 'nests'.

The results obtained were compared with the manufacturer's stated contents and with those provided by the official method described in *The British Pharmacopoeia* [18], which is based on the reaction with iodide and the back titration of iodine formed by thiosulfate employing starch as an indicator.

2. Results and discussion

3.1. Preliminary tests and pre-optimisation of chemical variables

3.1.1. Influence of pH

The oxidising power of chloramine-T depends strongly on pH. In fact, this variable dictates the nature of the reaction intermediates formed in the hydrolysis and reduction of the analyte, which include R-SO$_2$–NCl, R-SO$_2$–NHCl, R-SO$_2$–NCl$_2$, HClO and OCl$^-$ [30,31]. Hahn et al. [32] used electrochemical separation techniques such as capillary electrophoresis, and electro-analytical techniques including voltammetry and coulometry, to investigate the hydrolysis and reactivity of chloramine-T at various pH values. They reported various pH-dependent hydrolysis equilibria that yielded toluene, sulfonamide, hypochlorite, dichloramine-T, hypochlorous acid or even chlorine species at various redox potentials. Based on these findings, our initial tests were focussed on determining the influence of pH on (a) the stability of chloramine-T solutions and (b) the maximum analytical signal (i.e. the highest oxidising power of the chloramine-T solution).

The FI manifold initially tested was identical with that finally adopted (Fig. 1), with Q_1=1.4 ml min^{-1}, Q_2=5ml min^{-1}, L_1=1 m, L_2=1.47m and V=878 μl. The first few injections were of 10^{-4} M chloramine-T at pH 1.0–11.6, adjusted with dilute sulphuric acid or sodium hydroxy as required. Despite the strong dependence of the oxidizing power of chloramine-T on pH the peak height values thus obtained were independent of pH throughout the range tested. This behavior can be due to the fact that the analytical signal is likely to be related to the overall concentration of oxidants (mainly chloramine-T and different amounts of its hydrolysis products) exhibiting sufficient oxidizing power and rapid enough reaction for the oxidation of iodide to iodine. In addition, the acidic medium resulting from the in situ mixing of the iodine solution and sulphuric acid conditioned the medium for the redox reaction between the iodide ion and chloramine-T, whatever the pH of the chloramine-T solution. For simplicity, such a solution was prepared in de-ionized water, with no special pre-treatment. Under these conditions, the chloramine-T solution was found to remain stable for at least 1 week provided it was protected from ambient light.

3.1.2. Influence of iodide concentration

The influence of the iodide ion concentration was determined by changing the KI concentration over the range 0.2265–1.0276 M (6 values). At each concentration, the experimental data obtained by injecting chloramine-T solutions at concentrations up to 100 μg ml^{-1} were subjected to linear and parabolic fitting. Quadratic fitting provided the best results, with excellent regression coefficients; the fits occasionally coincided with their theoretical counterparts. This result appears to be associated with the detection system used for both the indicator reaction and the biamperometric detection of the tri-iodide formed [24]. Increasing the iodide concentration did not improve calibration linearity; in fact, it only increased sensitivity to a minor extent, from a slope of 0.3203 to 0.3920 for 0.2265 and 1.0276 M, respectively. On the other hand, the oxidation of iodide by dissolved oxygen, which was especially favoured at higher iodide concentrations, gave rise to increasingly large baseline oscillations. For all these reasons, and also for economy, a KI concentration of 0.2M was adopted for further work.

3.1.3. Influence of ionic strength

The effect of ionic strength on the detection system was examined by using a NaCl solution at concentrations up to 2M as carrier for chloramine-T; the salt was also added, at concentrations up to 3.9 M, to the KI solution. In both cases, the ionic strength was found not to influence the analytical signal; its sole effect being a slight worsening of the baseline with increase in the salt concentration.

3.1.4. Influence of acid medium

The effect of the acid used was assessed by using solutions of acetic, phosphoric, nitric, hydrochloric and sulphuric acids, all at 1M. Sulphuric acid was found to give the highest sensitivity (linear slope, 0.5101 mA $(\mu g\ ml^{-1})^{-1}$), but with little difference in nitric (0.4425) and hydrochloric (0.4502) acids. In contrast, acetic acid was found to give little response to the changes in the chloramine-T concentration.

The sulphuric acid concentration was optimised by examining its effect over the range 0.5–2.5 M. Above 1M, the signal reached a plateau, so 1.5 M H_2SO_4 was adopted for subsequent work.

3.1.5. Optimisation of FI variables

These parameters were optimised by using the modified simplex method (MSM) and for this purpose were included the flow rate of the carrier ($Q2$), and the oxidant and acidic media, which were both kept at the same value, $Q1$, the inserted volume (V), the length of mixing of the oxidising reagent with the acid medium ($L1$) and the reactor length ($L2$). The applied potential was 100mV and a series of 15 insertions of a 15 $\mu g\ ml^{-1}$ chloramine-T solution per vertex was made. Table 1 summarises the results obtained with both simplexes. The optimum vertex was selected from the three that provided the best analytical signal (in mA), using 25 insertions per vertex in order to obtain the best compromise among peak height, reproducibility and throughput.

The best results were obtained at high carrier flow rates, low reductant flow rates, short reactor lengths and intermediate sample volumes. This combination of FI parameter values resulted in minimal dispersion of the injected sample. Also, the fact that the optimum values of the simplex were associated with short residence times reveals that the redox reaction between iodide and chloramine-T is a fast.

Table 1. Optimisation of FI variables[a]

Variable	Simplex 1	Simplex 2	
	Interval studied	Interval studied	Selected value
Q_1 (ml min^{-1})	0.3–3.5	0.9–1.5	1.1
Q_2 (ml min^{-1})	2.4–5.7	3.0–5.4	4.1
L_1 (cm)	15–120	35–55	44.1
L_2 (cm)	20–200	55–90	66
V (μl)	78–373	78–373	213

[a] Applied potential: 100mV; testing solution: 15$\mu g\ ml^{-1}$ chloramine-T.

3.1.6. Polarization potential

The polarization potential can affect both the sensitivity and selectivity of the method. The selected working potential directly affects the number of species that can be electrolysed, the linear response range and the reversibility of the indicating system.

A study of the influence of the applied potential over the range of 50–250 mV revealed that peak height increased significantly with increase in potential up to 150 mV; however, the most favourable $\Delta\mu A/\Delta E$ ratio was obtained at 100 mV, which was thus adopted for subsequent experiments (Fig. 2).

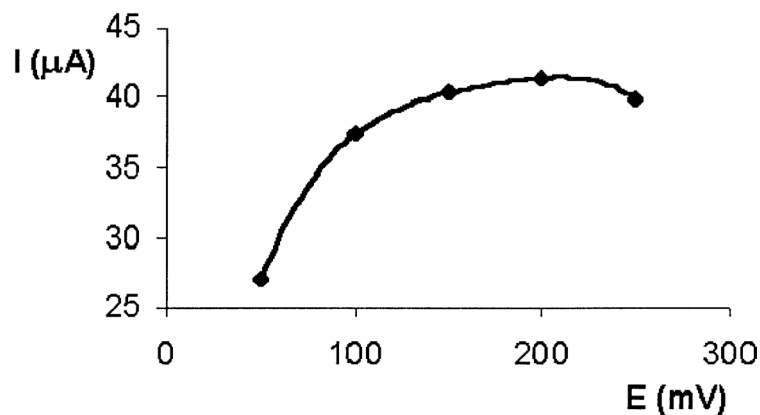**Figure 2.** Influence of applied potential. (values current for 45 μg ml^{-1} chloramine-T).

4. Analytical applications

The calibration graph was linear up to 65 μg ml^{-1} chloramine-T and conformed to the equation I (μA)= −0.16 + 0.6509 [chloramine-T] (μg ml^{-1}), with r=0.9998 (n=6) and a RSD of 2.8% for the slope (8 measurements). The 2σ limit of detection was 0.5 μg ml^{-1} and the sample insertion frequency 220 h^{-1}. The RSD for a series of 74 insertions of 40 μg ml^{-1} chloramine-T was 0.8%.

The tolerance of the proposed method to species that might accompany the analyte in pharmaceutical and veterinary preparations, in addition to waste waters, was studied. Since chloramine-T was not accompanied by any substance in the commercially available preparations tested, analyses were focussed on the ions usually present in waste water, and also on urea. Table 2 shows the results. As can be seen, the sole anion tested that interfered with the proposed method was nitrite, by virtue of its reducing character. However, because it decomposes very rapidly, its concentration in water can hardly reach the levels required to interfere seriously with the method. Cations such as Fe^{3+}, Cu^{2+} and Al^{3+} decreased the analytical signal. Saturated solutions of these cations at pH 6 were found to pose no interference.

The proposed method was applied to the analysis of two commercially available preparations, viz. a pharmaceutical (Clorina) and a veterinary drug (Halamid), in addition to a galenic formulation [26] and four water samples of various origins. One water sample was collected from a well in the city of Moncada, Valencia, eastern Spain, and was doped with a known amount of chloramine-T; the results were validated by simultaneously using a reference method. Two water samples collected from irrigation ditches in Moncada and Burjassot (Valencia), were similarly processed. As these samples gave only a blank signal, chloramine-T in a doped sample was determined from three calibration solutions prepared for the standard addition method. Finally, the waste water sample used was collected from washings from the use of the disinfectant Halamid on 'nests' in a rabbit farm in Navarrés (Valencia).

Table 2. Study of interfering compounds[a]

Possible interference	Concentration ($\mu g\,ml^{-1}$)	Error (%)
Al^{3+}	Saturated at pH 6	+0.8
Al^{3+}	100	+2.3
Ca^{2+}	500	−1.8
Cd^{2+}	500	+2.3
Co^{2+}	500	−2.1
Cu^{2+}	Saturated at pH 6	−1.5
Cu^{2+}	100	+3
Fe^{3+}	Saturated at pH 6	−0.3
Fe^{3+}	50	+0.8
K^{+}	500	+0.15
Mg^{2+}	500	−0.9
Mn^{2+}	200	−3
Na^{+}	500	+0.2
NH_4^{+}	500	+1.4
Zn^{2+}	500	+0.7
Cl^{-}	500	−0.8
CO_3^{2-}	500	+1.5
NO_2^{-}	0.1	−3
NO_3^{-}	500	+0.5
PO_4^{3-}	500	+0.4
SO_4^{2-}	500	+0.2
Urea	500	+1.3

[a] Values are relative to a chloramine-T solution of 35 $\mu g\,ml^{-1}$.
All ion solutions were prepared from nitrate salts for testing cations
and from sodium salts in case of anions. The obtained signal for
a solution containing the cation or anion and chloramine-T was
compared with that yielded by a solution at the same concentration
and pH in absence of the cation or anion.

The chloramine-T recoveries obtained by the proposed FI method and the reference method are shown in Table 3, which also gives the relative errors in each case.

Table 3. Determination of chloramine-T in pharmaceutical, galenic, veterinary and waste water samples

Sample	% Recovery proposed FIA method (ds)	% Recovery reference method (ds)	Error (%)[a]
Clorina, (from Squibb Industria)	100.1 ± 0.8	98.4 ± 0.3	+1.7
Halamid, (from Fort Dodge)	100.74 ± 0.11	99.5 ± 0.3	+1.2
Galenic preparation 1:10	99.17 ± 0.28	99.5 ± 0.7	−0.3
Spiked ground water (from Moncada)[b]	100.4 ± 0.5	98.3 ± 0.6	+2.1
Spiked water from irrigation ditches (from Moncada)[b]	103.2 ± 2.3[b]	98.4 ± 0.6	+5
Spiked water from irrigation ditches (from Burjassot)[b]	100.2 ± 2.3[b]	97.9 ± 0.4	+2.4
Waste water from rabbit 'nests' (from Navarrés)[b]	2.793 ± 0.026[c]	2.74 ± 0.09[c]	+2.4

[a] Error(%): error against reference method; [b] Determined by standard addition; [c] Concentration in $\mu g\,ml^{-1}$. The treatment was performed using 3 g of chloramine-T per l of water.

5. Conclusions

A FI-biamperometric procedure for the determination of chloramine is proposed. The method uses I_2/I^- as the indicating redox system with excess of the reduced form and two platinum electrodes polarised at low potential providing a high degree of selectivity and fast sample throughput. The high selectivity and robustness of the method were confirmed

by a study of the influence of interfering species as well as by the application to real samples in the environmental, pharmaceutical and veterinary fields.

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