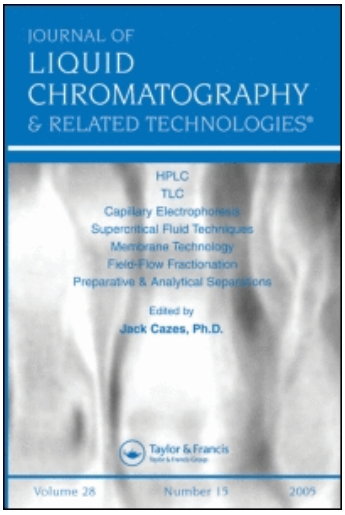

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Capillary Electrophoretic Enantiomeric Separations of Nonsteroidal Anti-inflammatory Compounds Using the Macrocyclic Antibiotic Actaplanin A and 2-Methoxyethanol

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CAPILLARY ELECTROPHORETIC ENANTIOMERIC SEPARATIONS OF NONSTEROIDAL ANTI-INFLAMMATORY COMPOUNDS USING THE MACROCYCLIC ANTIBIOTIC ACTAPLANIN A AND 2-METHOXYETHANOL

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ABSTRACT

Actaplanin A, a macrocyclic antibiotic, was examined as a chiral selector in capillary electrophoresis (CE) for the enantioseparation of several racemic nonsteroidal anti-inflammatory compounds. The chiral selectivity of this macrocyclic antibiotic was evaluated as a function of the run buffer pH, chiral selector concentration, and organic modifier composition. Optimized conditions using 15-30% of 2-methoxyethanol and 0.5 mM actaplanin A in 40 mM phosphate buffer (pH 6) were successful in separating all of the enantiomers from the racemic compounds tested in this study.

INTRODUCTION

Enantiomers of chiral drugs often possess distinctly different characteristics in their pharmacological activity, and in most cases the effectiveness and possible toxicity of a pharmaceutical drug may be affected by the presence of an unwanted enantiomer. Therefore, it is important in the pharmaceutical laboratory to resolve enantiomeric drugs and quantitate the undesired enantiomer.

One of the techniques used for such separations of optical isomers is CE. The use of CE in the separation of enantiomers is of great interest because of the rapid separations and high resolutions attained utilizing only small volumes of sample and run buffer. Addition of a chiral selector to the free buffer solution has emerged as the method of preference for achieving chiral separations by CE. Among various chiral additives, cyclodextrins have been most commonly used because of their ability to resolve a large number of chiral compounds.[1-4] Other additives such as surfactants, proteins, bile salts, and crown ethers have also been applied.[5-7]

Recently, several macrocyclic antibiotics have been examined as chiral additives and have been shown to be very successful in achieving enantioseparations. Such antibiotics include vancomycin, rifamysins, ristocetin, teicoplanin, and other vancomycin analogs such as A82846B and LY307599.[8-14] These macrocyclic antibiotics contain moieties capable of providing several potential interaction sites for chiral recognition between enantiomers such as numerous stereogenic centers, hydroxy groups, aromatic rings for π-π bonding, and amide bonds. Another feature in these macrocyclic antibiotics is the presence of multiple cavities which possibly serve as inclusion pockets.

Actaplanin A, evaluated in this paper, contains all of these chiral recognition criteria, therefore making this macrocyclic antibiotic a likely chiral selector candidate. The enantioselectivity of actaplanin A was evaluated by CE using the following six nonsteroidal anti-inflammatory compounds as the test analytes: ketoprofen, flurbiprofen, carprofen, fenoprofen, suprofen, and indoprofen.

EXPERIMENTAL

All separations were carried out using an automated Beckman P/ACE System Model 2100 capillary electrophoresis instrument (Fullerton, CA)

equipped with an on-column variable wavelength detector set at 254 nm. Analog data were collected on an in-house chromatography computer system from Hewlett Packard Model 1000 (Palo Alto, CA). The fused silica capillary (I.D. 50 μm, 57 cm total length) was purchased from Beckman (Fullerton, CA).

The capillary was first rinsed with 0.1 N NaOH for 5 minutes, followed by water for 10 minutes and then the run buffer for 10 minutes. All separations were carried out at ambient temperatures. Enantiomeric separations were performed with the detector at the cathode end, using applied voltage of 25 or 30 kV. Samples were injected into the capillary using a pressure injection for 5 seconds.

Actaplanin A hydrochloride was synthesized at Eli Lilly and Company (Indianapolis, IN). The nonsteroidal anti-inflammatory compounds ketoprofen, indoprofen, suprofen, fenoprofen, flurbiprofen, and carprofen, and the organic modifier 2-methoxyethanol were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Potassium dihydrogen phosphate was obtained from Mallinckrodt (Paris, KY). Phosphoric acid and sodium hydroxide were purchased from EM Science (Gibbstown NJ). Methanol and acetonitrile were obtained from Curtin Matheson Scientific (Houston, TX). Purified water was used from a Milli-Q® purification system from Millipore Corporation (Bedford, MA).

Sample solutions were prepared at 1 mg/mL in the run buffer. In order to enhance solubility, a small aliquot of organic modifier was added prior to the buffer for carprofen and fenoprofen when pH 6 buffer was used, and in all analytes when using buffer at pH 5. Run buffers and sample solutions were prepared daily and filtered through nylon Acrodisc® syringe filters (0.2 μm) from Gelman Sciences (Ann Arbor, MI).

RESULTS AND DISCUSSION

Actaplanins are gram positive antibiotics that are produced by *Actinoplanes missouriensis*. They are members of the glycopeptide family and are related to ristocetin and ristomycin. The core structure contains an amino sugar and several neutral sugars attached to a peptide group of aromatic amino acids. They differ only in their content and in distribution of attached sugar units.[15-16] The structure of actaplanin A is shown in Figure 1.

One of the problems associated with using actaplanin A, and most macrocyclic antibiotics in general, as chiral selectors is the UV absorbance of these compounds. The macrocyclic antibiotic actaplanin A absorbs strongly in

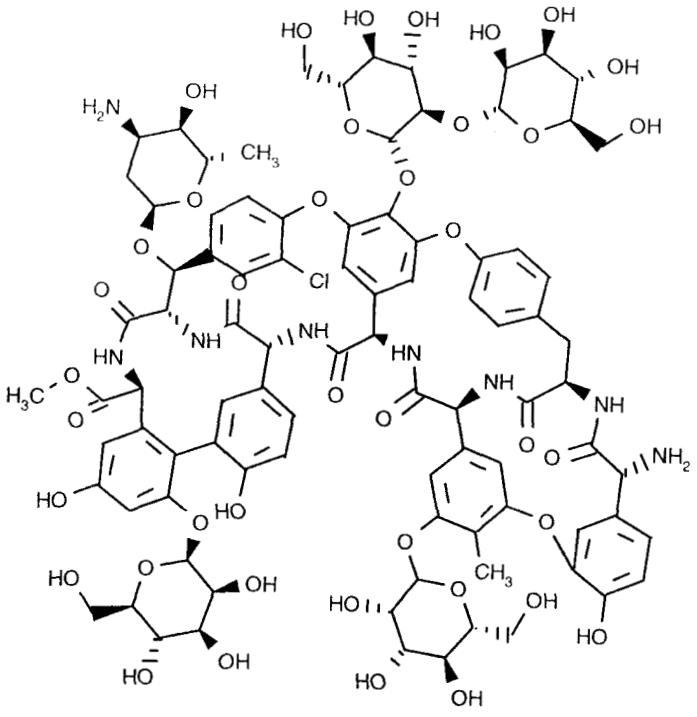


Figure 1. The structure of actaplanin A hydrochloride (MW =1970.27).

the lower UV range as shown in Figure 2. However, actaplanin A does not absorb strongly at 254 nm, allowing for the detection of the test analytes ketoprofen, flurbiprofen, fenoprofen, carprofen, suprofen, and indoprofen, shown in Figure 3.

It is well known that pH is a very effective experimental variable in manipulating chiral recognition and selectivity since the ionization of both the analyte and chiral selector can be controlled by this parameter. Previous reports using macrocyclic antibiotics as chiral selectors in CE indicate that the most successful chiral separations are achieved when using buffer of pH range between 5-7.[8-9] Therefore, this pH range using 40 mM potassium phosphate buffer containing actaplanin A was selected for evaluating the resolution of the test analytes. The concentration of actaplanin A was maintained at 2 mM, a typical concentration noted to be successful for other macrocyclic antibiotics evaluated using this technique.[8,10]

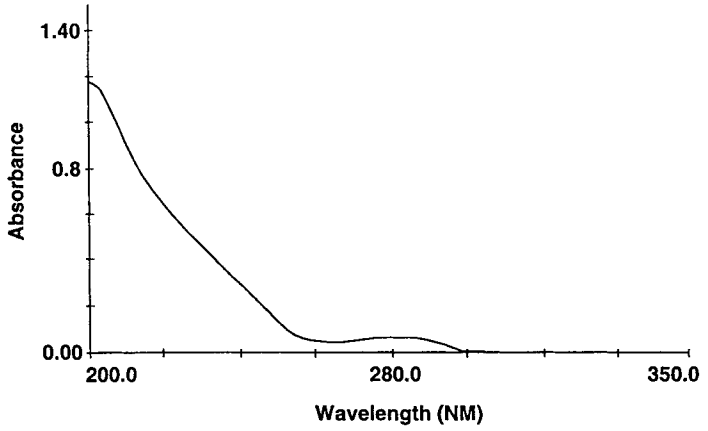


Figure 2. UV spectrum of actaplanin A hydrochloride in 40 mM phosphate buffer pH 6.

Table 1

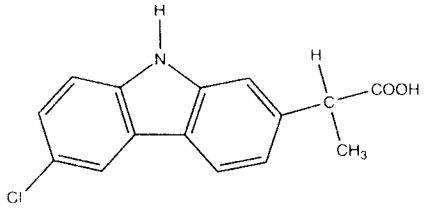
Effect of Buffer pH on the Resolution of the Enantiomers from Nonsteroidal Anti-Inflammatory Compounds Using 2mM Actaplanin A*

Compound	Resolution Value pH 5.0	pH 6.0	pH 7.0
Fenoprofen	1.9	2.1	1.7
Ketoprofen	3.2	4.2	2.2
Flurbiprofen	1.3	2.1	0.9
Indoprofen	1.3	1.2	0.0
Suprofen	0.7	0.4	0.0
Carprofen	0.0	0.0	0.0

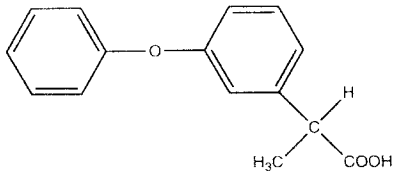
* Run Voltage +30 kV.

Table 1 lists the resolution values obtained for the six anti-inflammatory compounds using 2 mM actaplanin A in 40 mM phosphate buffer at pH 5, 6 and 7 with a run voltage of +30 kV. The resolution for ketoprofen, flurbiprofen, and fenoprofen enantiomers demonstrates that the greatest resolution is achieved at pH 6, whereas suprofen and indoprofen show

(a)



(b)



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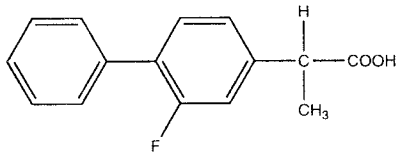

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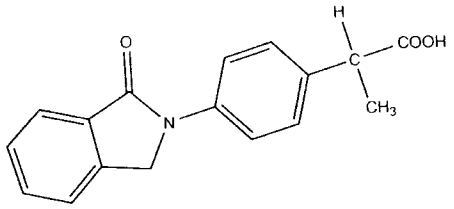
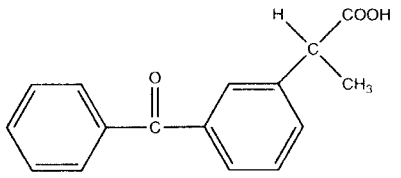


(e)



(f)

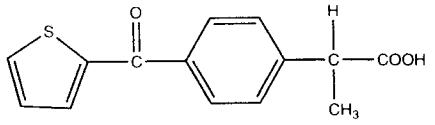


Table 2

Effect of Actaplanin A Concentration on the Enantiomeric Resolution of Group 1 Compounds Using Buffer pH 6.0*

Group 1	Resolution Value 0.5 mM	1.0 mM	2.0 mM
Ketoprofen	1.0	1.9	3.6
Flurbiprofen	0.4	0.7	1.7
Fenoprofen	0.9	2.1	3.1

* Run voltage +25 kV.

improved resolution at pH 5. There was no resolution obtained for the enantiomers of carprofen using these conditions. As the pH of the buffer is increased to pH 7, the analytes are negatively charged and migrate opposite to the electroosmotic flow (EOF) while the chiral selector at this pH still possesses a positive charge (mobility of 0.6 x 10^{-4} cm^2/Vs) allowing it to interact electrostatically with the analytes and move in the direction of the EOF.

Even though charge to charge interactions play an important role, there are other parameters that can influence enantioresolution as well, such as the EOF. The EOF in the presence of the actaplanin A chiral selector in the run buffer increased from 2.2 x 10^{-4} cm^2/Vs at pH 5 to 4.6 x 10^{-4} cm^2/Vs at buffer pH 7. Due to this increase in the EOF, the analytes experienced less time for interaction with the chiral selector, actaplanin A.

Once the optimum pH was defined for each of the compounds, further optimization steps were accomplished by investigating a range of actaplanin A concentrations from 0.5 mM to 2 mM, in an attempt to enhance enantiomeric separation. Based on resolution results from the pH study, the compounds were divided into two groups for further testing: pH 6 buffer was used for ketoprofen, flurbiprofen, and fenoprofen (group 1) and pH 5 buffer was used for indoprofen, suprofen, and carprofen (group 2). While the pH of the 40 mM phosphate buffer was maintained at pH 6, the concentration of actaplanin A was increased from 0.5 to 2.0 mM using a run voltage of +25 kV for group 1 compounds. Resolution values for this set of experiments is shown in Table 2.

Figure 3. (left) The nonsteroidal anti-inflammatory compounds: (a) carprofen, (b) fenoprofen, (c) flurbiprofen, (d) indoprofen, (e) ketoprofen and (f) suprofen.

Table 3

Effect of Actaplanin A Concentration on the Enantiomeric Resolution of Group 2 Compounds Using Buffer pH 5.0*

Group 2	Resolution Value 0.5 mM	1.0 mM	2.0 mM
Suprofen	0.0	0.8	1.0
Indoprofen	0.7	1.3	1.9
Carprofen	0.0	0.0	0.0

* Run voltage +25 kV.

All three compounds were separated with greater than baseline resolution (1.5) at the high concentration of 2 mM actaplanin A. Only partial separation was obtained for the flurbiprofen enantiomers using 1.0 mM actaplanin A and for all of the group 1 compounds using 0.5 mM actaplanin A.

As expected, the increased amount of actaplanin A enhanced the complex of analyte-selector formation and improved the resolution. Actaplanin A concentrations above 2 mM would be expected to improve resolution even more since there is increased time for interaction between the analytes and the chiral selector as well as increased amount of actaplanin A available, but concentrations above 2 mM resulted in longer migration times and noisier, erratic baselines. The EOF of the system using 40 mM phosphate buffer pH 6 was determined for methanol to be 4.4×10^{-4} cm^2/Vs. The addition of 1.5 mM actaplanin A to the 40 mM phosphate buffer (pH 6) reduced the EOF to 2.5×10^{-4} cm^2/Vs, while the EOF was further decreased to 0.97×10^{-4} cm^2/Vs with the addition of 3.0 mM actaplanin A to the 40 mM phosphate buffer (pH 6). The increased migration times at higher concentrations of actaplanin A is a result of the EOF slowing considerably. Previous reports on other antibiotics as chiral selectors have indicated that the decrease in EOF is due to possible interactions of the antibiotic with the walls of the capillary.[8,12,17]

Next, using the 40 mM phosphate buffer at pH 5, group 2 compounds were tested. For this set of experiments only the indoprofen enantiomers showed complete baseline resolution using 2 mM actaplanin A as shown in Table 3. Partial separation was achieved with 1.0 mM actaplanin A for the suprofen enantiomers, whereas the carprofen enantiomers could not be separated under any of these conditions.

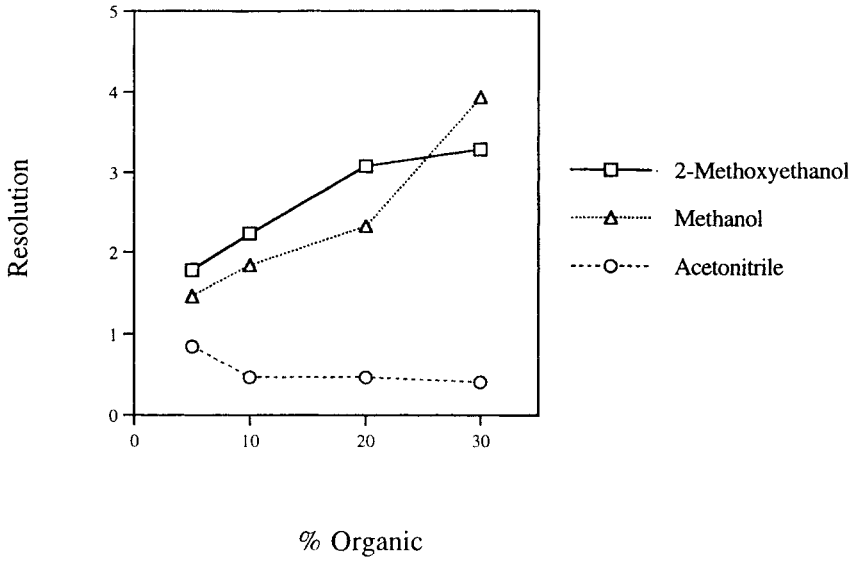


Figure 4. Effect of organic modifier on the resolution of ketoprofen enantiomers. Aqueous buffer component: 40 mM phosphate buffer (pH 6) containing 0.5 mM actaplanin A hydrochloride.

The addition of organic modifiers to the run buffer has been noted to affect enantioselectivity and migration times when using rifamycin B as a chiral selector.[14] Therefore, in order to enhance the enantioseparation using a minimal amount of actaplanin A, the organic modifiers acetonitrile, methanol and 2-methoxyethanol were tested ranging from 5-30% in 40 mM phosphate buffer pH 6.0 containing 0.5 mM of the macrocyclic antibiotic. From Tables 2 and 3, ketoprofen was the sample that yielded the best overall resolution values and was therefore selected for this set of experiments.

As shown in Figure 4, increasing the organic content of 2-methoxyethanol and methanol resulted in an increase in resolution of the ketoprofen enantiomers. However, in the case of acetonitrile, resolution was diminished with increasing the organic modifier from 5% to 30%. Migration times also increased as the percent organic increased for all three of the organic modifiers. The electropherogram, shown in Figure 5, illustrates this effect using 2-methoxyethanol for the separation of the ketoprofen enantiomers.

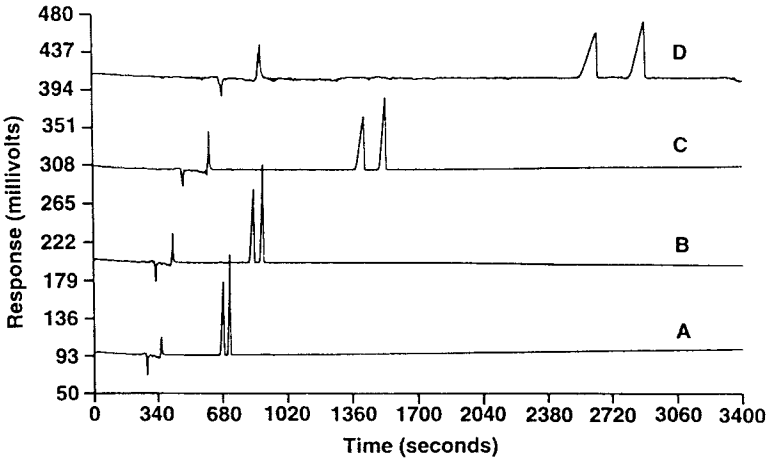


Figure 5. Electropherograms showing the effect of increasing 2-methoxyethanol concentration on the resolution of ketoprofen enantiomers. The percent 2-methoxyethanol is (A) 5%, (B) 10%, (C) 20% and (D) 30%. The aqueous buffer component is 40 mM phosphate buffer (pH 6) containing 0.5 mM actaplanin A hydrochloride.

The type of the organic modifier used was very important in altering the enantioselectivity for this macrocyclic antibiotic chiral selector. The improvements in resolution with 2-methoxyethanol and methanol were more dramatic than increasing the actaplanin A concentration and buffer pH. This was also the case reported with rifamycin B but not for vancomycin when 2-propanol was studied.[8] Acetonitrile and methanol have been frequently used in CE buffers, however, to our knowledge this is the first report of using 2-methoxyethanol, although it has been reported to be an excellent solvent for selectivity studies in HPLC and over-pressured layer chromatography (OPLC).[18-19]

The selectivity difference of 2-methoxyethanol compared to other alcohols may be due to the presence of the methoxy group which increases the polarity of this molecule and in turn may result in the enhancement of charge to charge interactions within the system. This ethylene glycol ether provided a unique selectivity alternative to traditional solvents and proved to be an excellent organic modifier for these CE enantioseparations. When examining baseline noise and peak shape, 2-methoxyethanol gave better overall results than methanol.

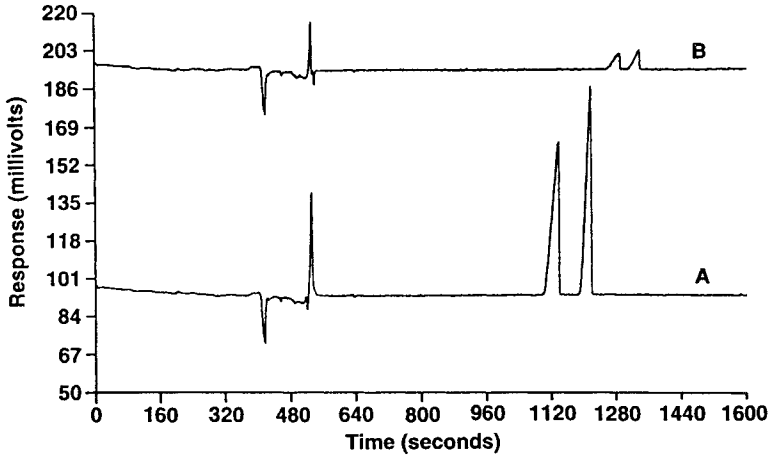


Figure 6. Electropherograms showing the separation of (A) ketoprofen and (B) fenoprofen enantiomers using 15:85 (v/v) 2-methoxyethanol/40 mM phosphate buffer (pH 6) containing 0.5 mM actaplanin A hydrochloride; run voltage +25 kV.

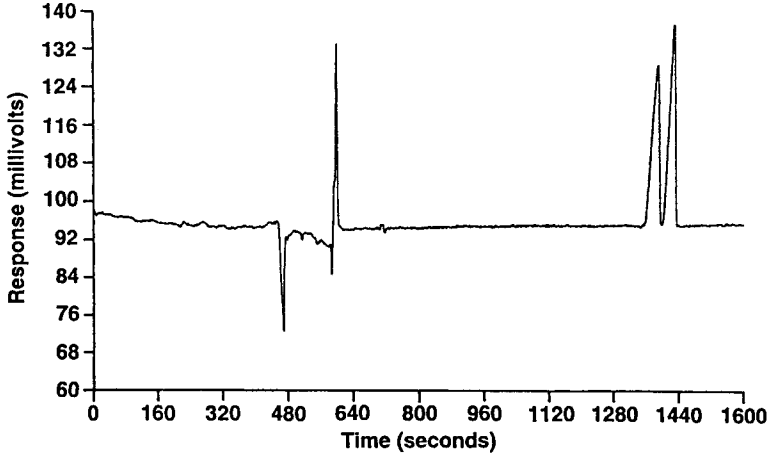


Figure 7. Electropherograms showing the separation of suprofen enantiomers using 20:80 (v/v) 2-methoxyethanol/40 mM phosphate buffer (pH 6) containing 0.5 mM actaplanin A hydrochloride; run voltage +25 kV.

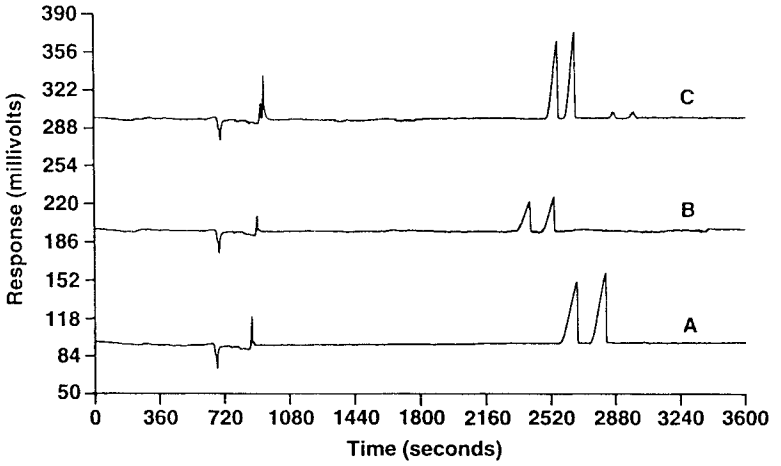


Figure 8. Electropherograms showing the separation of (A) flurbiprofen, (B) indoprofen and (C) carprofen enantiomers using 30:70 (v/v) 2-methoxyethanol/40 mM phosphate buffer (pH 6) containing 0.5 mM actaplanin A hydrochloride; run voltage +25 kV.

We attribute the enhancement in resolution of the ketoprofen enantiomers in these experiments to be a combination of a decrease in EOF through dynamic modification of the capillary wall or change in viscosity, a decrease in conductivity, an increase in analyte solubility and the unique selectivity of 2-methoxyethanol. Based on these parameters, 2-methoxyethanol was selected as the organic modifier of choice for further evaluation of the other test analytes.

Next, the remaining anti-inflammatory compounds were tested by maintaining the aqueous buffer at 40 mM phosphate pH 6 containing 0.5 mM actaplanin A while increasing the concentration of the organic modifier, 2-methoxyethanol. The enantiomers of fenoprofen were separated using only 15% of 2-methoxyethanol. Figure 6 illustrates the separation of the fenoprofen and ketoprofen enantiomers using 15% of 2-methoxyethanol in the run buffer comprising 40 mM phosphate pH 6 containing 0.5 mM actaplanin A. The enantiomers of suprofen were separated using 20% of 2-methoxyethanol (Figure 7), whereas the enantiomers of flurbiprofen, indoprofen, and carprofen required 30% of 2-methoxyethanol (Figure 8) in order to achieve baseline resolution. The resolution values and migration times for the resolved enantiomers are shown in Table 4.

Table 4

Resolution and Migration Times for the Enantiomers of the Nonsteroidal Anti-Inflammatory Compounds Using Buffer pH 6 with 0.5 mM Actaplanin A and 2-Methoxyethanol

Compound	Resolution	Migration 1 (Minutes)	Migration 2 (Minutes)	% Organic
Fenoprofen	2.1	18.9	20.3	15
Ketoprofen	2.8	21.4	22.2	15
Flurbiprofen	2.3	44.3	47.0	30
Indoprofen	2.7	40.0	42.2	30
Suprofen	1.3	23.1	23.8	20
Carprofen	1.9	42.5	44.1	30

Using optimized conditions, all six nonsteroidal anti-inflammatory compounds were separated into their respected enantiomers using only 0.5 mM actaplanin A in the run buffer. The use of such small amounts of this chiral selector will allow for improved sensitivity and detection of other racemic compounds at lower wavelengths.

CONCLUSION

Actaplanin A has been shown to be another successful macrocyclic antibiotic that can be effectively used as a chiral selector. In particular, the utilization of actaplanin A was successfully evaluated in the chiral resolution of the six nonsteroidal anti-inflammatory compounds ketoprofen, flurbiprofen, fenoprofen, carprofen, indoprofen, and suprofen, by adjusting several experimental parameters such as buffer pH, antibiotic concentration, and organic content. One of the most effective parameters for enhancing the enantiomeric separations was the use of 2-methoxyethanol. All of the enantiomers of the model compounds were separated requiring only 15-30% of 2-methoxyethanol, as well as, the use of only a very small amount of the chiral selector (0.5 mM) actaplanin A.

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