Challenging applications of capillary electrophoresis in biopharmaceutical analysis

After decades of development and refinement, the separation of proteins by capillary electrophoresis (CE) has moved beyond the R&D laboratory into the manufacturing quality control laboratory (QC).[1] Here, Giuseppe Peddio and Federica Bisceglia discuss the advancements in CE technology for development applications and batch release for biopharmaceutical products.

Introduction

Since its introduction in 1980, CE has been one of the most powerful techniques applicable as a choice method in the biopharmaceutical industry for the characterisation and QC testing of biomolecules such as proteins, peptides as well as monoclonal antibodies (mAbs) and high molecular weight polysaccharides. In addition to the intrinsic advantages of CE, such as high separation efficiency, high resolution, low sample volume requirements, short separation time and full automation possibility, it exhibits attractive possibilities for the efficient analysis of intact large molecules, providing some information about isoforms, degradation products and impurities of biopharmaceuticals and charge heterogeneity.[2] Over time, CE has advanced to become a superb complement to high-performance liquid chromatography (HPLC), and in many cases has also evolved as an automated and quantitative replacement for conventional slab gel electrophoresis methods such as SDS-PAGE and isoelectric focusing (IEF).[3]

Summary of CE applications in quality control of biopharmaceuticals	
Identity	Charge variants
Quantitation	Heterogeneity
Isoelectric point	Isoforms pattern
Purity	Glycoforms
Molecular weight	Stability

What kind of CE-based methods can be applied in the QC of biopharmaceuticals?

Several approaches using CE are applicable to assess the quality of biopharmaceuticals:


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- Capillary zone electrophoresis (CZE) is the simplest and most widely used method, which allows the analysis of ionised or ionisable compounds and is based on the differences in their electrophoretic mobility.[4] Analytes are simply separated according to their charge/hydrodynamic radius ratio (size) and migrate towards anode or cathode according to their charges. The separation can be accomplished by the presence of surfactants in the running buffer. Several CZE-based methods have been reported for the QC of biopharmaceuticals in terms of quantification, purity and heterogeneity analyses as well as stability studies.
- Capillary isoelectric focusing (cIEF) is similar to IEF–PAGE and separates proteins and peptides according to their isolectric point (pI) values with a 'high-resolution' response. Protein isoforms, proteins whose separation by other methods can be problematic, such as immunoglobulins and haemoglobins, and dilute biological solutions have all been successfully analysed by cIEF.[5] In comparison with the other CE modes, cIEF and especially imaging cIEF (icIEF) demonstrates the best resolution for the analysis of proteins and peptides;[4] they also play an important role in the charge heterogeneity analysis of biopharmaceuticals[6] and the development of biosimilars.[7]
- Capillary gel electrophoresis (CGE) under reducing or non-reducing conditions involves the separation of molecules according to their size. It has been developed according to the principles of sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE).[3] The main advantages of CGE over SDS-PAGE include ease of handling and automation, higher resolution, greater reproducibility, shorter separation time and more accurate determination of molecular weight; making it a superior technique in the QC of biopharmaceuticals.[8,9] Among others, CGE under reducing conditions allows the separation of heavy and light chain subunits and product related impurities.
- Capillary electrokinetic chromatography (CEKC) could be

a suitable method for separation of protein stereoisomers and peptides with similar charge-to-size ratios and different hydrophobicity. Among different CEKC modes, micellar electrokinetic chromatography (MEKC), which separates analytes using micellar pseudo-stationary phases of surfactants,[10] has been applied more commonly for the QC of biopharmaceuticals.

Other approaches, even if less frequently applied in the QC of biopharmaceuticals, include affinity capillary electrophoresis (ACE),[11] capillary electrochromatography (CEC) and CE-MS. CE-MS has been advanced for the characterisation, identification and QC of biopharmaceutical products owing to its high sensitivity and selectivity.

Gene therapy challenges

The theoretical risk associated with potential oncogenicity and infectivity due to a host cell oncogene, infectious retroviral or other viral genome sequence could raise concerns from a regulatory or safety standpoint. For this reason, the analysis of gene therapy products is a crucial and integral part of the manufacturing process. There are several technical difficulties in characterising gene therapy products, mainly due to the matrix complexity and very low concentration of analytes. CE is considered the most powerful tool, providing automated separation of viral proteins with high resolution and reproducibility; moreover, CE combined with LIF-detection represents a platform-based approach to characterise gene therapy products according to authorities' requests, providing the highest sensitivity and resolution.[12]

Capillary electrophoresis as an orthogonal technique: when should it be considered?

Recently, the use of dissimilar or orthogonal chromatographic systems to develop methods for drug impurity profiling is challenging and gaining importance. Although this approach is very powerful and can solve most problems, it does not always guarantee satisfactory separation for all impurity mixtures.[13] Capillary electrophoresis, on the other hand, offers high separation efficiency and can be considered a suitable technique for biomolecules being used orthogonally in the specificity assessment of HPLC methods.[14] CE is based on a different mechanism of separation and therefore provides selectivity differences toward HPLC. CE has the capacity to determine polar and neutral compounds, which may co-elute in the »

Advantages of working in a Biosafety Level-2 environment with capillary electrophoresis

Researchers, biomedical as well as QC laboratories, often work with live and potentially dangerous microorganisms (GMMO, Adeno-associated virus, lentivirus and plasmids). The main advantage of working with CE systems in a BLS-2 compliant environment is that a wider variety of agents and products can be safely handled. Under these conditions, samples can be managed and analysed without additional sample pre-treatment and inactivation that could cause modification or sample loss. Furthermore, this ensures that the test can be performed efficiently without the need for additional preparation steps. Analysis time is a key factor in QC release testing and ensuring that turnaround time achieved.

EXPERT VIEW Q&A

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The role of capillary electrophoresis in GMP testing for biologics products

Giuseppe: What kind of GMP testing could be supported by CE?

Federica: Capillary electrophoresis (CE) is one of the most powerful techniques for characterisation and quality control of biomolecules, guaranteeing intrinsic advantages such as high separation efficiency, high resolution, small sample amount, short separation time, and full automation possibility; it enables analyses of intact peptides and proteins, providing information about isoforms, degradation products, impurities and analysis of their charge heterogeneity. Application areas include identity tests, like isoelectric point and molecular weight determination, quantitative tests together with purity and stability, and characterisation tests such as heterogeneity, isoform patterns, glycoforms and charge variant determinations.

Giuseppe: What about CE application to gene therapy products?

Federica: There is a huge variety of gene therapy products such as plasmid DNA, viral vectors, human gene editing technology and patient-derived cellular products. CE coupled with laser induced fluorescence (LIF) detector represents a platform-based approach to mitigate technical difficulties in characterising gene therapy products. Adeno-associated viruses could be characterised for capsids purity and impurities or partial and empty capsids ratio, plasmids for purity testing and degradation monitoring, whereas lentivirus characterisation could be driven to genomic purity and host cell DNA fragments determination. The most suitable approach should be defined depending on sample characteristics and the desired goal.

Giuseppe: What are the main advantages of CE over LC (Liquid Chromatography)?

Federica: CE represents a 'green' analytical separation method, compared with LC which can generate significant volumes of chemical waste, including organic solvents.

CE separation media are aqueous buffers; typically, few millilitres are needed for sequences and for sample injection. Moreover, time consuming operations usually required for sample pre-treatment before chromatographic analysis can usually be avoided. Another advantage is the high-resolution efficiency, due to uniform velocity of the flow across the entire capillary.

profile generated by a conventional reversed-phase HPLC method. Finally, CE has also shown greater efficiency in separating isomers compared to chromatographic analyses.[14]

Conclusions

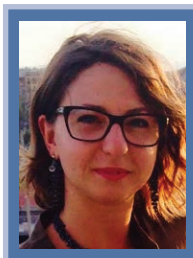
The molecular complexity of biopharmaceuticals requires a large variety of analytical methods to characterise these products.

In this context, CE and its modalities have shown to play a unique role in the overall QC testing strategy in the biopharmaceutical industry during all steps of production (process development, QC, release and stability testing): "the process is the product".[15]

In summary, the versatility, simplicity, low sample consumption, fast separation and high resolution of CE allows for the extensive, complete and reliable characterisation of therapeutic proteins and cell & gene therapy products.

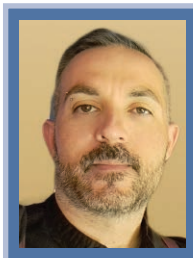
Table 1: Overview of the characteristics and applications of CE

Application		CE mode	Detector
Large Molecules: mAbs, Proteins, Peptides, Antibody-drug conjugates (ADC), Recombinant fusion Proteins Hormones, Degradation products, Aggregates, Neutral-monosaccharides, Amino-monosaccharides Oligosaccharides	Protein purity, and aggregation status	CGE	UV or PDA
	Charge variants distribution and heterogeneity	CZE	UV or PDA
		cIEF	UV or PDA
		icIEF	UV
			LIF
	pI determination	cIEF	UV
		icIEF	UV
			LIF
	Migration time and profile comparison	CZE	UV or PDA
		MECK and CECK	UV or PDA
		cIEF	UV
		icIEF	UV
			LIF
	Post translational modifications (PTMs): glycosylation, oxidation, phosphorylation, deamidation	CZE	UV, PDA or LIF
Gene therapy: Adeno-associated virus (AAV) lentivirus plasmids	AAV → capsids purity and impurities	CGE	LIF
	AAV → full, partial and empty capsids ratio	cIEF	
	Lentivirus → genomic purity	CGE	
	Lentivirus → Host cell DNA fragments	CGE	
	Plasmids → purity testing and degradation monitoring	cIEF	
		CGE	



Federica Bisceglia, PhD

Federica is a Project Leader of the chemical testing team in the Biologics Department at Eurofins BioPharma Product Testing (BPT) in Milan, Italy. After her master's degree in medical and pharmaceutical biotechnologies from University of Pavia, she obtained a PhD in chemical and pharmaceutical sciences focused on CE analysis for the study of aggregation process of proteins. Federica has been working in the pharmaceutical field for more than five years and at Eurofins BPT since 2020 where she is responsible of new methods development, transfer and validation of analytical methods for the characterisation of biotherapeutic products, raw materials, as well as quality control testing including several techniques such as CE, HPLC, LC-MS, IC and UV.



Giuseppe Peddio, PhD

Giuseppe holds a chemistry and pharmaceutical technologies degree and a PhD in neuroscience from the University of Cagliari. He has extensive experience as a bioanalytical researcher through chromatography method development and validation, mainly related to pharmacokinetic studies by LC-MS and LC-MS/MS. In his role as GLP Laboratory Manager, he has been involved in studies of bioequivalence in collaboration with the National Research Council for drug product and impurities qualitative/quantitative characterisation. Since 2017 he has been working at Eurofins BioPharma Product Testing; for the last four years as manager of a GMP- chemical laboratory working on biologics products characterisation and analysis.

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