

# Chapter 2

## Coated Capillaries for CE-MS of Therapeutic Protein

James Q. Xia

### 2.1 Introduction to Capillary Coating

Bare fused-silica capillary is so far the most commonly used type of capillary in CE-UV analysis. Under the running conditions using high salt buffer and detergents as background electrolytes, bare fused-silica capillaries are great tools for separating biological molecules. For example, in the case of reduced monoclonal antibodies, CE-SDS analysis has become a routine assay for the purity test of IgG therapeutics [1, 2]. However, for protein CE-MS analysis, coated capillaries become necessary—basic proteins tend to be absorbed on the inner surface of bare fused silica capillary. This necessitates the use of capillary coating to minimize protein absorption [3–6].

### 2.2 Types of Capillary Coating

There are hundreds of capillary coating methods being reported in the past two decades. A few types of capillaries commonly used for protein CE-MS analysis are polyethylenimine (PEI) [3, 7], polyvinyl alcohol (PVA) [8], fluorocarbon (FC), polybrene–dextran sulfate–polybrene (PB-DS-PB) and linear polyacrylamide (LPA) coated capillaries [5]. Table 2.1 lists out the specific applications of these types of coated capillaries in protein CE-MS analysis.

---

J.Q. Xia (✉)

CMP Scientific, Corp., 760 Parkside Ave, STE 211, Brooklyn, NY 11226, USA  
e-mail: james.xia@cmpscientific.com

**Table 2.1** Commonly used coated capillaries for protein CE-MS analysis

Coating chemistry	Charge type
Polyvinyl alcohol (PVA)	Neutral
Polyethylenimine (PEI)	Cation
Fluorocarbon (FC)	Neutral
Linear polyacrylamide (LPA)	Neutral
Polybrene-dextran sulfate-polybrene (PB-DS-PB)	Cation (triple layer)
Bovine serum albumin (BSA)	Cation (protein)

Besides the permanently coated capillaries shown in Table 2.1, there are also dynamic coating methods to modify the capillary surface without putting a permanent coat on the capillary [4, 6, 9]. One way of achieving dynamic coating is to introduce amine chemicals into the running buffer [6, 10]. For example, 2 mM Triethylenetetramine (TETA) can be added to acetic acid buffer to create a layer of amine cations on the capillary surface. Another type of dynamic coating can be done by coating the capillary with a polymer layer that does not bind covalently to the capillary surface silanol groups [9]. At the end of each analysis, sodium hydroxide or ammonium hydroxide can be used to rinse off the coating layer. After that, the capillary can be coated again with the polymer for the next injection. The advantage of dynamic coating is that the capillary surface can be regenerated, thus minimizing the effect of protein absorption or coating layer being damaged during injections [6, 9].

## 2.3 Challenges in Coating and the Use of Coated Capillaries

Changes on the capillary surface under electrophoresis may result in poor reproducibility. It is not well documented how electrophoresis affects the surface chemistry. The stability of capillary coating also depends on the running buffer that has been chosen to run the CE-MS experiments. In the case of protein analysis, the minute amount of proteins that gets absorbed on the capillary surface can build up and gradually affect electroosmotic flow. All these troubles make it difficult to select the right types of coated capillaries and optimize running conditions. In some cases, the coated capillary consumables can be cost-prohibitive due to the process that get involves to make the capillary useful for CE-MS coupling—this makes it even more challenging to use coated capillaries for routine protein analysis.

In our experience, linear polyacrylamide (LPA) capillaries have given consistent CE-MS results. It should be noted that the LPA capillaries need to be conditioned well before one can use it to carry out sample analysis. One of such

conditioning steps is to rinse the capillary with 10 % acetic acid thoroughly (usually rinse under 40 psi overnight). Without adequate aging, sometimes the unpolymerized residue can bleed off the capillary and cause high mass spectrometer background for an extended period. Given the fact that many types of commercially available coated capillaries are polymer based, in general we recommend that users use the same rinsing procedure to condition the coated capillaries before sample analysis. Such rinsing steps can also be done using syringe pumps, if the CE instrument is not readily available.

The running buffer for using the neutral coated and cation coated capillaries can be quite different. However, it is usually recommended to start testing out separation with 10 % acetic acid. For a 100 cm long, 50  $\mu\text{m}$  ID capillary, the BGE with 10 % acetic acid generates a CE current of about 10  $\mu\text{A}$ , which falls into the suitable working range for CE-MS hyphenation. On the other hand, the viscosity of 10 % acetic acid is such high that it provides sufficient sieving effect on separating large protein molecules. The authors have been using acetic acid up to 40 % and found that in general higher percentage of acetic acid gives better separation resolution. However, some types of coated capillary do not stand long time use when soaked in 30–40 % acetic acid while electrified under 30 kV. Therefore it is recommended that 10 % acetic acid is used as the initial step for CE-MS method development.

In terms of long term storage, after each use, we recommend rinsing the coated capillary with water for half an hour, and then store the capillary in water at 4  $^{\circ}\text{C}$  to prevent microbial growth. Unlike the conditions for storing bare fused silica capillary, drying the coated capillary for long term storage is not recommended.

## **2.4 Case Study of Coated Capillaries Used for Protein CE-MS Analysis**

### ***2.4.1 Analysis of Recombinant Human Erythropoietin (RHuEPO) Using Neutral and Cationic Dynamic Coating***

Two types of dynamic capillary coatings, UltraTrol<sup>TM</sup> low normal and high reverse, were evaluated for the separation of recombinant human erythropoietin and novel erythropoiesis-stimulating protein glycoforms by CE-MS [11]. However, in our experience we have observed that the low normal coating tended to bleed off the capillary, causing issues for mass spectrometer detection. As previously mentioned, this is a common problem associated with dynamic coating methods using polymers.

### ***2.4.2 Analysis of Recombinant Human Chorionic Gonadotropin***

For this highly glycosylated protein, the identification of over 20 glycoforms have been reported using polyvinyl alcohol (PVA) coated capillary [7, 8]. The PVA coated capillaries are commercially available through Agilent Technologies.

### ***2.4.3 Analysis of Recombinant Human Growth Hormone***

The use of polybrene–dextran sulfate–polybrene capillary on recombinant human growth hormone analysis is an interesting application of using a triple layer coating method for the analysis of glycosylated proteins [4].

### ***2.4.4 Analysis of Reduced Monoclonal Antibody Using LPA Capillary***

LPA capillary has been used in the industry for capillary electrophoresis for decades and is well known for its stability under strong electrical field. One of the most common analytical tasks in therapeutic monoclonal antibody characterization is the CE-SDS assay of reduced monoclonal antibody. Quantitative results of the peaks of heavy chain and light chain can be used as the basis for antibody purity release assay. It is thus important that a reliable CE-MS workflow for the analysis of reduced monoclonal antibody is established. It has been demonstrated that LPA capillaries are suitable for the CE-MS analysis of the intact form and subunits of IgG molecules [5]: heavy chain and light chain of the IgG molecules were separated on the LPA capillary by more than three minutes, with peak width of about 45 s. This high separation efficiency is allowed for the subsequent detection of posttranslational modifications [5].

### ***2.4.5 Analysis of Interferon— $\beta$ 1 Using Cross-Linked Polyethylenimine Coating***

Instead of using regular polyethylenimine coated capillary, a cross-linked polyethylenimine coating has been recently reported to be used on the quantitative CE-MS analysis of intact Interferon- $\beta$ 1 proteoforms [12].

### 2.4.6 Capillary Coating by Bovine Serum Albumin

Protein coating on the fused silica surface has been reported to generate highly reproducible CE-MS analysis [13]. Caution should be taken when applying this type of coating, in that protein coatings are usually reversible. The performance of protein coated capillary may gradually deteriorate. The electroosmotic flow on the capillary thus needs to be frequently monitored in order to minimize protein sample loss by absorption to capillary surface.

## References

1. Salas-Solano O et al (2006) Optimization and validation of a quantitative capillary electrophoresis sodium dodecyl sulfate method for quality control and stability monitoring of monoclonal antibodies. *Anal Chem* 78:6583–6594
2. Michels DA, Brady LJ, Guo A, Balland A (2007) Fluorescent derivatization method of proteins for characterization by capillary electrophoresis—sodium dodecyl sulfate with laser-induced fluorescence detection. *Anal Chem* 79:5963–5971
3. Horvath J, Dolnik V (2001) Polymer wall coatings for capillary electrophoresis. *Electrophoresis* 22:644–655
4. Haselberg R, Brinks V, Hawe A, de Jong GJ, Somsen GW (2011) Capillary electrophoresis–mass spectrometry using noncovalently coated capillaries for the analysis of biopharmaceuticals. *Anal Bioanal Chem* 400:295–303
5. Han M, Rock BM, Pearson JT, Rock DA (2016) Intact mass analysis of monoclonal antibodies by capillary electrophoresis–mass spectrometry. *J Chromatogr B* 1011:24–32
6. Righetti PG, Gelfi C, Verzola B, Castelletti L (2001) The state of the art of dynamic coatings. *Electrophoresis* 22:603–611
7. Thakur D et al (2009) Profiling the glycoforms of the Intact  $\alpha$  subunit of recombinant human chorionic gonadotropin by high-resolution capillary electrophoresis–mass spectrometry. *Anal Chem* 81:8900–8907
8. Belder D, Deege A, Husmann H, Kohler F, Ludwig M (2001) Cross-linked poly(vinyl alcohol) as permanent hydrophilic column coating for capillary electrophoresis. *Electrophoresis* 22:3813–3818
9. Chiu RW, Jimenez JC, Monnig CA (1995) High molecular weight polyarginine as a capillary coating for separation of cationic proteins by capillary electrophoresis. *Anal Chim Acta* 307:193–201
10. Gelfi C et al (2001) Protein analysis by capillary zone electrophoresis utilizing a trifunctional diamine for silica coating. *Anal Chem* 73:3862–3868
11. Giménez E, Benavente F, Barbosa J, Sanz-Nebot V (2008) Analysis of intact erythropoietin and novel erythropoiesis-stimulating protein by capillary electrophoresis-electrospray-ion trap mass spectrometry. *Electrophoresis* 29:2161–2170
12. Bush DR, Zang L, Belov AM, Ivanov AR, Karger BL (2016) High resolution CZE-MS quantitative characterization of intact biopharmaceutical proteins: proteoforms of interferon- $\beta$ 1. *Anal Chem* 88:1138–1146
13. Man Y et al (2013) Adsorptive BSA coating method for CE to separate basic proteins. *Chromatographia* 76:59–65

Capillary Electrophoresis-Mass Spectrometry

Therapeutic Protein Characterization

Xia, J.Q.; Zhang, L. (Eds.)

2016, IX, 74 p. 28 illus., 21 illus. in color., Hardcover

ISBN: 978-3-319-46238-7