Operating and Instruction Manual

Version 5.1

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Introduction to CElixir™

CElixir is an easy to use, pre-made capillary coating and buffer system that produces highly reproducible and reliable Electro-osmotic Flow (EOF) in Capillary Electrophoresis (CE) on bare fused silica capillaries It is used for small molecules, peptides, proteins and chiral compounds

Compared to classical CE, the double layer, coating technology produces an increased EOF while preventing adsorption of the analyte to the capillary wall. This results minimized analyte loss and a migration time that more related to electrophoretic mobility in the given CElixir background electrolyte.

- **Intended Uses:**

CElixir dynamic coatings/buffer systems are designed for use in Capillary Electrophoresis for the separation of positively charged molecules and/or amphoteric substances.

- **Dynamic Coatings Produce Precise, Stable EOF**

The CElixir coatings are dynamic (removed between each run) and utilize a coating system which, when applied to the surface of a bare fused silica capillary, produces a stable and highly reproducible EOF anywhere between pH 1.7 and 10.5. Once the silica capillary is properly conditioned, migration time drift is nearly eliminated permitting reproducible quantitative analysis.

CElixir dynamic coating solutions exclude the need for additives to modify capillary walls or use of coated capillaries as seen in standard CE runs.

Double Layer, Dynamic Coating
Definition

The properties of the CElixir™ dynamic coating system achieves its uniform EOF characteristics and other properties by a stable bond being formed between the Initiator Solution (A) (poly-cation) 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 one of the Accelerator Solutions (B) (poly-anion) which contains the background electrolyte (BGE) in a buffered solution. The layer that is now formed with the Accelerator Solution reagent (see figure 3) and the previously modified capillary wall (figure 2), exposes an abundance of negatively charged sites to the lumen of the capillary. This very high density of charge participates in producing a very stable and enhanced flow (EOF). Analyte adsorption is virtually eliminated. This method reduces analyte diffusion by increasing electro-osmotic velocity and can decrease the time for analysis.

Figure (1) Bare Fused Silica After NaOH Rinse

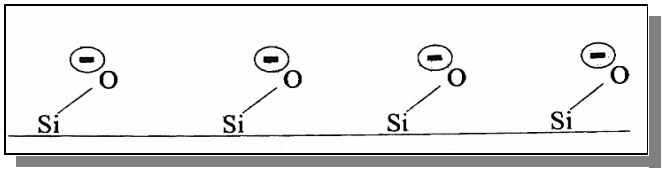


Figure (2) Capillary Wall After Initiator Solution (A) is applied

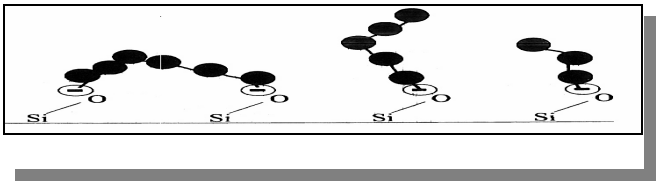
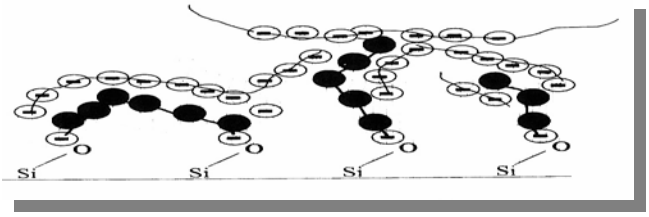


Figure (3) Capillary Wall after Accelerator Solution (B) is applied



Reagents and Materials Needed

Capillary	Fused Silica Capillary: typically 75 µm inner diameter and 60 cm length.
CE Grade Water::	In 2 vials: one for rinse and one for a water plug.
NaOH	0.1 M in 1 vial.
Initiator	CElixir™ Initiator in 1 vial.
Accelerator	CElixir™ Accelerator in 3 vials: 1 for rinse 2 for separation.
Waste	Empty vial

Vials, capillaries, ultra pure water and NaOH are not included in the kit but can be purchased separately from MicroSolv.
Use vials compatible with your CE instrument. It is recommended not to overfill the vials. Mount the capillary according to your CE instrument instruction manual.

Preparing To Run The CElixir Dynamic Coating System

Using CElixir™ Solutions

The CElixir Solutions are ready to use and should be maintained at room temperature while not in use to avoid absorption of oxygen. The bottle should be kept closed to avoid air adsorption or oxidation There is no need to filter any of the CElixir Solution (A) before coating procedures and they are ready to use out of the bottle.

Using the Capillary: Fused Silica Capillary made for CE

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 and a detection window can easily be created.

The capillary should be cut to your desired length with an appropriate ceramic cutter or a cleaving stone that is available from MicroSolv. Care should be taken to ensure that square cuts to the inlet and outlet end are made. The capillary should be rinsed for at least 5 minutes with 0.1N NaOH, followed by 2 minutes with de-ionized water. It is recommended that 2mm of polyimide are removed from the inlet and the outlet ends of the capillary. Wipe with clean Kimwipe. This will improve injection precision from run to run.

When storing capillaries, rinse with water for 5 minutes at 20 psi and cover the ends of the capillary. Store in a safe place to avoid cracking or breaking.

- *Cleaving Procedure for Bare Fused Silica Capillaries*

A true perpendicular cut to the end of the capillary is important to the success of any CE run. Care must be taken to be sure 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 30º angle to the capillary.
2. Draw or slide the edge of the cleaving stone across the capillary. Make sure that you penetrate 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.
5. Remove 2mm of the polyimide from the inlet and the outlet ends of the capillary. Wipe with a clean kimwipe.

Preparation of your Sample for use with CElixir

If possible, dissolve your sample in CE grade water, (18 meg-ohm de-ionized water). Sample concentration should generally not exceed 0.1mg/ml when using 50µm capillaries and 0.2mg/ml for 75µm 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. For these types of samples, it may be an alternative to use CElixirOA for Anions and Organic Acids.

If no chargeable group is present, dissolve the sample in an organic solvent (preferably Acetonitrile, Methanol or Propanol), then add the solution to water, keeping the percent of organic as low as possible yet to maintain solubility. For these compounds, CElixir-SDS should be considered as an alternative reagent system.

Other buffers compatible with CElixir Accelerator can be used as the sample solvent. The CElixir Accelerator Solution (B) can be used as your sample diluent but you must be careful to match the pH of the sample diluent and separating solution (buffer).

Selecting the pH of your Sample and CE Method.

"Changes in the EOF as pH Changes"
Standard CE Method vs.CE Method w/CElixir™

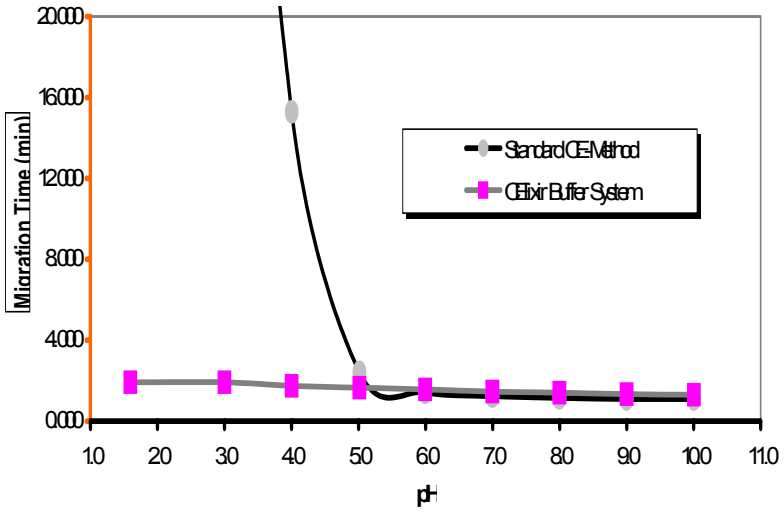


Figure (4)

- *Run to Run Precision with CElixir at any pH*

As pH changes with a standard CE Method, the EOF (as determined by a marker) decreases dramatically in the acidic range. Below pH 5, one can see the small difference in pH will induce a large variation of the migration time of the EOF marker. With CElixir™, migration time of the marker (EOF) remains essentially the same from pH 2.0 to pH 10.0. This means that pH does not effect the EOF with CElixir™ and as a consequence produces very precise, reproducible results.

- *Ionize Your Samples*

The Accelerator solution (B) to use is determined by the pKa of your samples. Normally, the best pH for the Accelerator is a pH where your analyte is most ionized. Normally one to two pH units above or below the pKa is optimal.

- Unknown pKa

If you do not know the pKa of your samples, it is highly recommended that you run a short and quick scouting of all six Accelerator solutions. Since you can use all six different solutions on the same capillary, this does not take much time and provides a very useful profile of your samples. Use the pH where you get the separation that best meets your objectives. Each run can be further optimized.

Hint:
Acids Ionize Basic Compounds
Bases Ionize Acidic Compounds

Preparation for Method

Using the following steps it is very simple to prepare your CE Instrument to use CElixir™.

1. Follow the manual and procedures of the CE instrument manufacturer to install the capillary in the instrument. Use a new, properly conditioned capillary.

2. Follow all the procedures in the manual for normal operations. All CE instruments should be maintained & thoroughly cleaned on a routine basis per the instructions of the instrument manufacturer.

3. The CE Unit should be stabilized and running at your desired operating temperature. If you are developing a new method we recommend a temperature of 25ºC as a starting point. However, other methods could be better performed at other temperatures.

4. Filling Autosampler Vials and Instrument Positions
Refer to your instrument Operators Manual for general operation. Load you instrument with the samples to be analyzed and the buffers and reagents.

- Place CElixir and Reagent in Vials

NaOH 0.1M	1 vial in buffer inlet position	
Initiator (A)	1 vial in buffer inlet position	
Water	2 vials in buffer inlet position	(one for water plug, one for rinse)
Accelerator B)	3 vials:	
	2 in buffer inlet position:	
	one for rinse	
	one for separation	
	1 in buffer outlet position	
	For separation	
Empty vial	1 vial in buffer outlet position	

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".

Hint: 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).

Running The Method™

Create a Program according to your CE instrument Manual

Step 1. *Coat the Capillary with Initiator (A).* Rinse the capillary using the Initiator (A) vial listed above as the inlet and using the Empty Vial as the outlet vial. Typically run a volume equal to one capillary volume or run for 0.2 minutes (12 seconds) to 0.5 minutes (30 seconds) at 20 psi.

Step 2. *Coat the capillary with Accelerator (B).* Rinse the capillary using the Accelerator (B) vial "for rinse" in the inlet position and the Empty Vial as the outlet vial. Typically run a volume equivalent to 2 capillary lengths or run for 0.5 minute to 1 minute at 20 psi.
Step 3. *Inject the Sample*. Using the sample Vial as the inlet vial and the Accelerator (B) vial "for rinse" as the outlet vial. Typically inject for 5 seconds at 0.5 psi.

Step 4. *Inject a Water Plug .* Using the water Vial "for water plug" as the inlet vial, inject for 10 seconds at 0.1 psi using vial with Accelerator (B) "for rinse" as the outlet vial.

Step 5. *Separate*. Using the Accelerator vial "for Separation" in the inlet position and the Accelerator vial "for Separation" in the outlet position. A good starting point is 25 kV for 20 minutes when working with a capillary of 60 cm total length.
Use a one minute ramping time (time needed for the current to reach 25 kV). Experimental conditions should be adapted for the specific separation. It is recommended to check the current and keep it below 100 µA.

Step 6 . *Auto-zero*. Include an auto-zero command before your peak of interest.

Step 7. *Remove the Coating and Remaining Sample*. After separation, using the NaOH vial as the inlet vial and the Empty vial as the outlet vial, perform a rinse of the capillary. Rinse for 0.5 minute at 20 psi which is typically a volume equivalent to 1 capillary length.

Step 8. *Rinse the Capillary*. Using the Water vial "for rinse" as the inlet vial and the Empty Vial as the outlet vial, rinse the capillary. Rinse for 0.5 minutes at 20 psi. which is typically a volume equivalent to 1 capillary length.

Step 9. End of separation program. New separation can start Step 1.

For Sample Programs, visit our website
@www.mtc-usa.com

Hints:
(1 psi = 0.06895 bar)
The same buffers and reagents vials can be used until the levels in the vials are too low or until the separation buffers are depleted (no more optimal separation).
Do not refill the vials but change all buffers and reagents vials.

Suggestions for Optimal Results

1. Setting Run Times. To detect the analyte when you do not know the migration times, your selected initial run times should be set to at least 20 minutes. This will help to ensure analyte detection. You can optimize run times based on initial migration times after first run.
2. Changing Voltage. Recommended starting voltage is 20kv for a 60cm long capillary. If this is not sufficient, you can vary the voltage to be between 15-25 kV when using a 50µm capillary or 10-20 kV when using a 75µm capillary. It is recommended to check the current and to keep it below 100uAmps.

3. Ramping Voltage. It is very important that you ramp the voltage from zero to your set point over a period of one minute.
4. Selecting Detector Wavelength. Ideally, work at the wavelength closest to the maximum absorbency of the analyte. You should adjust all experimental conditions for specific needs. It is recommended to apply “auto-zero” at 2 minutes in every run.
5. Use of SDS with CElixir. The use of Sodium Dodecyl Sulfate and other charge surfactants are not recommended with CElixir.
6. Achieving Chiral Separations. After determining your optimal separation pH, add neutral cyclodextrin to Accelerator Solution (B), beginning with ß-cyclodextrin then with di-methyl-ß-cyclodextrin, etc. You can use up to 1g of the cyclodextrin in 24g (37mM) of Accelerator Solution (B) buffered to pH 2.5 or up to 1.3g of cyclodextrin (48mM) at all other pH’s
7. Adapting the Accelerator (B) pH. The pH of the Accelerator Solution can be easily adjusted by following the chart below.

CElixir Buffer	**To Increase pH**	**To Decrease pH**
pH 2.5	Add CElixir pH 8.2	Add 1N H_3PO_4
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	NA	Add CElixir pH 2.5
pH 9.2	Add 1N NaOH	Add 1N H_3PO_4

NOTE: CElixir™ should never be diluted with water, as this will adversely affect performance and dilution of the essential active components.

8. Disregard first run of the day and new capillaries. It is good laboratory practice when using CElixir to disregard the first run of each day and the first few runs on a new capillary.
9. Storage of CElixir. CElixir is best stored at room temperature (18C—26C). There is not need to filter CElixir if it is capped properly after each use. DO NOT REFRIGERATE CElixir.

Suggestions for Increasing Resolution

1. Adapt the pH of the Accelerator (run buffer). By finding a different pH you may find resolution increases many fold.
2. Increase your capillary length. This will increase your run time as well. It is suggested that you increase your data collection window.
3. Add an organic modifier. Check pH after addition. Maximum suggested addition is up to 20%. Examples: Methanol, Ethanol, Acetonitrile, Isopropanol Methoxyethanol, Ethylene Glycol. Run times will be longer, increase data collection window as is appropriate.
4. Add neutral or amphoteric, surfactant additives. Do not use SDS with CElixir. Examples: Neutral-Brij 35, Tween 80 Amphoteric-CHAPS, CHAPSO
5. Add neutral cyclodextrins to the Accelerator Run Solution. CD can be used to increase resolution for many molecules; not just for chiral separation.
6. Combine 4 and 5.
7. 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. *Complete solubility is very important*.
8. Try several runs of the same method but each time increase injection time of your sample in 1-sec intervals. Repeat this until resolution or overloading occurs.
9. Reduce applied voltage to reduce Joule Heating if it is suspected. Run an Ohm’s law plot and re-run method with different (Lower) voltage and current.
10. Dilute sample and increase 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.

Suggestions for Improving Peak Shape

1. Re-run your method with the same parameters but use Accelerator Solution (B) at the next lowest pH.
2. Dissolve your sample at a lower concentration and re-run the method.
3. Add up to 25% w/w of MeOH or ACN modifier to Accelerator Solution (B) and re-run the method.
4. Decrease thermostat temperature if solubility and viscosity is not a concern. Re-run the method.
5. Ensure capillary end is cut squarely. 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 XX.
6. Reduce detector time constant. Re-run the method.
7. Ensure level of inlet and outlet liquids are filled to same heights and are level. Re-run the method.

Suggestions if there is a Sudden Loss of Resolution

1. As you run your method, BGE and Buffer depletion can occur. Empty your vials and fill with fresh solutions. Re-run your method.
2. Between your runs, the CElixir method calls for a wash of the capillaries with NaOH for one minute (Step 7) … If you are losing resolution increase the rinse time in Step 7 in 20 seconds intervals. ie. first time is 1 minute & 20 seconds. Increase time in increments. Re-run the method.
3. 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.
4. Check the capillary for breaks by visually inspecting them. If capillaries look broken, or the polyimide coating looks cracked, replace the capillary and re-run the method.
5. Replace the capillary with a new one. Re-run method

Suggestions if there is a Voltage Loss During the Run

1. Wash the capillary surface with a 2-minute (2 capillary lengths) wash of 0.1N NaOH. Then reapply both Initiator and Accelerator Solutions. Re-run your method.
2. Check 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
3. Check level of all rinse, solution, buffer and sample vials. If they are low, replace them with fresh, full vials. Re-run the method.
4. Check the capillary for blockage at capillary ends. If you suspect there may be a blockage, replace the capillary with a new one. Filter all samples with a .45µ syringe filter before re-running the method.
5. 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.

Warnings and Precautions:

CElixir contains Sodium Azide (5ug/ml) to prevent bacterial growth and it may form explosives compounds in metal drain lines. When disposing of reagents, follow company SOP for disposal. Flushing with large volumes of water prevents explosive compounds from forming.

Technical Support

MicroSolv is committed to providing technical support and our staff is available from 9:00AM to 5:30PM EST.

Technical Support No.: 1-888-248-4972

International Phone No.: 1-732-578-1777

FAX: 1-732-578-9777

E-mail: Technial.service@MTC-USA.com Website: www.MTC-USA.com

CElixir™ Complete Method Development Kit
Includes:
Catalog No. 06200-CE

1 ea. CElixir™ Initiator Solution (A), 80ml
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 2.5
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 4.3
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 6.2
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 8.2
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 9.2

CElixir™ Low Range Method Development Kit
Includes:
Catalog No. 06201-CE

1 ea. CElixir™ Initiator Solution (A), 80ml
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 2.5
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 4.3
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 6.2

CElixir™ High Range Method Development Kit
Includes:
Catalog No. 06202-CE

1 ea. CElixir™ Initiator Solution (A), 80ml
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 8.2
1 ea. CElixir™ Accelerator Solution (B), 50ml pH 9.2

Replacement Solutions:

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