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Journal of Chromatography A, 1084 (2005) 152–159
JOURNAL OF CHROMATOGRAPHY A
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Effects of non-ionic surfactants on isotachophoretic separations of 2-arylpropionic acids

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Abstract

Non-ionic surfactant (Brij 35, Tween 20, Tween 80 and Tergitol NPX) modified capillary isotachophoresis was investigated for the separation of 2-arylpropionic acids (fenoprofen, flurbiprofen, ibuprofen, ketoprofen and naproxen) and benzoic acid and its derivatives (salicylic, acetylsalicylic and gallic acids). The relative step height (RSH) values of analytes were found to be dependent on the type and concentration of the surfactant. The strength of the affinity of the 2-arylpropionic acids to the non-ionic micelles was found to be as follows: flurbiprofen > fenoprofen > ibuprofen > naproxen > ketoprofen. In general, the RSH values of 2-arylpropionic acids increase with an increase in the concentration of surfactants. However, the RSHs of benzoic, salicylic and gallic acids are not considerably affected. Separation of all acids was obtained with the Tween 20 (1.5%, w/v) in the leading electrolyte 10 mmol L^{-1} hydrochloric acid/L-histidine (pH 6.0). Changes in the fluorescence intensity of fenoprofen, flurbiprofen and naproxen were also investigated in micellar media (Tween 20, Tween 80 and Brij 35). The strength of the affinity of the 2-arylpropionic acids to the Tweens micelles was found to be as follows: flurbiprofen > fenoprofen > naproxen, which is consistent with the isotachophoretic results. On the contrary, the strength of the affinity to the Brij micelles was found to be as follows: fenoprofen > naproxen > flurbiprofen.

Keywords: Non-ionic surfactant; Micelles; Organic acids; Capillary isotachophoresis; Fluorescence spectrometry

1. Introduction

In capillary electrophoretic (CE) techniques, surfactants are usually applied for the modification of inner capillary surface in order to prevent the undesirable electroosmotic flow and/or to suppress the adsorption of analytes on the capillary surface [1,2]. Micellar electrokinetic chromatography (MEKC) with ionic surfactants has been proved to be a powerful technique for the separation of non-charged substances [3–5]. Non-ionic surfactant micelles were used in MEKC for the separability improvement of some charged analytes as dansyl aminoacids [6], naphthalenesulfonates [7], nitrophenols [8], tricyclic antidepressants [9], tetracycline antibiotics [10], various drugs [11], bromide and nitrate [12]. Non-ionic surfactants can also be employed in MEKC with a combination of ionic surfactants as mixed micelles of non-ionic and ionic surfactants show significantly different selectivity from the ionic surfactant micelles [13,14]. Recently, the influence of micelles of non-ionic surfactant in the leading electrolyte on the separation of dinitrophenyl and dansyl derivatives of amino acids and position isomers of nitrophenols by isotachophoretic focusing (ITF) was studied [15]. The results showed that micelles improved the separation efficiency and selectivity in ITF.

2-Arylpropionic acids (fenoprofen, flurbiprofen, ibuprofen, ketoprofen and naproxen) are non-steroidal anti-inflammatory drugs used in the treatment of rheumatoid arthritis and for mild to moderate pain as relievers. The separation of 2-arylpropionic acids has been investigated by many techniques, such as HPLC [16], capillary zone electrophoresis (CZE) [17], capillary electrochromatography (CEC) [18] and MEKC [19]. CZE with a background electrolyte containing a cyclodextrin as chiral selector is the most frequently

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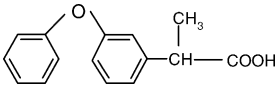
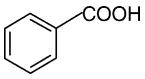
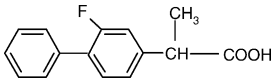
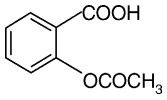
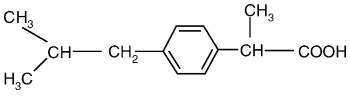
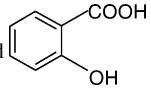
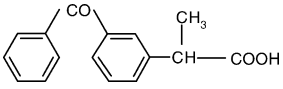
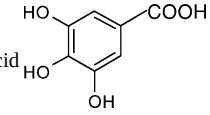
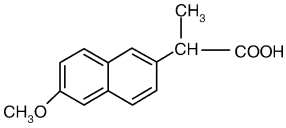
doi:10.1016/j.chroma.2004.08.139

Table 1
Test solutes and their literature [26–32] pK_a and log $P_{o/w}$ values

2-Arylpropionic acid	pK_a	log $P_{o/w}$	Benzoic acid and its derivatives	pK_a	log $P_{o/w}$
Fenoprofen 	4.50 [26]	–	Benzoic acid 	4.20 [29]	1.87 [31]
Flurbiprofen 	4.27 [26]	4.16 [27]	Acetylsalicylic acid 	3.82 [30]	1.19 [27]
Ibuprofen 	4.55 [26]	3.51 [28]	Salicylic acid 	3.11 [29]	2.26 [31]
Ketoprofen 	4.60 [26]	3.12 [28]	Gallic acid 	4.47 [29]	0.91 [32]
Naproxen 	4.08 [26]	3.21 [28]			

used operating mode of CE in resolving of 2-arylpropionic acids enantiomers [20–22]. Capillary isotachophoresis is a convenient alternative for the separation and determination of 2-arylpropionic acids [23–25]. As the pK_a values of the corresponding acids and the ionic mobilities of their anions are close in aqueous solutions, there are limited possibilities for optimizing the isotachophoretic separations using differences in the ionic mobilities or pK_a values. We showed that the effective mobilities of 2-arylpropioonic acids are influenced via the formation of complexes with either β-cyclodextrin or heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin [23]. However, the complete separation was not obtained as using concentrations of cyclodextrins higher than 0.01 mol L^{-1}, the mobility of analytes in isotachophoretic steady state becomes too low and they migrate within the zone of terminating ion.

In this study, non-ionic surfactants (Brij 35, Tween 20, Tween 80 and Tergitol NPX) were investigated as modifying reagent in the leading electrolyte to separate 2-arylpropionic acids, benzoic acid and benzoic acid derivatives by capillary isotachophoresis. Test solutes and their literature pK_a and log $P_{o/w}$ values [26–32] are given in Table 1. The structures of the non-ionic surfactants used are given in Table 2. The interactions of 2-arylpropionic acids with the surfactants were also investigated by the change in fluorescence intensity. The changes both in the mobility and the intensity were compared with each other.

2. Experimental

2.1. Apparatus

2.1.1. Isotachophoresis

Isotachophoretic separations were performed using a ZKI 02 isotachophoretic analyzer (Villa Labeco, Spišská Nová Ves, Slovakia) operated in the single-capillary mode. The analyzer was equipped with a high voltage power supply, delivering the stabilized driving current in the range of 0–500 μA with a maximum voltage of 15 kV, a sample valve of 30 μL fixed volume, a 0.3 mm i.d. capillary tube made of fluorinated ethylene–propylene copolymer of a 160 mm length to the detector and a contact conductivity detector. All measurements were made at constant driving current 80 μA. During detection, the driving current was reduced to 40 μA. During the experiments, the voltage increased from 2 to 7 kV. The isotachopherograms were evaluated by a personal computer software package supplied with the analyzer.

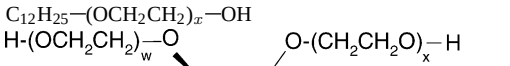
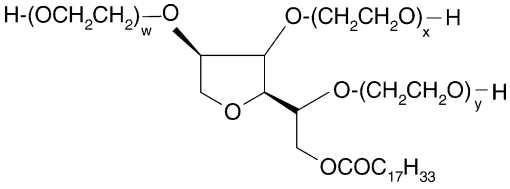
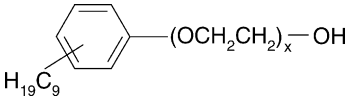
2.1.2. Fluorescence spectrometry

All fluorescence measurements were done on a Perkin-Elmer LS 50 luminescence spectrometer equipped with a xenon discharge lamp (equivalent to 20 kW for 8 μs duration) and 1 cm × 1 cm quartz cell. The LS 50 spectrometer was interfaced with a Epson PC AX2 microcomputer supplied with FL Data Manager Software (Perkin-Elmer) for spectral acquisition and subsequent manipulation of spectra.

2.2. Chemicals

The chemicals used were of analytical reagent grade. Fenoprofen calcium, flurbiprofen, ibuprofen, ketoprofen, naproxen sodium, benzoic acid, gallic acid, polyoxyethylene(10) lauryl ether and Tergitol NPX were obtained from Sigma–Aldrich (Deisenhofen, Germany). Hydrochloric acid, salicylic acid, acetylsalicylic acid, D-glucose, D-fructose, β-alanine, caproic acid and Brij 35 were obtained from Lachema (Brno, Czech Republic). L-Histidine, 4-morpholine propanesulfonic acid (MOPS), Tween 20 and Tween 80 were purchased from Merck (Darmstadt, Germany).

Table 2
Structures of the non-ionic surfactants used

Name	Structure
Brij 35 (polyoxyethylene(23) lauryl ether, x = 23)	
Tween 20 (polyoxyethylene(20) sorbitan monolaurate, x = 20)	
Tween 80 (polyoxyethylene(20) sorbitan monooleate, x = 20)	
Tergitol NPX (polyoxyethylene(9) nonylphenol, x = 9)	

'x' means average number of oxyethylene groups.

Deionized and double distilled water was used to prepare all solutions.

A stock solutions containing 0.2 mmol L^{-1} of analyte dissolved in double distilled water was proposed. Working standard solutions were freshly prepared from the stock solutions diluting with water or with 10 mmol L^{-1} hydrochloric acid adjusted with L-histidine to pH 6.0.

2.3. Isotachophoretic operating conditions

The leading electrolyte containing 10 mmol L^{-1} hydrochloric acid adjusted with L-histidine to pH 6.0 and a certain amount of surfactant (0–3%, w/v) was used. The terminating electrolyte was 5 mmol L^{-1} MOPS. The driving current was initially 80 μA. During detection, the current was reduced to 40 μA.

2.4. Fluorescence spectra measurements

A solution containing analyte, 10 mmol L^{-1} hydrochloric acid adjusted with L-histidine to pH 6.0, and a certain amount of surfactant (0–2%, w/v) was prepared, and the fluorescence spectra was measured.

3. Results and discussion

3.1. Isotachophoresis

To demonstrate the influence of the concentration of surfactants used on the effective mobility and the resolution of the compounds investigated, the relative step height (RSH) = $(h_X - h_L)/(h_T - h_L)$ (where h_X is the step height of the compound, h_L is the step height of the leading ion and h_T is the step height of the terminating ion) were calculated and plotted against the concentration of the non-ionic surfactants.

Fig. 1 illustrates the variation of the RSHs of the 2-arylpropionic acids, benzoic acid and benzoic acid derivatives obtained in a leading electrolyte containing varied concentration of surfactants at pH 6. The concentration of surfactants was varied in the range 0–3.0% (w/v). As show in Figs. 1 and 2A, in the absence of non-ionic surfactants using the leading electrolyte pH 6, 2-arylpropionic acids were not separated. In addition, the RSH difference of benzoic acid and salicylic acid (gallic acid and acetylsalicylic acid) is not significant and the system does not resolve them. However, the separability of the 2-arylpropionic acids was improved by adding non-ionic surfactants into the leading electrolyte. By increasing the amount of surfactant in the leading electrolyte, the RSH of all the analytes increased (decreased effective mobility). Although increasing the concentration of the surfactant enhanced the separability of these anions, higher concentrations of Brij 35 and Tween 80 make demands due to the decrease in differences between the flurbiprofen zone and the terminator zone. Based on our results, it is appeared that the optimum experimental conditions for the separation of the five 2-arylpropinic acids can be obtained if 1–1.5% (w/v) Tween 20 is used.

The strength of the affinity of the micelles to the 2-arylpropionic acids investigated was found to be as follows: Brij 35 > Tween 80 > Tween 20 > Tergitol NPX. The strength of the affinity of the 2-arylpropionic acids to the non-ionic micelles was found to be as follows: flurbiprofen > fenoprofen > ibuprofen > naproxen > ketoprofen. It has been pointed out

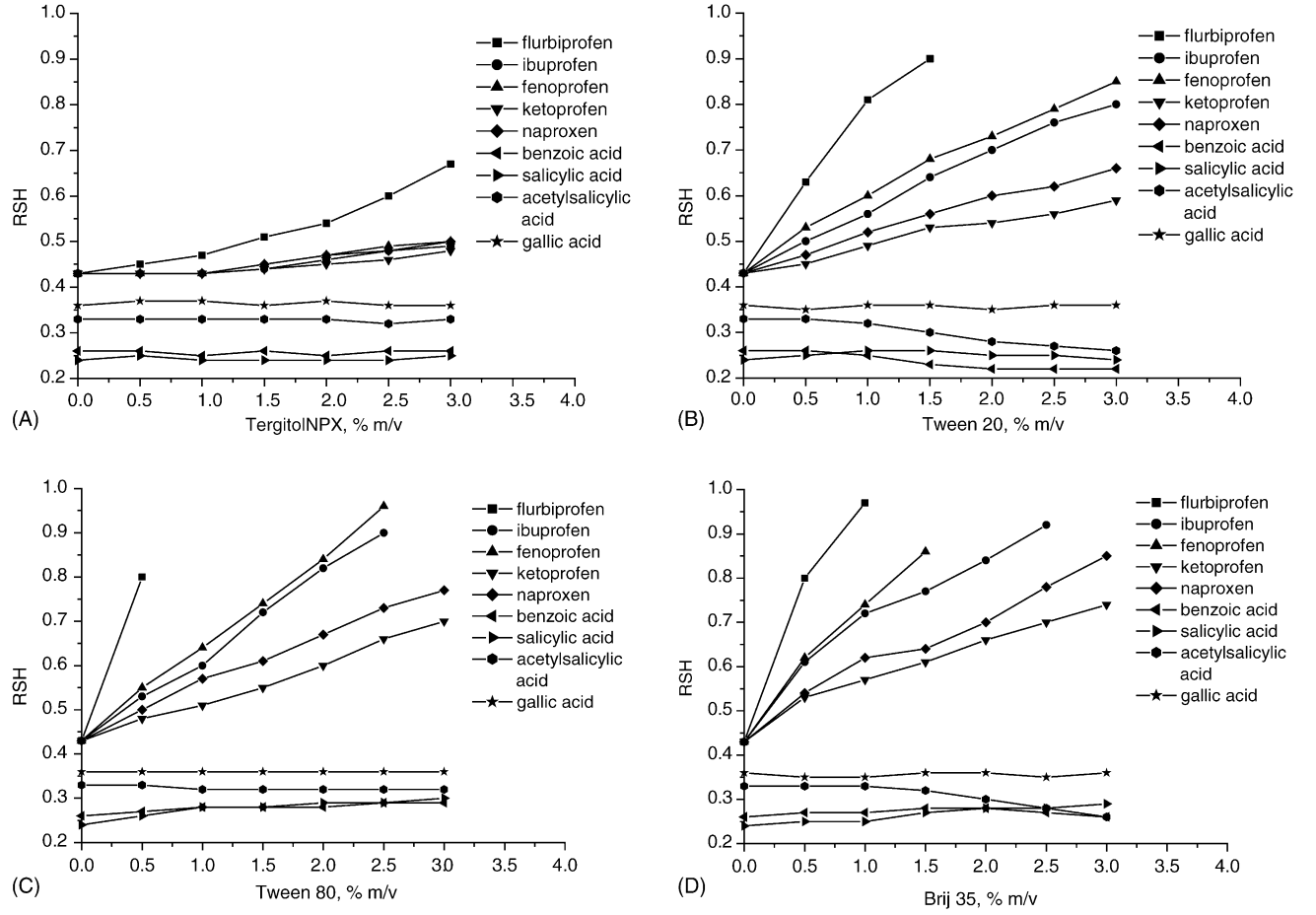


Fig. 1. Relative step heights (RSHs) of analytes as a function of surfactants concentrations (%, w/v): (A) Tergitol NPX, (B) Tween 20, (C) Tween 80, (D) Brij 35. Isotachophoretic conditions: the leading electrolyte, 10 mmol L^{-1} hydrochloric acid adjusted with L-histidine to pH 6.0; the terminating electrolyte, 5 mmol L^{-1} MOPS; the driving current, 40 μA.

in the literature that the octanol–water partition coefficient, $\log P_{o/w}$, has some predictive possibility for MEKC retention order. In our case, the migration order of 2-arylpropionic acids is in agreement with their octanol–water partition coefficients, $\log P_{o/w}$. Since the $\log P_{o/w}$ of flurbiprofen is the highest of all, very strong affinity with increasing concentration of Brij 35 and Tween 80 was observed for flurbiprofen, compared to other drugs. Non-ionic micelles used here possess a hydrophobic core and a hydrophilic surface. Since the 2-arylpropionic acids are charged and reasonably

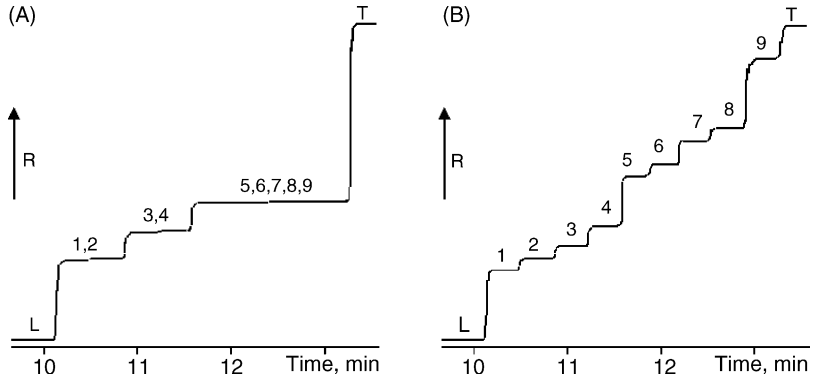


Fig. 2. Isotachopherograms for nine kinds of anions in the absence (A) and presence of non-ionic surfactant (B). The leading electrolyte: (A) 10 mmol L^{-1} hydrochloric acid adjusted with L-histidine to pH 6.0, (B) 10 mmol L^{-1} hydrochloric acid adjusted with L-histidine to pH 6.0 + 1.5% (w/v) Tween 20. The terminating electrolyte, 5 mmol L^{-1} MOPS; the driving current, 40 μA; sample solution: 0.1 mmol L^{-1} of each. (1) Benzoic acid, (2) salicylic acid, (3) acetylsalicylic acid, (4) gallic acid, (5) ketoprofen, (6) naproxen, (7) ibuprofen, (8) fenoprofen, (9) flurbiprofen.

hydrophobic, analyte–micelle interactions may be due to adsorption on the polar surface as well as inclusion in the hydrophobic core. For the separation of 2-arylpropionic acids, the difference in hydrophobicity may be much more important since they are approximately fully dissociated at pH 6.0. Pai and Liu [18] prepared a wall-coated capillary column containing L-histidine functional groups and employed that for the capillary electrochromatographic separation of nonsteroidal anti-inflammatory drugs. In this work, methanol, ethanol and 1-propanol were tested as organic modifiers of the electrolyte. It was found that higher concentration of the modifier caused a decrease in the effective mobilities of all analytes. The elution order was: ketoprofen > naproxen > ibuprofen > fenoprofen > flurbiprofen, which is consistent with the isotachophoretic results.

Furthermore, the influence of the concentration of surfactants on the effective mobilities of benzoic, gallic, salicylic and acetylsalicylic acids was compared. The influence on the effective mobility was smaller if any in comparison to that of 2-arylpropionic acids. It should be noted that under optimum conditions found for 2-arylpropionic acids (1.5% (w/v) Tween 20 in the leading electrolyte), the benzoic acid and its derivatives were also separated (Fig. 2B). Moreover, we must conclude from the comparison of Table 1 and Fig. 1 that the migration order of the benzoic acid and its derivatives does not follow the same migration order as for their $\log P_{o/w}$ probably due to low hydrophobicity of the analytes. The results implies that depending on the structure of the analytes and the surfactants, various chemical interactions additional to hydrophobic interactions, such as dipole interaction and hydrogen bonding, may occur between them in the partitioning process. Considering the pK_a of the benzoic acid and its derivatives it is obvious that separation can be improved at low pH, where the sample components are only partially dissociated. Therefore, the pH of the leading electrolyte used for the separation of the benzoic acid and its derivatives was optimized to 2.8. Using the leading electrolyte containing 10 mmol L^{-1} hydrochloric acid adjusted with β-alanine to pH 2.8 and a certain amount of surfactant (0–3%, w/v) and the terminating electrolyte 5 mmol L^{-1} caproic acid, the resolution was better than with the leading electrolyte pH 6. However, at pH 2.8, there was no significant effect of the surfactants on the RSHs of the benzoic, salicylic and gallic acids (data not shown).

3.2. Fluorescence spectrometry

Surfactant micelles have been used in spectrometry to enhance luminescence by compartmentalizing or solubilizing fluorophores, e.g. dansyl amino acids and poly nuclear aromatic hydrocarbons [33]. The absorbance and fluorescence spectra of naproxen are well studied in homogeneous [34] and heterogeneous (e.g. cyclodextrins, polyethylene glycol) [35] media. Fluorescence has become the preferred detection technique in HPLC for the determination of flurbiprofen [16].

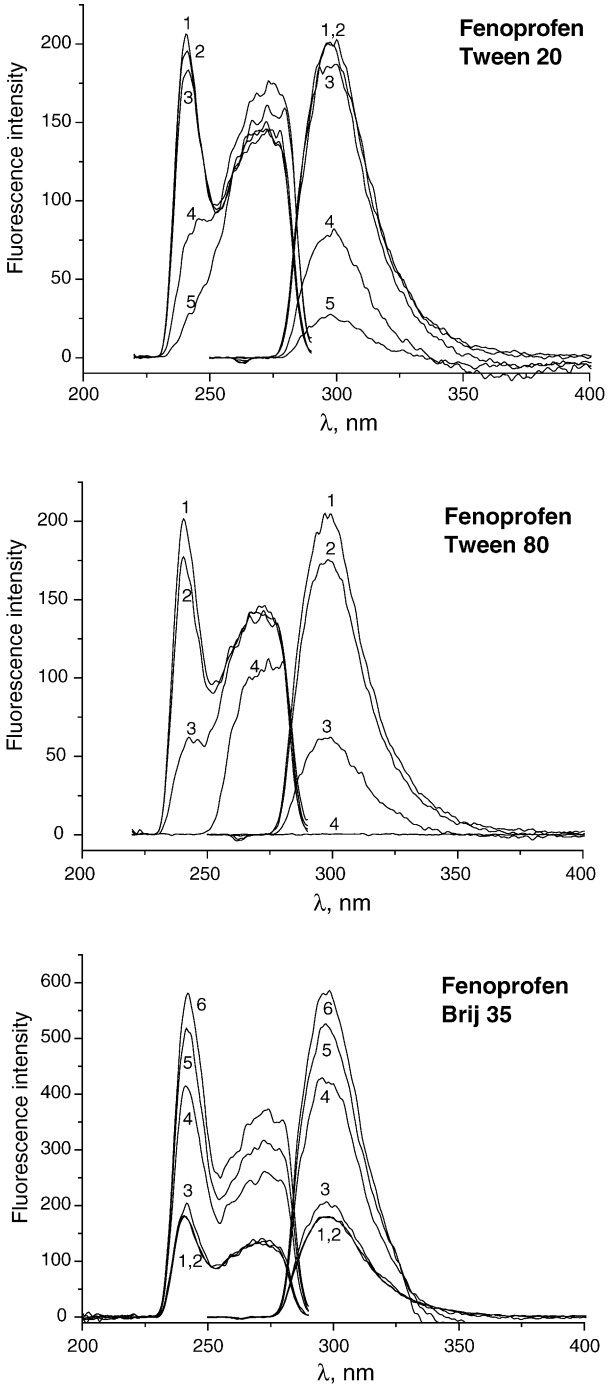


Fig. 3. Corrected excitation and emission spectra of fenoprofen at different Tween 20, Tween 80 and Brij 35 concentrations: (1) 0% (w/v), (2) 0.005% (w/v), (3) 0.05% (w/v), (4) 0.5% (w/v), (5) 1% (w/v), (6) 2% (w/v) of surfactant. Buffer: 10 mmol L^{-1} HCl adjusted with L-histidine to pH 6.0. Fenoprofen: 24 mg L^{-1}. λ_{ex} = 241 nm, λ_{em} = 298 nm.

In this work, we have investigated the excitation and emission spectra for fenoprofen, flurbiprofen and naproxen in L-histidine. HCl buffer and in micellar media like Tween 20, Tween 80 and Brij 35. The micellar concentrations are kept above critical micellar concentration for all the micellar results except for 0.005% (w/v) Tween 20. Fluorescence spectra

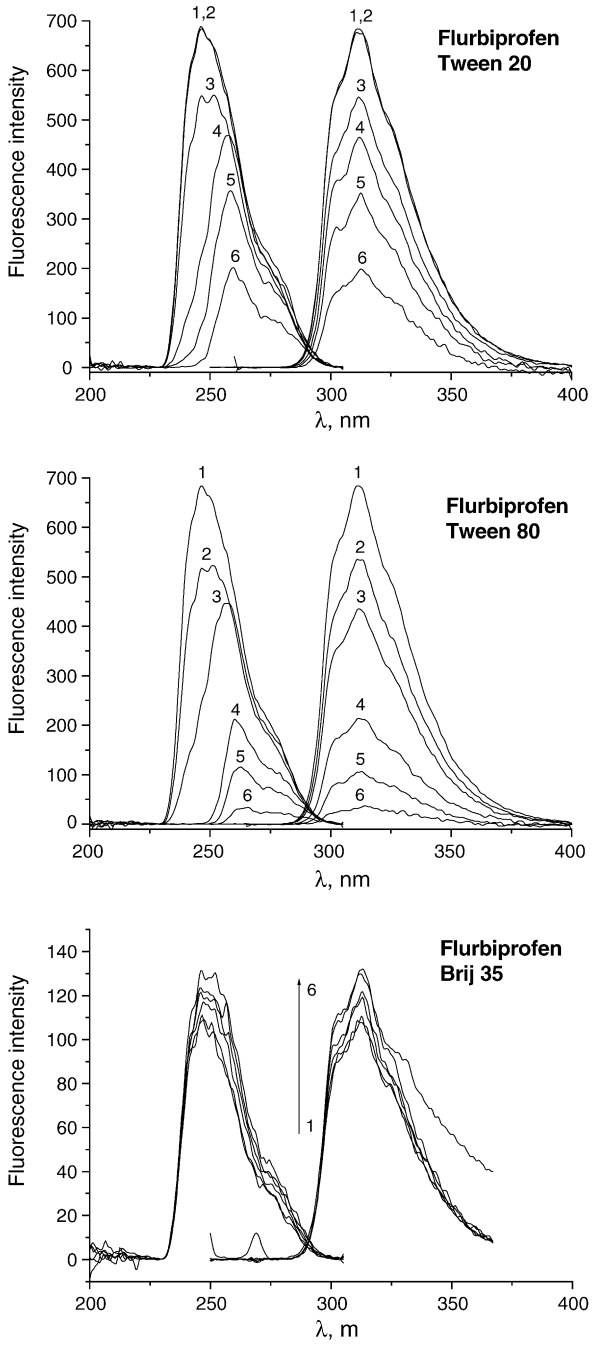


Fig. 4. Corrected excitation and emission spectra of flurbiprofen at different Tween 20, Tween 80 and Brij 35 concentrations: (1) 0% (w/v), (2) 0.005% (w/v), (3) 0.05% (w/v), (4) 0.5% (w/v), (5) 1% (w/v), (6) 2% (w/v) of surfactant. Buffer: 10 mmol L^{-1} HCl adjusted with L-histidine to pH 6.0. Flurbiprofen: 0.9 mg L^{-1} in Tweens, 0.09 mg L^{-1} in Brij 35. λ_{ex} = 246–258 nm (Tween 20), λ_{ex} = 246–262 nm (Tween 80), λ_{ex} = 246 nm (Brij 35), λ_{em} = 312 nm.

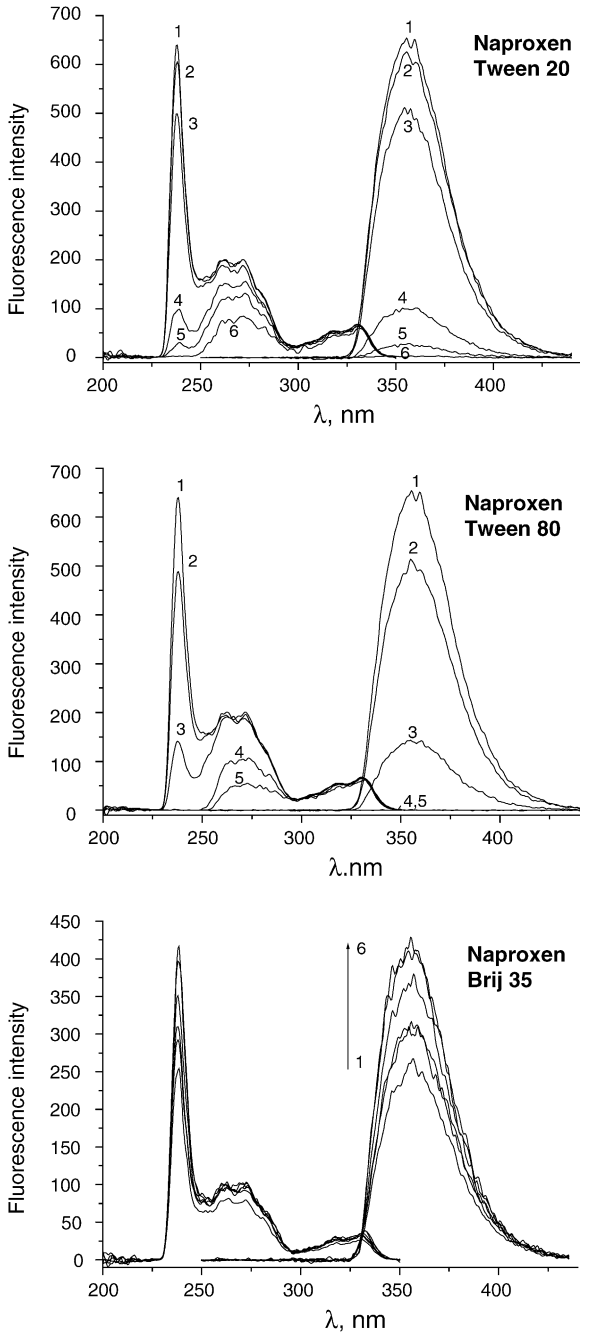


Fig. 5. Corrected excitation and emission spectra of naproxen at different Tween 20, Tween 80 and Brij 35 concentrations: (1) 0% (w/v), (2) 0.005% (w/v), (3) 0.05% (w/v), (4) 0.5% (w/v), (5) 1% (w/v), (6) 2% (w/v) of surfactant. Buffer: 10 mmol L^{-1} HCl adjusted with L-histidine to pH 6.0. Naproxen: 0.5 mg L^{-1} in Tweens, 0.2 mg L^{-1} in Brij 35. λ_{ex} = 238 nm, λ_{em} = 357 nm.

of fenoprofen, flurbiprofen and naproxen in L-histidine. HCl buffer at different concentrations of Tweens and Brij 35 are shown in Figs. 3–5. From Figs. 3 and 5, one can see that for fenoprofen and naproxen, no changes in the excitation and emission maxim are observed in all of the surfactants, which indicates that the anions are not incorporated in the hydrophobic core of the surfactant micelles. For flurbiprofen, distinct changes in the excitation maxim are observed with increasing concentration of Tween 20 and Tween 80 (Fig. 4). This effect was more evident for Tween 80 than Tween 20.

In general, the fluorescence intensity significantly decreased with increasing concentration of Tweens. Such changes in spectra can be attributed to the existence of an

interaction between the drug and the micelle. The fluorescence intensity decreased with increasing number of the Tweens, i.e. with increasing length of the surfactant non-polar tail. This corroborates the findings of isotachophoretic studies. The strength of the affinity of the 2-arylpropionic acids to the Tweens micelles was found to be as follows: flurbiprofen > fenoprofen > naproxen, which is consistent with the isotachophoretic results.

The non-ionic micelles Tweens are compounds of a fixed length of 20 polyoxyethylene polar head groups, a sorbitan moiety and a variable length of polymethylene chain in the molecule (monolaurate and monooleate in Tween 20 and Tween 80, respectively). On a comparative basis, Tween 20 and Brij 35 (both having identical non-polar tails and almost equal number of polyoxyethylene residues in the head group) have unequal quenching powers, quenching is effective in the Tweens only. This indicates the role of the sorbitan moiety in Tweens, which is absent in the Brij 35. The assistance of the quenching process by a carbohydrate moiety has been observed in the aqueous solutions of D-glucose and D-fructose (results are not presented).

Since, in Tweens, the number of polyoxyethylene groups is fixed and the polymethylene non-polar tail varies in length, the drug–micelle interactions with Brij 35, in which the non-polar tail is fixed and the polyoxyethylene head group varied, would be interesting to obtain a better understanding of the involvement of the polyoxyethylene moiety under different chemical conditions. The strength of the affinity of the 2-arylpropionic acids to the Brij 35 micelles was found to be as follows: fenoprofen > naproxen > flurbiprofen. The fluorescence intensity for fenoprofen significantly increased with increasing concentrations of Brij 35. On the other hand, the fluorescence intensity for flurbiprofen was almost identical even in the presence of Brij 35 at higher concentrations. Comparing the fluorescence intensity in Brij 35 (polyoxyethylene(23) lauryl ether) with those of polyoxyethylene(10) lauryl ether, the values are found to be almost identical, which suggests that the analyte anions should be bound to the polyoxyethylene moiety of the micelles.

4. Conclusions

The separation of benzoic, salicylic, acetylsalicylic, gallic acid and five 2-arylpropionic acids is effectively achieved by isotachophoresis with the use of Tween 20 micelles. The migration order of 2-arylpropionic acids depends primarily on the hydrophobic interactions of each analyte with the Tween micelles. However, electrostatic interactions may also play a significant role. The RSH values of these analytes were quite reproducible, with relative standard deviation varying in the range 1.1–1.8 ($n = 5$). The limits of detection (LODs) at a current equal to 40 μA determined with conductivity detection were in the range $1–2.10^{-6}$ mol L^{-1}. The LODs obtained by the proposed isotachophoretic method were almost equal to those obtained by CE methods [18,19,22–25]. Previous results have shown that such LODs are acceptable to the analysis of pharmaceutical [25] and some biological samples [19,22–24]. In the case of pharmaceutical samples, isotachophoresis is a very useful technique, given that there is no problem of sensitivity, the analysis is quick, easy and cheap. Therefore, the proposed method will be applied to the determination of 2-arylpropionic acids in pharmaceutical samples.

Another interesting aspect of this work is the fluorescence intensity increase of fenoprofen in presence of Brij 35 micelles. Fenoprofen is weakly fluorescent in neat aqueous media due to rapid rotation of phenyl rings, which provides a non-radiative path for decay of the excited state. However, in micellar media with, e.g. higher local viscosity, which decreases collision-induced non-radiative decay the fluorescence quantum yield increases with a subsequent increase in analytical sensitivity.

Acknowledgements

This research was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences No. 1/9128/02.

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