ELSEVIER

Journal of Chromatography A, 745 (1996) 225–232

JOURNAL OF
CHROMATOGRAPHY A

Peak splitting observed during capillary electrophoresis of α- and β-naphthols in borate buffer

Alma L. Revilla[a], Josef Havel[a,*], Petr Jandik[b]

[a]Department of Analytical Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 , Brno, Czech Republic
[b]Thermo Bioanalysis Corporation, 355 River Oaks Parkway, San Jose, CA 95161, USA

Abstract

β-Naphthol is an undesirable by-product in the synthesis of α-naphthol for various uses in the pharmaceutical and dyestuff industries. We have developed a new procedure for the determination of traces of β-naphthol in the presence of a large excess of α-naphthol. The new method utilizes direct UV detection at 230 nm in a 44.5 cm$\times$75 μm I.D. fused-silica capillary. The separation is carried out at 15 kV in a 50 mM borate electrolyte and pH of 9.3. During the research for optimum conditions, we were able to observe an occasional splitting of the peak belonging to β-naphthol. Under certain conditions, we could see up to three peaks for that compound. The realization that the peak splitting could be observed reproducibly under alkaline conditions and with increased borate concentration (higher than 50 mM) helped us to elucidate the mechanism behind the peak splitting phenomena. The artifact was caused mainly by complexation reactions between β-naphthol and borate polymers.

Keywords: Buffer composition; Peak splitting; Naphthols; Borate

1. Introduction

α-Naphthol and β-naphthol (αN and βN, respectively) are weak acids with their respective pK_a values at 9.34 and 9.51 [1]. They are needed as synthetic precursors to azo dyes and other mostly pharmaceutically relevant compounds. In many of the processes involved, a very high purity of the naphthols is required. A 5% trace of βN in an αN reagent, for example, causes a color instability of the resulting azo dyes [2]. For the synthesis of cephalosphorines, the required purity of αN is at less than 0.5% of the βN content.

At present, the mixtures of αN and βN can be analyzed mostly by chromatographic methods such as high-performance liquid chromatography (HPLC) [3], picochromatography [4], or without any separation by spectrophotometry using mathematical procedures of component deconvolution [5].

Capillary zone electrophoresis (CZE) with its high separation efficiency appears to offer an excellent potential for an improvement of hitherto difficult separations such as that of the positional naphthol isomers. The high separation efficiency was demonstrated by separating organic ions and their deuterated analogues which differ slightly [6,7].

There are relatively many reported CZE separations of (poly)hydroxy compounds using borate buffer [8–14], but the role of borate in the separation is not explained. It is known that borate ions form complexes with polyhydroxy compounds such as oligoalcohols, o-diphenols and hydroxycarboxylic

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00512-2

acids [15]. With *cis*-diols, borate is used as buffer additive for separation of catechols [16] and carbohydrates [17]. Furthermore, capillary electrophoresis (CE) separation of nucleotide isomers via complexation with cyclodextrin and borate was reported recently [18].

The aims of this work were:

(1) Find conditions for a robust separation of αN and βN by CE. Evaluate the possibility of quantitation of βN traces in αN. Improve reproducibility of βN determination in comparison with spectrophotometric methods [5].

(2) Elucidate the role of borate in the separation of βN from αN. Based on the understanding of the nature of borate–naphthol interactions, define A set of conditions under which the expected artifacts can be avoided. The artifacts caused by borate hydroxy group interactions were reported previously in connection with an HPLC separation of catecholamines [19].

2. Experimental

2.1. Chemicals

Naphthol samples were reagent grade (Merck, Darmstadt, Germany). Additionally, βN was recrystallized prior to use to assure the highest possible purity. Mesityl oxide (MSO) was from Fluka (Buchs, Switzerland). The other reagents were analytical grade supplied by Lachema (Brno, Czech Republic). Tridistilled water used was produced in a commercial apparatus from Heraeus (Hanau, Germany). Standard pH buffers were from the Institute of Serum and Vaccines (Prague, Czech Republic).

2.2. Instrumentation

A SpectraPHORESIS 2000 CE system (Thermo Separation Products, San Jose, CA, USA) was utilized for all CZE experiments. The PC1000 Version 3.0 software provided a complete system control and was also used for data acquisition and evaluation.

An uncoated fused-silica capillary of 44.5 cm total length × 75 μm I.D. (length to detector 36.8 cm) was used.

A pH meter PMH64 (Radiometer, Copenhagen, Denmark) with saturated calomel electrode and glass electrode was chosen for the pH measurements.

A UV2 Quartz Series spectrophotometer (ATI-Unicam, Cambridge, UK) with a 1 cm path-length quartz cell was used for the absorbance measurements.

2.3. Conditions

The sampling was by hydrodynamic injection for 1.0 s (calculated volume of 4 nl). The CZE separations were performed by applying the constant voltage in a range of 30 to 5 kV. The UV detection was carried out either at 230 nm or by scanning the range between 200 to 340 nm. Constant temperature was set at 30°C. The electroosmotic flow (EOF) was determined by injections of 0.1% MSO (v/v) under the same conditions as for the separation of samples. Occasionally we utilized the peak of water as an EOF marker.

Several buffer were used: borate, phosphate, acetate and mixtures of those. Borate buffer was prepared from sodium tetraborate and/or boric acid. The pH was adjusted with HCl and/or NaOH. All buffer solutions were degassed by vacuum filtration.

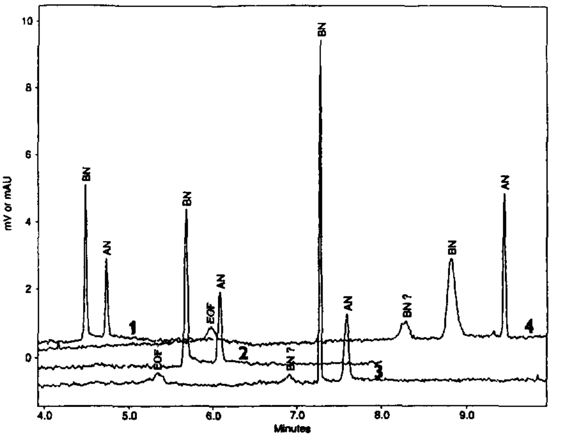

Fig. 1. Electropherograms for the mixture of αN ($2.47 \cdot 10^{-5}$ *M*) and βN ($2.44 \cdot 10^{-5}$ *M*) at different borate concentration (*M*): (1) 0.01; (2) 0.025; (3) 0.05 and (4) 0.075. Conditions: pH 9.2; 10 kV; 1.0 s hydrodynamic injection.

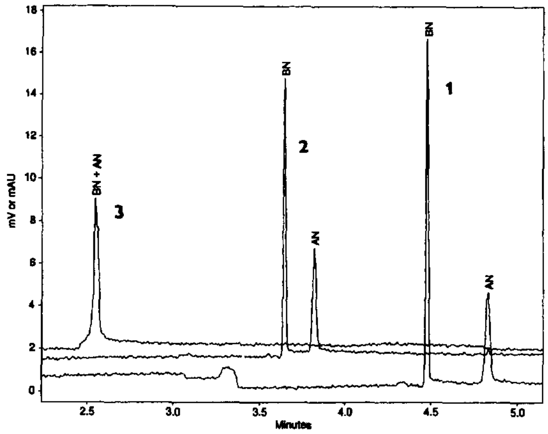

Fig. 2. Electropherograms for the mixture of αN ($3.44\cdot10^{-5}$ *M*) and βN ($4.16\cdot10^{-5}$ *M*) at different pH values: (1) 9.3; (2) 9.0 and (3) 8.0. CE conditions: 0.05 *M* borate; 15 kV; 1.0 s hydrodynamic injection.

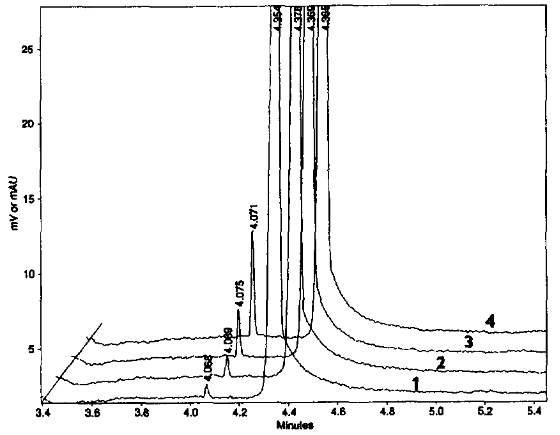

Fig. 4. αN is at $6.15\cdot10^{-4}$ *M* in all four electropherograms. The βN concentration is as follows: (1) $2.04\cdot10^{-6}$ *M*; (2) $2.72\cdot10^{-6}$ *M*; (3) $6.80\cdot10^{-6}$ *M* and (4) $1.70\cdot10^{-5}$ *M*. CE conditions: 0.05 *M* borate; pH 9.3; 15 kV; 1.0 s hydrodynamic injection.

3. Results and discussion

We started our research for optimum analytical conditions by comparing separations of the two analytes at pH 9 in 10 m*M* borate and 10 m*M* phosphate electrolyte buffers respectively. We decided to try these two particular electrolyte buffers based on several literature references [8–11] reporting separation of azo dyes and phenolic compounds. Both electrolyte systems generated a separation of the two naphthol isomers. However, in our judgement, the resolution was not yet sufficient for analyzing disparate concentrations of the naphthols and additionally in the case of phosphate we observed some excessively noisy baselines.

Later we extended our investigation to mixtures of

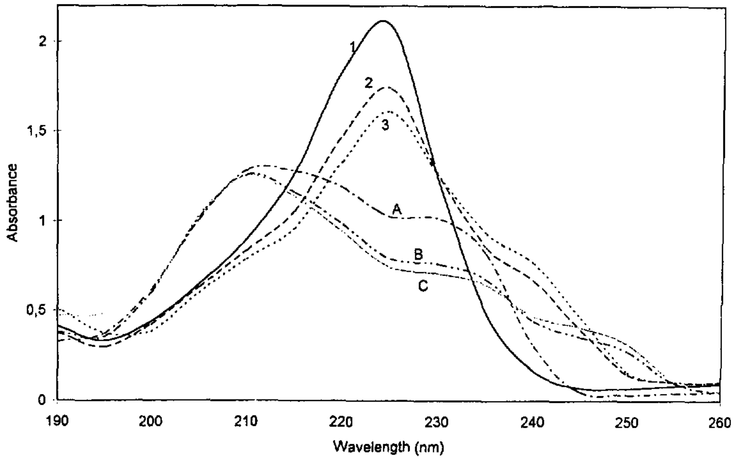

Fig. 3. UV spectra at different borate concentration: (A) deionised water; (B) 0.01 *M* and (C) 0.1 *M* for αN $1.05\cdot10^{-4}$ *M* and (1) deionised water; (2) 0.01 *M* and (3) 0.1 *M* for βN $1.98\cdot10^{-5}$ *M*.

Table 1
Statistical result for the calibration curves: α-Naphthol; β-Naphthol

α-Naphthol[a]		β-Naphthol[b]	
Concentration (M)	Area	Concentration (M)	Area
$5.01\cdot10^{-6}$	777	$2.05\cdot10^{-6}$	1 030
$1.00\cdot10^{-5}$	1 449	$3.44\cdot10^{-6}$	1 410
$5.01\cdot10^{-5}$	8 022	$5.13\cdot10^{-6}$	1 880
$1.00\cdot10^{-4}$	18 649	$1.03\cdot10^{-5}$	3 140
$5.01\cdot10^{-4}$	93 075	$2.57\cdot10^{-5}$	8 293
$1.00\cdot10^{-3}$	183 992	$5.13\cdot10^{-5}$	14 748
		$1.03\cdot10^{-4}$	27 642
		$5.13\cdot10^{-4}$	140 864

[a] Squared $r=0.99992$, $b=-274.2$, $M=1.84\cdot10^{8}$.
[b] Squared $r=0.99990$, $b=495.9$, $M=2.73\cdot10^{8}$.

acetate with phosphate and also to single compound acetate electrolytes. At a pH near to 5 and with separation voltages between 5 and 30 kV we obtained only various degrees of insufficient resolution.

Because of these preliminary results, we subsequently decided to investigate the influence of borate electrolyte concentrations on the resolution of naphthol isomers.

The use of borate as working buffer was studied for concentrations ranging from 0.01 to 0.075 M. In our judgement, the best resolution, sensitivity and a minimum of artifacts (baseline shifts, unknown peaks) were observed at 0.05 M borate and 10 kV (trace 3, Fig. 1).

We then evaluated the influence of pH on the separation.

Using 0.05 M borate, several pH values near to 9 were tested (Fig. 2). Note that all separations in Fig. 2 were generated in the relatively narrow pH range of 8.3 to 9.3. At pH 10 and 15 kV the current became excessive (>200 μA). Decreasing the voltage to 7 kV to reduce the current, led to unacceptably long migration times for the peaks of interest (>10 min.).

At this point we also conducted experiments in a micellar medium. Additions of sodium dodecyl sulphate (SDS) were made to 50 mM borate electrolyte with the objective of improving the separation. Surprisingly, this only led to peak broadening

Table 2
Statistical results for samples

	Area	Concentration (M)		% error	% βN in αN
		estimated	real		
Pure solutions					
αN	140 424	$7.63\cdot10^{-4}$	$7.03\cdot10^{-4}$	8.5	
	6 701	$3.65\cdot10^{-4}$	$3.52\cdot10^{-4}$	3.7	
	2 443	$1.47\cdot10^{-5}$	$1.41\cdot10^{-5}$	4.2	
βN	20 411	$7.29\cdot10^{-5}$	$7.63\cdot10^{-5}$	−4.4	
	10 736	$3.75\cdot10^{-5}$	$3.81\cdot10^{-5}$	−1.6	
	1 457	$3.51\cdot10^{-6}$	$3.44\cdot10^{-6}$	2	
Mixtures					
αN	128 541	$6.99\cdot10^{-4}$	$6.15\cdot10^{-4}$	13.6	11.06
βN	21 269	$7.60\cdot10^{-5}$	$6.80\cdot10^{-5}$	11.8	
αN	125 865	$6.84\cdot10^{-4}$	$6.15\cdot10^{-4}$	11.2	5.52
βN	12 122	$4.20\cdot10^{-5}$	$3.40\cdot10^{-5}$	23.5	
αN	120 236	$6.54\cdot10^{-4}$	$6.15\cdot10^{-4}$	6.3	2.76
βN	5 056	$1.90\cdot10^{-5}$	$1.70\cdot10^{-5}$	11.8	
αN	138 625	$7.53\cdot10^{-4}$	$6.15\cdot10^{-4}$	22.4	1.11
βN	2 399	$6.95\cdot10^{-6}$	$6.80\cdot10^{-6}$	2.2	
αN	134 026	$7.29\cdot10^{-4}$	$6.15\cdot10^{-4}$	18.5	0.44
βN	1 119	$2.27\cdot10^{-6}$	$2.72\cdot10^{-6}$	−16.5	

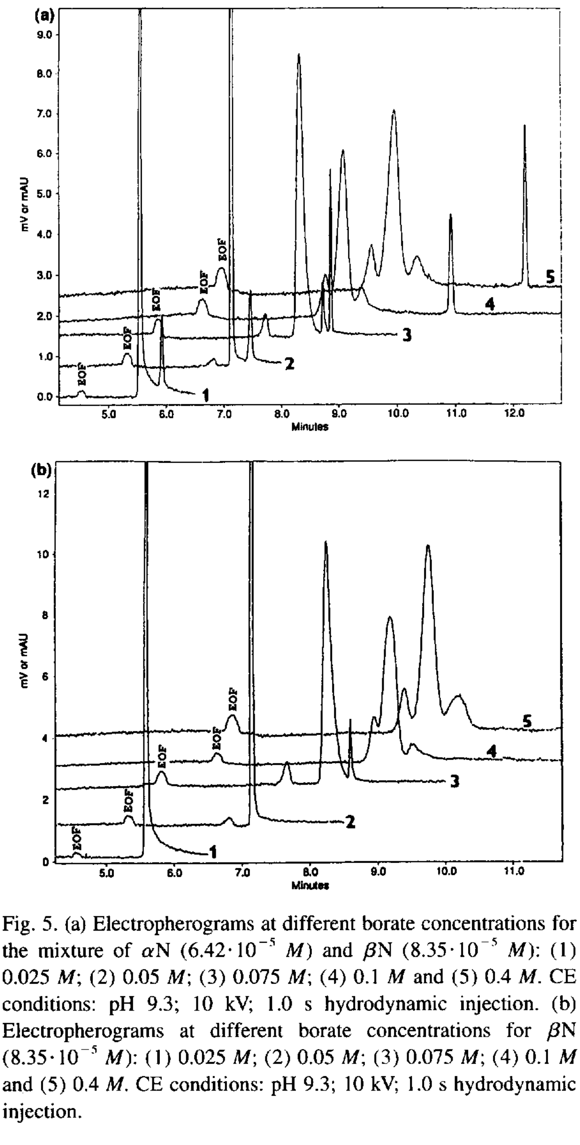

Fig. 5. (a) Electropherograms at different borate concentrations for the mixture of αN ($6.42 \cdot 10^{-5}$ *M*) and βN ($8.35 \cdot 10^{-5}$ *M*): (1) 0.025 *M*; (2) 0.05 *M*; (3) 0.075 *M*; (4) 0.1 *M* and (5) 0.4 *M*. CE conditions: pH 9.3; 10 kV; 1.0 s hydrodynamic injection. (b) Electropherograms at different borate concentrations for βN ($8.35 \cdot 10^{-5}$ *M*): (1) 0.025 *M*; (2) 0.05 *M*; (3) 0.075 *M*; (4) 0.1 *M* and (5) 0.4 *M*. CE conditions: pH 9.3; 10 kV; 1.0 s hydrodynamic injection.

at pH 9.3, 15 kV and SDS concentrations between 0.5–25 m*M*.

Starting with the preliminary experiments, all our electropherograms were detected at 230 nm. As seen in the UV spectra depicted in Fig. 3, the highest sensitivity for the determination of βN can be predicted for that wavelength. Also, at 230 nm, the absorption coefficient of βN is considerably higher than that of αN, this circumstance is in a good agreement with our goal to measure traces of βN in the presence of much larger levels of αN.

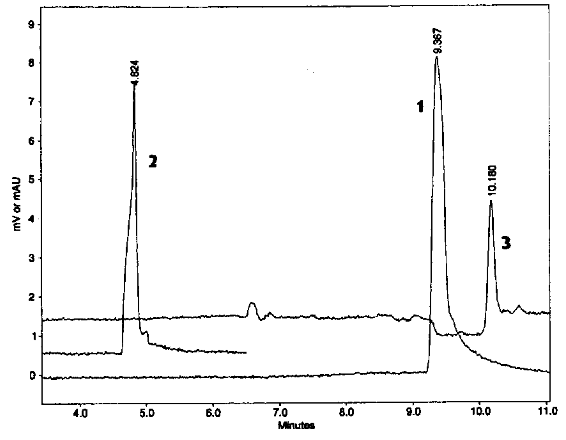

Fig. 6. Electropherograms for αN at different pH values: (1) 4.1; (2) 7.1 and (3) 9.3. CE conditions: 0.4 *M* borate; 10 kV; 1.0 s hydrodynamic injection. αN concentration of $6.42 \cdot 10^{-4}$ *M* for pH 4.1 and 7.1 and $8.99 \cdot 10^{-5}$ *M* for pH 9.3.

3.1. Optimal conditions and possibility of artifacts

Four examples of separation traces of αN under optimized conditions (0.05 *M* borate buffer, pH 9.3, 15 kV, 1.0 s hydrodynamic injection) are illustrated in Fig. 4. We have found that concentrations of αN exceeding $6.15 \cdot 10^{-4}$ *M* may affect the separation and lead to an increasingly unreliable quantitation. The calibration results from single injections of αN and βN are presented in Table 1. The data represents experimental evidence of a good correlation between concentration on the one hand and measured peak areas on the other hand. An experimental evaluation of accuracy for the determination of both analytes is summarized in Table 2. The table presents the results for either of the analytes alone and for different levels of βN in combination with varying levels of excess of αN. Because of the artifacts discussed below, the borate concentration in the carrier electrolyte should always be kept at 0.05 *M* or lower (at pH 9.3).

3.2. Chemical interactions leading to artifacts

We have first noticed the emergence of multiple peaks for βN in the experimental series shown in Fig. 1. Additional evidence of chemical changes occurring with αN and βN is contained in Fig. 3. In comparing pure water solutions to increasing con-

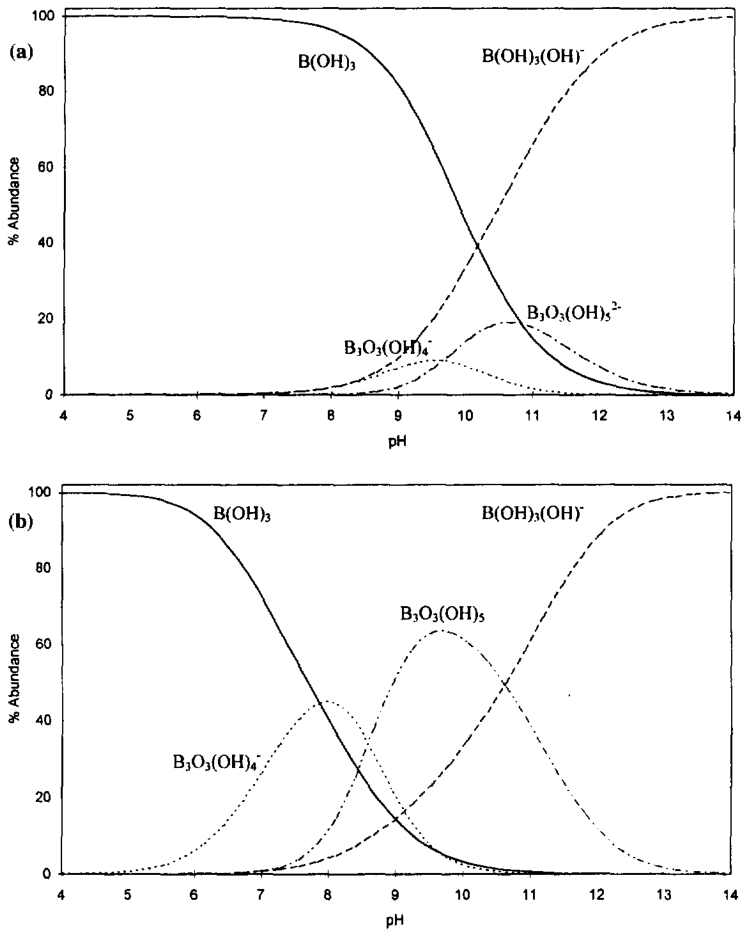

Fig. 7. (a) Distribution diagram for borate species at total concentration of 0.05 M. (b) Distribution diagram for borate species at total concentration of 0.4 M.

centrations of borate, it is possible to observe the formation of shoulders in the UV spectra at ca. 240 and 250 nm for αN and βN, respectively. Since the multiple peak formation and the changes in the UV spectra are both borate concentration dependent, the possibility of a reaction between the naphthols and borate is considered next. The occurrence of multiple reaction paths for the reactions between boric acids and polyhydroxy compounds has been reported in the literature [15]. A previous, combined electrochemical and chromatographic investigation resulted in an elucidation of the borate–catechol complexation as the main influence leading to a chromatographic peak splitting in the HPLC assays of catecholamines [19].

Regarding borate and/or boric acid, it is known that there may be several trimeric anionic species, in addition to the monomeric borate anion and boric acid, present in aqueous solutions [20].

The formation of trimers was confirmed [20] for total borate concentrations exceeding 50 mM. The reactions occurring in such concentrated solutions are as follows:

$$B(OH)_3 + H_2O \Leftrightarrow B(OH)_4^- + H^+$$
$$\log K_{11} = -9.00$$

$$3B(OH)_3 \Leftrightarrow B_3O_3(OH)_4^- + H^+ + 2H_2O$$
$$\log K_{13} = -6.84$$

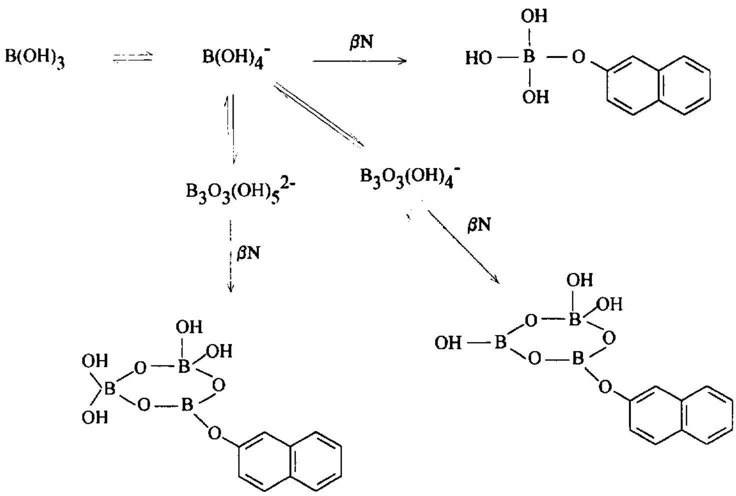

Fig. 8. Structural formulas for the possible products of reactions between βN and borate trimer.

$$3B(OH)_3 \Leftrightarrow B_3O_3(OH)_5^{2-} + 2H^+ + H_2O$$
$$\log K_{23} = -15.44$$

To further investigate contributions to the peak artifacts, we have generated another series of αN–βN separations, this time in broader borate concentration range than in Fig. 1, reaching from 0.025 to 0.4 *M*. Note that the concentrations of αN and βN were higher in Fig. 5 than in Fig. 1. As expected, the multiple peak formation is much more evident in Fig. 5 than in Fig. 1. Also, it is quite remarkable than in addition to the previously reported peak splitting [19], we were able to observe well defined, multiple peaks for a single analyte. Comparing the separations of the dual component mixture (Fig. 5a) with those obtained under otherwise identical conditions and with a single component solution of βN (Fig. 5b), it is clear that only the βN and not αN is the species producing multiple peaks.

Keeping the borate concentration constant at 0.4 *M*, we have also investigated the role of pH (Fig. 6). As seen in Fig. 6, the presence of artifacts can be detected even for the αN peak at the two lower pH values. The deformed and/or split peaks are a strong indication of several different forms of αN–borate electrolyte. Similarly to αN, peak splitting is also observed for βN at the lower pH value albeit not as clearly as for αN (electropherograms not shown).

In order to consider the possible reaction paths leading to the observed peak splitting and multiple peak formation, we have first constructed pH dependent distribution diagrams for two different concentrations of boric acid (Fig. 7a and b). Predictably, the higher concentration of boric acid leads to a higher abundance of trimeric borate species. The relative abundances of the various borate species depending on pH and borate concentration can help us to predict the relative importance of the three main reaction paths formulated in Fig. 8 for βN. The existence of three different reaction paths seems to be in agreement with three different peaks due to βN at 0.4 *M* borate and pH 9.3 in Fig. 5. However, the existence of multiple different complexed forms of a naphthol does not always have to lead to a formation of multiple well defined and separated peaks under the conditions of CE. This is evident from the separation results for αN in the entire pH range and for βN under acidic conditions in our study.

4. Conclusions

In this work we present optimized conditions for the separation of traces of βN in the presence of excess concentrations of αN. We also discuss various artifacts leading to peak splitting and formation of multiple, well separated peaks for a single analyte species. Experimental evidence is presented in support of our conclusions regarding the borate–naphthol formation as the main source of the observed artifacts.

Acknowledgments

We thank WATREX Corporation (Prague, Czech Republic) for the kind support for this work. One of us, A.R., would like to thank the U.N.A.M. (National Autonomous University of Mexico) and especially D.G.A.P.A. for the support given.

References

[1] Handbook of Chemistry and Physics, J. Pharm. Sci., 65 (1976) 380.
[2] V.F. Borodkin, Chemistry of Organic Dyes, SNTL, Prague, 1987.
[3] M. Vrchlabský, N. Pollaková, St. Navrátilová and P. Kuthan, Proc. Colorchem' 94, Vth International Conference, Research Institute for Organic Syntheses (VÚOS), Czech Republic, 1994, p. 115.
[4] N. Pollaková, M. Vrchlabský, V. Kahle, J. Haruda and P. Kuthan, Scripta Fac. Sci. Nat. Masaryk Univ. Brun., 25 (1994) 3.
[5] A. Revilla, G. Vargas and J. Havel, submitted for publication (Quím. Anal.).
[6] S. Terabe, T. Yashima, N. Tanaka and M. Araki, Anal. Chem., 60 (1988) 1673.
[7] M.M. Bushey and J.W. Jorgenson, Anal. Chem., 61 (1989) 309.
[8] S.M. Burkinshaw, D. Hinks and D.M. Lewis, J. Chromatogr., 640 (1993) 413.
[9] S. Suzuki, M. Shirao, M. Aizawa, H. Nakazawa, K. Sasa and H. Sasagawa, J. Chromatogr. A, 680 (1994) 541.
[10] P. Blatný, C.H. Fischer and E.J. Kenndler, Anal. Chem., 67 (1995) 351.
[11] K.N. Tapley, J. Chromatogr. A, 706 (1995) 555.
[12] R.A. Wallingford and A.G. Ewing, J. Chromatogr., 441 (1988) 299.
[13] J. Hjertén and M. Zhu, J. Chromatogr., 327 (1985) 157.
[14] M. Quilliam, P. Thibault and Zhao Jian-Ying, Anal. Chem., 61 (1989) 1053.
[15] M. Bartušek, Folia Fac. Sci. Nat. Masaryk Univ. Brun., 25 (1984) 19.
[16] R.A. Wallingford, P.D. Curry and A.G. Ewing, J. Microcolumn Sep., 1 (1989) 23.
[17] S. Hoffsltetter-Kuhu, A. Paulus, E. Gassman and H.M. Widma, Anal. Chem., 63 (1991) 54.
[18] T. Tadey and W.C. Purdy, J. Chromatogr. B, 657 (1994) 365.
[19] F. Degel, P. Zuman, P. Jandik and G. Gilch, Clin. Chem., 33 (1987) 108.
[20] N. Ingri, G. Lagerström, M. Frydman and L.G. Sillén, Acta Chem. Scand., 11 (1957) 1034.