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Separation of fatty alcohol polyethoxylates by capillary electrophoresis through easy electroosmotic flow control with a quaternary diammonium salt

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Abstract

An optimised protocol for the fast and reliable analysis of fatty alcohol polyethoxylates (FAEs), with resolution between the alkyl chain and ethylene oxide (EO) oligomers, is reported. Phthalic and maleic anhydrides were used to quickly form the hemiesters, which were separated by capillary electrophoresis. Effective reduction of the electroosmotic flow to very low values was achieved by previously rinsing the capillary with a quaternary diammonium salt, which remains strongly adsorbed to the silica wall. The phthalic hemiesters of a FAE mixture with 10 carbon atoms in the alkyl chain, and an average EO number of 6, were separated in a background electrolyte (BGE) containing 25 mM borate buffer of pH 9.0 in water. The same capillary pre-treatment was used to separate mixtures of the maleic hemiesters of hydrophobic FAEs, having 12–16 carbon atoms in the alkyl chain and an average EO number of 3. Full resolution of all the oligomers was achieved by using a finely tuned BGE in which a 35% borate buffer was mixed with 65% acetonitrile.
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1. Introduction

Fatty alcohol polyethoxylates (FAEs) constitute a class of non-ionic surfactants that is widely used in household cleaners, cosmetics, pesticides, textile lubricants and in other domestic and industrial applications [1]. Owing to the faster biodegradability and lower toxicity of the metabolites, FAEs are preferred over the corresponding phenol polyethoxylates. FAEs are industrially obtained as complex mixtures of oligomers with the general formula:

$$CH_3(CH_2)_x(OCH_2CH_2)_nOH$$

where n is the number of ethylene oxide units (EOs). They are characterised by both the average ethoxylation degree and the hydrophobic cut, with typical ($x + 1$) values ranging from 8 up to 18. Fast and selective analytical methods are required in the related industries for supporting quality control of raw materials and manufactured products, as well as for investigating market trends. Selective and sensitive methods are also required in environmental studies.

A variety of NP-HPLC and RP-HPLC isocratic and gradient methods for the separation of FAEs with resolution of both types of homologues, i.e., those due to the length of the alkyl chain and those associated to the EO distribution, have been described. Owing to the lack of a chromophore, chromogenic and fluorogenic pre-column derivatisation with phenyl isocyanate [2,3], naphthyl isocyanate, naphthoyl chloride [4,5] and 3,5-dinitrobenzoyl chloride [6–9] are frequently used. The polyoxyethylene chain forms weak association complexes with potassium ion, thus allowing the 3,5-dinitrobenzoyl chloride derivatives to be also separated on a cation-exchange column [10,11].

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Underivatised FAEs can be analysed in industrial samples using either a refractive index [12–15] or evaporative-light scattering detection (ELSD) [13,16–20]. Supercritical fluid chromatography with ELSD has been also described [21]. However, a loss of sensitivity has been observed with ELSD for the oligomers with a low ethoxylation degree ($n < 3$), which seems to be due to volatilisation of the lighter and less polar oligomers in the spray chamber [22]. The chromatographic procedures for the determination of FAEs in both industrial [22,23] and environmental samples [24] have been revised.

Matrix-assisted laser desorption/ionisation, electrospray and atmospheric pressure chemical ionisation mass spectrometry (MALDI-, ESI- and APCI-MS), without or with previous HPLC separation, are today increasingly preferred to characterise FAEs and other ethoxylated surfactants in both industrial and environmental samples. Thus, the quantification of FAEs in diluted aqueous environmental samples by HPLC–thermospray MS was early described [25]; then, aromatic and aliphatic polyethoxylated surfactants were characterised in environmental samples by HPLC–APCI-MS [26,27], and HPLC–ESI-MS was used to quantify the surfactants in sewage, river and drinking water [28], and in hydroponic plant growth media [29]. The determination of the oligomer distribution in aromatic and aliphatic polyethoxylated surfactants by MALDI-MS, thin-layer chromatography and reversed-phase HPLC have been compared [30]. In spite of the increasing attention paid to the MS detection techniques, HPLC with both ELSD and UV–vis detection is still very useful for routine applications, as well as to optimise the separation conditions to be used with the more complex MS detection, and UV–vis detection is also used in capillary electrophoresis (CE) for the same purposes. Further, it has been shown that previous derivatisation of FAEs by placing a permanent positive charge on the analites is useful to avoid response problems arising from the large sensitivity differences among the oligomers within a given solute class in HPLC–MS [31].

As described for other surfactant classes, FAEs can be separated with oligomer resolution by CE in the presence of a large concentration of an organic solvent that hinders micellisation. UV–vis detection can be implemented using previous esterification with phthalic anhydride, which has the advantage of providing a chromophore and a charge at a time [16,32]. Separation of the non-charged 3,5-dinitrobenzoyl chloride derivatives by solvophobic association with a charged surfactant, such as sodium dodecyl sulphate (SDS), has been also described [16]. The separation of FAEs by both HPLC and CE have been compared [16,33,34].

A highly effective electroosmotic flow (EOF) control can be achieved with dynamic capillary coatings, usually implemented by adding a low concentration of a cationic surfactant, a cationic polyelectrolyte or a soluble non-ionic polymer to the background electrolyte (BGE) [35,36]. A different approach, based on the previous rinsing of the capillary with a quaternary diammonium salt, is used in this work. Over the years at the *Politecnico di Milano*, we have prepared a number of silica wall modifiers, all of which members of a family of quaternarised aliphatic heterocycles [37–40]. A number of them, i.e., those with an iodinated alkyl chain, are able of spontaneously alkylating the silanols in alkaline media, thus forming a covalent bond. The compound used in this work, 1,4-di(4-aza-1-azonia-bicyclo[2.2.2]octane)butane diiodide (M7C4M7), lacks a terminal iodinated alkyl tail, thus being unable of forming a covalent bond with the silanols; nevertheless it sticks tightly to the silica walls, which is probably due to the optimal distance between the two quaternary nitrogens in the two rings. This distance is maintained fixed at 0.75 nm by both the repulsion between the charges and the short C_4 chain between the rings [39]. This has been claimed to be the average distance between two ionised silanols at alkaline pHs along the silica surface. Since rinsing of the capillary is performed before separation, the BGE is not perturbed by the presence of M7C4M7. Using this capillary pretreatment, simple, fast, fine and reproducible EOF control was achieved, and the oligomers of complex FAE mixtures were well-resolved in short running times.

2. Materials and methods

2.1. Reagents, instrumentation and working conditions

Methanol (MeOH), acetonitrile (MeCN) (Scharlab, Barcelona, Spain), *n*-dodecanol, *n*-tetradecanol (Sigma–Aldrich, Steinheim, Germany, and Fluka, Buchs, Switzerland), other analytical grade reagents and deionised water (Barnstead deionizer, Sybron, Boston, MA, USA), were used. The EOF modulator, 1,4-di(4-aza-1-azonia-bicyclo[2.2.2]octane)butane diiodide (M7C4M7), was synthesised as reported [39]. The molecular structure of M7C4M7 is shown in Fig. 1.

An HP^{3D} capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) provided with a diode-array spectrophotometric detector and fused-silica capillaries (Composite Metal Services, Ilkley, UK) of 33.5–45 cm (25–36.5 cm effective length) and 50 μm i.d. (363 μm o.d.) were used. Separations were performed at 25/45 °C under 20/25 kV using the positive or the negative polarity, as indicated in the figure legends. Detection was set at 214 nm.

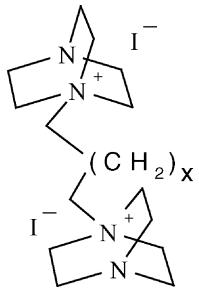

Fig. 1. Molecular structure of M7C4M7 ($x = 4$).

New capillaries were treated with 1 and 0.1 M NaOH and water at 60 °C (10 min each). Daily before use, the capillary was rinsed with 0.1 M NaOH (20 min) and water (10 min). The preconditioning protocol to be used between runs was as follows: an auxiliary nitrogen supply was used to wash the capillary at 500 kPa for 2 min with 0.25 M phosphoric acid (thus to remove all traces of the capillary wall modifier), then with the modifier solution (4 mM M7C4M7 in 25 mM borate buffer of pH 9.0) for 2 min, then finally at 500 kPa for 3 min with the running buffer. The sample was injected at 1 kPa for 10 s, followed by a buffer plug (0.5 kPa, 5 s). During the separations, the capillary ends were submerged in the 25 mM borate buffer of pH 9.0, but the anodic vial solution was also doped with 1 mM M7C4M7 when pre-treated capillaries were used. Before injection, all solutions were filtered through a 0.45 μm pore-size nylon membrane (Albet, Germany). After each working session, the capillary was flushed with water for 10 min.

The samples analysed were Findet 1018, of moderate hydrophobicity (x = 10 and nominal average n = 6), and Findet 1214 of increased hydrophobicity (x = 12–14 with a small amount of x = 16 and nominal average n = 3) (Kao Corp., Barcelona, Spain). A mixture containing 35% of 25 mM borate buffer of pH 9.0 in water and 65% MeCN was used to separate the oligomers of Findet 1214. Since micelles are not formed at low concentrations when x < 12, a simpler 25 mM borate buffer of pH 9.0 in plain water was used for Findet 1018.

2.2. Sample derivatisation procedures

The formation of the hemiesters with both the phthalic and maleic anhydrides was investigated. These reactions, that introduce a charge and a chromophore group in the FAE molecules, are depicted in Fig. 2. According to literature, phthalic anhydride affords efficient derivatisation of FAEs in the presence of pyridine and imidazol at 100 °C in 60 min [32], or using MeCN at 100 °C in 8 h [16]. We tried the direct melting of the FAE samples with the anhydrides. For this purpose, 1 g of sample was mixed with 1 g of either the phthalic or maleic anhydride and, respectively, heated at 120 °C in a sealed vial. After cooling, the mixture was diluted with 5 ml MeCN and stored at −25 °C. From these solutions, 20 μL aliquots were diluted with 400 μL of the running buffer and injected. The formation of the hemiesters was completed in less than 4 h and less than 90 min for the phthalic and maleic anhydrides, respectively. Phthalic anhydride was used for Findet 1018, but owing to the shorter reaction time, the maleic anhydride was preferred for Findet 1214. Maleic hemiesters have a lower molar absorptivity than the corresponding phthalic hemiesters; however, a low limit of detection is not necessary to analyse industrial samples.

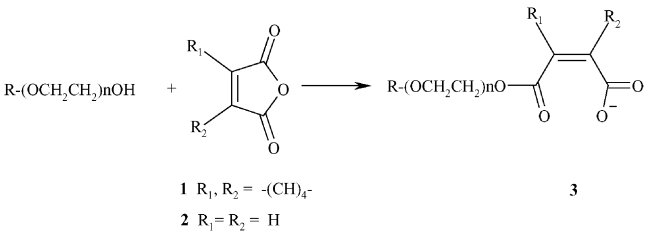

Fig. 2. Derivatisation of FAEs using either phthalic (**1**) or maleic (**2**) anhydride to yield a hemiester (**3**).

3. Results and discussion

The electropherogram of Fig. 3A, was obtained by injecting the derivatised Findet 1018 in an untreated capillary. Owing to the strong normal EOF (from anode to cathode), positive polarity (cathode at the detector end) was required to observe the derivatives. As shown in the figure, most of the oligomers migrated as a large unresolved envelope, only

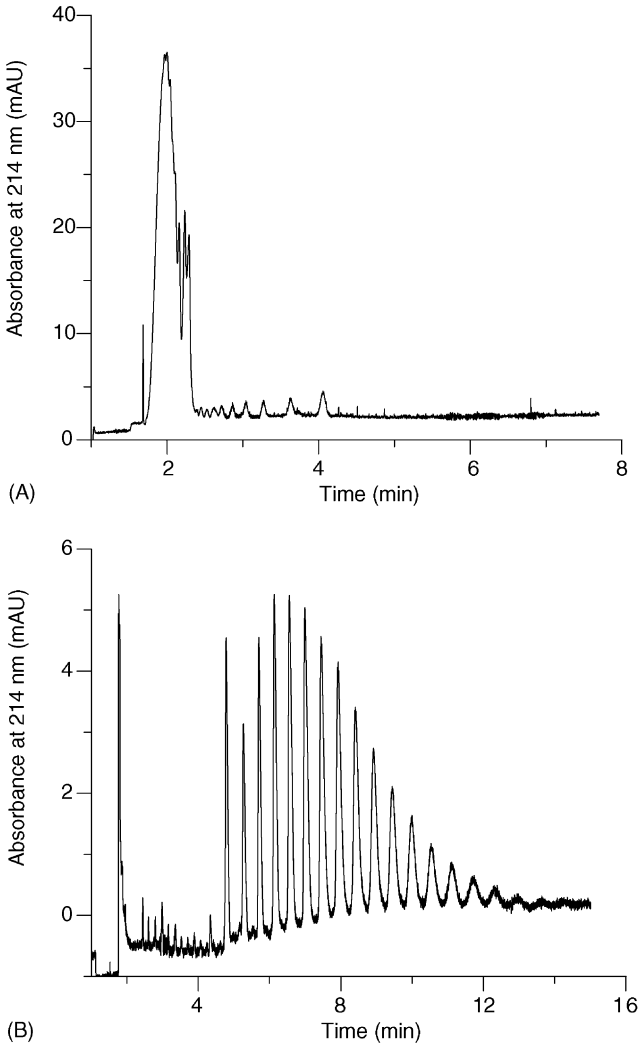

Fig. 3. Electropherograms of Findet 1018 obtained in a 25 mM borate buffer of pH 9.0 prepared in water. Separations performed under +20 kV using an untreated capillary (A) and under −20 kV using a capillary previously rinsed with 4 mM M7C4M7 (B). Other conditions: L = 33.5 cm, T = 25 °C.

a few low molecular mass oligomers (those moving faster against the EOF) being resolved at long migration times.

Conversely, when the capillary was pre-conditioned with M7C4M7, negative polarity (anode at the detector end), was required for the peaks to be observed (Fig. 3B). With negative polarity, an inverted EOF would have dragged the non-ionic solutes (as the di-esterified fraction) to the detector, and the corresponding peaks would have appeared at long migration times, after the peaks of the anionic solutes. Since these peaks were not observed, then a reduction of the EOF to a small positive value, rather than an EOF inversion, was produced by M7C4M7. By injecting dimethylformamide as EOF marker, in fact, an electroosmotic mobility of 1×10^{-4} cm^2 s^{-1} V^{-1} was observed (8×10^{-4} cm^2 s^{-1} V^{-1} was obtained for an untreated capillary).

Under the conditions of Fig. 3B, a large peak which did not interfere with the peaks of the derivatives, and that was attributed to phthalic acid (the UV spectrum at the peak location agreed with this assignment), was observed at 2 min. This peak was followed by the peaks of the derivatives, spanning from 4.5 to 14 min, with an excellent resolution between all the peak pairs. The small peaks between 2 and 4.5 min were probably due to derivatised polyethylene glycols, which are always present as impurities in FAEs. The first large peak at 4.8 min is the derivative of the unethoxylated decylalcohol, which is followed by the peaks of the derivatives of the successive EO homologues, from n = 1 up to n = 18. The average EO number (moles of EO units/mol of product), calculated as the weighted average of the corrected peak areas, was n = 5.2. In contrast with the electropherogram of Fig. 3A, with peaks having non-linear migration behaviour, the electropherogram of Fig. 3B, shows a quasi-linear migration of the successive EO oligomers. This is an advantage, since it makes peak identification easier and represents the best balance between analysis time and resolution for all the pairs of the successive oligomers.

Owing to its higher hydrophobicity and lower critical micellar concentration, the addition of an organic modifier to the BGE was required to separate the Findet 1214 derivatives. In a number of preliminary experiments performed both at 25 and 45 °C, dioxane, methanol, ethylene glycol monobutyl ether and MeCN were tried at several concentrations ranging from 40 up to 70%. The best separations were achieved at 45 °C with 65% (v/v) MeCN. This value was proved to be rather critical, with important resolution losses at 60 and 70% MeCN. As discussed next, the reason was the fine tuning achieved with 65% MeCN for the separation of the two oligomer classes, i.e., those due to the different lengths of the alkyl chain and those associated to the EO distribution.

As shown in the electropherogram of Fig. 4, in the newly optimised conditions a quasi-linear migration of the EO oligomers of Findet 1214 was obtained. The peak of the maleic acid excess appeared first (the UV spectrum at the peak location agreed with this assignment), followed by an intertwined repeated pattern that corresponded to the three EO distributions, i.e. those of the (x + 1) = 12, 14 and 16

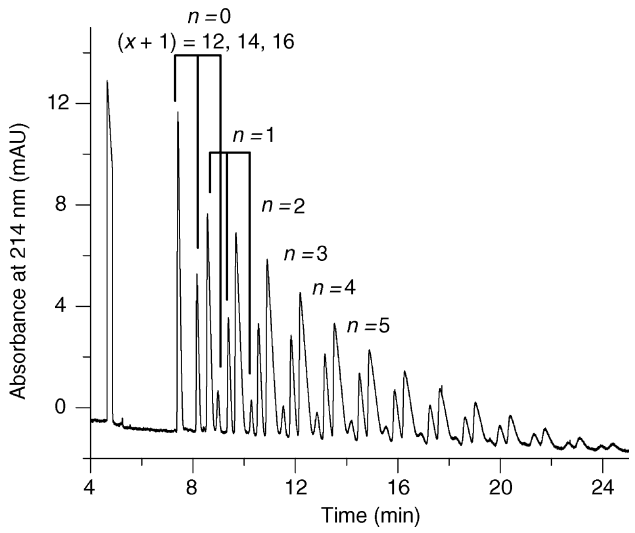

Fig. 4. Electropherogram of Findet 1214 obtained in a 25 mM borate buffer of pH 9.0 diluted with MeCN (35:65). Separation performed under −25 kV after capillary preconditioning with 4 mM M7C4M7. Other conditions: L = 45 cm, T = 45 °C.

alkyl homologues. Along each series, the well-resolved EO oligomers were distinguished up to n = 13. Peak integration along the series showed a relative abundance of 59, 32 and 9% for (x + 1) = 12, 14 and 16, respectively. For the EO distributions, the weighted average of the corrected peak areas (moles of EO units/mol of product), yielded n = 3.3, 3.1 and 2.7 for the (x + 1) = 12, 14 and 16 series, respectively. Derivatised polyethylene glycols were not observed in this sample. The composition agreed with the results obtained by RP-HPLC with ELSD (although the peaks of the oligomers with n < 2 were not observed) using reported conditions (C18 column and 70% MeCN as isocratic mobile phase) [16,22].

4. Conclusions

The ability of a previous capillary rinsing with M7C4M7 to provide effective EOF control has been demonstrated. Also, as a result of the quasi-linear increase of the migration times with both the EO number and the alkyl chain length, and of the fine tuning achieved through control of the MeCN concentration, complex mixtures of FAEs oligomers were fully resolved within short running times. Although it remains to be proven, some interaction of the EOF modulator with the analytes, such as a weak association of the quaternary nitrogens with the EO chains, or as ion-pairing, could also contribute to the separation success. These two interaction mechanisms have been indeed, respectively reported for FAEs with potassium ions [10,11], and for other anionic analytes with a variety of counter ions [40]. In comparison to HPLC, capillary zone electrophoresis with M7C4M7 capillary pre-treatment ensures a shorter running time and a simpler operative protocol.

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References

[1] H.P. Fiedler, Lexicon der Hilfsstoffe für Pharmazie, Kosmetic und angrenzende Gebiete, Edition Cantor, Aulendorf, 1989.
[2] M.C. Allen, D.E. Linder, J. Am. Oil Chem. Soc. 58 (1981) 950.
[3] T.M. Schmitt, M.C. Allen, D.K. Brain, K.F. Guin, D.E. Lemmez, Q.W. Osburn, J. Am. Oil Chem. Soc. 67 (1990) 103.
[4] K. Lemr, M. Zanette, A. Marcomini, J. Chromatogr. A 686 (1994) 219.
[5] M. Zanette, A. Marcomini, E. Marchiori, R. Samperi, J. Chromatogr. A 756 (1996) 159.
[6] A. Nozawa, T. Ohnuma, J. Chromatogr. 187 (1980) 261.
[7] K. Lemr, J. Chromatogr. A 732 (1996) 299.
[8] C. Sun, M. Baird, H.A. Anderson, D.L. Brydon, J. Chromatogr. A 771 (1997) 145.
[9] K. Lemr, J. Sevzík, J. Hlavác, J. Chromatogr. A 1021 (2003) 19.
[10] T. Okada, Anal. Chem. 63 (1991) 1043.
[11] T. Okada, J. Chromatogr. 609 (1992) 213.
[12] M. Kudoh, J. Chromatogr. 291 (1984) 327.
[13] Y. Mengerink, H.C.J. De Man, S. Van der Wal, J. Chromatogr. 552 (1991) 593.
[14] D. Cho, J. Hong, S. Park, T. Chang, J. Chromatogr. A 986 (2003) 199.
[15] B. Trathnigg, C. Rappel, J. Chromatogr. A 952 (2002) 149.
[16] K. Heinig, C. Vogt, G. Werner, Anal. Chem. 70 (1998) 1885.
[17] G.R. Bear, J. Chromatogr. 459 (1988) 91.
[18] W. Miszkiewicz, W. Hreczuch, A. Sobczynska, J. Szymanowski, Chromatographia 51 (2000) 95.
[19] W. Miszkiewicz, J. Szymanowski, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 1013.
[20] T. Kamiusuki, T. Monde, K. Omae, K. Morioka, T. Konakahara, Chromatographia 51 (2000) 390.
[21] S. Brossard, M. Lafosse, M. Dreux, J. Chromatogr. 591 (1992) 149.
[22] W. Miszkiewicz, J. Szymanowski, Crit. Rev. Anal. Chem. 25 (1996) 203.
[23] K. Rissler, J. Chromatogr. A 742 (1996) 1.
[24] A. Marcomini, M. Zanette, J. Chromatogr. A 733 (1996) 193.
[25] K.A. Evans, S.T. Dubey, L. Kravetz, I. Dzidic, J. Gumulka, R. Mueller, J.R. Stork, Anal. Chem. 66 (1994) 699.
[26] M. Castillo, F. Ventura, D. Barceló, Waste Manage. 19 (1999) 101.
[27] K.A. Krogh, K.V. Vejrup, B.B. Mogensen, B. Halling-Sorensen, J. Chromatogr. A 957 (2002) 45.
[28] C. Crescenzi, A. di Corcia, R. Samperi, A. Marcomini, Anal. Chem. 67 (1995) 1797.
[29] L.H. Levine, J.L. Garland, J.V. Johnson, Anal. Chem. 74 (2002) 2064.
[30] G.A. Cumme, E. Blume, R. Bublitz, H. Hoppe, A. Horn, J. Chromatogr. A 791 (1997) 245.
[31] J.C. Dunphy, D.G. Pessler, S.W. Morrall, K.A. Evans, D.A. Robaugh, G. Fujimoto, A. Negahban, Environ. Sci. Technol. 35 (2001) 1223.
[32] R.A. Wallingford, Anal. Chem. 68 (1996) 2541.
[33] K. Heinig, C. Vogt, G. Werner, J. Chromatogr. A 745 (1996) 281.
[34] C. Vogt, K. Heinig, Fresenius J. Anal. Chem. 363 (1999) 612.
[35] J.E. Melanson, N.E. Baryla, C.A. Lucy, Trends Anal. Chem. 20 (2001) 365.
[36] C.M. Boone, E.Z. Jonkers, J.P. Franke, R.A. de Zeeuw, K. Ensing, J. Chromatogr. A 927 (2001) 203.
[37] R. Sebastiano, C. Gelfi, P.G. Righetti, A. Citterio, J. Chromatogr. A 894 (2000) 53.
[38] R. Sebastiano, C. Gelfi, P.G. Righetti, A. Citterio, J. Chromatogr. A 924 (2001) 71.
[39] B. Verzola, R. Sebastiano, P.G. Righetti, C. Gelfi, M. Lapadula, A. Citterio, Electrophoresis 24 (2003) 121.
[40] R. Sebastiano, M. Lapadula, P.G. Righetti, C. Gelfi, A. Citterio, Electrophoresis 24 (2003) 4189.