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# Electrokinetic supercharging for on-line preconcentration of seven non-steroidal anti-inflammatory drugs in water samples

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**Abstract**

The development of new sensitive methods for the analysis of non-steroidal anti-inflammatory drugs (NSAIDs) in water samples is of great importance. In this work, seven NSAIDs were separated within 9 min using 15 mM sodium tetraborate (pH 9.2) containing 0.1% (w/v) hexadimethrine bromide (HDMB) and 10% (v/v) methanol. Field-amplified sample injection (FASI) was examined and found to improve the detection limits by 200-fold providing detection limits of 0.6–2.0 μg/L, but these are insufficient for the determination of NSAIDs as environmental pollutants in water samples. To improve the sensitivity further, electrokinetic supercharging (EKS) was examined. The optimum EKS method involved hydrodynamic injection leading electrolyte (100 mM NaCl, 30 s, 50 mbar), electrokinetic injection of the sample (200 s, −10 kV) and finally injection of the terminating electrolyte (100 mM 2-(cyclohexylamino) ethanesulphonic acid, CHES, 40 s, 50 mbar). With this method, the sensitivity was improved by 2400-fold giving detection limits of 50–180 ng/L. The developed method was validated and then applied to the analysis of wastewater samples from a local sewage treatment plant. The detection limits were found to increase by approximately 10-fold, however, this is still lower than levels previously found in wastewater samples from European and Mediterranean cities. The proposed method has the advantage of simplicity and achieving sensitivity through high-preconcentration power without the use of off-line chromatographic sample cleanup.


*Keywords:* Capillary electrophoresis; Electrokinetic supercharging; Non-steroidal anti-inflammatory drugs; Field-amplified sample injection; Water samples

## 1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used widely for several decades for the treatment of different inflammatory disorders, pain relief, and also for their antipyretic effect; some NSAIDs are available without prescription. Because of their high solubility and poor degradability in water, elimination of NSAIDs in sewage treatment plants (STPs) is rather low and consequently they are able to penetrate through all natural filtration steps and enter groundwater as well as drinking water [1]. The continuous environmental input of such drugs may lead to a relatively high long-term concentration and thereby to promote continuous, but unnoticed adverse effects on aquatic and terrestrial organisms [2]. Different toxicological studies have been performed showing the possible environmental hazards of NSAIDs, for example ibuprofen stimulates the growth of the cyanobacterium *Synechocystis*, inhibits the growth of duckweed *Lemna minor* [3] and results in steroidogenesis in rainbow trout [4]. Chronic exposure to diclofenac leads to renal damage in brown trout [5] and rainbow trout [6]. More significantly, it has been found that a mixture of NSAID analgesics was toxic for certain aquatic organisms at concentrations at which the single compounds showed no or only little effects [7].

Because of its advantageous high separation efficiency and fast analysis time, capillary electrophoresis (CE) has been proven to be a useful technique for the separation and determination of NSAIDs in a range of sample matrices. Different CE modes have been described for the analysis of NSAIDs, including; capillary zone electrophoresis (CZE) [8–23], capillary electrochromatography [24,25], micellar electrokinetic capillary chromatography (MEKC) [8,26–31], microemulsion electrokinetic chromatography (MEEKC) [32,33], and isotachophoresis (ITP) [34,35]. While excellent separations can be obtained by these approaches, the major disadvantage of CE is its low concentration detection limit. This is due to the very small optical path length for spectrophotometric detection (typically 50–100 μm) and the limited amount of sample

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that can be introduced into the capillary (typically <1 μL). This drawback is very important when low limits of detection are required, such as for the analysis of many environmental and biological samples. This can be overcome by on-line concentration and various electrophoretic and chromatographic approaches have been developed to improve the sensitivity of CE [36,37]. A number of these approaches have been applied to the analysis of NSAIDs, including field-amplified sample injection with sample matrix removal using electroosmotic flow (EOF) pumping (FAEP), large volume sample stacking with EOF pumping (LVSEP), LVSEP with anion selective exhaustive injection (ASEI) [21,38,39], stacking with reversed migrating micelles (SRMM), SRMM-ASEI, and field-enhanced sample injection with reverse migrating micelles (FESI-RMM) [31]. Stacking with these approaches has been shown to improve the sensitivity of the method (based on peak area) by factors ranging between 100 and 1800-fold, giving detection limits as low as 100 ng/L. However, even this level of sensitivity requires an additional solid phase extraction and enrichment step when dealing with real water samples. A system that could achieve the detection limits required for environmental analysis of NSAIDs without the need for off-line sample processing would obviously have significant advantages.

A recent on-line preconcentration method for CE that has great potential is that of electrokinetic supercharging (EKS). This is the combination of electrokinetic injection under field-amplified conditions (field-amplified sample injection, FASI) and transient isotachophoresis (tITP) and was first described for the analysis of rare-earth ions [40,41] by the group of Hirokawa. EKS was developed to extend the range of FASI and is performed by hydrodynamic injection of a leading electrolyte, followed by EKI of the analytes, and finally hydrodynamic injection of a terminating electrolyte. Upon applying the separation voltage the diffuse band of analytes introduced during electrokinetic injection is stacked between the leading and the terminating electrolytes by tITP until the ITP stage destacks and the analytes are allowed to separate by conventional CE. EKS is an exceptionally simple but powerful approach to on-line sample preconcentration and has been shown to improve the sensitivity of analytical response by several orders of magnitude.

In the current work, the separation and preconcentration of seven NSAIDs by CE has been examined, with emphasis on the development of a simple and rapid CE method for the determination of these NSAIDs in environmental water samples. Towards this end, co-EOF separations have been performed using an EOF reversal agent and the separation optimized by variation of the composition of the electrolyte (electrolyte concentration and methanol content). After selectivity optimization, the potential of FASI and EKS for on-line enrichment of NSAIDs has been examined. The results presented in this work provide the lowest detection limits for these NSAIDs without using off-line solid phase extraction for sample enrichment, and the preconcentration procedure takes less time than previously published methods for these analytes.

## 2. Experimental

### 2.1. Standards and reagents

Naproxen was purchased from Fluka (Buchs, Switzerland), while diclofenac, diflunisal, fenoprofen, ibuprofen, indomethacin, ketoprofen, 2-(cyclohexylamino) ethanesulphonic acid (CHES), and hexadimethrine bromide (HDMB) were from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide (98%) and disodium tetraborate decahydrate were from BDH (Kilsyth, Australia). Sodium chloride was from M&B Pronalys Analytical Reagents (West Footscray, Australia). Methanol (HPLC-Grade) was from Ajax Finechem (Seven Hills, Australia). Water was treated with a Millipore (North Ryde, Australia) Milli-Q water purification system.

A stock standard solution of 1 mg/ml of each drug was prepared in methanol. A mixed standard solution of the seven NSAIDs was prepared at a concentration of 0.1 mg/ml in methanol. The working standard solutions were prepared daily by diluting the stock standard solution with Milli-Q water. All solutions were stored in dark containers at 4 °C.

The working background electrolyte (BGE) solution had a concentration of 15 mM of disodium tetraborate (pH 9.2) containing 10% methanol and 0.1% HDMB unless otherwise stated. The buffer solutions were prepared freshly each day, sonicated for 5 min and filtered through a 0.45 μm membrane filter.

### 2.2. Instrumentation

Electrophoretic separations were performed using an Agilent $3^D$ CE (Agilent Technologies, Waldbronn, Germany) equipped with a UV diode-array detection (DAD) system operating at 214 nm. Separations were carried out using fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 85 cm total length (76.5 cm effective length) and 50 μm i.d. The capillary temperature was set at 25 °C.

New capillaries were flushed with 1 M sodium hydroxide for 120 min, with Milli-Q water for 20 min, and with the BGE for 10 min. Each day the capillaries were equilibrated by rinsing with 1 M sodium hydroxide for 10 min, with HDMB (1%, w/v) for 10 min, and with the BGE for 5 min.

### 2.3. Field-amplified sample injection

The analytes dissolved in Milli-Q water were injected into the capillary electrokinetically with a negative voltage (−3 kV) for 40 s. All NSAIDs are weakly acidic (Fig. 1) and under the conditions used these analytes were negatively charged and migrated into the capillary by a combination of electrophoretic migration and EOF.

### 2.4. Electrokinetic supercharging

A small volume of the leading electrolyte (100 mM sodium chloride) was introduced into the capillary by hydrodynamic injection at 50 mbar for 30 s, then the sample was injected electrokinetically by a negative voltage (−10 kV) for 200 s, and