Journal of Chromatography, 545 (1991) 403–406
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 174

Determination of p*I* by measuring the current in the mobilization step of high-performance capillary isoelectric focusing

Analysis of transferrin forms

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ABSTRACT

In high-performance isoelectric focusing in capillaries, the focusing pattern of a sample is obtained during the mobilization step. A current parameter can be assigned to each peak in this pattern. Keeping the experimental conditions unchanged, these current parameters characterize the positions of the respective substances in the pH gradient and therefore they can be used for the determination of the isoelectric points of the substances.

INTRODUCTION

In high-performance capillary isoelectric focusing, the focused pattern of the sample is detected in an electrophoretic (mobilization) step [1–3]. Mobilization of the pH gradient formed in the focusing step is achieved by applying an anion or a cation in the catholyte or anolyte, respectively, and then the focused substances and ampholytes pass the detection site in an electrophoretic process [2,3].

The flux of the mobilizing ions from the electrolytes into the capillary tube during the mobilization is governed by the relationships.

$$N'_{X^{n+}} = I' \cdot (u'_{X^{n+}}) \cdot (n'_{X^{n+}})/\kappa' \text{ (for the cations)}$$
$$N'_{X^{n-}} = I' \cdot (u'_{X^{n-}}) \cdot (n'_{X^{n-}})/\kappa' \text{ (for the anions)}$$

where κ' is the conductivity in the anolyte or catholyte used for the mobilization, n' is the number of ions (X^{n-} or X^{n+}) in the catholyte or anolyte, respectively, u' is the mobility of the ions and I' is the current in the tube (primed symbols refer to the mobilization step) [2]. The electrophoretic migration of the mobilizing ions into the tube results in an increase in the current.

0021-9673/91/$03.50

In this paper, experimental data are presented that show that measuring the current can be used for the evaluation of capillary isoelectric focusing experiments.

EXPERIMENTAL

Iron-free and iron-containing transferrin samples were used in high-performance isoelectric focusing experiments as has been described [3,4]. The current was recorded either with a two-channel recorder or manually. All experiments were repeated 2–5 times to control the reproducibility.

RESULTS AND DISCUSSION

Electropherograms of a typical capillary isoelectric focusing experiment and the change in the current are shown in Fig. 1, which demonstrates that the transferrin

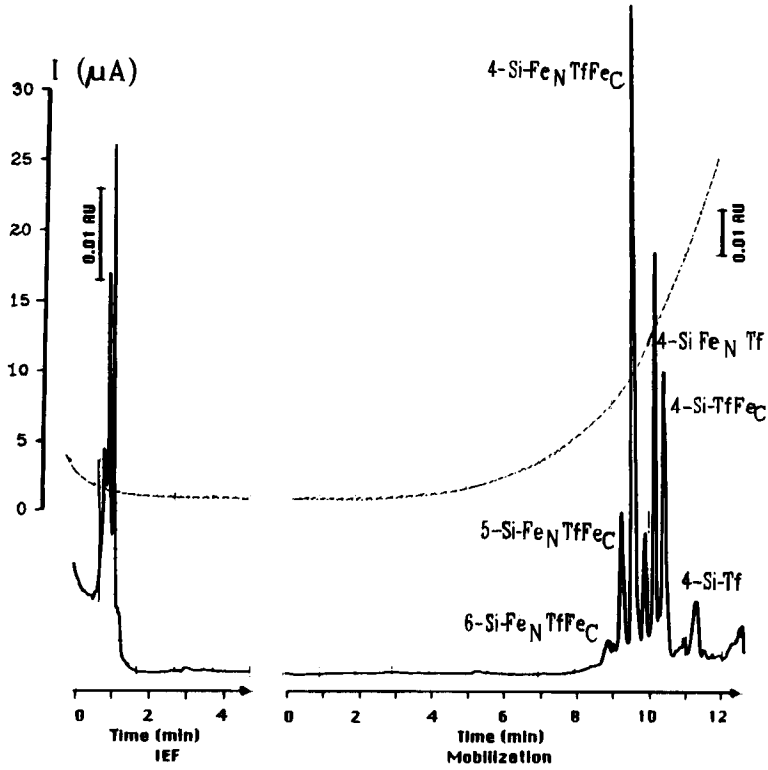

Fig. 1. High-performance capillary isoelectric focusing experiment of a transferrin sample. Si = sialo; Tf = transferrin; Fe_NTf and TfFe_C = monoferric transferrin forms containing iron at the N- or C-terminal lobe, respectively; Fe_NTfFe_C = diferric transferrin. The current (dotted line) decreases in the focusing step as a result of the immobilization of all the substances in the pH gradient. After a certain time the current reaches a 'plateau value', which shows the formation of the steady state in the capillary. On replacing the electrolyte at one end of the capillary the current increases, indicating the electrophoretic migration of the mobilizing ion into the tube. Experimental conditions: tube length, 185 mm; detection point, 155 mm; tube diameter, 0.1 mm; voltage, 5000 V; protein concentration, 1 mg/ml; ampholyte, 2% BioLyte 5/7; anolyte 20 m*M* H_3PO_4 (focusing)–20 m*M* NaOH (mobilization); catholyte, 20 m*M* NaOH; the protein was dissolved in distilled, deionized water.

TABLE I

CURRENT AND p*I* VALUES CHARACTERIZING TRANSFERRIN FORMS

Transferrin forms[a]	Current[b]	p*I*[c]
6-Si-Fe_NTfFe$_C$	8.7 ± 0.2	5.25
5-Si-Fe_NTfFe$_C$	9.5 ± 0.2	5.35
4-Si-Fe_NTfFe$_C$	10.5 ± 0.2	5.45
4-Si-Fe_NTf	14.0 ± 0.4	5.75
4-Si-TfFe$_C$	15.5 ± 0.4	5.85
4-Si-Tf	21.0 ± 0.5	6.10

[a] Si = sialo; Tf = transferrin; Fe_NTfFe$_C$ = diferric transferrin; Fe_NTf and TfFe$_C$ = monoferric transferrins containing iron at the N and C terminal binding site, respectively.

[b] The current values were obtained in experiments described in ref. 3. Experimental conditions: tube length, 185 mm; detection point, 155 mm; tube diameter, 0.1 mm; voltage, 5000 V; protein concentration, 1 mg/ml; ampholyte, 2% BioLyte 5/7; sample dissolved in distilled, deionized water; anolyte, 20 m*M* H_3PO_4 (focusing)–20 m*M* NaOH (mobilization); catholyte, 20 m*M* NaOH.

[c] Data extracted from Fig. 2 in ref. 5.

forms can be characterized with a current parameter. If the experimental conditions (*i.e.*, the length and diameter of the tube, the compositions of the sample and the electrolytes and the applied voltage) were kept constant the change in current was the same in separate runs. A summary of the measured current values and the p*I* values obtained from the literature [5] characterizing transferrin forms are given in Table I (note that the two monoferric transferrin forms are mixed up in Fig. 2 in ref. 5). Fig. 2 shows a p*I*–current curve obtained using the data in Table I.

Why is this curve important? The determination of the p*I* of focused substances is one of the purposes of performing isoelectrofocusing experiments. Generally, internal standards and their 'retention time' values (*i.e.*, the time parameter of the peaks in the electropherograms) are used to construct a calibration graph in the evaluation. Using the curve in Fig. 2, however, the p*I* values can be calculated without having internal standards in each run, *i.e.*, the current values characterizing peaks in the electropherograms can be used for the calculation of their p*I* values. According to the experimental errors in Table I, the p*I* values can be determined with an error of about 0.03 pH unit.

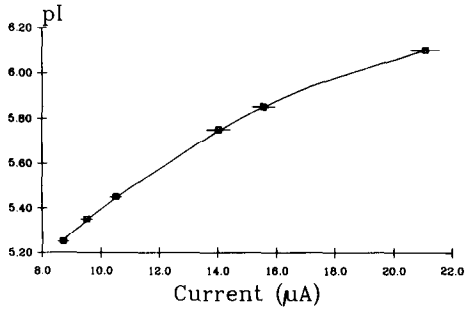

Fig. 2. Calibration graph for p*I* determination using the current as a parameter in high-performance isoelectric focusing experiments. Experimental conditions as in Fig. 1.

TABLE II

CALCULATED p*I* VALUES OF TRANSFERRIN FORMS

Isoform[a]	Molecular forms[a,b]			
	Tf	$TfFe_C$	Fe_NTf	Fe_NTfFe_C
2-Si	n.d.	6.01 ± 0.03	5.90 ± 0.03	5.75 ± 0.03
3-Si	n.d.	5.92 ± 0.03	5.85 ± 0.03	5.60 ± 0.03
4-Si	*6.10*	*5.85*	*5.75*	*5.45*
5-Si	6.03 ± 0.03	5.76 ± 0.03	5.60 ± 0.03	*5.35*
6-Si	5.92 ± 0.03	5.61 ± 0.03	5.46 ± 0.03	*5.25*

[a] Abbreviations as in Table I.

[b] The numbers in italics were obtained from ref. 5 and used for the calibration graph (Fig. 2). n.d. = Not determined.

Table II gives the isoelectric points of transferrin isoforms that overlap in the pH gradient and therefore cannot be analysed in one run. When experiments were repeated using different experimental conditions (longer, shorter or narrower capillaries, etc.), the p*I* values of these isoforms obtained were the same (but not the current parameters, of course).

Finally, an interesting observation is that the 'retention time' in the mobilization step is dependent on the duration of the focusing step (compare, *e.g.*, the positions of the iron-free 4-Si-transferrin component in the Figs. 1a, 1b and 5 in ref. 3). However this was not observed with the above-mentioned 'current' parameter. The explanation of this phenomenon is not known.

These experimental results may make it possible to perform theoretical calculations for capillary isoelectric focusing runs and thus to determine the isoelectric points of substances from measured current values without using any internal standards in the experiments.

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