Chiral Separation of Ruthenium Bipyridyl Complexes and β-Lactams by Capillary Electrophoresis

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Abstract

The goal of this project was to separate the enantiomers of twelve racemic β-lactams using capillary electrophoresis (CE) following the work of Jiang et al. and then to apply this technique to chirally separate a series of ruthenium bipyridyl complexes. These ruthenium complexes had already been successfully separated with high performance liquid chromatography (HPLC), but never with CE. Sulfated α-cyclodextrin (SAC) served as the chiral selector for the β-lactams, and was able to give at least a partial separation for eight of them. Two of four ruthenium bipyridyl complexes were separated using sulfated β-cyclodextrin (SBC). Higher concentrations of cyclodextrin in the running buffer usually resulted in longer migration times and better resolution for the enantiomers, due to greater residence time of the analyte with the chiral selector.

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Introduction

Capillary electrophoresis (CE) is a relatively new technique that can be useful for the separation of biologically important chiral and nonchiral molecules. A great number of medicines, such as aspirin and mifepristone (the "morning-after" pill), are made from one enantiomer of a chiral compound, with the other enantiomer usually having very different effects [1]. To avoid these other effects, it is necessary to separate chiral molecules either with high performance liquid chromatography (HPLC) or CE. While HPLC has been the most popular technique in past years, an increasing number of reports are being written on enantioselective CE approaches, with promising results. CE is beneficial in its use of very small samples of analyte and its relative efficiency when compared with other methods of enantiomeric separation [2].

Electrodes are placed in two buffer solutions so that the ends of the capillary, which are also in solution, have opposite charges (see Figure 1). By applying a current, there will be a bulk movement through the capillary known as the electroosmotic flow (EOF) [2]. This is due to a buildup of surface charge on the interior wall of the capillary. Cations are attracted to the cathode, or the negatively charged electrode, while anions move toward the anode, or the positively charged electrode. Since the EOF is greater than these other forces affecting the solutes, all molecules within the capillary eventually flow toward the detection end at the cathode. CE is able to separate molecules depending on their charge and size, since these properties allow them to travel through the capillary at different velocities. The molecules are then detected by using their ultraviolet absorption. The greater absorbance of the analyte, compared with that of the running buffer, causes it to show up as a peak on an electropherogram.

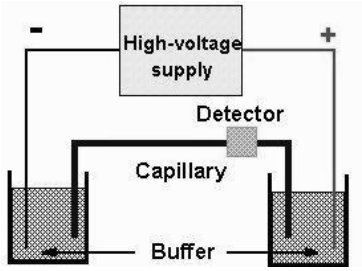

Figure 1 – CE mechanism

Cyclodextrins (CDs) are cyclic glucose oligosaccharides widely regarded as excellent chiral selectors that come in a variety of forms [4]. They have minimal ultraviolet absorbance, making them very useful for CE since this technique depends on UV light for analyte detection. The center of a CD molecule is hydrophobic and the outside surface is hydrophilic. While α-CD consists of six glucopyranose units (see Figure 2), β-CD has seven and γ-CD has eight. Thus, α-CD is the smallest CD and is suitable for the separation of β-lactams. Since CDs have a basket shape, it is preferable to choose a CD close in size to the analyte, as this strategy allows for the best complexation to occur. However, it is not always possible to predict the particular bonding pattern between an analyte and the chiral selector, making it essential to try different kinds of CDs.

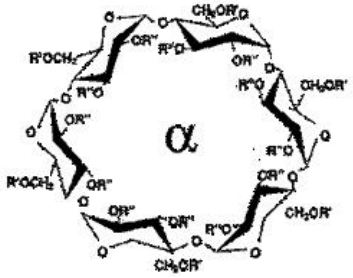

Figure 2 – Alpha cyclodextrin

Procedure

Either a Beckman P/ACE 2050 or P/ACE 2100 (Fullerton, CA, USA) was used to carry out all separations. Data was analyzed using Beckman System Gold software. The capillaries used for these experiments were purchased from Polymicro Technologies (Phoenix, AZ, USA), with an inner diameter of 50 μm and an outer diameter of 358 μm. The capillary length was 37 cm (30 cm from inlet to detector). The buffer for both separations was a mixture of a 2.5 mM solution of NaH_2PO_4 and a 2.5 mM solution of Na_2HPO_4 (which had a final pH of 7.2). SAC and SBC were both obtained from Aldrich Chemical Company (Milwaukee, WI, USA). The wavelength of detection was 214 nm for all experiments. The chiral β-lactam compounds were donated by Dr. Antal Peter of the Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary, and the ruthenium bipyridyl complexes were synthesized by Arthi Krishnan at the University of Texas at Arlington.

The capillary was first rinsed with a 0.1 M basic solution of NaOH and then water for five minutes each. After this initial conditioning and before each sample was run, the capillary was washed for 0.5 minutes with base, 0.5 minutes with water, and 3.0 minutes with the buffer solution. Each sample was then injected into the capillary for 5.0 seconds before running the buffer solution through for the separation. Nitrogen gas provided the pressure for these injections.

The twelve racemic β-lactams are as follow:

(“A”) *cis*-6-azabicyclo[3.2.0]heptan-7-one
(“B”) *cis*-7-azabicyclo[4.2.0]octan-8-one
(“C”) *cis*-7-azabicyclo[4.2.0]oct-3-en-8-one
(“D”) *cis*-7-azabicyclo[4.2.0]oct-4-en-8-one
(“E”) *cis*-8-azabicyclo[5.2.0]nonan-9-one
(“F”) *cis*-9-azabicyclo[6.2.0]decan-10-one
(“G”) *cis*-9-azabicyclo[6.2.0]dec-4-en-10-one
(“H”) *cis*-3,4-benzo-6-azabicyclo[3.2.0]heptan-7-one
(“I”) *cis*-4,5-benzo-7-azabicyclo[4.2.0]octan-8-one
(“J”) *cis*-5,6-benzo-8-azabicyclo[5.2.0]nonan-9-one
(“K”) *exo*-3-azatricyclo[$4.2.1.0^{2.5}$] nonan-4-one
(“L”) *exo*-3-azatricyclo[$4.2.1.0^{2.5}$] non-7-en-4-one

The ruthenium bipyridyl complexes are as follow:

(1) $[Ru(Phen)_3](PF_6)_2$
(2) $[Ru(Phen)_3](Cl)_2$
(5) $[Ru_2(Phen)_4(tpphz)](PF_6)_4$
(6) $[Ru_2(Phen)_4(tpphz)]Cl_4$
tpphz = tetrapyrido[3,2-a:2’,3’-c:3’’,2’’-h:2’’’,3’’’-j]phenazine

See Appendix A for molecular structures.

Results and Conclusions

While higher concentrations of selector resulted in a better separation of the enantiomers, it also decreased the EOF and therefore resulted in longer retention times. In many cases, enantiomeric separation of the analyte could not be achieved when the concentration of selector was below a certain point. Optimization of the separations may eventually be reached with enough time to test various selectors and concentrations, as well as differing buffers and pH values.

Eight of the twelve β-lactams were at least partially separated by a 100 mM concentration of SAC. These results did not exactly match the conclusions drawn by Jiang et al., most likely due to the disparities between different batches of cyclodextrins. For this reason it is not yet possible to obtain reproducible results in CE. Irregularities in the selectors are caused by different batch-to-batch substitution patterns and by varying amounts of water in the sulfated α-CD, since it is a sodium salt hydrate [3]. Thus it is very difficult to create multiple buffer solutions with the exact same concentrations of the exact same chiral selector using different batches of CD. While most of the β-lactams could be separated using a potential of 8.00 kV, some responded more favorably to a voltage of 7.00 kV. In Appendix B, the data reported on β-lactams “G” and “H” (see Procedure section) were generated using 7.00 kV, while the other β-lactams were separated with 8.00 kV. A lower voltage generally gives better resolution for the separations although it gives a slower EOF as well [3]. More combinations of selector concentrations and voltages could have been tested to optimize the separations, if time had permitted.

Because some β-lactams were separated while others with similar structures were not, it shows that molecular modeling does not prove a good predictor for chiral separations. It is very difficult to anticipate the results of a CE separation since it is usually unknown how a certain analyte and selector will interact with each other. There are many variables to be taken into account, most especially voltage, pH of the running buffer, concentration of the chiral selector, and type of selector used.

The ruthenium bipyridyl complex monomer was enantiomerically separated with a 10 mg/mL concentration of SBC and a voltage of 7.00 kV. While at least a partial separation of both $[Ru(Phen)_3](PF_6)_2$ and $[Ru(Phen)_3](Cl)_2$ was achieved, neither molecule had a complete baseline separation. By adjusting the various factors affecting the experiments, a better resolution could probably be gained. Although a few different concentrations of

hydroxypropyl γ-CD were tested and did not appear to separate the ruthenium complexes, higher concentrations of this selector and altered testing conditions may cause it to be a better separator. Time constraints limited the number of tests that could be performed, and so a chiral separation of the dimer could not be found. Another issue arising in attempts to separate the dimer is the possibility that trace amounts of the monomer may exist in the dimer molecule.

The process of CE is important in that it is able to separate enantiomers which would otherwise behave similarly under nonchiral conditions. This is very helpful to industry, especially in the areas of medicine and pharmacology. There are still many components of CE yet to be explored and perfected, but it does show great potential for the future.

Works Cited

1. Armstrong, Daniel W. The University of Texas at Arlington. Personal Communication.

2. Heiger, David N. High Performance Capillary Electrophoresis. 2nd ed. France: Hewlett Packard, 1992. 186-200.

3. Jiang, Chunxia, Daniel W. Armstrong, and Peter Antal. *Enantiomeric Separation of a Series of β-lactams using Capillary Zone Electrophoresis*.

4. Wan, Hong, and Lars G. Blomberg. "Chiral Separation of Amino Acids and Peptides by Capillary Electrophoresis." Journal of Chromatography A 875 (2000): 43-88.

Acknowledgements

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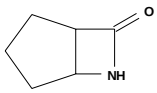
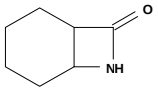
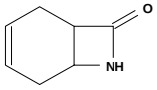
Appendices

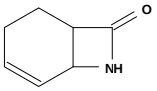
Appendix A

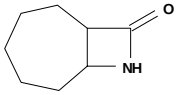
β-Lactams:

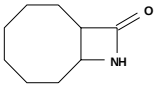
"A" 

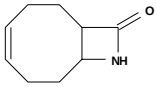
"B" 

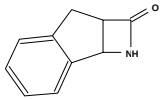
"C" 

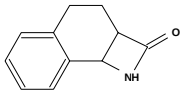
"D" 

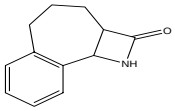
"E" 

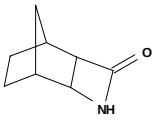
"F" 

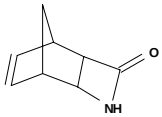
"G" 

"H" 

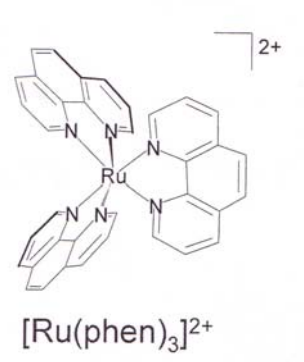
"I" 

"J" 

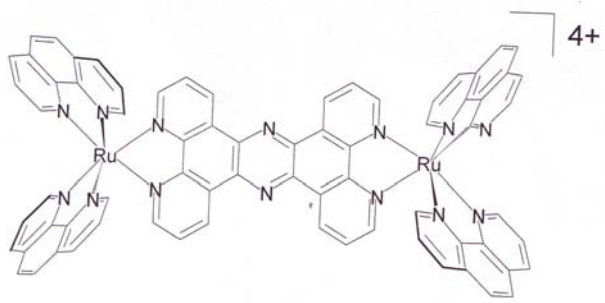
"K" 

"L" 

Ruthenium bipyridyl complexes:



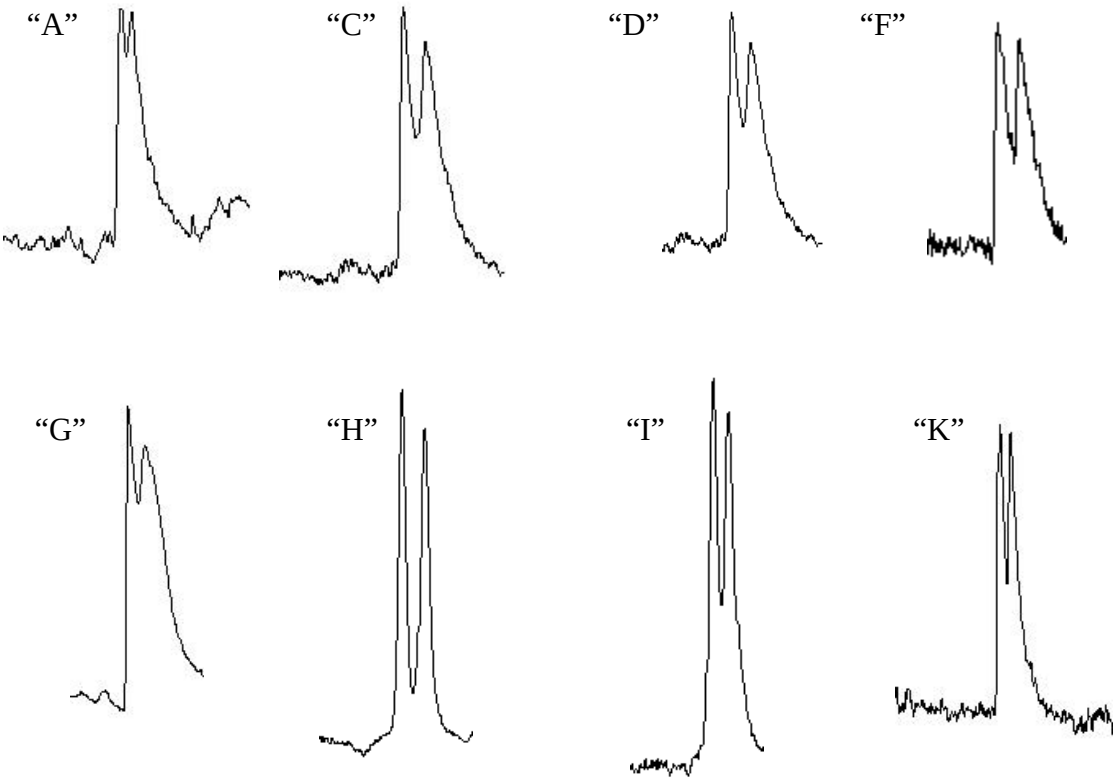
$[Ru(phen)_3]^{2+}$



$[Ru_2(phen)_4(tpphz)]^{4+}$

Appendix B

β-Lactam separations:



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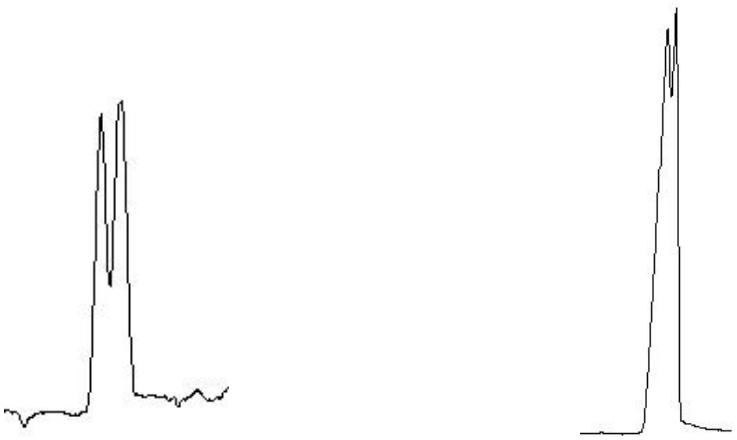

(1) $[Ru(Phen)_3](PF_6)_2$ (2) $[Ru(Phen)_3](Cl)_2$