

# MIAPE: Capillary Electrophoresis

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**This module identifies the minimum information required to report the use of capillary electrophoresis in a proteomics experiment, in a manner compliant with the aims as laid out in the 'MIAPE Principles' document (latest version available from <http://psidev.sf.net/gps/miape/>).**

## Introduction

The term capillary electrophoresis (CE) describes a group of high performance separation techniques that employ narrow-bore fused-silica capillaries (coated or uncoated) for the separation of charged species through the application of an electric field. Alternative modes to the basic capillary zone electrophoresis (CZE), include micellar electrokinetic chromatography (MEKC), isotachopheresis (ITP), capillary gel electrophoresis (CGE) and capillary electrochromatography (CEC).

In classical CZE two processes take place; the method by which the species are separated, dependant upon their charge to size ratio, and electroosmosis, which is the method by which the mobile phase is transported through the capillary.

These techniques can be utilised to separate (complex) mixtures of molecules such as proteins, peptides, inorganic ions, sugars or lipids. Samples are usually on-line injected into the capillary by pressure, vacuum or voltage. The separation is then achieved by application of a voltage across the length of the capillary containing buffered electrolyte.

The approach is quick, efficient, and allows atomisation, low solvent and sample consumption and on line detection. For whichever CE techniques is employed, this document specifies a minimal amount of description that needs to be provided, to support the comprehension and corroboration of the work performed. For a full discussion of the principles underlying this specification, please refer to the MIAPE 'Principles' document, which can be found on the MIAPE website<sup>†</sup>.

This module specifies that experimenters provide a point of contact for the 'owner' of the data set, report the specific technique being deployed (*e.g.*

CZE, CIEF, CGE, EKC, *etc.*), list the equipment and parameters used and describe the manner of data collection and processing. They do not require a description of the manner of preparation of the sample, nor do they address the 'fate' of the processed sample (whether collected, or passed directly to another instrument). Items falling outside the scope of this module may be captured in complementary modules, which can be obtained from the website<sup>†</sup>. Note that subsequent versions of this document may have altered scope, as will almost certainly be the case for all the MIAPE modules.

The following section, detailing the reporting requirements for all forms of capillary electrophoresis, is subdivided as follows:

1. General features of the experiment
2. Description of sample
3. Description of equipment
4. Run processes
5. Detection processes
6. Electropherogram data processing

## Reporting requirement for capillary electrophoresis

### 1. General Features

- 1.1.1 Date stamp
- 1.1.2 Responsible person or role
- 1.1.3 Experiment type
- 1.1.4 Experiment aim

### 2. Sample

- 2.1.1 Sample name(s)
- 2.1.2 Sample solution

### 3. Equipment

- 3.1 Capillary
  - 3.1.1 Description of capillary

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<sup>†</sup> <http://psidev.sf.net/miape/>

- 3.1.2 Capillary manufacture
- 3.1.3 Capillary dimensions
- 3.2 Other equipment
  - 3.2.1 Model name, number and manufacturer
- 3.3 Control and data collection software
  - 3.3.1 Name, manufacturer and version

#### **4. Run Processes**

*A run is made up of various steps, each of which needs to be defined as specified in sections 4.2, 4.3 and 5; there are also parameters that should be specified across the whole run (4.1). Pre- and post-conditioning should be described in the same manner as other steps, but omitting section 4.3.*

- 4.1 Run descriptors
  - 4.1.1 Duration of data collection
  - 4.1.2 Temperature of capillary
  - 4.1.3 Temperature of samples
- 4.2 Step descriptors
  - 4.2.1 Step name
  - 4.2.2 Step conditions
  - 4.2.3 Flush and wash solutions
  - 4.2.4 Background electrolyte
- 4.3 Sample injection
  - 4.3.1 Name of sample
  - 4.3.2 Volume of sample solution
  - 4.3.3 Concentration of sample
  - 4.3.4 Injection process
  - 4.3.5 Injection process parameters

#### **5. Detection**

- 5.1.1 Detection type
- 5.1.2 Detection specifics
- 5.1.3 Direction or indirect detection process
- 5.1.4 Detector calibration
- 5.1.5 Auxiliary data channels

#### **6. Electropherogram Data Processing**

- 6.1.1 Integration protocol
- 6.1.2 Integration specifics
- 6.1.3 Peak efficiency
- 6.1.4 Mobility
- 6.1.5 Resolution

#### **Summary**

The MIAPE: Capillary Electrophoresis minimum reporting requirements for the use of any of this broad family of techniques specify that a significant degree of detail be captured, for the equipment deployed, its manner of use, the sample analysed and the data processing performed. However, it is clear that providing the information required by this document will enable the effective interpretation and assessment of data and metadata from this area and potentially, support experimental corroboration.

Much of the information required herein may already be stored in an electronic format, or may be exportable from instrumentation; we anticipate further automation of this process.

These guidelines will evolve. To contribute, or to track the process to remain 'MIAPE-compliant', browse to the Proteomics Standards Initiative website at <http://psidev.sf.net/gps/miape>

**Appendix One.** The MIAPE: [Brief Catchy Title] glossary of required items.

<i>Classification</i>	<i>Definition</i>
<i>1. General features</i>	
<b>1.1.1 Date stamp</b>	The date on which the work described was initiated; given in the standard 'YYYY-MM-DD' format (with hyphens).
<b>1.1.2 Responsible person or role</b>	The (stable) primary contact person for this data set; this could be the experimenter, lab head, line manager <i>etc.</i> . Where responsibility rests with an institutional role ( <i>e.g.</i> one of a number of duty officers) rather than a person, give the official name of the role rather than any one person. In all cases give affiliation and stable contact information, which consists of (i) Name, (ii) Postal address and (iii) Email address.
<b>1.1.3 Experiment type</b>	The capillary electrophoresis experiment type; such as CZE, MEKC, CGE IEF, CEC.
<b>1.1.4 Experiment aim</b>	Quantitation, identification, size determination, pI determination, enantiomeric excess determination, etc...
<i>2. Sample</i>	
<b>2.1.1 Sample name(s)</b>	Name and concentration of sample(s) including any label, marker or tag applied that will be used for detection, such as fluorescent labels (by name only). From the sample described above identify a control, standard and test samples.
<b>2.1.2 Sample solution</b>	The components, with concentrations (excluding the sample) of the sample solution that is to be injected into the capillary ( <i>e.g.</i> background electrolyte). Manufacturer, order and lot numbers used
<i>3. Equipment – 3.1 Capillary</i>	
<b>3.1.1 Description of capillary</b>	Type of capillary being used, <i>e.g.</i> fused silica capillary, coated capillary <i>etc</i>
<b>3.1.2 Capillary manufacture</b>	If the capillary was purchased pre-made <i>e.g.</i> coated, with window or to pre-cut lengths, then include the model name, model number, manufacturer and lot number. If the capillary has been manufactured 'in house' then supplier of silica capillary, model number and lot number should be given and if coating protocol has been used a reference should be given to the method. If no published protocol is available a process should be given.
<b>3.1.3 Capillary dimensions</b>	The exact dimensions of the capillary employed: From inlet to detection window (effective length, cm); from inlet to outlet (total length, cm); and the inner and outer diameters of the capillary ( $\mu\text{m}$ ).

<i>3. Equipment — 3.2 Other equipment</i>	
<b>3.2.1 Model name, number and manufacturer</b>	Record the model name, model number and manufacturer for all equipment used.
<i>3. Equipment — 3.3 Control and data collection software</i>	
<b>3.3.1 Name, manufacturer and version</b>	Record the name, manufacturer and version of the control and data collection software.
<i>4. Run Processes — The protocol for a run normally follows the following order of steps; (i) preconditioning (carried out prior to the first use of a capillary, consisting of various flush steps, designed to clean/activate/coat the inner walls of the capillary); (ii) injection; (iii) separation; (iv) post-conditioning (again consisting of various flush steps). Each of these steps needs to be defined as specified in 4.2, 4.3 and 5; there are also parameters that should be specified across the whole run (4.1). Note that pre- and post-conditioning steps should be described in the same manner as other steps, but omitting section 4.3. Note also that if a sample stacking, electro focusing experiment has being carried out then this must be specified.</i>	
<i>4. Run Processes — 4.1 Run descriptors</i>	
<b>4.1.1 Duration of data collection</b>	Duration of data collection from detector and auxiliary data channels (see section 5 and 6).
<b>4.1.2 Temperature of capillary</b>	Controlled temperature of capillary (if controllable).
<b>4.1.2 Temperature of samples</b>	Controlled temperature of samples (if controllable).
<i>4. Run Processes — 4.2 Step descriptors</i>	
<b>4.2.1 Step name</b>	A descriptor of the individual steps involved in the run. For example, wash, flush, inject, and separate.
<b>4.2.2 Step conditions</b>	For each step described above in 4.3 the conditions applied to the capillary. To be given in terms of pressure or voltages versus time and vial location. This information should include voltage mode (positive/negative, step and hold, or gradient) if applicable.
<b>4.2.3 Flush and wash solutions</b>	For all steps carried out, solutions should be described in terms of components with concentrations.
<b>4.2.4 Background electrolyte</b>	For all steps carried out, the background electrolyte should be described in terms of components with concentrations.
<i>4. Run Processes — 4.3 Sample Injection.</i>	
<b>4.3.1 Name of sample</b>	Reference 2.1.1

<b>4.3.2 Volume of sample solution</b>	Volume of sample solution (preferred units?)
<b>4.3.3 Concentration of sample</b>	Reference 2.1.1
<b>4.3.4 Injection process</b>	Pressure or electrokinetic
<b>4.3.5 Injection process parameters</b>	Pressure or voltages versus time, as appropriate
<i>5. Detection — this section documents the process and the methods employed both to allow analytes to be detected and for the overall process to be monitored.</i>	
<b>5.1.1 Type of detection</b>	Details of detection method used, UV, DAD, LIF, conductivity, MS.
<b>5.1.2 Detection specifics</b>	Details of emission wavelength of laser if used, detection wavelengths, reference wavelengths or if MS TIC or SIM (and masses monitored) as applicable and data collection rate.
<b>5.1.3 Direction or indirect detection process</b>	The detection process applied when the sample generates a signal that is of higher or lower intensity than the background electrolyte.
<b>5.1.4 Detector calibration</b>	Has a detector calibration step been carried out (yes/no)?
<b>5.1.5 Auxiliary data channels</b>	Descriptions of the auxiliary channels set up to monitor current, power, voltage and pressure applied and values obtained for all steps. This is instructive as to whether the method is functioning properly.
<i>6. Electropherogram Data Processing</i>	
<b>6.1.1 Integration protocol</b>	<i>E.g. Gaussian, parabolic interpolation, etc.</i>
<b>6.1.2 Integration specifics</b>	Minimum peak width, threshold (or height reject), shoulder sensitivity, minimum area shoulder sensitivity.
<b>6.1.3 Peak efficiency</b>	Which model/equation used ensure values imputed from 3.2.1 and 4.2.
<b>6.1.4 Mobility</b>	Equation inputting ensure values from 3.2.1 and 4.4 are used.
<b>6.1.5 Resolution</b>	Resolution of the data, post-processing.