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The determination of benzalkonium chloride in eye-drops by difference spectrophotometry

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Abstract

A direct, extraction-free spectrophotometric method was developed for the determination of benzalkonium chloride (BAC) in various eye-drops. The procedure is based on ion-pair formation between BAC and 2′,4′,5′,7′-tetrabromofluorescein (eosin-Y) which decreases the absorbance and induces a bathochromic shift of the maximum in the eosin-Y spectrum. The effects of pH, excess of reagent and ionic strength on the ion-pair formation have been studied in detail. At pH 4.40 and 9.62, the working curve is linear in the 1.98×10^{-6} to 2.40×10^{-5} M (0.7–8.5 μg cm^{-3}) concentration range; however, the sensitivity drops to about one third in the basic solution. At pH 4.40, the analytical signal is stable for more than 60 min, while at pH 9.62 the signal changes in time and reaches the maximum value 3 min after mixing the reagent and the sample. When the active substance is β-5-isopropyl-2′deoxyuridine and the sample contains typical additives, the reproducibility of the analytical signal at pH 4.40 is R.S.D. = 2.36% ($n = 81$). In the case of such samples, the linearity of the method is somewhat dependent on the composition, but generally acceptable at the 50–150% concentration levels. Eye-drops containing tobramycin, an aminoglycoside-type antibiotic, as the active substance were analyzed at pH 9.62. This was necessary to avoid strong interference from the analyte in acidic solution. In this case the linearity of the method is limited to a narrower concentration range; however, the recovery is still acceptable at the 100% level. © 1998 Elsevier Science B.V.

Keywords: Benzalkonium chloride; Eye-drop; Spectrophotometric method; Tobramycin

1. Introduction

Quaternary ammonium compounds, such as benzalkonium chloride, cetylpyridinium bromide, etc., are often used as preservatives in pharmaceutical products. A vast amount of literature data is available for their determinations. However, in most cases two main types of analytical methods are used. A relatively new technique based on the application of surfactant sensitive/selective electrodes has been recently reviewed by Vytřas [1].

The well-established spectrophotometric method is ion-pair extraction. In this case, an ion-pair is formed between the quaternary ammo-

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nium salt and an anionic dye such as bromthymol blue, bromphenol blue, methyl orange, etc. At a specific pH, the ion-pair is extracted into an organic solvent which is immiscible with water. In addition to the traditional batch procedures, FIA (flow injection analysis) methods are also frequently used with spectrophotometric [2–4], indirect atom absorption [5] and ion-selective electrode detectors [6].

In both classical spectrophotometry and FIA, methods without solvent extraction are the subject of interest. In this respect, the work of Lowry [7] should be mentioned. In that paper, benzalkonium chloride and chlorhexidine were determined with bromthymol blue at pH 7.5 by measuring the absorbance decrease of the free dye ion at the 610 nm absorption maximum. Stevens and Eckardt [8] have determined polyquaternium-1 in contact lens cleaner liquids by using trypan-blue reagent. Their method is based on the bathochromic shift in the spectrum of the dye due to the presence of the quaternary ammonium salt. The absorbance was a linear function of the concentration in the 5–15 μg cm^{-3} region. Zhebentyajev and Tahit used 2′,4′,5′,7′-tetrabromo-3,6-dichloro-fluorescein for the determination of cetyltrimethylammonium bromide, cetylpyridinium bromide and tetradecylpyridinium bromide in aqueous solution [9]. At 543–547 nm, the linear absorbance range was between 0.4 and 6 μg cm^{-3} analyte concentration. An excellent review of spectrophotometric determination of quaternary ammonium compounds in pharmaceutical products is given in the monograph of Görög [10].

Our main objective was to develop a fast, precise and extraction-free analytical method for the determination of benzalkonium chloride (BAC) in various eye-drops. As a basis, we used the procedure given by Zhebentyajev and Tahit [9]. Earlier, in a significantly modified form and by using eosin-Y (2′,4′,5′,7′-tetrabromo-fluorescein-disodium salt) instead of 2′,4′,5′,7′-tetrabromo-3,6-dichloro-fluorescein, this method has been successfully adapted for the determination of ion-pairing agents adsorbed on chromatographic silica-gel layers [11,12].

2. Experimental

2.1. Reagents

All reagents were of analytical grade quality. The model solutions for the artificial tears and eye-drops without benzalkonium chloride were kindly provided by the manufacturers (Biogal Pharmaceutical, Debrecen, Hungary and Humán Pharmaceutical, Gödöllő, Hungary) of the corresponding commercial products. The benzalkonium chloride was of European Pharmacopoeia quality [13]. In this study, eye-drops A and C, and artificial tear samples from B_1 to B_3 have been analyzed. Besides benzalkonium chloride, sample A also contained β-5-iso-propyl-2′-deoxyuridine (epervudin, 4 mg cm^{-3}), nicotinic acid amide (8 mg cm^{-3}, and sodium chloride (4 mg cm^{-3}). In samples B_1–B_3, the additional components were polyvinyl alcohol (14 mg cm^{-3}), and phosphate (B_1) or acetate (B_2) buffers. In sample B_3, hydroxypropylmethylcellulose (3.2 mg cm^{-3}), dextran and disodium EDTA were added. Sample C contained tobramycin (3 mg cm^{-3}), disodium–EDTA (0.1 mg cm^{-3}) and sodium chloride (8 mg cm^{-3}). In each product the concentration of BAC was 0.1 mg cm^{-3}.

Eosin-Y (Aldrich, Steinheim, Germany) was used in 1×10^{-3} mol dm^{-3} (0.6918 mg cm^{-3}) aqueous solution. This reagent was stable for at least 4 months when stored at room temperature in a dark glass container. Britton-Robinson buffer solution (pH = 9.62) was prepared by adding 75 cm^3 of 0.2 mol dm^{-3} NaOH solution to 100 cm^3 of 4×10^{-2} mol dm^{-3} acetic acid–phosphoric acid–boric acid solution. All reagent solutions and samples were prepared with doubly distilled water.

2.2. Instrumentation

Spectrophotometric measurements were made with a Hewlett-Packard 8453 diode-array spectrophotometer (Hewlett-Packard, Palo Alto, CA) by using 1 cm cuvettes.

2.3. Methods

2.3.1. Samples A and B_1–B_3

An 0.30 cm^3 aliquot of the undiluted sample was thoroughly mixed with 7.20 cm^3 water, 0.50 cm^3 of 1 mol dm^{-3} acetic acid, 1.00 cm^3 of 0.2 mol dm^{-3} sodium acetate and 1.00 cm^3 of 1×10^{-3} mol dm^{-3} eosin-Y solutions. The absorbance of the final solution was measured against water at 550 nm within 1 h. The same experiments were repeated with blank solutions which contained all components except BAC. The analytical signal was obtained as the difference between the absorbances of the corresponding solutions with and without BAC (ΔA_{550}). The concentration of BAC was determined by using the working curve method. The calibration curves were determined by using at least three to five points. The standards were diluted from 2–3 mg cm^{-3} aqueous stock solution of BAC such that the concentration of the sample was always within the concentration range of the standard solutions. The stock solution of BAC was stable for at least 2 months when stored in a polypropylene container at room temperature.

2.3.2. Eye-drops C

To 0.30 cm^3 undiluted sample, 1.70 cm^3 water, 7.00 cm^3 Britton-Robinson buffer (pH = 9.62) and 1.00 cm^3 of 1×10^{-3} mol dm^{-3} eosin-Y solutions were added. The absorbance was measured at 550 nm after 3 min. The analytical signal (ΔA_{550}) was obtained again as the difference in the absorbances of the corresponding solutions with and without BAC.

3. Results and discussion

3.1. The spectrum of eosin-Y in the presence of BAC

BAC is not an absorbing species in the visible region. In contrast, at pH 4.40, by using acetate buffer eosin-Y has an absorption maximum at 515 nm (Fig. 1(A)). In the presence of BAC, the intensity of the 515 nm peak decreases and at about 562 nm a shoulder develops in the spectra. This spectral change was attributed to ion-pair formation between the reagent and the analyte. In the analytical procedure, the reagent is used in excess. Thus, at 515 nm the measured absorbance is mainly due to the free eosin-Y and the relatively small absorbance change is not suitable for analytical purposes. Similar spectra can be obtained at pH = 9.62 using Britton-Robinson buffer (Fig. 1(B)). It should be noted that Fig. 1 shows the spectrum of an eosin-Y solution which is about 5 times less concentrated than the solutions used for the analysis. In the 550–562 nm region, the absorbance of eosin-Y is considerably smaller, thus 550 nm was selected for the analysis. The apparent molar absorptivity of the measure species is $(4.08 \pm 0.10) \times 10^4$ at pH = 4.40, and $(1.47 \pm 0.07) \times 10^4$ at pH = 9.62.

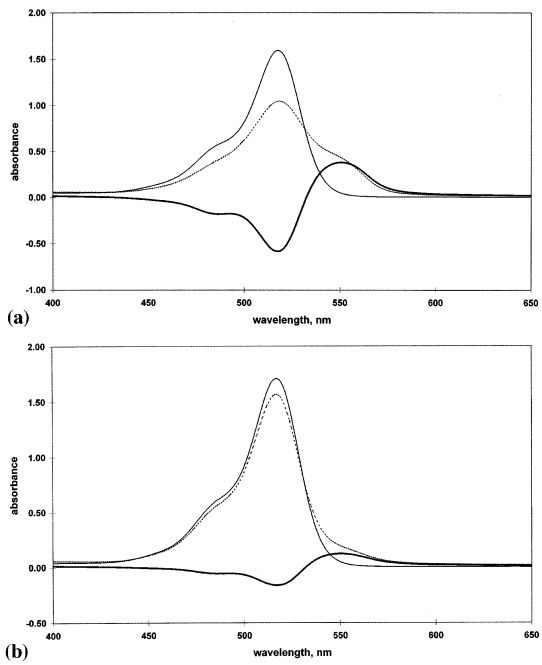

Fig. 1. (A) The spectra of eosin-Y and eosin-Y–BAC solutions at pH = 4.40 (in acetate buffer). (——) Eosin-Y, 2.06×10^{-5} mol dm^{-3}; (– – –) 2.06×10^{-5} M eosin-Y and 1×10^{-5} M BAC; (double solid line) the difference spectrum. (B) The spectra of eosin-Y and eosin-Y–BAC solutions at pH = 9.62 (in Britton Robinson). (——) Eosin-Y, 2.06×10^{-5} mol dm^{-3}; (– – –) 2.06×10^{-5} M eosin-Y and 1×10^{-5} M BAC; (double solid line) the difference spectrum.

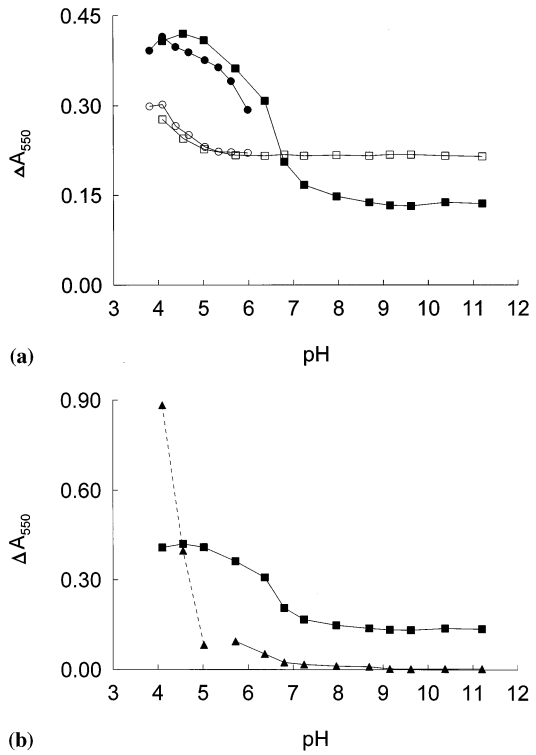

Fig. 2. The pH dependence of ΔA_{550} for eosin-Y, eosin-Y + BAC and eosin-Y + tobramycin solutions in two different buffers. (A) (○) Eosin-Y (1×10^{-4} mol dm^{-3}); (●) eosin-Y + BAC (3.47 μg cm^{-3}) in acetic acid/acetate buffer; (□) eosin-Y; (■) eosin-Y + BAC in Britton-Robinson buffer. (B) (△) Eosin-Y + tobramycin in Britton-Robinson buffer; (- - -) 22.5 μg cm^{-3}; (——) 90 μg cm^{-3} tobramycin.

3.2. The effect of pH

The ion-pair formation with quaternary ammonium compounds may strongly depend on the pH. Therefore, the pH effect was investigated by using two different buffer solutions. As shown in Fig. 2, the two curves for BAC are practically the same in acetic acid/acetate and Britton-Robinson buffers in the pH 3.8–6.0 region. Precipitation was observed below pH 3.40. In the absence of BAC, the absorbance of eosin-Y is constant in the pH 5.2–11.2 region. According to Fig. 2, the optimum pH range for the spectrophotometric measurements is pH 4.1–5.0. Further measurements were made at pH 4.40 in the presence of acetic acid/acetate buffer.

3.3. The effect of the reagent excess

The ion-pair formation as a function of eosin-Y concentration was studied at pH 4.40. The eosin-Y/BAC concentration ratio was calculated by using a $C_{22}H_{40}ClN$ molecular structure ($M_r = 354.0$) for BAC [13]. The results showed that the reagent needs to be used in a 5–15 times excess for the analysis.

3.4. The effect of sodium chloride

The ion-pair formation may also show significant ionic strength dependence. One of the major components in eye-drops is sodium chloride which is used to set the isotonic concentration of these solutions. The concentration of NaCl is 4 mg cm^{-3} in eye-drop A and 8 mg cm^{-3} in eye-drop C. It follows that the reaction mixtures prepared for photometric measurements contain 3 μg cm^{-3} BAC, and 120 μg cm^{-3} (product A) or 240 μg cm^{-3} (product C) sodium chloride. The effect of sodium chloride was studied in the 0–720 μg cm^{-3} concentration range (eight points). The observed ΔA_{550} values did not show any trend as a function of sodium chloride concentration. The standard deviation of the results at various NaCl concentrations was 3.39%.

3.5. The stability of the analytical signal

The variation of the absorbance difference (ΔA_{550}) in time was tested with reaction mixtures prepared from samples A, B_1–B_3 and C at 25 ± 0.1°C. The pH was 4.40 and 9.62 for samples A and B_1–B_3 and C, respectively. For samples A and B_1–B_3, the analytical signal was constant for at least 60 min. A continuous decay of ΔA_{550} was observed for sample C. In this case, with respect to repeatability, the exact timing of the absorbance measurement is of crucial importance. The experiments were designed so that the absorbance of the reaction mixtures was always measured 3 min after mixing.

Table 1
Linearity (recovery) studies for model solutions of eye-drop A

BAC concentration in the model solution			R.S.D. (%)	*n*
Theoretical concentration level[a] (%)	Measured concentration level (%)	Recovery (%)		
50.18	51.80	103.23	2.33	9
74.77	73.33	98.08	1.45	8
99.36	99.33	99.97	2.26	9
148.53	152.66	102.78	2.38	8

[a] 100% Of the theoretical value = declared value (in this case 0.1 mg cm^{-3}) corrected with quantity weighted into the model solution.

3.6. The stability of the BAC stock solution

The stability of a 2.54 mg cm^{-3} BAC stock solution was monitored for 2 months. The solution was stored in a transparent polypropylene vessel at room temperature (23–25°C). The stock solution was sampled and analyzed four times during the 2 months period. As a reference, freshly prepared solutions were also analyzed. In each case, five parallel experiments were run by using the analytical procedure given for eye-drops A and B_1–B_3. The stock solution was stable within the studied interval. The results obtained with the high-performance liquid chromatography (HPLC) techniques support this conclusion [14].

3.7. The stability of the reagent (eosin-Y) solution

The stability of a 1×10^{-3} mol dm^{-3} eosin-Y solution was studied with standard BAC solution and by using the method given for samples A and B_1–B_3. The reagent was stored in a dark bottle at room temperature and was sampled and analyzed 1, 2 and 4 months after preparation. The results obtained for the aged and freshly prepared reagent solutions were compared. There was no difference between the results obtained with fresh and 4 months old reagents.

3.8. The cleaning procedure of the laboratory glassware

In the analysis of minor components, the use of properly cleaned laboratory equipments and glassware is of primary importance. This is particularly true in the determination of surface active materials. Trace amounts of the detergents left over from the cleaning process may falsify the results. Hence, the analytical procedure was validated with respect to the cleaning procedure as follows. The test tube, which was used to prepare the reaction mixtures, was thoroughly cleaned and rinsed 20 times with doubly distilled water. Two drops of commercial detergent, which contained sodium lauryl sulphate as the main component, were then added to the test tube. The detergent was diluted with distilled water. The test tube was rinsed 14 times. Samples were taken and analyzed after every rinsing step. According to the results, the glassware needs to be rinsed five to six times in order to eliminate potential interference from that detergent. The cuvettes were cleaned with an acetone–water mixture.

3.9. The repeatability of the analytical signal

As discussed above, the analytical signal (ΔA_{550}) is essentially the difference between the absorbances obtained with the same solution in the presence and absence of BAC. Each absorbance value was obtained as the average of three to seven measurements.

The repeatability was tested by analyzing nine blank solutions and nine samples prepared from standard BAC stock solution. The BAC concentration in these samples was the same as in the eye-drops. The reaction mixtures were prepared and measured in a random order. For the blank and sample measurements, R.S.D. values of 1.34 and 1.49% were obtained, respectively. By comparing each blank absorbance value with each sample absorbance value ($n = 81$), the repeatability of the analytical signal was R.S.D. = 2.36%.

Table 2
Linearity (recovery) studies for model solutions of artificial tears B_1–B_3

BAC concentration in B_1 model solution			R.S.D. (%)	*n*
Theoretical conc. level[a] (%)	Measured conc. level (%)	Recovery (%)		
49.74	53.22	106.99	3.17	7
74.60	76.53	102.59	2.42	6
99.47	98.32	98.84	2.43	9
124.34	123.29	99.16	3.87	7
149.21	146.00	97.85	3.66	8
BAC concentration in B_2 model solution			R.S.D. (%)	*n*
Theoretical conc. level (%)	Measured conc. level (%)	Recovery (%)		
49.74	54.95	110.48	3.63	6
74.60	75.91	101.75	1.90	7
99.47	96.58	97.09	2.64	9
124.34	125.20	100.69	2.92	8
149.21	149.90	100.46	2.37	7
BAC concentration in B_3 model solution			R.S.D. (%)	*n*
Theoretical conc. level (%)	Measured conc. level (%)	Recovery (%)		
49.74	54.64	109.85	2.81	7
74.60	77.90	104.43	0.79	7
99.47	98.12	98.64	1.07	9
124.34	126.88	102.04	2.32	7
149.21	153.31	102.75	1.08	6

3.10. The concentration dependence of the analytical signal

The concentration dependence was studied at pH 4.40 and 9.62 by varying the BAC concentration in the 0.7–8.5 μg cm^{-3} range. In both cases, the absorbance was a linear function of the concentration:

$$y_{pH=4.40} = 0.119x + 0.0006 \quad (r = 0.9995)$$

$$y_{pH=9.62} = 0.0378x + 0.0155 \quad (r = 0.9990)$$

This confirms that the data obey the Lambert-Beer law in the applied concentration range.

3.11. The results for samples A and B_1–B_3

3.11.1. Linearity

In order to study the linearity of the method, model solutions were prepared in which the concentration of BAC was 50–150% of the nominal value in the samples (four to five points). The samples were analyzed as described in Section 2. The results are summarized in Tables 1 and 2. According to these data, in the case of product A systematic error was not observed. In the case of B_1–B_3 artificial tear products, a 7–10% positive error was found at 50% concentration level. The

Table 3
The repeatability of the analysis for products A, B_1–B_3 and C

Sample	R.S.D. (%)			
	Day 1 (I)	Day 1 (II)	Day 2	Day 3
Eye-drops A	2.38	1.35	3.34	2.71
Artificial tears B_1	3.37	1.97	2.25	2.17
Artificial tears B_2	2.64	2.85	2.73	3.07
Artificial tears B_3	1.07	2.53	1.89	2.33
Eye-drops C	2.30	3.78	1.49	1.82

results seem to be correct between 75 and 150% concentration level. The source of the positive error was not studied further.

3.11.2. Repeatability

The repeatability of the method was studied with model solutions at 100% nominal concentration of BAC. The samples were analyzed in two sets of experiments on the day of preparation. On the 2nd and 3rd day 1–1 set of measurements were made. In each set, the analysis was repeated nine times. As shown in Table 3, the repeatability of the applied analytical procedure is acceptable.

3.12. The results for eye-drops C

One of the main components of these eye-drops is an antibiotic compound, tobramycin. They also contain Na_2EDTA and sodium chloride as additives. Because of the protonation of the amino groups, the aminoglycoside-type tobramycin causes significant interference at pH 4.40. The pH dependence of the absorbance of the tobramycin–eosin-Y system as well as the corresponding curves for the eosin-Y–BAC system and eosin-Y are shown in Fig. 2. The concentration of tobramycin is 3 mg cm^{-3} in the original solution and 90 μg cm^{-3} in the reaction mixtures. In Fig. 2, the curve (- - -) corresponds to a 4 times smaller tobramycin concentration. As shown, in the pH 4–5 region, the absorbance of the tobramycin system is much higher than the absorbance of the BAC solutions. At $pH > 9.5$, the absorbance of the eosin-Y–BAC solution is constant and the absorbance of the tobramycin system is zero. Accordingly, the analysis was made at pH 9.62. Under these conditions, the analytical signal is a linear function of the BAC concentration in the 0.7–8.5 μg cm^{-3} region; however, the sensitivity is about 3 times smaller than at pH 4.40.

3.12.1. Linearity

The linearity of the method was studied at 50–150% of the nominal BAC concentration of the eye-drops. Tobramycin and Na_2EDTA were used in the same concentrations as in the samples. The results are shown in Table 4. While at 100 and 150% the results agreed with the theoretical

Table 4
Linearity (recovery) studies for model solutions of eye-drop C

BAC concentration in the model solution			R.S.D. (%)	*n*
Theoretical conc. level[a] (%)	Measured conc. level (%)	Recovery (%)		
50.00	55.96	111.92	2.09	7
75.00	79.06	105.41	2.56	6
100.00	100.33	100.33	2.30	9
150.00	148.41	98.94	2.27	6

concentration, from 12 to 5.5% positive error was observed at 50–75% concentration levels.

3.12.2. Repeatability

The analysis was made as described above for samples A and B_1–B_3 at 100% nominal concentration. The repeatability of the analysis of eyedrops C is practically the same as in the case of samples A and B_1–B_3.

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