



***OPERATING AND  
TROUBLE  
SHOOTING MANUAL***



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# Introduction to CELixir<sup>®</sup>

CELixir<sup>®</sup> is an easy to use, capillary coating system that produces highly reproducible and reliable Electro-Osmotic Flow (EOF) in Capillary Zone Electrophoresis (CZE).

The speed of your EOF will be increased over routine CZE due to CELixir<sup>®</sup> and most analytes will exhibit a reduced affinity to adsorb to the capillary wall. This unique product prevents analyte loss or adsorption to the negatively charged capillary surface by shifting the adsorption equilibrium to the CELixir background electrolyte (BGE).

## Unchanging EOF

CELixir<sup>®</sup> enhances the analysis of small molecules, peptides, proteins and chiral compounds by High Performance Capillary Electrophoresis (HPCE). The CELixir<sup>®</sup> solutions provide a dynamic polyanionic/polycationic coating system which when applied to the surface of a bare fused silica capillary, produces a stable and highly reproducible EOF anywhere between pH 2.5 and 9.3. The CELixir<sup>®</sup> coated surface of the fused silica capillary is highly propagated with negative charges producing robust CE methods where resolution and analyte velocities are highly increased. Once the silica capillary is properly conditioned, migration time drift is nearly eliminated permitting reproducible quantitative analysis.

CElixir<sup>®</sup> can facilitate the analysis and separation of neutral and charged (anionic and cationic) species in a single run while the capillary is operated at a pH of 2.5 to 4.3. Thus, runs where positively charged analytes are attracted to the negatively charged surface of the capillary are eliminated and a concurrent increase in resolution and reproducibility is achieved with CElixir<sup>®</sup>. Our dynamic coating solutions excludes the need for additives to modify capillary walls or use of coated capillaries as seen in standard CE and CEC runs.

## Coating Definition

The proprietary properties of the CElixir<sup>®</sup> Dynamic Coating System achieves its uniform EOF characteristics by a stable bond formed between the Initiator Solution (A) (polycation) and the capillary wall (see figure 2). This step covers the capillary wall with an excess of positive charges. The coated wall is then interacted with The Accelerator Solution (B) (polyanion) which also contains the background electrolyte (BGE) in a buffered, bulk flow solution. The layer that is now formed with the Accelerator Solution reagent (see figure 3) and the previously modified capillary wall, exposes an abundance of negatively charged sites to the lumen of the capillary. This very high density of charge participates in producing an extremely stable and enhanced

flow (EOF). Analyte adsorption is virtually eliminated by the shifting of the adsorption equilibrium to the BGE. This method reduces analyte diffusion by increasing electro-osmotic velocity and decreasing the time for analysis.

### **Superior Sample Stacking**

The robust EOF created by the CElixir<sup>®</sup> system permits the use of higher molality buffers (50-150mM) which ordinarily would lengthen the separation times due to reductions in solute mobility and the EOF. The higher buffer concentrations provide for superior sample stacking improving both peak shape and sensitivity of detection. Effects from electrodispersion are reduced as well as elimination of the characteristic saw-toothed peaks that often occur at low buffer concentrations. CElixir<sup>®</sup>'s configuration also permits a high degree of reproducibility in capillary to capillary and inter-run separations.

Figure (1)

Bare Fused Silica After NaOH Rinse

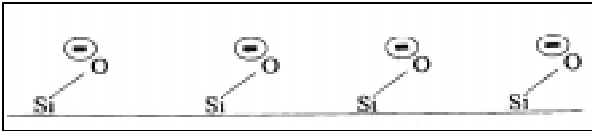


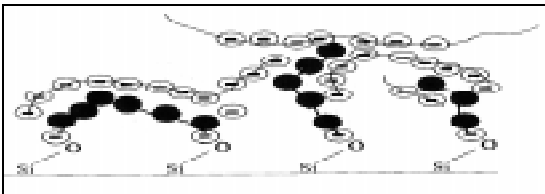
Figure (2)

Capillary Wall After Initiator Solution (A) is applied



Figure (3)

Capillary Wall after Accelerator Solution (B) is applied



## The Capillary Surface Coating can be removed

CElixir is a dynamic coating system and may be removed from the capillary at will. Fill the capillary with 0.1N NaOH with a pressure/rinse cycle. Let the capillary stand filled for at least one minute, then fill it with 0.1N HCl. Let the capillary stand filled again for one minute. Repeat these two steps once more, then follow with a CEwater rinse for at least one minute, followed by 0.1N NaOH for another minute.

### CElixir<sup>®</sup> HPCE Column Kit includes

- 1 ea. CElixir<sup>®</sup> Initiator Solution (A), 80ml
- 1 ea. MicroSolvCE Fused Silica Capillary, 50µm ID x 2m
- 1 ea. CElixir<sup>®</sup> Accelerator Solution (B), 240ml Buffered
- 1 ea. CEwater<sup>®</sup> Ultra Pure Water, 80ml
- 1 ea. Cleaving Stone for cutting capillary to desired length
- 1 ea. Instruction and Trouble Shooting Manual

## **Replacement Solutions and Capillaries:**

06025-CE-20	Initiator Solution (A) 20ml
06025-CE-80	Initiator Solution (A) 80ml
06125-CE-240	Accelerator Solution (B) pH 2.5 240ml
06125-CE-50	Accelerator Solution (B) pH 2.5 50ml
06143-CE-240	Accelerator Solution (B) pH 4.3 240ml
06143-CE-50	Accelerator Solution (B) pH 4.3 50ml
06162-CE-240	Accelerator Solution (B) pH 6.2 240ml
06162-CE-50	Accelerator Solution (B) pH 6.2 50ml
06182-CE-240	Accelerator Solution (B) pH 8.2 240ml
06182-CE-50	Accelerator Solution (B) pH 8.2 50ml
06192-CE-240	Accelerator Solution (B) pH 9.2 240ml
06192-CE-50	Accelerator Solution (B) pH 9.2 50ml
05080-W	CEwater $\hat{O}$ 80ml
04050-C	MicroSolvCE $\hat{O}$ Fused Silica Capillary 100cm, 50 $\mu$ ID, 375 $\mu$ OD.
04051-C	MicroSolvCE $\hat{O}$ Fused Silica Capillary 10m, 50 $\mu$ ID, 375 $\mu$ OD.

See MicroSolv Technology Corporation's catalog of supplies and accessories for HPCE. Ask your local distributor for a copy or contact us at [WWW.MicroSolvTech.com](http://WWW.MicroSolvTech.com).

# Preparation for Run

## **CElixir Solutions**

The CElixir Solutions should be maintained at room temperature before use. There is no need to filter the Initiator Solution (A) before coating procedures. It is recommended that all aliquots of Accelerator Solution (B) be filtered with a 0.45um low-binding syringe filter to reduce any dissolved oxygen that may be present.

## **MicroSolvCE Fused Silica Capillary**

CElixir HPCE Column kits includes 2 meters of a bare fused silica capillary. These capillaries are designed with a high degree of available silanol sites which makes them excellent for dynamic coating procedures. The capillaries are coated with Polyimide for strength and flexibility.

The capillary should be cut to your desired length with an appropriate ceramic cutter or the cleaving stone that is included with the kit. For initial method development, we recommend starting with a length of 30-42cm to detection. Care should be taken to ensure that a square cut to the inlet end is performed. The capillary should be rinsed for at least 5 minutes with 0.1N NaOH, followed by 2 minutes with CEwater, ultra pure water.

When storing MicroSolvCE<sup>®</sup> capillaries, follow the procedure provided on the inside panel of each MicroSolvCE<sup>®</sup> Capillary Case.

## **Cleaving Procedure for MicroSolvCE<sup>®</sup> Capillaries**

A true perpendicular cut to the end of the capillary is vital to the success of any CE run. Care must be taken to ensure that the proper cut is made. Follow this procedure to assure good cuts.

1. While holding the capillary over a large diameter surface under slight tension, place the cleaving stone at approximately 30° angle to the capillary.
2. Draw or slide the edge of the cleaving stone across the capillary. Make sure that you penetrate the polyimide to make a “slice” in the coating.
3. Pull the capillary horizontally until it breaks.
4. If the capillary will not “break”, the polyimide has not been cut. Repeat the above steps.

## **Preparation of your Sample**

Dissolve your sample in CEwater<sup>®</sup> . Sample concentration should generally not exceed 1mg/ml when using 50 $\mu$ m capillaries and 0.2mg/ml for 75 $\mu$ m capillaries. If your sample is not very soluble or is insoluble in water, and an amine or nitrogen group is present, add a proportionately small volume of HCl to help dissolve it. If the molecule contains a phenolic or carboxylic group, add a proportionately small volume of NaOH to help solubilize it. If no chargeable group is present, dissolve the sample in an organic solvent (preferably Methanol), then add the solution to CEwater<sup>®</sup> , keeping the percent of organic as low as possible yet to maintain solubility.

## Selecting the pH of your Sample and CE Method.

### *Changes in the Speed of EOF as pH Changes Standard CE Method vs. CELixir<sup>®</sup> Method*

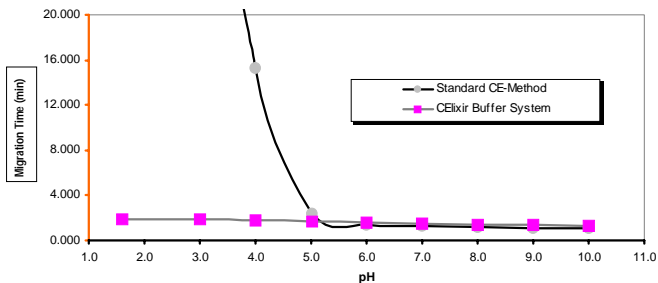


Figure (3)

As the pH changes with a standard CE Method, the EOF (as determined by a marker) decreases greatly in the acidic range. With CELixir<sup>®</sup>, the change in the migration time of the marker (EOF) remains essentially the same from pH 2.0 to pH 10.0. This means that the pH does not effect the EOF when using CELixir<sup>®</sup>.

The above chart shows the effect of pH on EOF with standard CE methods. While developing your method

using CElixir<sup>®</sup> , keep in mind the very small changes you should expect when you use different Accelerator Solutions (B). This will allow you to use CElixir<sup>®</sup> and run your samples at any pH within the range.

## **HPCE Unit Preparation**

Using the following steps it is very simple to prepare your HPCE Unit to use CElixir<sup>®</sup> .

1. Follow the manual and procedures of the HPCE instrument manufacturer to install a capillary in the instrument. Follow all the procedures in the manual for normal operations.
2. The HPCE Unit should be stabilized and running at your desired operating temperature. If you are developing a new method we recommend a temperature of 25°C to start.
3. In your HPCE instrument's autosampler, fill your inlet and outlet reservoirs with Accelerator Solution (B) with the pH of your choice. This will replace your normal "run buffer".

If you are resolving racemic mixtures or chiral compounds, the desired cyclodextrin should be added to the Accelerator Solution (B) in the "run buffer vial".

4. Fill a vial position with 0.1N NaOH. Fill two additional vials: One with Initiator Solution (A), another with CEwater<sup>®</sup> , which is provided in the kit.

Any pH can be achieved in the Accelerator Solution (B) by mixing different Accelerator Solutions of different pH's or by adding appropriate amounts of acid or base. For Instance, to get a pH of 7.2 as your "run buffer", you add corresponding amounts of Accelerator Solution pH 6.2 and Accelerator Solution pH 8.2. See Figure (4) on page 24.

# Method of Analysis

1. Prepare a new capillary. The capillary should be cut to the desired length with an appropriate ceramic cutter or the cleaving stone that is included with the kit. Care should be taken to ensure that a square cut to the inlet end is performed. Follow the cleaving procedure supplied on page 8 to cut with the cleaving stone. Make a 2mm detection window by using the MicroSolvCE<sup>®</sup> electric *Window Maker*<sup>®</sup>. If you do not have a window maker, use a method approved by your laboratory.
2. Apply Initial Coating to the Capillary. Rinse the cut capillary for one minute or one capillary length with Initiator Solution (A).
3. Select the proper pH for Accelerator Solution (B). If the pI or pK of the sample is known, perform the analysis with CElixir<sup>®</sup> Accelerator Solution (B) of the pH that is just below the pI or pK value of the sample. If the pI or pK is unknown, start with CElixir Accelerator Solution (B) pH 2.5. then try pH 4.2, etc. until initial resolution is achieved.
4. Apply the Final Coating to the Capillary. Rinse the capillary for two minutes or two capillary lengths with Accelerator Solution (B) buffered to the pH determined in step 3.
5. Inject the sample. Following the proper procedures of the instrument manufacturer, inject a sample volume of about 2% of the capillary volume.

6. Insert a Water Plug. Inject CEwater plug for 1 second. (Recommended for Beckman™ PACE units or other units where the outlet end of the capillary is not immersed during injection in outlet buffer reservoir).
7. Apply Voltage for Separation. Apply 20-25 kV when using a 50µm capillary or 10-15 kV when using a 75µm capillary.
8. Selecting a Detector Wavelength. Ideally, work at the wavelength closest to the maximum absorbency of the analyte. Ensure that the marker recommended by the instrument manufacturer absorbs at that wavelength to determine EOF. It is recommended that a ramping time of 20 seconds be used if your unit is capable of ramping.
9. Setting Run Times. To detect the analyte when the migration times are unknown; set your initial run times to at least 30 minutes. This will help to ensure analyte detection. You can optimize run times based on initial migration times after the first run.
10. Final Step for CElixir and Care of Capillary  
Rinse the capillary for at least 1 minute by running NaOH 0.1N through it. Repeat steps 2 through 9 for each subsequent injection. Follow recommended procedure of capillary manufacturer for storage.

## **To Increase or To Get Initial Resolution**

The following suggestions will help you increase your resolution with CELixir<sup>®</sup> after you have followed the above procedure.

- a. Increase the capillary length.
- b. Add an organic modifier; Check pH after each addition. Suggested modifiers:  
Methanol, Ethanol, Acetonitrile, Isopropanol  
Methoxyethanol, Ethylene Glycol.
- c. Add neutral or amphoteric, surfactant additives.  
Do not use SDS with CELixir<sup>®</sup> . Suggested surfactants:  
Neutral- Brij 35, Tween 80      Amphoteric-  
CHAPS, CHAPSO
- d. Combine b and c.

## **To Achieve Chiral Separations Using CELixir<sup>®</sup>**

After determining your optimal separation pH, add a chiral agent to Accelerator Solution (B), beginning with  $\beta$ -cyclodextrin then with dimethyl- $\beta$ -cyclodextrin, etc. You may use up to 1g in 24g (37mM) of Accelerator Solution (B) buffered to pH 2.5 or up to 1.3g (48mM) at all other pH's.

**NOTE: CELixir<sup>®</sup>**  
**Solutions should not be**  
**used with SDS**

# Method Trouble-Shooting

## 1. If The Marker is Not Detected:

*Check the following Before Calling the MicroSolv Help Desk.*

- a. Ensure that the marker that you used absorbs at the wavelength you are using. If you do not know if the marker absorbs at your wavelength, change the wavelength of your detector and re run your method.  
If that does not provide enough information, change the marker and re run the method.
- b. If there was no injection specified in your method, a marker may not have injected. You should re run the method and verify that a marker is injected.
- c. If the polarity of your marker is not the opposite of the HPCE system, the marker will not migrate. Change the polarity of the instrument or change to a different marker.
- d. Verify that the correct sample vial with the marker in it is specified in your instrument.

- e. The marker may not be well dissolved. Mix the marker thoroughly and vigorously. Dissolve the marker in Accelerator Solution (B) if it is not detected. Re run the method this time with your marker dissolved in Solution (B).
- f. If the marker concentration is too low the instrument may not detect it. Increase the concentration or the marker volume sufficiently and re run the method. Increase the injection volume from the marker vial.
- g. Ensure that the marker is stable at the method's run pH. Check with the manufacturer of the Marker to verify proper pH.
- h. Check the inlet and outlet reservoirs to ensure that Accelerator Solution (B) is present during the run. Running the method with only Initiator Solution (A) applied to the capillary wall may result in reversed polarity and the opposite order of migration.
- i. A broken or plugged capillary will cause all migration to stop including the marker. Plugged capillaries are usually plugged at the ends. Follow the cleaving procedure and re cut the capillary ends. Re run the method.

## 2. If your Sample Peak is Not Well Resolved:

*Check the following before calling the MicroSolv Help Desk.*

- a. Check to make sure that your sample is completely dissolved and that it has not precipitated out of solution. If the sample is precipitating in the vial, lower the concentration of the sample or follow the Sample Preparation Procedure on page 9 trying other variations. Complete solubility is very important for good results.
- b. Increase the length of the capillary by cutting a new capillary. This time cut it longer. The more capillary, the greater the resolution.
- c. Try several runs of the same method but each time increase the injection time of your sample in 1-sec intervals. Repeat this until resolution or overloading occurs.
- d. Reduce applied voltage to reduce Joule Heating if it is suspected. Run Ohm's law plot and re run method with different (Lower) voltage and current.
- e. Dilute the sample and increase the injection time. By doing both of these at the same time load the same amount of sample as your method calls for but the sample will be more dilute.
- f. Add up to 25% W/W of organic modifier to Accelerator Solution (B) and re run the method.

- g. Use amphoteric surfactant additive or cyclodextrins. Do not use SDS with CELixir Solutions.
- h. Combine steps f and g.
- i. Change pH of Accelerator Solution. Either select a different CELixir Solution, combine two to achieve the pH and selectivity you need or add appropriate amounts of acid or base to solutions. (**See section on Accelerator B modification**).

### **3. If Your Sample Peak is Tailing:**

*Check the following before calling the MicroSolv Help Desk.*

- a. Re run your method with the same parameters but use Accelerator Solution (B) at the next lowest pH.
- b. Dissolve your sample at a lower concentration and re run the method.
- c. Add up to 25% W/W of MeOH or ACN modifier to Accelerator Solution (B) and re run the method.
- d. Decrease thermostat temperature if solubility and viscosity is not a concern. Re run the method.

- e. Ensure capillary is cut squarely and correctly. If you cannot determine if a square cut has been performed, it is recommended that you re cut the capillary following the Cleaving Procedure on page 8.
- f. Reduce detector time constant. Re run the method.
- g. Ensure the level of the inlet and outlet liquids are filled to equal heights and are level. Re run the method.

**4. After Achieving Good Resolution there is a Subsequent Loss of Resolution:**

*Check the following before calling the MicroSolv Help Desk.*

- a. As you run your method, BGE and buffer depletion can occur. Empty the vial with Accelerator Solution (B) and now fill the vial with fresh Solution (B). Re run your method.
- b. Between your runs, the CElixir method calls for a wash of the capillaries with NaOH for one minute (see METHOD OF ANALYSIS, Final Step... If

you are losing resolution increase the wash time in the Final Step of 1 minute in 20 seconds intervals. First time is 1minute 20 seconds. Increase time in increments. Re run the method.

- c. Check sample and other vials for evaporation or insufficient volume for proper injection. There must be a sufficient level of liquid above the end of the capillary to ensure proper injection. If evaporation of CElixir persists, contact the MicroSolv help desk.
- d. Check capillary for breaks by visually inspecting them. If capillaries appear broken, or the Polyimide looks cracked, replace the capillary and re run the method.
- e. Replace the capillary with a new one. Re run method.

## **5. If you experience a Voltage Loss during the Run:**

*Check the following before calling the MicroSolv Help Desk.*

- a. Wash the capillary surface with a 2-minute (2 capillary lengths) wash of 1.0 N NaOH. Then re apply both Initiator and Accelerator Solutions. Re run your method.

- b. Check the capillary for breakage, cracking and bubble formation. If you suspect any of these, replace the capillary with a new one following the proper preparation procedures above. Re run the method.
- c. Check the level of all rinse, solution, buffer and sample vials. If they are low, fill them to full. Re run the method.
- d. Check the capillary for blockage at the capillary ends. If you suspect there may be a blockage, cut the ends of the capillary following the cleaving procedure. Filter all samples and other solutions with a .45 $\mu$  filter before re running the method.
- e. Assure there are no bubbles within the sample vial from previous injections. If a bubble gets into the capillary, it can cause a drop in the current.

# CElixir<sup>®</sup> pH Adjustment Accelerator Solution (B)

<b>CElixir Solution (B)</b>	<b>To Increase pH</b>	<b>To Decrease pH</b>
pH 2.5	Add CElixir pH 8.2	Add 1N H <sub>3</sub> PO <sub>4</sub>
pH 4.3	Add 1N NaOH	Add 1N HCl
pH 6.2	Add CElixir pH 8.2	Add CElixir pH 2.5
pH 8.2	N/A	Add CElixir pH 2.5
pH 9.2	N/A	Add 1N H <sub>3</sub> PO <sub>4</sub>

NOTE: CElixir<sup>™</sup> solutions should never be diluted with water, as this will adversely affect buffer performance and dilution of the essential active component

## Storage of CElixir<sup>®</sup>

CElixir<sup>®</sup> Solutions should be capped tightly and stored at room temperature. Sodium Azide (>0.005%) may be added to prevent bacterial contamination. Check the expiration date of the solution before using it.

## Technical Support

We are here to provide you with the support you need to successfully implement the CElixir<sup>®</sup> in all your separations. Our technicians are available from 9:00 AM to 5:30 PM Eastern Standard Time in the USA and from 9:00 AM to 5:00 PM GB Time.

Technical Support Phone No.:	1-732-389-8852
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E-mail:	<a href="mailto:MicroSolv2@aol.com">MicroSolv2@aol.com</a>
Website:	<a href="http://www.MicroSolvTech.com">www.MicroSolvTech.com</a>

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