ELSEVIER

International Journal of Pharmaceutics 113 (1995) 263–266

international journal of pharmaceutics

A colorimetric ion-pairing analytical method for quaternay ammonium compounds using eosin-Y and Triton X-100

Leo J. Schep [a], David S. Jones [a,*,1], I. Tucker [a], M.G. Shepherd [b]

[a] *School of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand*
[b] *School of Dentistry, University of Otago, P.O. Box 913, Dunedin, New Zealand*

Received 5 January 1994; modified version received 3 May 1994; accepted 1 June 1994

Abstract

Colorimetric assays were developed for cetylpyridinium chloride, cetrimide and benzalkonium chloride when complexed with excess eosin-Y (9.0 μg/ml) and Triton X-100 (0.025% v/v), using a wavelength of 534 nm. Standard curves were linear ($P > 0.05$) with $r > 0.99$ over the range 0.2–3.0, 0.3–3.0 and 0.7–15.0 μg/ml for cetylpyridinium chloride, cetrimide and benzalkonium chloride, respectively.

Keywords: Quaternary ammonium compound; Eosin-Y; Triton X-100, Ion-pair; Absorbance

The predominant approaches to the analysis of quaternary ammonium compounds have involved either ultraviolet spectroscopy (e.g., cetylpyridinium chloride, benzalthonium chloride) or analysis of complexes of the quaternary ammonium compounds with an anionic agent (Petrocci, 1983). However, though widespread in use, these methods require large samples and are low in sensitivity (in the range 50–250 μg/ml). Other approaches have employed gas chromatography (Suzuki et al., 1989), liquid scintillation counting (Bonesvoll and Gjermo, 1978) and high-pressure liquid chromatography (Collins and Deasy, 1990) with the minimum quantifiable concentrations of 100, 35 and 2.0 μg/ml, respectively. However, a major drawback of the use of gas chromatography is thermal decomposition of quaternary ammonium compounds (Ng et al., 1985). HPLC can only be employed on those agents that possess a chromophore (such as cetylpyridinium chloride and benzalthonium chloride) and the expense of tracer-labelled quaternary ammonium compounds prohibits the use of scintillation counting as a routine method.

Further analytical complications occur when assessing the uptake of these compounds onto micro-organisms. Quaternary ammonium compounds induce the leakage of cytoplasmic materials into the environment which bathes the cells and this interferes with the analysis of cetylpyridinium chloride and benzalthonium chloride at 260 nm.

Chawner and Gilbert (1989) developed a colorimetric assay for alexidine in which the anionic

* Corresponding author.
[1] Present address: School of Pharmacy, Queen's University of Belfast, Medical Biology Centre, 97, Lisburn Road, Belfast, U.K.

SSDI 0378-5173(94)00206-1

dye eosin-Y was complexed with this biguanide and the absorbance of the ion-pair examined at 540 nm. This assay was useful for concentrations in the range 4–20 μg/ml. Calatayud and Falcó (1986) employed bromocresol green (as an anionic ion-pairing agent) in the presence of Triton X-100 for the analysis of chlorhexidine. The detection limit was reported to be 8.5 μg/ml.

This paper reports a sensitive colorimetric method for the analysis of three quaternary ammonium compounds (cetylpyridinium chloride, cetrimide and benzalkonium chloride) following ion-pairing with eosin-Y in the presence of Triton X-100.

Cetylpyridinium chloride, cetrimide and benzalkonium chloride were purchased from Sigma Chemicals (St Louis, U.S.A.). Iso-octylphenoxypolyethoxyethanol (Triton X-100), borate buffer tablets, and disodium hydrogen orthophosphate anhydrous were obtained from BDH (Poole, U.K.). Citric acid was purchased from Ajax Chemicals Ltd (Auburn, Australia). Tetrabromo® fluorescein (eosin-Y) was purchased from May and Baker (Dagenham, U.K.).

All spectrophotometric measurements were performed using a Hewlett Packard 8452 diode array spectrophotometer linked to an NEC powermate 1 plus 286 computer. A Hannah HI 1280 pH meter was used to measure pH.

A least-squares linear regression statistical analysis program was written for statistical evaluation of linearity. Essentially, the software tests for non-linearity by comparing the lack of fit mean square with the pure error (between replicates) mean square. The software was written in Turbo Pascal 5.0 (Borland International, Scotts Valley, CA) and the statistics calculations were derived from Draper and Smith (1966) and Bolton (1984).

The quaternary ammonium compounds were analysed using colorimetry following ion-pairing with eosin-Y in the presence of Triton X-100. Varying concentrations of Triton X-100 (0.0075–0.2% v/v) over the pH range of 6.0–9.2 at room temperature, were mixed with eosin-Y (9.0 μg/ml) and cetylpyridinium chloride (5.0 μg/ml). The absorbance at the λ_{max} of the resulting ion-pair was assessed. The effects of eosin-Y (6.0–18.0 μg/ml) and cetylpyridinium chloride concentrations in the presence of Triton X-100 (0.025% v/v) at pH 8.0 were also determined. Absorbance and λ_{max} values were assessed as above.

Table 1
The effects of pH[a] and Triton X-100 concentration on the absorbance of the cetlypyridinium chloride (5.0 μg/ml)-eosin-Y (9.0 μg/ml) ion-pair

Triton X-100 (% v/v)	Absorbance (534 nm)			
	pH 6.0	pH 7.0	pH 8.0	pH 9.2
0.2	0.044	0.0	0.07	0.07
0.1	0.11	0.11	0.13	0.15
0.05	0.21	0.22	0.23	0.26
0.025	0.33	0.34	0.34	0.35
0.015	0.33	0.35	0.37	0.31
0.0075	0.11	0.31	0.32	0.31

[a] McIlvaine's buffer (pH 6.0–8.0) and borate buffer (pH 9.2).

Optimal conditions for analysis were obtained using excess eosin-Y (9.0 μg/ml) and 0.025% v/v Triton X-100 and these concentrations were employed for the production of the appropriate calibration curves for each quaternary ammonium compound. All calibration curves were produced by dilution of three independent stock solutions of the relevant quaternary ammonium compound and the absorbance of the solutions plotted as a function of concentration.

The effects of pH and Triton X-100 concentration on the ion-paired absorbance of cetylpyridinium chloride (5.0 μg/ml) and eosin-Y (9.0 μg/ml) are shown in Table 1. The range of pH values examined were selected to ensure that eosin-Y existed primarily in the anionic form, the pKa for eosin-Y being approx. 4.2 (Fompeydie et al., 1979, Mchedlov-Petrosyan et al., 1985), and hence ion-pairing with cetylpyridinium chloride was promoted. In addition, excess eosin-Y was used, thus promoting ion-pairing. Optimal absorbance occurred whenever Triton X-100 was present at either 0.015 or 0.025% (v/v). In further studies, 0.025% was used.

At a constant concentration of Triton X-100 (0.025% v/v), the effect of eosin-Y (6.0, 9.0, 12.0, 15.0, 18.0 μg/ml) on absorbance (534 nm) of the produced ion-pair was determined, using a range of cetylpyridinium chloride concentrations (0.3, 0.5, 1.0, 3.0, 5.0 μg/ml) (Table 2). Eosin-Y at 9.0

Table 2
The effects of eosin-Y and cetylpyridinium chloride concentration (0.3–5.0 μg/ml) on the absorbance (534 nm) in the presence of Triton X-100 (0.025%) at pH 8.0

Eosin-Y (μg/ml)	Absorbance (534 nm)				
	0.3	0.5	1.0	3.0	5.0
6.0	0.00	0.00	0.00	0.13	0.17
9.0	0.02	0.03	0.06	0.17	0.32
12.0	0.00	0.03	0.05	0.13	0.26
15.0	0.00	0.00	0.07	0.20	0.30
18.0	0.00	0.00	0.07	0.23	0.30

μg/ml represents the best ion-pair concentration for cetylpyridinium chloride analysis. This may be due in part to the range of cetylpyridinium chloride concentrations assessed since the cetylpyridinium chloride to eosin-Y molar ratio did not exceed 1 : 1.

Therefore, employing the following conditions: eosin-Y (9.0 μg/ml) and 0.025% v/v Triton X-100, pH 8.0, calibration curves were prepared for cetylpyridinium chloride, cetrimide and benzalkonium chloride. Fig. 1 shows a typical cetylpyridinium chloride/eosin-Y ion-pair spectrum, with λ_{max} at 534 nm, measured against a reference solution containing eosin-Y (9.0 μg/ml) and Triton X-100 (0.025% v/v). The spectra for benzalkonium chloride and cetrimide showed similar profiles with the same λ_{max}, differing only in their absorbances. Linearity was observed for all three agents ($P > 0.05$ and $r > 0.99$) over the concentration ranges 0.2–3.0 μg/ml for cetylpyridinium chloride, 0.3–3.0 μg/ml for cetrimide and 0.7–15 μg/ml for benzalkonium chloride.

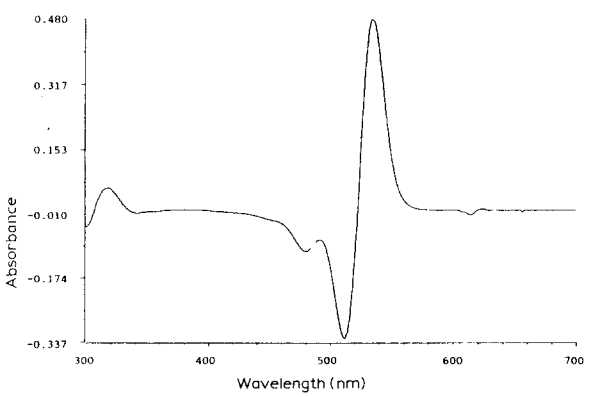

Fig. 1. A spectrum of cetylpyridinium chloride (5.0 μg/ml)-eosin (9.0 μg/ml) ion-pair in the presence of Triton X-100 (0.025%) at pH 8.0.

In addition, calibration curves of the three quaternary ammonium compounds were also prepared in the absence of Triton X-100. Linearity was observed for all three agents; however, the range of concentration for linearity and the sensitivity of each assay was decreased (2.0–3.0, 1.0–3.0, and 3.0–15.0 μg/ml for cetylpyridinium chloride, cetrimide and benzalkonium chloride, respectively.

The ion-pairing of cationic surfactants to anionic dyes for the purpose of chemical analysis has been reported for over 30 years (Petrocci, 1983). Although widely used, assays utilizing this interaction have suffered from low sensitivities. For example, Lowry (1979) developed an ion-pair assay for assessing benzalkonium chloride concentration in different pharmaceutical formulations using complexation with bromothymol blue, an anionic dye, and analysis at 610 nm. However, this assay was only sensitive to 50 μg/ml. Chin and Lach (1965) reported an assay which was capable of detecting three quaternary ammonium compounds (cetylpyridinium chloride, benzalkonium chloride, and benzalkonium chloride) at concentrations as low as 2.0 μg/ml. In this assay the quaternary ammonium compounds were ion-paired with picric acid, this complex was back-extracted into chloroform, the solvent evaporated, and the ion-pair reconstituted in buffer. This was performed in order to separate and assess ion-paired dye concentrations from unbound species, thus removing false absorbance values; however, inaccuracies resulting from incomplete extraction and emulsion formations with the hydrocarbon have been reported (Walters et al., 1983). Other workers have used gravimetric analysis and titrametry but these approaches have been designed for the quantification of quaternary ammonium compounds at concentrations exceeding 5.0 mg/ml.

Calatayud and Falcó (1986) reported an ion-pair assay for the determination of chlorhexidine, using the anionic dye bromocresol green. Triton X-100 was added (0.2% v/v, final concentration) and the absorbance of the complex measured at 630 nm. The blanking solution contained bromo-

cresol green and Triton X-100. These workers reported a lowest detectable concentration of 8.5 μg/ml, and linearity over the range 8.5–25 μg/ml. The sensitivities of the assay reported in this paper, dependent on the quaternary ammonium compound assessed, yielded values ranging between 0.2 and 15 μg/ml. This is due both to the use of eosin-Y as the ion-pairing agent and the sensitivity of the spectrophotometer. Eosin-Y has previously been used as an ion-pairing agent in the colorimetric analysis of the antimicrobial agent alexidine by Chawner and Gilbert (1989), who examined the adsorption of alexidine onto the surface of *E. coli*. Eosin-Y has also been utilised to assess chlorhexidine in saliva by means of fluorescence spectroscopy (De Vries and Arends 1991), but only with a minimum quantifiable concentration of 6.7 μg/ml.

The assay developed in this present study offers distinct advantages over previously reported methods. The use of Triton X-100 both overcomes the problems associated with the use of chloroform (Walters et al., 1983) and, greatly improves the sensitivities of the analyses. Cytoplasmic materials extracted from *C. albicans* blastospores do not interfere with the assay (unpublished results). Due to the ease of analysis, this assay can be employed to rapidly assess quaternary ammonium compound concentrations in a variety of preparations, however, their analysis in the presence of dyes or other cationic agents in products may prove troublesome.

References

Bolton, S., *Pharmaceutical Statistics*, Dekker, New York, 1984.

Bonesvoll, P. and Gjermo, P., A comparision between chlorhexidine and some quaternary ammonium compounds with regard to retention, salivary concentration and plaque-inhibiting effect in the human mouth after mouth rinses. *Arch. Oral. Biol.*, 23 (1978) 289–294.

Calatayud, J.M. and Falcó, P.C., Spectrophotometric determination of chlorhexidine with bromocresol green by flow-injection and manual methods. *Anal. Chim. Acta*, 189 (1986) 323–328.

Chawner, J.A. and Gilbert, P., A comparative study of the bactericidal and growth inhibitory activities of the bis-biguanides alexidine and chlorhexidine. *J. Appl. Bacteriol.*, 66 (1989) 243–252.

Chin, T.F. and Lach, J.L., Spectrophotometric determination of some quaternary compounds. *J. Am. Pharm. Assoc., Sci. Ed.*, 54 (1965) 1550–1551.

Collins, A.E. and Deasy, P.B., Bioadhesive lozenge for the improved delivery of cetylpyridinium chloride. *J. Pharm. Sci.*, 79 (1990) 116–119.

Draper, N.R. and Smith, H., *Applied Regression Analysis*, Wiley, London, 1966.

De Vries, J. and Arends, R.J., Determination of chlorhexidine in saliva and in aqueous solutions. *Caries Res.*, 25 (1991) 410–414.

Fompeydie, D., Onur, F. and Levillain, P., Spectroscopic and potentiometric study of successive ionizations of eosin. *Bull. Soc. Chim. Fr.*, 9 (1979) 375–380.

Lowry, J.B., Direct spectrophotometric assay of quaternary ammonium compounds using bromothymol blue. *J. Pharm. Sci.*, 68 (1979) 110–111.

Mchedlov-Petrosyan, N.O., Mindrina, V.F. and Nikishina, L.E., Effect of solvent on the ionization of hydroxyxanthene dyes. *Inz. Vyssh. Uchebn. Zaved. Khim. Khim. Teknol.*, 28 (1985) 29–32.

Ng, L., Hupé, M. and Harris, A.G., Direct gas chromatographic method for determining the homologue composition of benzalkonium chloride. *J. Chromatogr.*, 351 (1985) 554–559.

Petrocci, A.N., Surface-active agents: quaternary ammonium compounds. In Block, S.S. (Ed), *Disinfectant, Sterilization and Preservation*, Lea & Febiger, Philadelphia, 1983, pp. 309–329.

Suzuki, S., Nakamura, Y., Kaneko, M., Mori, K. and Watanabe, Y., Analysis of benzalkonium chlorides by gas chromatography. *J. Chromatogr.*, 463 (1989) 188–191.

Walters, T.H., Furr, J.R. and Russell, A.D., Antifungal action of chlorhexidine. *Microbios*, 38 (1983) 195–204.