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Importance of capillary characterization and capillary electrophoresis Molecular recognition elements

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ABSTRACT
Since the introduction of modern capillary electrophoresis (CE) by Jorgenson and Lukacs in 1981, CE has evolved into a highly mature and versatile separation technique. After a first decade of development studies and instrument commercialization, CE took its place among established analytical techniques and, for instance, became the method of choice for fast high-resolution DNA sequencing in the nineties of the last century. Although with a considerably smaller footprint than liquid and gas chromatography, CE remains to play an essential role in contemporary analytics. For example, with the strong advent of biopharmaceuticals, CE has shown particularly useful for routine quality control of therapeutic proteins, such as monoclonal antibodies. Current CE applications range from determination of small inorganic ions to characterization of high-molecular-weight biomolecules, and even particles and intact cells. The research field of CE remains very active, as exhibited by a steady and significant flow of scientific reports on theory, separation modes, new instrumentation and applications of CE techniques in various areas.
The present review provides a brief cross section of new developments in the broad field of CE.
Keywords: - Capillary Electrophoresis, Chromatography, High-resolution DNA Sequencing, Molecular Recognition Elements.

I. INTRODUCTION

Since the introduction of capillary electrophoresis (CE) in 1967 by Hjerten and coworkers. CE techniques can also be used to separate and quantify drugs, their corresponding impurities, and metabolites Whereas in the beginning the optimism to completely replace HPC with CE was rather high due to the tremendous selectivity, nowadays pharmaceutical industries, licensing authorities and the pharmacopoeias do not make use of CE with some very rare exceptions [9]. capillary electrophoresis (CE) has been shown to be an economical, efficient, and rapid separation technique that has been widely applied in pharmaceutical analysis. Compounds that cannot be separated by liquid chromatography (LC) are often resolved by CE because of its selectivity through buffer concentration, additives, and pH tuning [41].

Two different capillary electrochromatography (CEC) stationary phases, Hypersil phenyl and Hypersil C18, have been characterised with respect to their ability to separate [12]. introduction of modern capillary electrophoresis (CE) by Jorgenson and Lukacs in 1981, CE has evolved into a highly mature and versatile separation technique. After a first decade of development studies and instrument commercialization, CE took its place among established analytical techniques and, for instance, became the method of choice for fast high-resolution DNA sequencing in the nineties of the last century.[40] Capillary Electrophoresis has emerged as an alternative method for evolution of MREs. Molecular Recognition Elements (MREs) are compounds capable of binding a variety of inorganic and biological molecules. These MREs have a variation of applications including incorporation into biosensors, medicine discovery, diagnostic testing, and medicinal. MREs are evolved via a generalized method known as the Systematic Evolution of Ligands by Exponential Enrichment, or SELEX.[47]

MER (Molecular Recognition Elements): -

Molecular Recognition Elements (MREs) are organic molecules such as DNA, RNA, or polypeptides capable of binding a target with high affinity and specificity. The broad range of

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applications paired with ease of production of MREs ensures that they will m be a topic a great interest for many years to come. The evolution of MREs is a process known as the Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Capillary Electrophoresis SELEX, or CESELEX, is a method of selection which has begun to take footing in many traditional SELEX labs. Capillary electrophoresis offers several benefits when compared to traditional selection methods. These include increased target binding affinity and fewer rounds of selection. Capillary electrophoresis has allowed for development of MREs with high specificity and unique binding properties. When paired with the technique's efficiency, these benefits will likely aid in development of MREs for applications which were previously inconceivable.[47]

MRE selection: -

MREs bind with their target in a lock and key modelusing non covalent interactions such as hydrogen bonding and dipole dipole interactions. In many ways, they are comparable to antibodies. However, unlike antibodies, they can be easily created and selected against without the use of a living organism. MREs can be formed from DNA, RNA, or amino acids. DNA MREs are remarkably stable under varying conditions and can be readily synthesized. RNA MREs have additional means of synthesis, but are not exceptionally stable.[47]

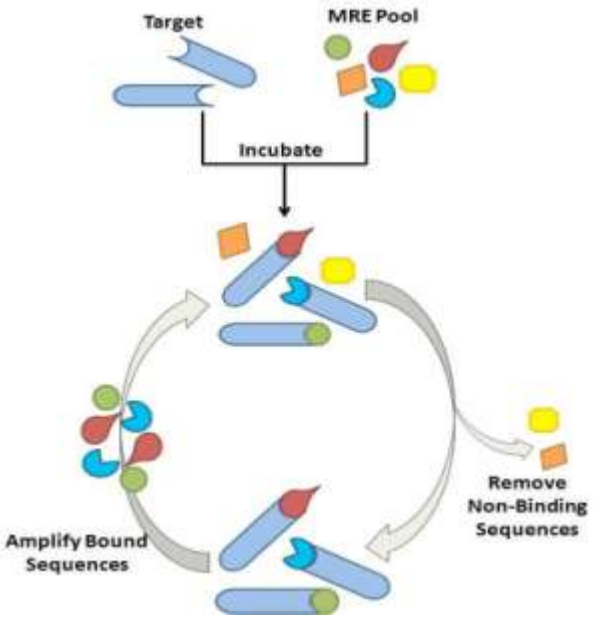

Figure 1. Generalized MRE Selection Process The MRE pool and target are first incubated together where molecules with high affinity will bind.

Amino acid MREs provide increased variability of the pool and the prospect of alternative selection methods. The target and potential application will determine the type of molecule used for selection.

For example, DNA MREs would be the best suited for biosensors due to their stability and ease of regeneration. Amino acid MREs are ideal for creating novel "proteins" for therapeutic use and can bind larger targets with ease. RNA MREs have similar applications to DNA MREs, but are notably useful therapeutically.[47] They are capable of mimicking small interfering RNA, thus silencing the expression of a given protein. The researcher will specify their known primer regions and then the length in nucleotides of the random region. The random region is generated viastandard phosphoramidite synthesis. In this synthesis, each nucleotide base is added sequentially to a growing chain. In traditional synthesis, the base to be added is predetermined and a very pure solution of this is added to the reaction[47].

Oligosynthesis consists of four steps. First, a phosphoramidite monomer is immobilized onto a surface and the 5' dimethoxytrityl (DMT) group is removed – thus activating the monomer. Next, through a condensation reaction, the next base to be added attaches to the 5' end of the

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growing chain. The resulting compound contains an unstable trivalent phosphate group which is then oxidized to the stable pentavalent phosphate. Finally, any unreacted 5' hydroxyl groups are acetylated in a process known as "capping" which prevents internal base deletions. The process is repeated beginning at the de‗ tritylation step until an oligonucleotide of the desired sequence or length is formed. This process is fully automated, and custom oligonucleotides can be ordered for next day delivery.[47]

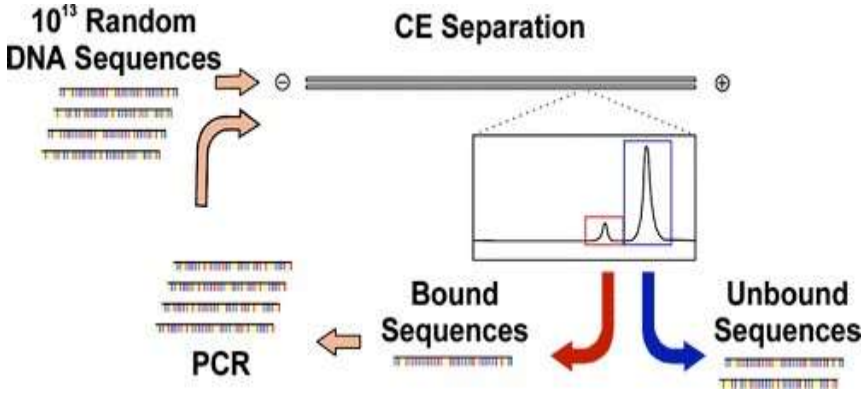

Figure 2. Generalized Representation of CE‗SELEX ‗ The peak in the red box corresponds to the unbound fluorescently tagged MRE pool, which elutes first. The second peak corresponds to the MRE/Target complex which is to be collected.

Small molecules can have potential MRE binding sites made unavailable due to biotinylating. Other targets cannot be biotinylated as easily. For these targets, an alternative method of selection which does not require target immobilization is preferable. There are several free solution selection methods, but the most prominent of these is capillary electrophoresis. Capillary electrophoresis SELEX does not require target immobilization, and therefore this technique decreases non‗specific binding to the immobilization surface and increases overall affinity of the MRE for the target. During CE‗SELEX, the MRE pool is combined with a target in buffer solution and loaded via a pressure plug into a small capillary.[47]

One end of the capillary is placed in a source vial containing the cathode, while the other is placed in a waste vial containing the anode. As current is passed through the solutions, molecules migrate at different speeds through the capillary based on their charge to mass ratios. This migration is monitored at the capillary window by a UV absorbance or fluorescence sensor. This sensor is capable of detecting the target, MRE, and bound target/MRE complexes. A very small fraction containing the MRE with bound target can then be collected as the pool elutes. The collected MREs can then be amplified and subjected to further rounds of selection until an MRE with high affinityand specificity for the target has evolved, the MRE pool has been fluorescently tagged, and this emission is being detected.[47]

MRE Optimization: -

The collected fraction of MREs is amplified and sent to one or more oligo‗ houses such as Integrated DNA Technologies (IDT) or Invitrogen for separation, sequencing, and synthesis. Once a potential MRE candidate has been sequenced, it will undergo a series analyse. Sequencing provides vital information about the binding motifs of each MRE. Various programs have been developed which can readily predict the tertiary structure or folding of an MRE when given a particular sequence of amino acids or nucleotides.[47]

CAPILLARY RISE METHOD: -

- **Principle: -** The principle of capillary rise measurements consists of dipping a fabric sample in a probe

liquid in order to study the wicking behavior of the porous medium. In the present work, a capillary rise experimental installation has been developed to record simultaneously the flow front position and the uptake fluid mass.[49]

- **Instrumentation: -** The fabric sample is placed in a transparent rigid mold held

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vertically on a frame supported by a motorized linear stage.

- The thickness of the mold is controlled by calibrated spacers to set precisely the fiber volume fraction of the sample, and the bottom part of the cavity possesses an opening that allows a direct contact between the reinforcement and the test fluid. The latter is contained in a standard rheometer capsule placed on a micro balance.

- This liquid container exhibits a high surface area with respect to the total fluid volume absorbed by the fibers, which allows minimizing buoyancy force variations during wicking as a result of a decrease in the liquid level.

- During the experiment, a constant displacement speed of 0.01 mm/s is first applied to the linear stage.

- The control software stops the motor once the fabric touches the liquid surface.

- Images of the imbibition are recorded by a 21 megapixels high definition digital camera, and the absorbed mass is measured with a resolution of 10g.

- The camera is remotely controlled, and each image capture of the capillary rise progression is taken at a specific rate of 1 image per 5 s. In order to follow the progression of the fluid, an inert fluorescent dye is mixed with the test liquid, and two 15Watts UV-black light bulbs are placed near the fabric sample.

- The whole experimental installation is placed in a closed dark room in order to prevent external light sources, air streams, or vibrations from perturbing the mass and image acquisitions.

- All the equipment (motor, balance, and camera) are controlled with a computer placed inside the dark room but remotely controlled from outside to prevent any disturbance when launching the experiments.

- This resulted in a fiber volume fraction of 36% for the tested sample. The test fluid was 99% pure hexadecane from Sigma-Aldrich, which possesses perfect wetting behavior with the fabric material.

- The images recorded during capillary rise tests were post-processed to detect the position of the flow front and characterize its temporal evolution.

Detection of the flow front was carried out with a Matlab® program following three main stages:-
-conversion of colored pictures into gray levels;
- conversion of gray levels into black and white;
- edge detection to locate the flow front position.

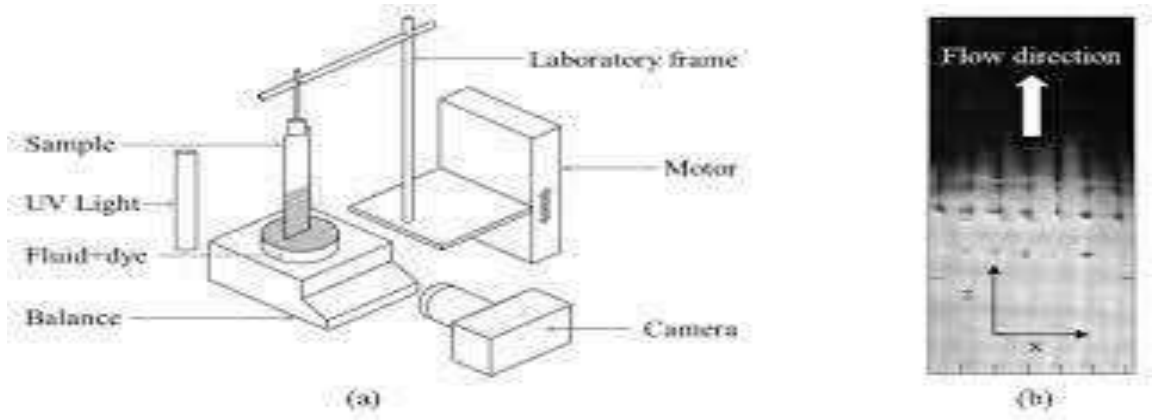

Figure 3. (a) Experimental setup for capillary rise measurements; (b) example of captured image

This resulted in a threshold value that varied during the progressive filling of the sample, which could in turn affect the detection of the flow front. Another source of error stemmed from the use of a semi-automatic camera with a variable shutter speed.[49]

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Firstly, in order to minimize the brightness variability between the images, the experimental setup was equipped with a new high resolution camera including a full frame captor and the possibility to control the brightness level in manual mode.
These features allowed collecting more accurate image data and hence improving the quality of the images for post-processing.
Secondly, a new conversion algorithm adapted to capillary images was devised. The gray signal was averaged in the x and plotted against the direction for every image captured during the capillary rise.[49]

Modeling of capillary rice: -

The progression of the fluid front during the capillary rise experiments was modeled using a simple approach based on the classical Lucas-Washburn law. The latter considers a HagenPoiseuille flow in which the effects of inertia and gravity can be neglected.[49]

Impregnation in RTM Mold of Varying Geometry

- **Manufacturing**

A laboratory scale RTM mold was devised to inject 2D plates of variable geometry at room temperature. The mold cavity has a total length of 370 mm and is divided in two zones of different cross-sections: a large one (110 mm wide) and a small one (55 mm wide). These two sections are separated by a transition zone with a length of 30 mm. The objective of the variable geometry is to show the influence of the impregnation velocity on the final void content.

Manufacturing experiments were carried out with fibrous beds made of 6 layers of TG15N fabric oriented in the weft direction. A fiber volume content of Vf = 38% was thus obtained given the mold cavity thickness of 3.175 mm.

The resin system was then degassed for 15 min under vacuum and placed in an injection pot. The latter was connected to a metering pump (85 MHP5 from Stenner®), which was subsequently used to deliver the mixture to the mold cavity. The pump was set manually in order to ensure the desired constant flow rate. No bleeding or consolidation pressure was applied after mold filling. The injection was stopped when the resin started to exit the mold cavity into the waste pot, and the part was cured at room temperature under atmospheric pressure[49].

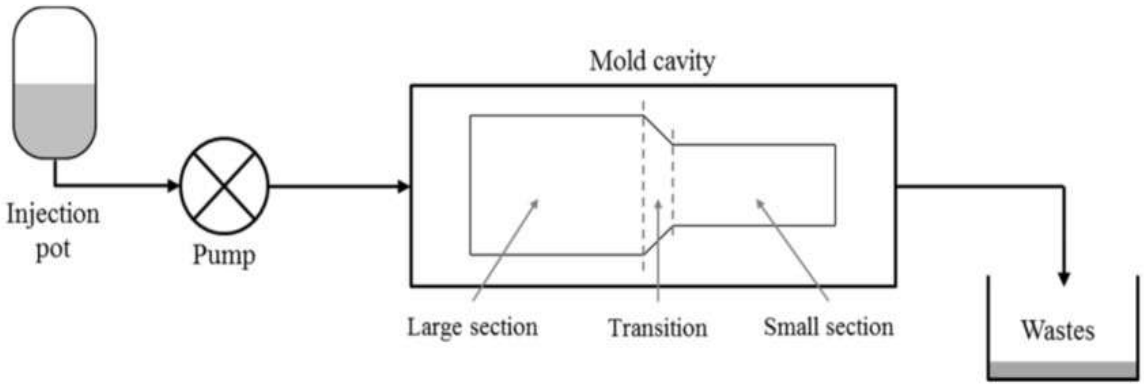

Figure4. SchematicsoftheexperimentalRTMmanufacturingsetup.

Themanufacturingexperimentswerecarriedoutwitht wodifferentconfigurations(convergentand divergent) by reversing the mold cavity. Three injection strategies were tested for eachconfiguration:

1. Maintainingtheoptimalimpregnationvelocitythr oughouttheplate(CSLandDLStests).
2. Maintainingtheoptimalimpregnationvelocityon lyinthesmallsection(CSandDStests).
3. Maintainingtheoptimal impregnationvelocityonlyinthelargesection(CL andDLtests)[49].

- **CEC-columnpreparation:-**

TheCEC-columnwaspreparedinour labsbyaslurrypackingmethodbasedonpreviouslydes cribedprocedures.
The manufacturing procedure was designed for a packed bed length of 25cm whereas longercapillariesup to50cmcouldbeproduced.Therefore,aslurryof20mgs tationaryphasein1mLacetone was prepared by sonification for 5 min and afterwards quickly

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transferred into a glass-linedsteeltubeusingasyringe.

The steel tube was then connected to the fused-silica capillary (50cm in length), which, on theother side,wasconnectedto aunioncontaining a0.5mmretainingsteelfrit.Polyimidehad beenremovedfrombothendsofthefused-silica capillaryusingamicroflame ModelBtorch.

For the final packing procedure a pneumatic HPLC pump was used pump head at a pressure ofapproximately850barfor 20min.Afterwardsthepumpwasturnedoffandthepressureallowedtodecreaseslowly.Thepackedbed wasdried at10 barbyconnectiontoanitrogenbottle.[9]

Using water in the slurry reservoir the first frit in the front region of the capillary was thermallysintered at packing pressure and a wire temperature below 4507C for 30 s. The pressure wasreleasedagain,thecapillaryturnedaround,andthesecondfritproducedinthesamewayathigh-pressure pump side. The excess of stationary phase was expelled by opening the bottom union ata residualpressureof400bar.[9]
The detection window was prepared by removing the polyimide coating directly after the outletfrit with hot, concentrated sulfuric acid on a grooved quartz block. Finally, the capillary wasflushed with ACN in reversed direction at 150 bar, to remove the other part of the superfluouscolumn material and stored in electrolyte until usage. Prior to the first injection, the capillary wasequilibratedwithrunningelectrolyte.
This was achieved by pressurizing the inlet vial with 10 bar, and ramping the voltage programstepwise vfrom 5 to 25 kV over 45 min. Then both vials were pressurized with 10 bar, and thechosenvoltage wasappliedtilbaseline andcurrentshoweda stable signal[9]

II. APPLICTION: -

1.Inorganic Compounds
2.Nanoparticles
3.Affinity
4.Nucleic Acid
5.Virus and Bacteria
6.Metabolites

1. InorganicCompound

Theanalysisofinorganicmaterialsremainsafieldofinterestinwhich CE has beenappliedforeitherquantitative,complexationorkineticanalysis.
Suchanalyseswerepredominantlyperformedusing(indirect)UV/Visabsorbancedetection, butC4D is increasingly applied. Saiz et al. utilized CE-C4D for the analysis of common cations andcompared the performance of the conductivity detection-compatible BGEs (MES/HIS, Lac/Hisand Lac/β-Ala) and emphasized that addition of 18-crown-6 and hydroxyisobutyric acid wereessentialforincreasingthedetectionsensitivity.
CE-C4Dwasalsousedforthedeterminationofbromateinwater.Utilizingelectromembraneextractionasasamplepretreatment,LODswereinthesub-ng/mLrange.[40]

2. Nanopaericles

CE has seen significant applied in the still expanding field of NPs, such as QDs and gold NPs,whichexhibithighstability,easeofchemicalsynthesisandlowtoxicity.CEwasmainlyusedforobtaining information on the size and surface characteristics of NPs and their interaction withbiomolecules.

EfficientCEseparationsofNPsoften requireadditionofstabilizerstotheBGE. Forexample,Poly(4-styrenesulfonate)(PSS)wasstudiedasanalternativeto SDSforimprovedseparationandsizedeterminationof AuNPs.

The addition of PSS along with a stepwise field gradient significantly improved the resolutionfor AuNPs with diameters ranging from 5 to 20 nm. Similar observations were made forstabilizerssuchasPluronicF-127,citrate,andcetyltrimethylammoniumallowingdifferencesinAuNPsurfacechemistry andsizetoberevealed.

In order to establish selective UV detection of titanium dioxide nanoparticles, they were boundtosinglestranded(ss)DNAfollowedbycoating withPEG73,whereasdetectionofzincoxideNPswasachievedby their interactionwithdithiothreitol inphosphatebuffer.

Theseapproachesled to13-27foldenhancedUVabsorbancesignal intensities,respectively.

CE wasalsousedfortheinvestigationofinteractions betweenproteinsandNPsusedforbiologicalapplications.

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CE-ICP-MS of functionalized Au nanorods interacting with serum proteins revealed metal-specific protein profiles for the differently functionalized AuNPs77. However, identification ofthe proteinswas notalways possible duetothelarge numberof possiblecandidate[40]

3.Affinity

CE has shown particular useful for the study of (bio)molecular interactions, providing shortanalysistimes,lowsamplesizerequirements,high separationefficiencies,andabilitytocoveralarge rangeofaffinities.Lietal.

employedaffinityCE(ACE) inordertostudythebinding ofsulfatedβ-CDtouranylcompoundsin aqueous solutions. ACE was also employed for establishing apparent binding constants ofcomplexes between enantiomers of acyclic nucleoside phosphonates (ANPs) and β-CD inaqueous alkalinemedium.

Estimationoftheequilibriumdissociationconstantsby nonlinearregressionandlinearized plotsshowed that the ANP-β-CD complexes are relatively weak. Limitations of ACE forquantification of the supramolecular interactions between the CD cavity and ionic liquids, andtheireffectonthe stabilityoftheinclusioncomplexes,were investigatedaswell85.

The effect of surface oxidation state on the intensity and mode of particle-protein conjugationwas quantitatively evaluated by CZE and ACE methods86. Partial filling (PF) ACE wascombined with adsorption energy distribution to determine the heterogeneity of interaction ofapoB-100containinglipoproteinsandtheirantibodies87.The interactionprovedhomogenousand PF-ACEresultswereinalignmentwithquartzcrystalmicro balanceexperiments.[40]

TheuseofCEforstudyingenzymaticactivityandinhibi tiongainedattention.

Theinhibitionofhumanneutrophilelastasewasstudied usingbothtransversediffusionoflaminarflow profiles(TDLFP)andmicroscale thermophoresiswithLIFdetection.

Twonaturalpentacyclictriterpenes, ursolicandoleanolicacidwereusedtovalidatethedevel opedCEassay.

Themethod enabled estimationoftheIC50 and Kivaluesoftheseinteractions, whichwerein agreement with those reported in literature. In a similar work, cIEF-LIF was compared to CZEforitsabilitytosimultaneouslystudycomposition andinhibition ofmultipleproteinkinases [40].

The method was successful regardless of structure and charge of the substrate peptides. The useof nanogels to physically constrain an enzyme in a separation capillary while performingelectrophoretically-mediatedmicroanalysis(EMMA)wasproposedforim provingsensitivityandseparationspecificity[40].

4.NeuclicAcid

Kanoatov and Krylov pointed out that an ACE experiment under physiological relevantconditions for the study of DNA-ligand interactions is feasible. With DNA molecules inphosphate buffered saline, they used a pressure-facilitated non-equilibrium CE of equilibriummixtures approach to attain insights in DNA-ligand kinetics95. Similarly, Tohala et al. usednative separation conditions to study the interaction between homopolymeric sequences andweakDNAbindingenantiomers.

StudiesfocusingontheCEanalysisofmicroRNA(miR NA)remainscarce.Recentadvancesinseparationperfo rmancesforlongnon-codingRNAmayalter this.

DirectseparationofmiRNAwithCEisnotalwaystrivia landoftenrequiresanadditivetothebuffer for enhanced performance. ssDNA can be used for such purposes as was shown byWegman et al., using a hybridization assay with miRNA-specific DNA probes labeled with afluorophoreforLIFdetection.

To separate the miRNA-DNA hybrids from each other and from the probe excess, an ssDNAbinding proteinaswella differentsizesofprobeswereincorporatedintheworkfl ow.[40]

Although often the identification of nucleic acids is done by polyacrylamide gel electrophoresis,or PAGE, and polymerase chain reaction, or PCR, analysis, the capability of attaining insights inpost-transcriptionalmodificationsislimited100.

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Recently,CE-MSwasusedtoidentifyandquantifythesemodifications101. Theauthorscoulddetect two endogenous human circulating miRNAs isolated from B-cell chronic lymphocyticleukemia serum.

TheCEseparationandfollowingMSanalysisprovided label-freequantitationandrevealed5'-phosphorylationand3'-uridylationasmodificationsofmiRNAs.[40]

5.VirusandBacteria

Van Trichtdeveloped a capillary gel electrophoresis (CGE) method for fast and selectivecharacterizationandquantificationofviralproteins ininfluenzavaccines.Dilutionofthegelbuffer allowed higher separation voltages which led to shorter run times and improvedefficiencies.
The CGE method allowed analysis of 100 samples in four days making it very suitable forquality control purposes. In order to enable characterization of low quantities of adeno-associated virus capsid proteins, Zhang et al. developed a head-column FASS method as anonline sample preconcentrationtechnique compatible withCGE103.

The effects of a short water plug, SDS concentration both in sample matrix and in the matrixexchangingsolutionaswellastheeffectofsampleinjectiontimewereinvestigated.WithLODsinthelow-pM range.[40]

The characterization of intact phytopathogen bacteria was investigated using cIEF, CZE andmatrix-assistedlaser-desorptionionization – time-of-flight.

Forty-threestrainsoftheDickeyaand Pectobacteriumspecieswereselectedamongofwhichsome that could not be classified with the traditional methods. In the case of cIEF the majorchallenge was thesimilarityonthepHvaluesofsome subspecies.

On the other hand, most of these species could be discriminated unambiguously by CZE. MostdiscriminatorypowerwasobtainedwithMALDITOF-MS asuniquemassspectralprofileswereobtainedforallrespective species orsubspecies.[40]

6.Metabolites

OneofthemajorapplicationsofCErelatestothedeterminationofmetabolitesinvarioustypesof biologically relevant samples. MS detection plays an important role since many metabolitescannot be optically detected without prior derivatization and it provides opportunities for theidentificationofunknowns.

Toaidinthelatter,achemoinformaticsapproachforrankingcandidatestructuresofunidentifiedpeaks was developed108. The approach uses information about the known metabolites detectedin samples containing unidentified peaks and was successfully applied to identify two unknowncompoundsobservedina CE-MSurinarymetaboliteprofile.

Anotherstudyfocusedmoreonbig-datahandlinginaSPE-CE-MSforidentifyingbiomarkers(in mice) related to Huntington's disease109. The workflow ensured significant data reductionpriortomultivariate curve resolutionasymmetricleastsquaresanalysis.

CationicmetaboliteprofilingbyCE-MSisroutinelyapplied, however,profilingofanionsproves more problematic. Yamamoto et al. showed that alkaline ammonia-based buffers (pH > 9)often used for these analyses react with polyimide outer coatings of fused-silica capillariesresulting in frequent capillary fractures and poor long-term performance110. By making minoradaptationstotheBGE,robusthigh-throughputprofilingofanionicmetaboliteswasachieved.[40].

III. SUMMARY AND CONCLUSION

Molecular Recognition Elements are versatile compounds which have potential uses in a varietyoffields.Thesecompoundswereoriginallyderivedby SELEXselectionswhichrequiredimmobilization of the target. These methods restricted use of small molecule targets and limitedthevarietyofevolvedMREs.CapillaryElectrophoresishascertainlytransformedSELEXselectionsandMREanalysis.Freesolutionselectionhasremediedmanyofthesepriorshort-comings. However, CE-SELEX does come with its own set of weaknesses which will beimproved upon in the future. This optimization will likely be achieved by technological advancescombined.
Capillary Electrophoresis has become an incredibly

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useful tool for both selection and studying ofMRE/target interactions. CE-SELEX has greatly reduced the time and increased the efficiency ofMREevolution.
NewmethodsofpartitioningthetargetandMREhavere sultedintheabilitytopredictandevolveMREswithspec ificaffinitiesforagiventarget.Capillaryelectrophoresi shasalsoprovidedanewmeansofmeasuringtarget/MR E.

REFERENCE

[1]. C. Borst , F. Belal, U. Holzgrabe ,"possibilities and limitations of capillaryelectrophoresisinpharmaceuticalana lysis",analyticalchemistry2013,526-530.
[2]. Helena Brunnkvist , Bo Kaelberg, Linda Gunnarsson, Ingrid Granelli, "Experimentaldesignasatoolwhenevaluatings tationaryphasesforthecapillaryelectrochroma tographic separationofbasic peptides",journalofchromatographyB.813,20 04,67-73.
[3]. Robert L.C. Voeten, Iro K. Ventouri, Rob Haselberg, and Govert Willem Somsen,"Capillaryelectrophoresis:Trendsan drecentadvances",Analyticalchemistry,2018.
[4]. LiWanga,YanSuna&QiLiua,"Determination ofpharmaceuticalsand personalcareproduct components in wastewater using capillary electrophoresis coupled with solidphase extraction" ,Analyticallatters,2015,48,944-954.
[5]. BrianaD.Vecchio,capillaryelectrophoresis:a methodforevolutionandanalysisofmolecularr ecognitionelements,51-69.
[6]. PhilippeCausse*,ChristopheRaveyandFranç oisTrochu,Capillarycharacterizationof fibrous reinforcement and optimization of injection strategy in resin transfermolding,Journalofcompositescience, 2018,2,19.
[7]. Meriem Dadouch, Yoann Ladner and Catherine Perrin *, Analysis pf monoclonalantibodiesbycapillaryelectrophor esis:samplepreparation,separation,anddetecti on,Separations,2021,8,4.
[8]. Martyna Piotrowskaa, Krzesimir Ciura b,#, Michalina Zalewskaa,#, Marta Dawidc ,Bruna Correiac , Paulina Sawickac , Bogdan Lewczuk d, Joanna Kasprzyke , LauraSolaf , Wojciech Piekoszewski g , Bartosz Wielgomas c , Krzysztof Walerona,∗ ,SzymonDziombac,∗,"Capillaryzoneelectrop horesisofbacterialextracellularvesicles:Apro ofofconcept",JournalofChromatographyA16 21(2020)461047.
[9]. FangguiYe, ShutingLi,andShulinZhao*,"Electrochromat ographicevaluationofdiol-bonded silica monolith capillary column for separation of basic compounds", Journal ofchromatographyofchromatographyscience, 2009,47.
[10]. Jandai*,jared lamp,qiangweixia*,andyingruzhang*,"capill aryisoelectric focusing-massspectrometrymethodforthesеparationan donlinecharacterizationofintactmonoclonala ntibodychargevariant",analyticalchemistry,2 018,90,2246-2254.
[11]. Philippe causse*, christophe ravey and francois trochu, "capillary characterization offibrousreinforcementandoptimizationofinj ectionstrategyinresintransfermolding",journa lofcomposites science,2018.
[12]. Qingai Chdn, Lijun Deng, Jinxin Chi, Min Liu, Xucong Lin *, and Zenghong Xie,"Sensitive profiling of trace neurotoxin domoic acid by pressurized capillaryelectrochromatographywithlaser-inducedfluorescencedetection",Royalsociety Ofchemistry,2017,7,53778.
[13]. Unita L. Peri- Okonny, emst kenndler, R. John Stubbs, Norberto A. Guzman,"Characterization of pharmaceutical drugs by a modified nonaqueous capillaryelectrophoresis-massspectrometrymethod",Electrophorosis,2 003, 24,139-150.
[14]. Rabah Gahoual,1 Alicia Burr,1 Jean-Marc Busnel,2 Lauriane Kuhn,3 PhillipeHammann,3AlainBeck,4Yannis-NicolasFrançois1*,andEmmanuelleLeize-Wagner1,"Rapid and multi-level characterization of trastuzumab using sheathless capillaryelectrophoresis-tandemmassspectrometry",Landesbioscience ,2013,1-12.
[15]. Tomoyoshi Soga,*,† Yoshiaki Ohashi,† Yuki Ueno,† Hisako Naraoka,† MasaruTomita,† Takaaki Nishioka†, "Quantitative metabolom analysis using capillaryelectrophoresismassspectrometry",J ournalofproteomeresearch,2003,2,488-494.
[16]. Robert L.C. Voeten, Iro K. Ventouri, Rob Haselberg, and Govert Willem

Somsen,"Capillaryelectrophoresis:Trendsan drecentadvances",Analyticalchemistry,2018.

[17]. Guor-tzo wei*, Chih-Fu Hsu, Fu-Ken Liu, and Fu-Min Chou, "The development ofreversed-phase capillary electrochromatography for the separation of steroids", JournaloftheChinesechemicalsociety,2005,52,741-751.