

# Separation of Chlorins and Carotenoids in Capillary Electrophoresis

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## Abstract

The present study reports the investigation of capillary electrophoresis (CE) for the separation of the photosynthetic pigments (chlorophyll derivatives as well as carotenoids) together. Various CE methods, such as micellar electrokinetic chromatography, capillary electrokinetic chromatography, and nonaqueous capillary electrophoresis (NACE) are tested, with coated and uncoated capillary columns to evaluate optimal separation conditions using diode array detection. The effect of different type and composition of organic solvents and surfactants on the separation is discussed. Detection limits are found in the range of 1.14–2.45 ppm. According to the system suitability results, the most effective separation is observed using NACE with Aliquat 336 as cationic surfactant in coated capillary and mixture of MeOH–ACN–THF (5:4:1, v/v/v) as solvent. Quantitative evolution is investigated, and recovery percentage values are found to be 96.7–102%.

## Introduction

Chlorins (i.e., chlorophyll derivatives) and carotenoids are basic classes of photosynthetic pigments. Chlorophylls are greenish pigments which contain a porphyrin ring. This is a stable ring-shaped molecule around which electrons are free to migrate. Because the electrons move freely, the ring has the potential to gain or lose electrons easily and, thus, the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll captures the energy of sunlight. Chlorophyll *a* is the most common component of this group, which is found in all oxygenic photosynthetic organisms, while chlorophyll *b* occurs only in green algae and in green plants. Carotenoids, another group of lipophilic natural pigments, have long isoprenoid chains with alternating double and single bonds and may contain numerous functional groups. All photosynthetic organisms contain carotenoids. Analysis and identification of these pigments in various sample matrices by

different spectroscopic and analytical techniques has already been described (1–8). The separation of pigments may be carried out by column chromatography, thin-layer chromatography, and high-performance liquid chromatography (HPLC) on various stationary phases, such as silica-gel, alumina, and reversed-phase materials (C18 and C30). The choice of the most suitable chromatographic method depends on the amount of sample, pigment composition, resolution, speed, and purity required. However, attention must be paid to reconditioning of the column used for HPLC applications after each injection by washing with less polar solvent for at least 15 min. Therefore, longer analysis time is needed for these applications. Because the pigments, most particularly carotenoids, are extremely reactive and consequently unstable due to their long system of conjugated double bonds, several precautions, such as protection from light and oxygen, use of low temperature and antioxidants, and analysis within the shortest time possible, should be taken into the consideration. On the other hand, neither porphyrins nor carotenoids can be analyzed directly by gas-liquid chromatography because of their involatility and/or thermal instability. A rapid analytical technique with high resolving power and sensitivity is therefore required for the analysis of these compounds.

Capillary electrophoresis (CE) is a powerful separation technique, which is performed in narrow tubes. Rapid analysis time, greater separation efficiency, and higher selectivity, simplicity, and adaptability to a variety of different application conditions make CE more advantageous, especially where other liquid-phase separation techniques are limited or impractical. Moreover, fused silica capillaries are far less expensive than chromatographic columns, easily washed between runs, and unlike packed columns, free of irreversible contamination of the matrix. The principle of separation by CE is based on differences in the mobilities of the analytes under the electrical field which is created by the application of high voltage between the electrodes replaced in a buffer solution (9).

There are a number of different ways of performing CE separations depending on the analytes of interest. Capillary zone electrophoresis (CZE) is the basic CE method which is applied particularly for the separation of small, charged molecules (10). Micellar electrokinetic chromatography (MEKC) is a widely used

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molecular structure of carotenoids and chlorins, fewer researchers have been interested in the new method development for their analysis in CE. To our knowledge, there is only one nonaqueous capillary electrophoresis (NACE) study for the analysis of hydrophobic porphyrins and their oligomers (26) and one study for the chlorin and carotenoid bonded protein separation by polyacrylamide gel electrophoresis in a single run (20). The present study reports the investigation of CE for the separation of chlorins and carotenoids together using model compounds. For this purpose, various CE methods, such as MEKC, CEKC, and NACE, were investigated using coated and uncoated capillary columns to obtain optimal separation conditions.

## Experimental

### Reagents

Chlorophyll *a*, chlorophyll *b*, hyamine (benzethonium chloride), ethylene imine polymer solution (PEI,  $M_r$  600000–1000000), sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ), methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF), sodium dodecyl sulfate (SDS), benzyldimethyltetradecylammonium chloride (BDMTDA), and cetyltrimethylammonium bromide (CTAB) were obtained from Fluka (Bucks, Switzerland). Astaxanthin, beta-carotene, and pyropheophorbide *a* methyl ester were from Sigma (St. Louis, MO). Aliquat 336 (Tricapryl methylammonium chloride) and methylpyrroporphyrin XXI ethyl ester were purchased from Aldrich (Milwaukee, WI). Lutein and disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) were obtained from Apin Chemicals Ltd. (Abington, Oxon, UK) and Riedel-de Haën (Seelze, Germany), respectively. Pheophorbide *a* stigmasteryl ester was synthesized in our laboratory (7).

### Instrumentation

CE investigations were performed on a Beckman Coulter (Palo Alto, CA) P/ACE MDQ system (Fullerton, CA) equipped with a photodiode-array UV detector from Beckman Technologies. The system was controlled with 32 Karat software; an uncoated fused silica capillary (Agilent, Switzerland) of 75- $\mu\text{m}$  i.d.  $\times$  375- $\mu\text{m}$  o.d., with total and effective lengths of 57 and 50 cm, respectively, was used at 25°C.

### Sample preparation

Pigment standards (beta-carotene, lutein, and astaxanthin for the carotenoid group, and chlorophyll *a*, *b*, pyropheophorbide *a* methyl ester, and methylpyrroporphyrin XXI ethyl ester for the chlorin group and derivatives) were dissolved in MeOH within the concentration range of 0.1–1.0mM and diluted to desired concentrations with separation buffer and filtered before injection. Stock solutions were kept in dark and + 4°C in the refrigerator.

### Conditioning of capillaries

Coated capillaries were prepared as follows: (i) a new capillary was rinsed with 1.0 N. NaOH (in MeOH) for 10 min and then with pure MeOH for 10 min; (ii) the capillary was rinsed with a 2% (w/v) polymer (PEI) solution in MeOH for 30 min, and left with polymer solution for 20 min; (iii) the capillary was rinsed with running electrolyte for 15 min. Uncoated capillaries were used after the following pre-treatment: a new capillary was rinsed first with MeOH for 5 min, and with 1.0 N NaOH for 20 min, then with pure water, and finally with background electrolyte (BGE) for 10 min at each step. Before each run, the capillary (both uncoated and coated) was rinsed with BGE for 2 min.

## Results and Discussion

### MEKC and CEKC studies

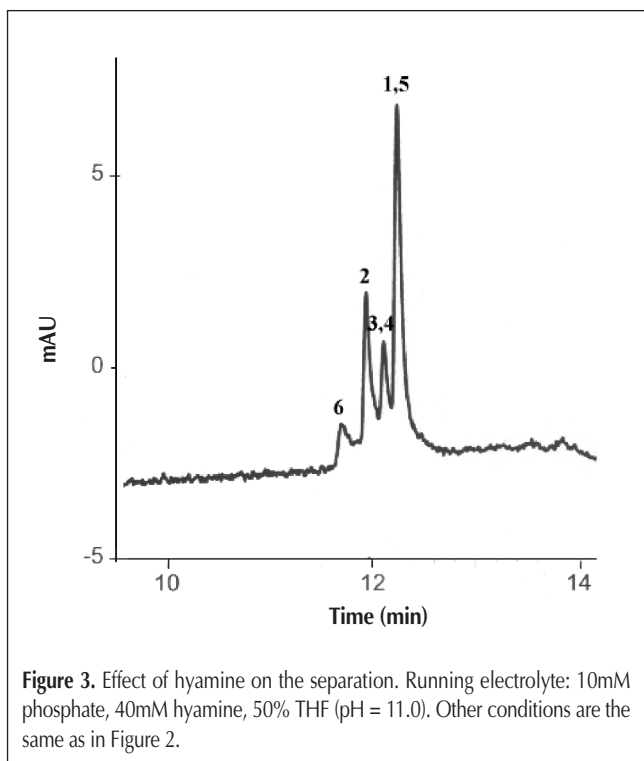
Chlorins and carotenoids are highly hydrophobic, neutral, and huge molecules, and their effective separation is difficult because of their similar properties. Therefore, CZE is not the most convenient CE method for this type of analytes. As a preliminary study, we tested the MEKC method described by Saitoh (14) for the separation of chlorophyll *a*, *c1*, and *c2* using SDS in the concentration range of 10–50mM. However, no separation was observed for our analytes under the same electrophoretic conditions as in the work of Saitoh. All analytes migrated with the same electrophoretic mobility. Various solvents such as MeOH, ACN, DMF, and THF were tested, respectively, in the range of 5–30% in order to avoid micelle distortion, which is observed with higher solvent ratios. Because of the solubility problem of the analytes investigated in this study, MEKC was not found to be as sensitive and efficient as expected. Organic solvent content was therefore increased in the buffer electrolyte to dissolve the analytes sufficiently and to increase the detection response. However, even in higher organic solvent content and with various solvents, all analytes migrated together when sodium dodecylsulfate (SDS) and cetyltrimethylammonium (CTAB) were used in the CEKC electrolyte. Because of the solubility limits of these surfactants in some organic solvents, different surfactants such as BDMTDA, hyamine, and Aliquat 336 were then investigated to find possible differences compared with SDS and CTAB monomers. In the literature, there is no study using BDMTDA and

**Table II. System Suitability Results\***

Peaks	Resolution	Selectivity	Theoretical Plate Numbers	Tailing
	$R_s = \frac{2(t_2 - t_1)}{(w_1 + w_2)}$	$\alpha_1 = \frac{k_2'}{k_1'}$	$N = 5.54 \left( \frac{t}{w_{1/2}} \right)^2$	$T = \frac{(a + b)}{2a}$
1			8870	1.0
2	0.9	1.18	11321	1.0
3	1.2	1.2	12144	0.97
4	2.45	1.43	14304	1.0
5	3.0	1.38	12027	1.0
6	1.6	1.13	19044	1.0

\* For equations:  $t$  = Retention time;  $w_{1/2}$  = Peak width at half peak height;  $k'$  = capacity factor;  $a$  = Distance from the leading edge to the midpoint (at 10% of peak height); and  $b$  = Distance to the trailing edge from the midpoint (at 10% of peak height).

Aliquat 336 as an additive in CE. On the other hand, only two studies related with hyamine have been reported, explaining that either it was only used as electroosmotic flow (EOF) modifier for the determination of anions (27), or a negative result was obtained for the analysis of urine proteins (28). Therefore, the use of these surfactants (Figure 1) in CE is completely new investigation. BDMTDA in the running electrolyte caused a partial separation of chlorophyll, porphyrine, and carotene classes. Therefore, the effect of the total concentration of BDMTDA over 5–40mM range was examined. All CE buffers were prepared containing 10mM sodium phosphate and 50% THF at pH = 11.0. Increasing the concentration of BDMTDA led to the best separation at 20mM, and higher concentration of BDMTDA did not improve the separation. It follows that the interaction of this cationic surfactant led to positively charged analytes that migrated after the EOF. This partial separation is shown in Figure 2. Another cationic surfactant, hyamine, was also tested to see if its more hydrophilic structure, coming from the ether units, may positively influence the separation of the pigments. In order to compare the results, running electrolyte was always



kept at same conditions as with other surfactants. Hyamine improved the separation within the pigment groups but with overlapping of components from different classes. The hyamine concentration range of 5–100mM was investigated to optimize the separation, and 40mM was found as the optimum surfactant concentration (Figure 3). Finally, Aliquat 336 was tested as a cationic surfactant because of its branched hydrophobic side chains. These hydrophobic interactions between Aliquat 336 monomers and pigment analytes made the electrophoretic separation more effective. 5–100mM concentration range of this surfactant in the buffer electrolyte was investigated, and 20mM was found as the best under these CEKC conditions (Figure 4).

### NACE studies

As an alternative to MEKC and CEKC, the separation of these pigments in NACE was studied by using bare fused and PEI coated silica columns. Similarly to CZE, NACE can only separate charged analytes, but the main advantage of using a nonaqueous system is that the strong interactions can be observed between the analyte and the additive. Solvophobic interactions are generally much weaker in nonaqueous solvents than in water, but ion–ion, ion–dipole, and dipole–dipole interactions are stronger in most nonaqueous solvents than in water (12). Such buffer systems also allow effective sample ionization for MS analysis with the high solvent volatility and the low surface tension (29–31). Another advantage of NACE buffers is the low electrical conductivity producing low Joule heat leading to higher separation efficiency (32). Several authors reported that the use of polyelectrolyte coatings was very effective in reducing solute adsorption onto the capillary wall in aqueous electrolytes (33–36). One of the main advantages of this type of coating is to set the direction and the magnitude of the EOF independently from the electrolyte pH. EOF is the main parameter for the separation of analytes in CE and, therefore, the repeatability of EOF should be taken into consideration. The use of coated capillaries is one of the most practical ways to control EOF. In nonaqueous electrolytes, there have been a few investigations on the approach of polyelectrolyte coatings by using polyvinyl alcohol (PVA) (37,38), polyethylene glycol (PEG) (37,38), polyacrylamide (PAA) (39), and C18 (40). In this work, PEI, which is effectively used for aqueous CE applications, was chosen as polyelectrolyte coating material for capillary column to increase the analyte–additive interactions in NACE by reducing the EOF (41–43). Depending on the physical properties affecting analyte–additive interactions (12), methanol [cohesion energy density ( $P_s$ ) = 928, dielectric constant ( $\epsilon$ ) = 33, donor numbers (DN) = 126, acceptor numbers (AN) = 41.3], and acetonitrile ( $P_s$  = 655,  $\epsilon$  = 38, DN = 59, AN = 18.9) were chosen as the solvent for NACE investigations. Preliminary studies with PEI coated capillary without any additive showed a simple retardation of the migration times but no separation was observed even with 4% PEI addition into the running electrolyte. For this reason, the most effective cationic surfactant was observed from the described CEKC studies, and Aliquat 336 was used to try to improve the method. As it can be seen in Figure 5A, with the

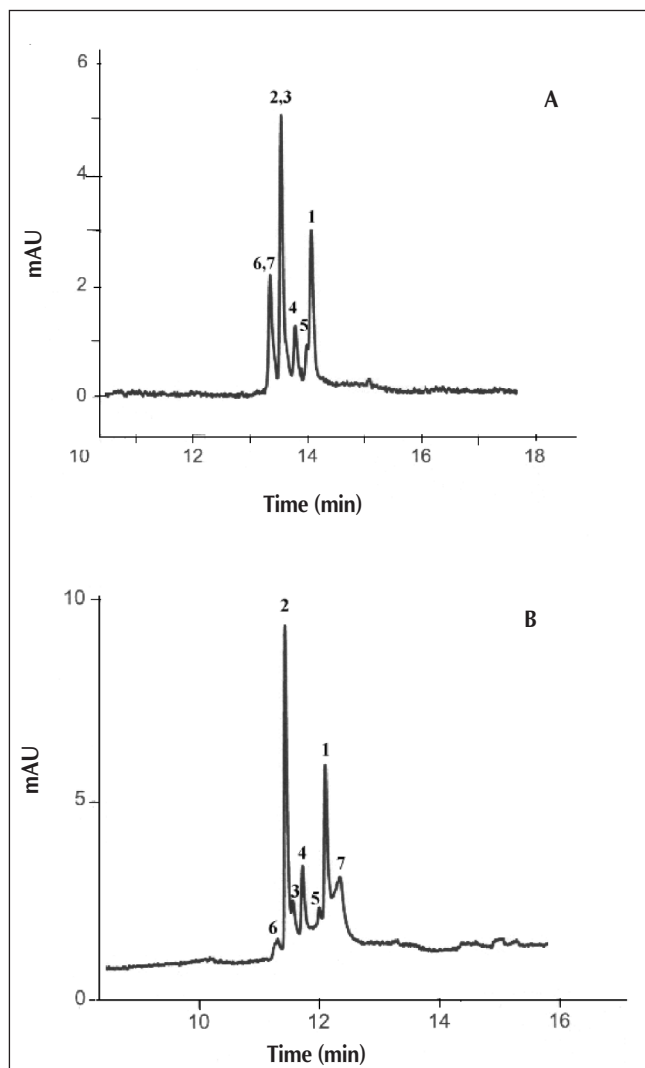
**Table III. Quantitative Analysis Results**

Analytes	Quantity (mg/L)	Added amount (mg/L)	Total amount (mg/L)	Recovery (%)
Beta-carotene	30	10	41.2 ± 0.5	97.1
Lutein	20	10	29.7 ± 0.3	101
Astaxanthin	20	10	29.4 ± 0.6	102
Pp-a Me ester	20	10	31.0 ± 0.4	96.7
Chlorophyll b	20	10	30.5 ± 0.5	98.3
Me-pp Et ester	20	10	29.6 ± 0.5	101.3

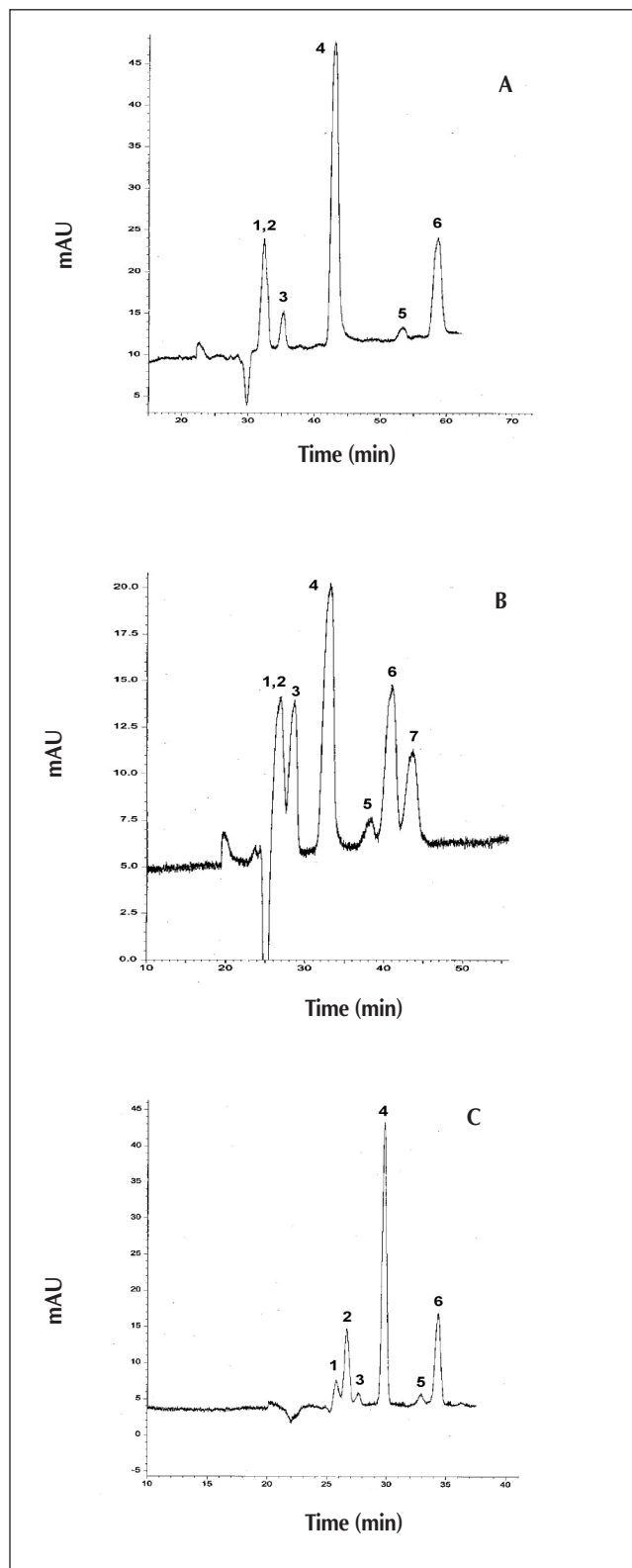
addition of 15mM Aliquat 336 into the 2% PEI containing running electrolyte, an effective separation could be observed except for beta-carotene/lutein pair. In order to improve the separation, different solvent compositions were tested. Figure 5B shows the differences observed by increasing ACN content from 50% to 70%. Resolution was slightly decreased when ACN content was increased because of a higher EOM. Finally, the best results were obtained when using a solvent mixture of MeOH-ACN-THF (5:4:1, v/v/v) and a lower concentration (10mM) of Aliquat 336 (Figure 5C). Pheophorbide *a* stigmasteryl ester (peak 7) does not appear in Figure 5A and Figure 5C because of its long retention time.

#### Method validation

Validation of the proposed NACE method was performed with the evaluation of linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ). The precision of the method was estimated by measuring the inter- and intraday repeatability of the same homogeneous sample and expressed as relative standard deviation (RSD). The accuracy of the method



**Figure 4.** Effect of Aliquat 336 on the separation, with the addition of 12mM (A) and 20mM Aliquat 336 into the buffer (B). Other conditions are the same as in Figure 2. Additional peak identification: Chlorophyll *a* (peak 7).



**Figure 5.** Effect of solvent composition on NACE separation. MeOH-ACN (1:1) with 15mM Aliquat 336 (A), MeOH-ACN (3:7) with 15mM Aliquat 336 (B), MeOH-ACN-THF (5:4:1) with 10mM Aliquat 336 (C).  $V = -28$  kV (cathodic side), PEI coated capillary. Peak identifications: Beta-carotene (peak 1), Lutein (peak 2), Astaxanthin (peak 3), Pyropheophorbide *a* methyl ester (peak 4), Chlorophyll *b* (peak 5), Methylpyrroporphyrin XXI ethyl ester (peak 6), Pheophorbide *a* stigmasteryl ester (peak 7). Other conditions are the same as in Figure 2.

was expressed as recovery (%) by spiking the standard mixture with three different concentration levels of each standard (one for lower limit, one for middle point, and one for upper limit). The recovery values were found in the range of 95–98% ( $\pm 2$ ). The linearity was tested by preparing standard solutions at ten different concentration levels ranging from 0.5 to 200 ppm. The calibration curves were linear in the concentration range of 5–100 ppm with the determination coefficient ( $R^2$ ) values between 0.9985 ( $\pm 0.02$ ) and 0.9997 ( $\pm 0.01$ ) for duplicate injections of the standards at each concentration. All validation parameters are given in Table I. System suitability tests are commonly used to verify resolution, column efficiency, and repeatability of a chromatographic system to ensure its adequacy for a particular analysis. Therefore, these analyses were completed by calculating resolution ( $R_s$ ), selectivity ( $\alpha$ ), and peak tailing factors (T) (Table II). All analyte peaks observed under the optimum separation conditions (in Figure 5C) have a good symmetry (tailing factors are  $\leq 1$ ) despite their long retention times. Because of the different absorption maxima of the pigments selected, diode array detection (DAD) was employed for the quantitative evaluation at the maximum absorbance of each pigment (Table III). Accurate and precise results were obtained with lower LOD and LOQ limits under the optimized NACE conditions [i.e. 2% PEI, 10mM Aliquat 336, MeOH-ACN-THF (5:4:1, v/v/v), Voltage = -28 kV].

## Conclusion

Between the various CE techniques, NACE is shown to be the most efficient for the separation of tetrapyrrolic and carotenoid pigments. The most effective separation results were observed using NACE with Aliquat 336 as cationic surfactant in PEI coated capillaries and a mixture of MeOH-ACN-THF (5:4:1, v/v/v) as solvent. This methodology is simple, rapid, and sensitive, and it can overcome the difficulties of analysing these compounds because of their non-volatile, thermally unstable, light-, and oxygen-sensitive properties.

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## References

1. R. Bushway. Separation of carotenoids in fruits and vegetables by high performance liquid chromatography. *J. Liq. Chromatogr.* **8**: 1527–1547 (1985).
2. F. Khachik, G.R. Beecher, J.T. Vanderslice, and G. Furrow. Liquid chromatographic artifacts and peak distortion: sample-solvent interactions in the separation of carotenoids. *Anal. Chem.* **60**: 807–811 (1988).
3. A.K. Roy, A.K. Banerjee, and J. Chakrabarti. Separation of carotenoids, chlorophylls, and related pigments. *J. Inst. Chem.* **63**: 79 (1991).
4. N.E. Craft, S.A. Wise, and J.H. Soares. Optimization of an isocratic high-performance liquid chromatographic separation of carotenoids. *J. Chromatogr.* **589**: 171–176 (1992).
5. P. Kowalowski, J. Burczyk, B. Smietana, A. Stolarczyk, K. Terminska, M. Zych, and M. Kopec. Optimization of chromatographic separation of carotenoids from inflorescences of *Calendula officinalis* (Asteraceae) orange variety. *Herba Polonica* **45**: 324–333 (1999).
6. E. Darko, B. Schoefs, and Y. Lemoine. Improved liquid chromatographic method for the analysis of photosynthetic pigments of higher plants. *J. Chromatogr. A* **876**: 111–116 (2000).
7. C. Riffe-Chalard, L. Verzeznassi, and F.O. Gülaçar. A new series of steryl chlorin esters: pheophorbide a steryl esters in an oxic surface sediment. *Org. Geochem.* **31**: 1703–1712 (2000).
8. L. Verzeznassi, C. Riffe-Chalard, and F.O. Gülaçar. Rapid identification of Mg-chelated chlorins by on-line high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **14**: 590–594 (2000).
9. J.W. Jorgenson and K. Lukacs. Zone electrophoresis in open-tubular glass capillaries: preliminary data on performance. *J. High. Resolut. Chromatogr. Chromatogr. Commun.* **4**: 230–231 (1981).
10. J.W. Jorgenson. Zone electrophoresis in open-tubular capillaries. *TrAC, Trends Anal. Chem.* **3**: 51–54 (1984).
11. S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, and T. Ando. Electrokinetic separations with micellar solutions and open-tubular capillaries. *Anal. Chem.* **56**: 111–113 (1984).
12. M.T. Bowser, A.R. Kranack, and D.D.Y. Chen. Analyte-additive interactions in nonaqueous capillary electrophoresis: A critical review. *Trends Anal. Chem.* **17**: 424–434 (1998).
13. M.T. Bowser and D.D.Y. Chen. The effect of complexation additives on analyte migration behavior in capillary electrochromatography. *Electrophoresis* **19**: 1452–1460 (1998).
14. K. Saitoh, H. Kato, and N. Teramae. Separation of chlorophyll -c1 and -c2 by micellar electrokinetic capillary chromatography. *J. Chromatogr. A* **687**: 149–153 (1994).
15. T. Watanabe, N. Kotokawa, A. Yamamoto, and S. Terabe. Separation by capillary electrophoresis of natural yellow pigments used in food. *Kuromatogurafi* **15**: 212–213 (1994).
16. T. Watanabe and S. Terabe. Analysis of natural food pigments by capillary electrophoresis. *J. Chromatogr. A* **880**: 311–322 (2000).
17. J.E. Melanson and C.A. Lucy. Enhanced detection of porphyrins by capillary electrophoresis-laser induced fluorescence. *Electrophoresis* **23**: 1689–1694 (2002).
18. X. Sun, X. Yang, and E. Wang. Chromatographic and electrophoretic procedures for analyzing plant pigments of pharmacologically interests. *Anal. Chim. Acta* **547**: 153–157 (2005).
19. Q. Li, C.K. Chang, and C.W. Huie. Investigation of solvent effects in capillary electrophoresis for the separation of biological porphyrin methyl esters. *Electrophoresis* **26**: 3349–3359 (2005).
20. K. Aizawa, F. X. Cunningham Jr., and E. Gantt. Enhanced recovery of chlorophyll and carotenoids with dextran-polyacrylamide gel electrophoresis. *Anal. Sci.* **13**: 253–256 (1997).
21. D. W. Dixon, G. Pu, and H. Wojtowicz. Capillary electrophoretic separation of cationic porphyrins. *J. Chromatogr. A* **802**: 367–380 (1998).
22. S.C.C. Chiang and S.F.Y. Li. Separation of porphyrins by capillary electrophoresis in fused-silica and ethylene vinyl acetate copolymer capillaries with visible absorbance detection. *Biomed. Chromatogr.* **11**: 366–370 (1997).
23. P. Andrighetto, T. Carofiglio, R. Fornasier, and U. Tonellato. Capillary electrophoresis behavior of water-soluble anionic porphyrins in the presence of  $\beta$ -cyclodextrin and its O-methylated derivatives. *Electrophoresis* **21**: 619–626 (2000).
24. R. Sáenz-López, P. Fernández-Zurbano, and M.T. Tena. Analysis of aged red wine pigments by capillary zone electrophoresis. *J. Chromatogr. A* **1052**: 191–197 (2004).
25. L. Del Giovine and F. Fabietti. Copper chlorophyll in olive oils:

- identification and determination by LIF capillary electrophoresis. *Food Control* **16**: 267-272 (2005).
26. M.T. Bowser, E.D. Sternberg, and D.D.Y. Chen. Development and application of a nonaqueous capillary electrophoresis system for the analysis of porphyrins and their oligomers (PHOTOFIN). *Anal. Biochem.* **241**: 143-150 (1996).
  27. A. Negro, E. Paz, and B. Rabanal. New electrolyte composition for determination of anions by capillary electrophoresis with indirect UV detection. *J. Liq. Chromatogr. Relat. Technol.* **26**: 709-722 (2003).
  28. A.G. Eppel, S. Nagy, A.M. Jenkins, R.N. Tudball, M. Daskalakis, N.D.H. Balazs, and W.D. Comper. Variability of standard clinical protein assays in the analysis of a model urine solution of fragmented albumin. *Clin. Biochem.* **33**: 487-494 (2000).
  29. C. Simo, H. Cottet, W. Vayaboury, O. Giani, M. Pelzing, and A. Cifuentes. Nonaqueous capillary electrophoresis-mass spectrometry of synthetic polymers. *Anal. Chem.* **76**: 335-344 (2004).
  30. M.L. Riekkola, M. Jussila, S.P. Porras, and I.E. Valko. Non-aqueous capillary electrophoresis. *J. Chromatogr. A* **892**: 155-170 (2000).
  31. Q. Yang, L. M. Benson, K. L. Johnson, and S. Naylor. Analysis of lipophilic peptides and therapeutic drugs: online-nonaqueous capillary electrophoresis-mass spectrometry. *J. Biochem. Bioph. Methods* **38**: 103-121 (1999).
  32. F.M. Matysik. Special aspects of detection methodology in non-aqueous capillary electrophoresis. *Electrophoresis* **23**: 400-407 (2002).
  33. J. K. Towns and F. E. Regnier. Polyethyleneimine-bonded phases in the separation of proteins by capillary electrophoresis. *J. Chromatogr.* **516**: 69-78 (1990).
  34. M. Chiari, M. Cretich, F. Damin, L. Ceriotti, and R. Consonni. New adsorbed coatings for capillary electrophoresis. *Electrophoresis* **21**: 909-916 (2000).
  35. N. Gonzalez, C. Elvira, J. San Roman, and A. Cifuentes. New physically adsorbed polymer coating for reproducible separations of basic and acidic proteins by capillary electrophoresis. *J. Chromatogr. A* **1012**: 95-101 (2003).
  36. C. Simo, C. Elvira, N. Gonzalez, J. San Roman, C. Barbas, and A. Cifuentes. Capillary electrophoresis-mass spectrometry of basic proteins using a new physically adsorbed polymer coating. Some applications in food analysis. *Electrophoresis* **25**: 2056-2064 (2004).
  37. D. Belder, K. Elke, and H. Husmann. Use of coated capillaries for nonaqueous capillary electrophoresis. *J. Microcol. Sep.* **11**: 209-213 (1999).
  38. D. Belder, K. Elke, and H. Husmann. Influence of pH\*-value of methanolic electrolytes on electroosmotic flow in hydrophilic coated capillaries. *J. Chromatogr. A* **868**: 63-71 (2000).
  39. Y. Esaka, S. Inagaki, D. Uchida, M. Goto, and K. Kano. Polyacrylamides as hydrophilic selectors in non-aqueous capillary electrophoresis. *J. Chromatogr. A* **905**: 291-297 (2001).
  40. K. Heinig, C. Vogt, and G. Werner. Determination of cationic surfactants by capillary electrophoresis. *Fresenius J. Anal. Chem.* **358**: 500-505 (1997).
  41. S.P. Porras, S.K. Wiedmer, S. Strandman, H. Tenhu, and M.L. Riekkola. Novel dynamic polymer coating for capillary electrophoresis in nonaqueous methanolic background electrolytes. *Electrophoresis* **22**: 3805-3812 (2001).
  42. F. Steiner and M. Hassel. Control of electroosmotic flow in non-aqueous capillary electrophoresis by polymer capillary coatings. *Electrophoresis* **24**: 399-407 (2003).
  43. W. Vayaboury, D. Kirby, O. Giani, and H. Cottet. Noncovalent coatings for the separation of synthetic polypeptides by nonaqueous capillary electrophoresis. *Electrophoresis* **26**: 2187-2197 (2005).

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