



QUICK SEARCH: [advanced]			
Author:	Keyword(s):		
Go			
Year:		Vol:	Page:

Institution: United States Patent and Trademark Office | [Sign In via User Name/Password](#)

Clinical Chemistry 43: 1798-1799, 1997;

(*Clinical Chemistry*. 1997;43:1798-1799.)

© 1997 American Association for Clinical Chemistry, Inc.

Oak Ridge Poster Sessions

Clinical Application of Capillary Isoelectric Focusing on Fused Silica Capillary for Determination of Hemoglobin Variants

Amin A. Mohammad^a, Anthony O. Okorodudu, Michael G. Bissell, Pat Dow, Gregg Reger¹, Annette Meier¹, Phil Guodagno¹ and John R. Petersen

¹ Helena Labs., Beaumont, TX;

^a author for correspondence: fax 409-772-9231

Hemoglobin is composed of two α and two β polypeptide chains. Any modifications in the amino acid sequence, which may either be congenital or acquired, affects the oxygen-carrying ability of hemoglobin, resulting in a series of hemoglobinopathies. To date, >600 structurally different human hemoglobins are known (1)(2). Identification of abnormal hemoglobin is very important in the differential diagnosis of hemoglobinopathies. Currently, most clinical laboratories have a battery of hemoglobin assays that includes gel electrophoresis, ion-exchange or affinity chromatography, and isoelectric focusing (1)(3). Of all the available methods, computer-operated cation-exchange HPLC is the most widely used for identifying and quantifying major and minor hemoglobins (4)(5)(6)(7). The main disadvantage of a HPLC system is expensive instrumentation and the high cost of columns and reagents. Although an excellent resolution is achieved with gel isoelectric focusing (8), it is labor intensive, time consuming, and not readily amenable for routine screening.

The use of capillary electrophoresis (CE) for identification of hemoglobin variants has been reported by several authors (9)(10)(11)(12). In 1994, Hempe and Craver demonstrated the applicability of CE for quantification and identification of hemoglobin variants in clinical samples (13). Capillary isoelectric focusing (cIEF) was performed on a dimethylpolysiloxane (DMS)-coated fused silica capillary having an i.d. of 50 μ m. The method is rapid, requires low sample volume, and gives excellent resolution for all major and most of the minor hemoglobin variants. The main

This Article

- [Extract FREE](#)
- [Alert me when this article is cited](#)
- [Alert me if a correction is posted](#)

Services

- [Email this article to a friend](#)
- [Similar articles in this journal](#)
- [Similar articles in ISI Web of Science](#)
- [Similar articles in PubMed](#)
- [Alert me to new issues of the journal](#)
- [Download to citation manager](#)
- [Get Permissions](#)

Citing Articles

- [Citing Articles via ISI Web of Science \(11\)](#)
- [Citing Articles via Google Scholar](#)

Google Scholar

- [Articles by Mohammad, A. A.](#)
- [Articles by Petersen, J. R.](#)
- [Search for Related Content](#)

PubMed

- [PubMed Citation](#)
- [Articles by Mohammad, A. A.](#)
- [Articles by Petersen, J. R.](#)

Related Collections

- [Molecular Diagnostics and Genetics](#)
- [Oak Ridge Conference](#)
- [Drug Monitoring and Toxicology](#)

drawback of this method is the use of DMS-coated capillary. In our laboratory we found that coated capillaries are not very stable and show considerable lot-to-lot variation, thereby resulting in irreproducible migration times. cIEF can also be done on a fused silica capillary provided electroosmotic flow (EOF) is eliminated or reduced. This can be achieved by addition of hydrophilic polymers such as methyl cellulose (MC) (11). MC (2 g/L) reduces EOF significantly but not to the extent seen in a neutral DMS-coated capillary, thus resulting in poor precision for migration times.

We have overcome this problem of irreproducible migration time on a fused silica capillary by introducing two external pI markers purchased from Bio-Rad bracketing the pI gradient 6.6 and 7.7 formed by the ampholine (pI 6.6–7.7). The cIEF is performed on a 57 cm x 50 μ m (i.d.) fused silica capillary. The electrophoresis is performed on a PrinCE system manufactured by Helena Labs., and data evaluation was done on Ceaser software. NaOH (20 mmol/L) and H₃PO₄ (100 mmol/L) constituted catholyte and anolyte solutions, respectively. The run buffer consisted of 50 mL/L ampholine prepared in 3 g/L MC solution. Sample preparation involved dilution of 50 μ L of citrated whole blood to 1 mL with hemolyzing reagent (10 mmol EDTA and 5 mmol KCN). Two-hundred microliters of hemolyzed blood is further diluted to 400 μ L with 2 g/L MC containing 30 mL/L ampholine and 2 μ L of individual pI markers (1 g/L). Before focusing, the capillary is flushed with run buffer for 1 min at 200 kPa (2000 mbar) pressure. A sample plug is injected for 0.4 min at 20 kPa (200 mbar) pressure, followed by injection of run buffer at 20 kPa (200 mbar) pressure for 0.4 min. Focusing is carried out at 30 kV for 5 min and separated hemoglobin variants are mobilized past the detector window by applying low pressure [8 kPa (80 mbar)] under an applied voltage of 30 kV.

Figure 1 shows the separation of the four most common hemoglobin variants (C, S, F, and A). The pI marker 7.7, being most alkaline, migrates first and the pI marker 6.6 is seen last. These two markers define the beginning and end of the pH gradient. We overcame the problem of irreproducible migration times on fused silica capillaries by relating the migration times of the individual hemoglobins to these two markers. This is done by defining a new term called migration index, which is the ratio of the distance of a hemoglobin variant from two markers and is calculated as shown in Eq. 1:

$$\text{Migration index} = t_{\text{hgb}} - t_{7.7} / t_{6.6} - t_{\text{hgb}} \quad (1)$$

where t_{hgb} , $t_{7.7}$, and $t_{6.6}$ are the migration times of the hemoglobin variant and pI markers 7.7 and 6.6, respectively. The migration index is a dimensionless quantity and is constant for a given set of conditions. This is readily seen from the CVs shown in Table 1. Furthermore, the migration index calculated for hemoglobins C, S, F, and A in a commercial control were used to create a calibration plot. The regression equation derived from this curve is then used to calculate the pI of the hemoglobin present in a patient's sample. Table 1 shows a comparison between the calculated and the literature pI for hemoglobins C, S, F, and A. By using this equation we are able to identify these four most commonly seen hemoglobins unambiguously in 100 patient samples previously identified by classical gel isoelectrophoresis. Because of the existence of a considerable EOF, we are unable to consistently resolve hemoglobin C from A₂ and E. Even with this shortcoming, the method is robust, fast, and can be used as a rapid screen for detecting abnormal hemoglobins in clinical samples. It makes use of easily available fused silica capillary, and the concept of using migration index instead of migration times for positive identification makes this method readily adaptable to any CE system available in the market.

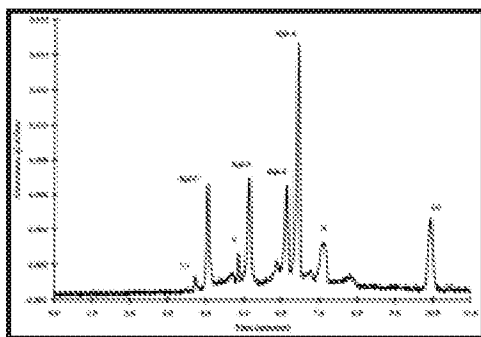


Figure 1. cIEF separation of hemoglobins C, S, F, and A.

X denotes degraded hemoglobin.

View larger version (16K):

[\[in this window\]](#)

[\[in a new window\]](#)

View this table: **Table 1.** Comparison between migration time, migration index, and pI.

[\[in this window\]](#)

[\[in a new window\]](#)

Footnotes

Clin. Chem. Div., Dept. of Pathol., Univ. of Texas–Medical Branch, Galveston, TX 77555-0551

References

- Schmidt RM. Laboratory diagnosis of hemoglobinopathies. Bick RL eds. Hematology: clinical and laboratory practices 1993:327-390 CV Mosby St. Louis, MO. .
- International Hemoglobin Information Center variant list. Hemoglobin 1994;18:77–183..
- Perrotta G. Hemoglobin separation and quantitation. Pesce AJ Kaplan LA eds. Methods in clinical chemistry 1987:1249-1257 CV Mosby St. Louis, MO. .
- Huisman TH. High-performance liquid chromatography as method to identify hemoglobin abnormalities. Acta Haematol 1987;78:123-126. [\[ISI\]](#)[\[Medline\]](#) [\[Order article via Infotrieve\]](#)
- Ou CN, Rognerud CL. Rapid analysis of hemoglobin variants by cation-exchange HPLC. Clin Chem 1993;39:820-824. [\[Abstract/Free Full Text\]](#)
- Deacon-Smith R, Lord I. Hemoglobin A₂ measurement using high performance liquid chromatography. Med Lab Sci 1992;49:138-140. [\[ISI\]](#)[\[Medline\]](#) [\[Order article via Infotrieve\]](#)
- van der Dijs FPL, van den Berg GA, Schermer JG, Muskiet FD, Landman H, Muskiet FAJ. Screening cord blood for hemoglobinopathies and thalassemia by HPLC. Clin Chem 1992;38:1864-1869. [\[Abstract/Free Full Text\]](#)
- Basset P, Beuzard Y, Garrel MC, Rosa J. Isoelectric focusing of human hemoglobin: its application to screening, to the characterization of 70 variants, and to the study of modified fraction of normal hemoglobins. Blood 1978;51:971-982. [\[Abstract/Free Full Text\]](#)

9. Zhu M, Rodriguez R, Wehr T, Siebert C. Capillary electrophoresis of hemoglobins and globin chains. J Chromatogr 1992;608:225-237. [\[ISI\]](#) [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
10. Zhu M, Wehr T, Levi V, Rodriguez R, Shiffer K, Cao ZA. Capillary electrophoresis of abnormal hemoglobins associated with alpha thalassemias. J Chromatogr A 1993;652:119-129. [\[ISI\]](#) [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
11. Molteni S, Frischknecht H, Thormann W. Application of dynamic capillary isoelectric focusing to the analysis of human hemoglobin variants. Electrophoresis 1994;15:22-30. [\[ISI\]](#) [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
12. Ishioka N, Iyori N, Noji J, Kurioka S. Detection of abnormal hemoglobin by capillary electrophoresis and structural identification. Biomed Chromatogr 1992;6:224-226. [\[ISI\]](#) [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
13. Hempe JM, Craver RD. Quantification of hemoglobin variants by capillary isoelectric focusing. Clin Chem 1994;40:2288-2295. [\[Abstract/Free Full Text\]](#)

This Article

- [Extract FREE](#)
- [Alert me when this article is cited](#)
- [Alert me if a correction is posted](#)

Services

- [Email this article to a friend](#)
- [Similar articles in this journal](#)
- [Similar articles in ISI Web of Science](#)
- [Similar articles in PubMed](#)
- [Alert me to new issues of the journal](#)
- [Download to citation manager](#)
- [Get Permissions](#)

Citing Articles

- [Citing Articles via ISI Web of Science \(11\)](#)
- [Citing Articles via Google Scholar](#)

Google Scholar

- [Articles by Mohammad, A. A.](#)
- [Articles by Petersen, J. R.](#)
- [Search for Related Content](#)

Publited

- [PubMed Citation](#)
- [Articles by Mohammad, A. A.](#)
- [Articles by Petersen, J. R.](#)

Related Collections

- [Molecular Diagnostics and Genetics](#)
- [Oak Ridge Conference](#)
- [Drug Monitoring and Toxicology](#)

[HOME](#) [HELP](#) [FEEDBACK](#) [SUBSCRIPTIONS](#) [ARCHIVE](#) [SEARCH](#) [TABLE OF CONTENTS](#)