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Capillary zone electrophoresis and micellar electrokinetic capillary chromatography of some non-steroidal antiinflammatory drugs (NSAIDs)*

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Abstract: The possibilities of capillary electrophoresis (CE) and micellar electrokinetic capillary chromatography (MEKC) were investigated for the qualitative analysis of some non-steroidal antiinflammatory drugs. In CE the influence of the pH of the buffer and its ionic strength were investigated for a test mixture of six compounds. Also the influence of organic modifiers was studied. The best conditions were applied to the separation of 15 drugs. In MEKC the influence of the concentration of SDS in buffers with pH ranges of 8.0–9.0 was investigated. The influence of an organic modifier, namely acetonitrile was discussed, whereby an interesting phenomenon of change in retention behaviour was noted. A combination of CE and MEKC allows the separation of the 15 above-mentioned compounds and forms an interesting alternative to HPLC.

Keywords: *Non-steroidal antiinflammatory drugs; capillary electrophoresis; micellar electrokinetic capillary chromatography.*

Introduction

Electrodriven analysis of drugs [1–6] is becoming more and more important in pharmacokinetic studies, to monitor intermediates during chemical manufacturing of drugs and in quality control of pharmaceutical preparations.

Non-steroidal antiinflammatory drugs (NSAIDs) are used for their antiinflammatory action and, in lower dosages, for their analgesic effect. This class contains the following chemical groups: arylacetic acid derivatives; arylpropionic acid derivatives; indolic acid derivatives; fenamic acid derivatives; and oxicams.

Till recently, most drug analyses, including those of the most common NSAIDs, were performed by HPLC [7–9]. Compared with HPLC, capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC) provide higher resolution, less sample and lower cost for reagents and columns. The analysis time, too, is usually shorter compared with HPLC.

Non-steroidal antiinflammatory drugs have not been yet widely studied; there are only few papers on capillary electrophoresis of some of these compounds [10–13].

Many factors affect efficiency and resolution in electrodriven separations; e.g. sample time, choice of buffer (pH and ionic strength), addition of organic modifier to the run buffer, amount of surfactant added to the buffer, etc. These parameters were studied in order to get the best experimental conditions for the separation of 15 NSAIDs, the structures of which are shown in Fig. 1.

For most practical applications of CZE and MEKC in drug analysis, the separation of the 15 NSAIDs will not be required. Optimization of the separation of the 15 NSAIDs however provides broad information of the CZE and MEKC behaviour of NSAIDs and general applicability of the best conditions.

Experimental

Apparatus

CZE and MEKC were performed on a Waters Quanta 4000 instrument. A fused silica capillary, 60 cm × 75 μm i.d. (52.5 cm to the detector) was used as a separation column.

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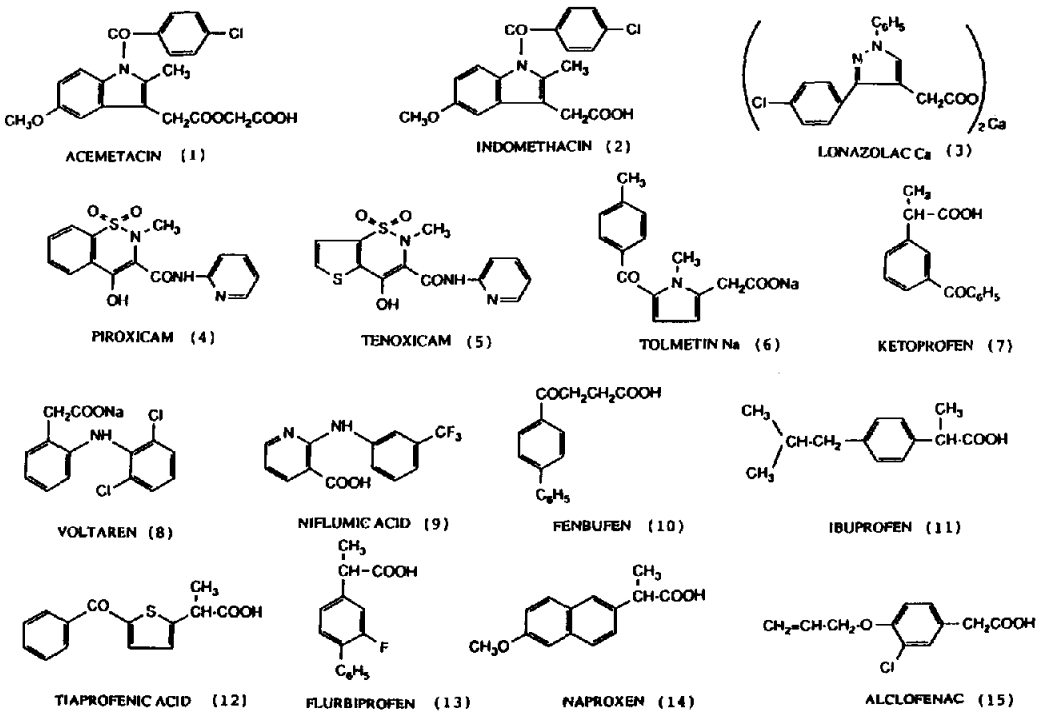

Figure 1
Structures of the NSAIDs.

Detection was performed by on-column UV absorption at 214 nm. Sample introduction was made by hydrostatic injection by raising the capillary 9.8 cm higher than the buffer reservoir for 5 s. The experiments were carried out at ambient temperature (*ca* 22°C). Formamide was used to measure the electroosmotic flow. A Hewlett–Packard Integrator (HP 3396 Series II) was used for data processing.

Reagents

Acemetacin was obtained from Tropon, alclofenac from Continental Pharma, fenbufen from Lederle–Cyanamid, flurbiprofen from The Boots Co., ibuprofen and ketoprofen from Bayer Leverkusen, indomethacin from Merck Sharp & Dohme, lonazolac calcium from Byk Belga, naproxen from UCB, niflumic acid from Qualiphar, piroxicam from Pfizer, tenoxicam from Roche, tiaprofenic acid from Erfa, tolmetin sodium from Cilag, voltaren from Ciba–Geigy. SDS was obtained from Janssen. The reagents used to prepare the buffer solutions were all of analytical grade. The buffers were prepared by mixing stock solutions in appropriate ratios to give the pH values of interest. Prior to use, both electrolytes and samples were sonicated for 5 min. The buffers and SDS solutions were freshly prepared every day. The samples were prepared in stock solutions in acetonitrile except for lonazolac calcium in acetone, tolmetin sodium in water, voltaren methanol, and then diluted with run buffer to obtain a final concentration of 0.025 mg ml^{-1} for each of the 15 drugs.

Results and Discussion

Capillary zone electrophoresis

CZE conditions were optimized with a test mixture of acemetacin, tenoxicam, tolmetin, niflumic acid, flurbiprofen and alclofenac. Sodium phosphate buffer was chosen to study the effect of ionic strength in the pH range 7.0–8.0. Phosphate buffers of 10, 20 and 30 mM were tested. The best results were obtained with 30 mM phosphate pH 7.0, although niflumic acid and flurbiprofen were not baseline separated. Figure 2 shows the electropherogram of the 15 drugs carried out in 30 mM phosphate buffer pH 7.0. The separation is incomplete and some drugs coelute: tolmetin and ketoprofen; voltaren and niflumic acid; fenbufen, ibuprofen and tiaprofenic acid.

The separation was not improved by using borate buffers, 50 and 75 mM at pH 8.5 and 9.0.

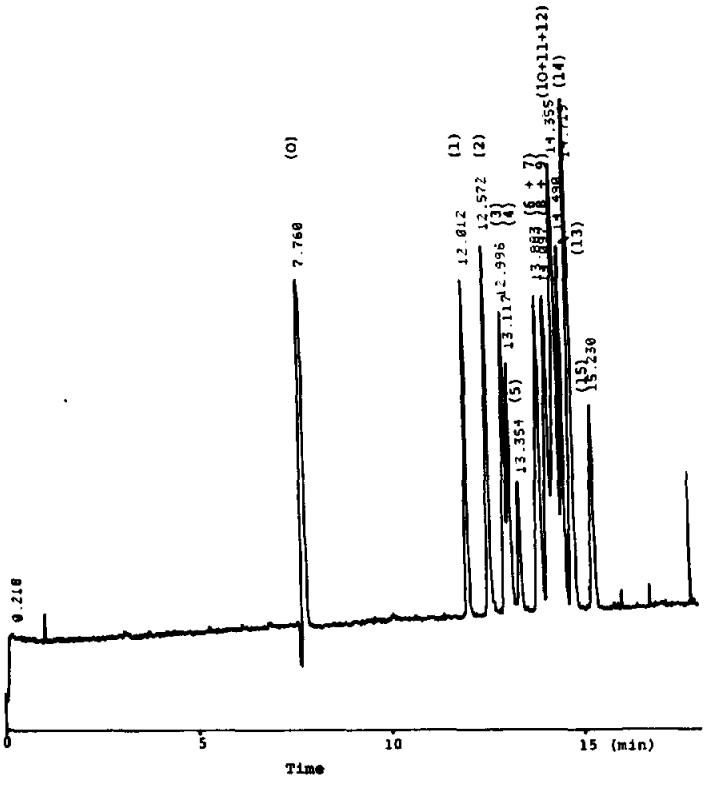

Figure 2
Electropherogram of 15 NSAIDs in 30 mM phosphate buffer pH 7.0. 0, formamide; 1–15, see Fig. 1.

The addition of organic modifiers to the run buffer for the separations of positional isomers of substituted benzoic acids [14] or human erythropoietin [15] improved the resolution. The effect of methanol and acetonitrile was therefore tested on the separation of the test mixture of the six drugs, analysed in 30 mM phosphate buffer pH 7.0. With 5–20% methanol, the separation did not improve, the only effect being a decrease in efficiency and longer migration times. Acetonitrile, on the other hand, had a better effect on the separation, without great loss in efficiency and with acceptable elution times. Figure 3 shows the separation of the 15 drugs analysed in 30 mM phosphate pH 7.0 with 20% acetonitrile. Compared with the analysis in pure buffer, a remarkable improvement in separation is obtained. Only two pairs of drugs coelute, namely ketoprofen–fenbufen and niflumic acid–ibuprofen.

Micellar electrokinetic capillary chromatography

Although MEKC was developed for the analysis of neutral compounds, it also proved useful in the separation of charged solutes [16–18].

The influence of pH and SDS concentration was studied in the separation of tenoxicam, alclofenac, tolmetin, niflumic acid, flurbiprofen and acemetacin. Borate buffers of 50 mM, pH 8.0, 8.5 and 9.0 with SDS concentrations ranging from 20 to 60 mM were evaluated. The best results were obtained with 40 mM SDS in 50 mM borate buffer pH 9.0. Figure 4 shows a chromatogram of the 15 drugs under these conditions. The elution order is completely altered in MEKC where micellar solubilization and electroosmotic mobility control the separation. As in CZE, there is coelution of some compounds: piroxicam–tenoxicam ketoprofen–alclofenac and naproxen–tiaprofenic acid–ibuprofen–tolmetin.

The improvement in resolution with an organic modifier in MEKC is known [19]. Since we are concerned with the separation of the chiral drugs of this class, the influence of methanol and acetonitrile was therefore tested on the MEKC separation of flurbiprofen, ibuprofen, ketoprofen, naproxen and tiaprofenic acid. The five compounds were analysed

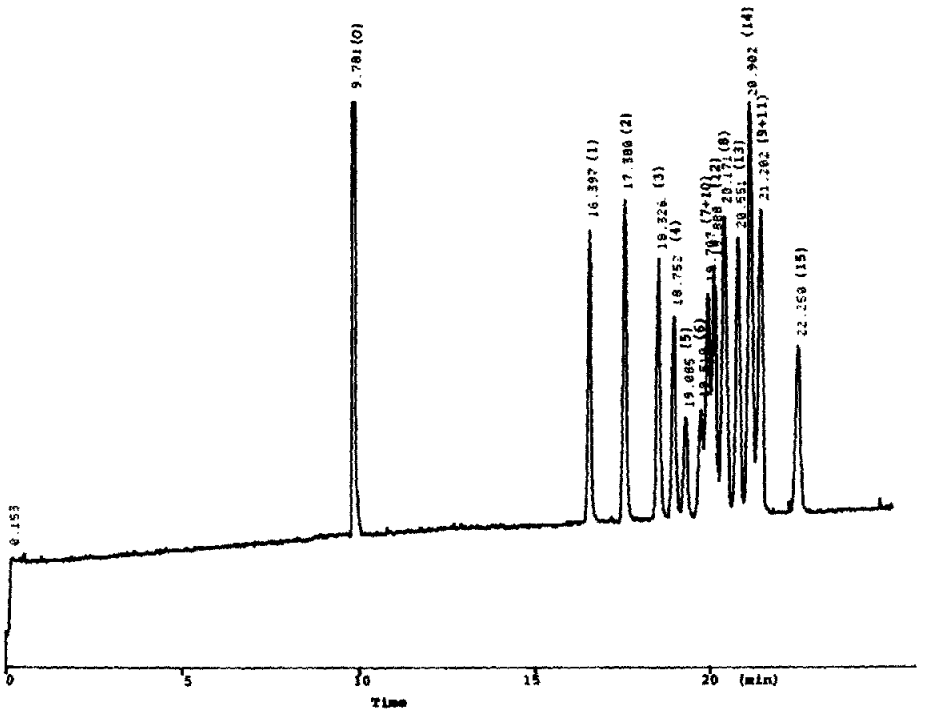

Figure 3
Electropherogram of 15 NSAIDs in 30 mM phosphate buffer pH 7.0 with 20% acetonitrile. 0, formamide; 1–15, see Fig. 1.

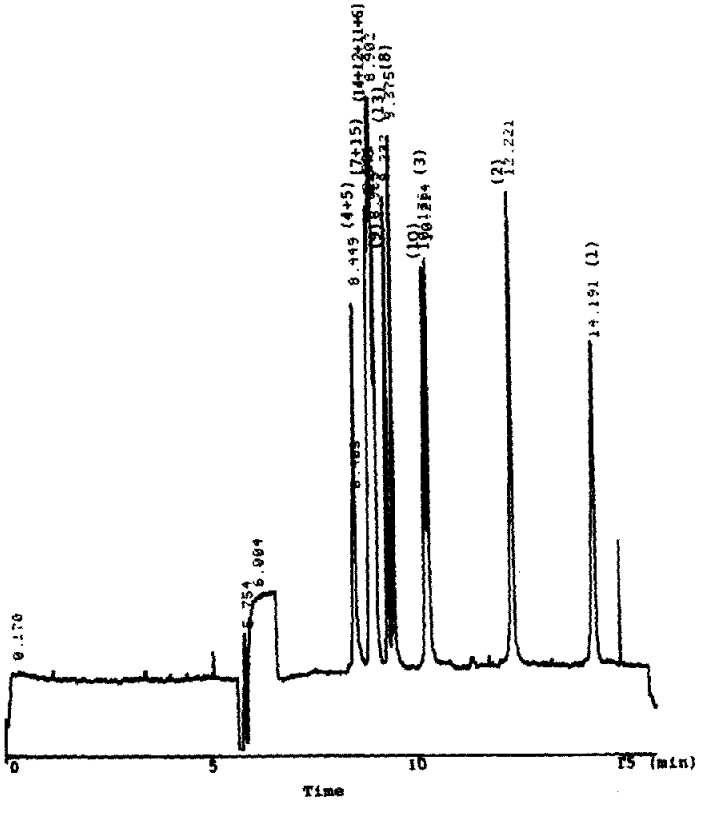

Figure 4
Chromatogram of 15 NSAIDs with 40 mM SDS in 50 mM borate buffer pH 9.0. 0, acetonitrile; 1–15, see Fig. 1.

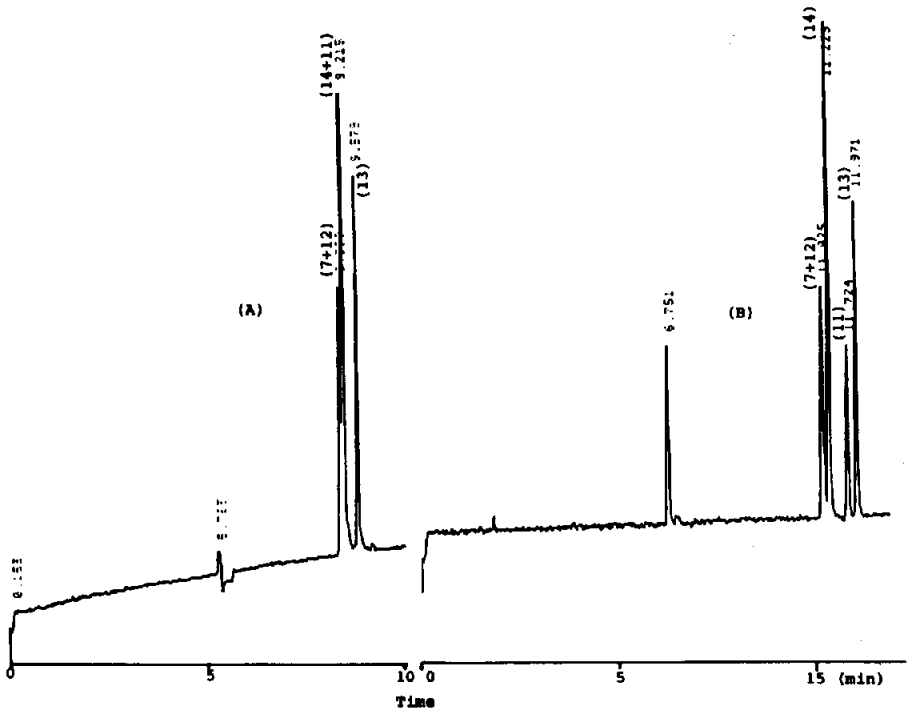

Figure 5
Comparison of the separation of some NSAIDs (A) with 40 mM SDS in 50 mM borate buffer pH 9.0 and (B) with 40 mM SDS in 50 mM borate buffer pH 9.0 + 10% acetonitrile. For peak assignment see Fig. 1.

by adding 5–20% methanol or acetonitrile to 40 mM SDS in 50 mM borate buffer pH 9.0. With methanol the separation did not improve from the one without methanol. For acetonitrile, the effect is positive up to 15%, whereas for 20% peaks were broad and the separation was not different from the one carried out in pure buffer. The best separation was obtained with 10% acetonitrile. The organic modifier improves the separation of ketoprofen and tiaprofenic acid from naproxen and alters the micellar retention of ibuprofen, making it more included in the pseudo-stationary phase, whereby a separation of naproxen and ibuprofen is accomplished. This effect is illustrated without [Fig. 5(A)] and with [Fig. 5(B)] the addition of acetonitrile.

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