

Library of Congress Cataloging-in-Publication Data

Capillary Electrophoresis / edited by Patrick Camilleri. — 2nd. ed.
p. cm. — (New directions in organic and biological chemistry)
Includes bibliographical references and index.
ISBN 0-8493-9127-X (alk. paper)
1. Capillary electrophoresis. I. Camilleri, Patrick. II. Series.
[DNLM: 1. Hepatitis B virus. QW 710 G285h]
QP519.9.C36C373 1997
5436'.0871—dc21

97-22491
CIP

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International Standard Book Number 0-8493-9127-X
Library of Congress Card Number 97-22491
Printed in the United States of America 2 3 4 5 6 7 8 9 0
Printed on acid-free paper

Helena Laboratories Corp.
Exhibit 1020
Page 1020-0001

electrophoresis and zwitterionic

Methylcellulose	Hjerten	<i>J. Chromatogr.</i> 347, 191 (1985)
Polyacrylamide (through Si-O-Si-C bonds)	Hjerten	<i>J. Chromatogr.</i> 347, 191 (1985)
Polyacrylamide (through Si-C bonds)	Novotny	<i>Anal. Chem.</i> 62, 2478 (1990)
3-glycidioxypropyltrimethoxysilane	Jorgenson	<i>Science</i> 222, 266 (1983)
Epoxy-diol, maltose	Poppe	<i>J. Chromatogr.</i> 480, 339 (1989)
Polyethyleneglycol	Poppe	<i>J. Chromatogr.</i> 471, 429 (1988)
Polyvinylpyrrolidone	McCormick	<i>Anal. Chem.</i> 60, 2322 (1988)
Aryl-pentafluoro(aminopropyltrimethoxy)silane	Swardberg	<i>Anal. Biochem.</i> 185, 51 (1990)
α -lactalbumin	Swardberg	<i>J. High Res. Chromatogr.</i> 14, 65 (1991)
Polyether	El Rassi	<i>J. Chromatogr.</i> 559, 367 (1991)
Hydrophilic, C1, C8, C18	Dougherty	<i>Supelco Reporter</i> , Vol. X, No. 3 (1991)
Ion exchangers, polyacrylamide	Engelhardt	<i>J. Microcol. Sep.</i> 3, 491 (1991)
Neutral, hydrophilic	Karger	<i>J. Chromatogr.</i> 652, 149 (1993)
Hydrophilic & hydrophilic polymers	Lee	<i>Anal. Chem.</i> 65, 2747 (1993)

Hence, the separation of protein species varying only in subtle structural differences would be difficult, if not impossible, to achieve.

If the optimum for the separation is found at around neutral pH conditions, strategies other than the extreme pH approach should be considered. One of these is to coat the capillary surface either dynamically (using the rinse capability of the CE instrument) or covalently (by using chemical derivatization procedures prior to inserting the capillary in the instrument). The goal of these procedures is to minimize, eliminate, or reverse the surface charge on the capillary wall.

Covalent coating is generally performed by attaching hydrophilic polymers to the silanol groups on the surface. Robust and reproducible covalent coatings are often not easily made in the laboratory. However, nowadays several companies offer such coated capillaries.⁵ They are typically developed for specific applications and are usable in a given pH range. Table 8.1 lists a selection of some of the coatings which have appeared in the CE literature.⁶

Instead of chemically modifying the capillary surface to avoid adsorption of the proteins, one can perform a "dynamic" coating by adding various substances to the electrophoresis buffer while using the rinse cycles of the CE instrument to equilibrate the columns. One simple way of doing this is to increase the electrophoresis buffer concentration or to add various salts to the buffer.^{6,7} As the salt and protein compete for adsorption sites on the capillary wall, the higher the salt-to-protein ratio, the higher the probability of salt adsorption. A drawback of this approach is that the higher the salt concentration, the higher the conductivity of the buffer, and therefore the higher the power induction (at a constant applied voltage) and temperature increase in the capillary tube. This is especially pronounced in CE instruments lacking the ability to adequately remove Joule heating, i.e., with simple configurations based on ambient air-cooling of the capillaries.

The power induction can be greatly reduced by using zwitterionic salts such as tricine, CAPS, CHES, and betaine instead of ionic salts such as phosphate and borate.¹⁸ The zwitterions yield very low conductivity, and therefore they may be used at rather high concentrations (up to more than 1 M, depending on their solubility) without causing high power inductions. As buffer components, zwitterions are frequently used in FSCE.

Cationic amines such as 1,4-diaminobutane (DAB) and 1,5-diaminopentane (DAP) have also been used to suppress protein-capillary surface interactions at concentrations up to 60 mM.⁸ As mentioned above for the "high salt" approach, the idea is that the amine competes with the protein for adsorption sites on the capillary wall. The larger the protein, the higher the concentration of cationic amine must be in order to obtain the desired effect.

electrophoresis and zwitterionic

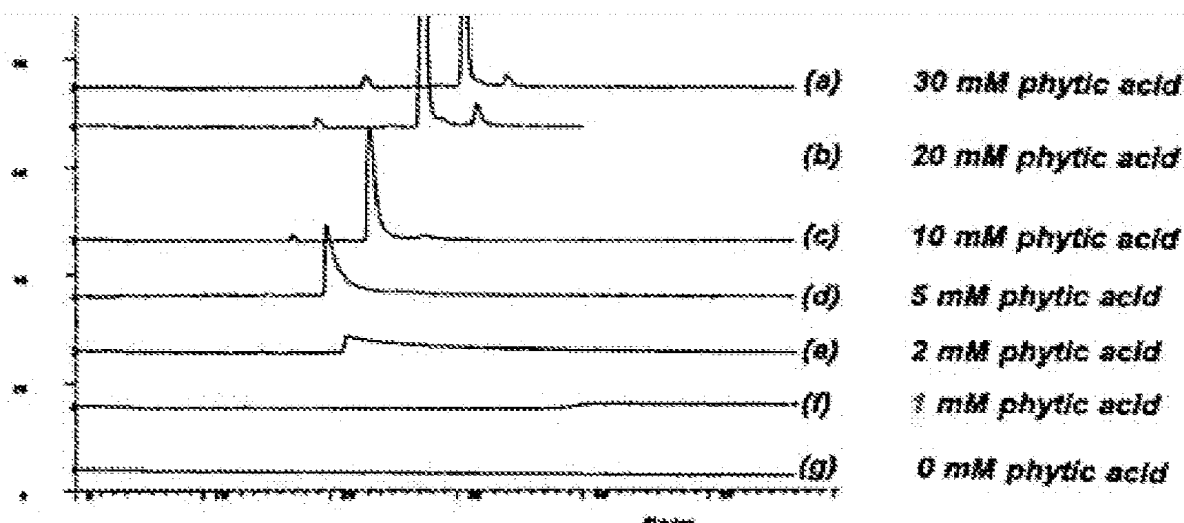


Figure 8.4 FSCE analysis of aprotinin by the use of phytic acid as ion-pairing reagent. Analysis was performed in 25 mM phosphate buffer at pH 7.0 with increasing amounts of phytic acid (0, 1, 2, 5, 10, 20, 30 mM, from bottom to top) added to the buffer. (From Okaf, G. N., et al., *Electrophoresis*, 16, 1917, 1995. With permission.)

Various cellulose derivatives (e.g., methylcellulose, hydroxyethylcellulose, and hydroxypropylmethylcellulose — generally added at concentrations below 1%), polyethylene oxides, and ethyleneglycol (10 to 30%) decrease the zeta potential (i.e., a measure of the electric potential at the shear plane set up in the surface region of the electrical double layer) on the surface of the capillary wall. These additives also increase the viscosity of the buffer, thereby decreasing the tendency for protein adsorption.

Protein adsorption may also be reduced by addition of cationic surfactants such as cetyltrimethylammonium bromide (CTAB) to the buffer. Increasing the concentration first reduces, then eliminates, and finally reverses the charge on the capillary surface, thus creating a positively charged surface (the latter condition also causes a reversal of the EOF).

Finally, one can perform ion-pairing CE in order to suppress or eliminate protein adsorption. The anionic ion-pairing reagent, which is added to the buffer, reacts with cationic sites on the protein molecule, thus making it electrophoretically net negatively charged. An example of that approach is shown in Figure 8.4,¹⁹ using phytic acid as the ion-pairing reagent. This molecule contains six phosphate groups with pK_a values in the range of 1.9 to 9.5, which allows one to manipulate its charge over a wide pH range. Aprotinin is a highly basic protein ($pI \sim 10.5$, MW 6511 Daltons, 58 amino acid residues). Initial FSCE analysis of aprotinin in a 25 mM phosphate buffer at pH 7.0 resulted in the protein being totally adsorbed onto the capillary surface. When phytic acid was added to the buffer, aprotinin eluted as a distinct peak — first severely tailed, but with increasing phytic acid concentrations the aprotinin peak became more symmetrical, even separating several aprotinin variants.

Ion pairing with phytic acid can also be used to optimize separation of peptides and predict their basic nature.²⁰ This is illustrated by the following example: Aprotinin was unfolded, reduced, pyridylethylated, and finally digested with endoproteinase Lys-c. In this way the expected five peptide fragments were formed: fragment 1, RNNFK ($pI \sim 11.4$); fragment 2, SAEDCMRTCGGA ($pI \sim 4.4$); fragment 3, AGLCQTVYGGCRK ($pI \sim 9.2$); fragment 4, ARIRYFYNAK ($pI \sim 11.3$); fragment 5, RPDFCLEPPYTGPCK ($pI \sim 5.9$). The digest was subjected to CE analysis in a 100 mM sodium phosphate buffer (pH 7.0) with varying concentrations of the dodecasodium salt of phytic acid. The mobilities of fragments 1, 4, and 3 (all with basic pI values) decreased with increasing concentration of phytic acid.