Talanta 84 (2011) 1361–1365

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Sensitive fluorimetric flow injection analysis for fluoride ion with a novel reagent, 2′,7′-dichlorofluorescein di-*tert*-butyldimethylsilyl ether

Mayu Nakaya, Mitsuko Oshima*, Toshio Takayanagi, Shoji Motomizu, Hiroki Yamashita

Graduate School of Natural Science and Technology (Department of Chemistry, Faculty of Science), Okayama University, 3–1–1 Tsushimanaka, Kitaku, Okayama 700–8530, Japan

ARTICLE INFO

Article history:
Available online 5 April 2011

Keywords:
Fluoride ion
2′,7′-dichlorofluorescein-di-*tert*-butyldimethylsilyl ether
Fluorimetric flow injection analysis
Water

ABSTRACT

A novel reagent, 2′,7′-dichlorofluorescein di-*tert*-butyldimethylsilyl ether (FCl_2TBS), was synthesized for fluoride ion and used for a sensitive fluorimetric flow injection analysis by detecting the recovery of fluorescence due to cleavage of Si–O bond. Four kinds of fluorescein di-*tert*-butyldimethylsilyl ether (FTBS) analogues were synthesized and FCl_2TBS was the best. By introducing chlorine to FTBS, stability of the reagent, reactivity and the baseline signals were improved. The FIA system was three lines. The sample solution (aqua medium) was injected in the carrier solution (water) and merged with the reagent solution (2.0×10^{-5} M FCl_2TBS acetone solution), then mixed with phosphate buffer solution (pH 7.5). The fluorescence intensities were measured at λ_{ex} 503 nm and λ_{em} 527 nm. The calibration graph had linear relationship between $(1.0–50.0) \times 10^{-6}$ M and the determinable limit was 1.0×10^{-6} M. The relative standard deviation of 12 measurements with 1.0×10^{-5} M F^- solution was 1.0% and the sample throughput was 13 h^{-1}. The developed method was successfully applied to river and tap water samples.

1. Introduction

Fluoride ion is widely contained in natural water and industrial effluent [1]. Fluoride ion is one of essential elements, and has an important biological role. For example, it is used such as dental care and clinical treatment for osteoporosis. On the other hand, excessive intake results in fluorosis and gives renal, gastrointestinal and immunological toxicity [2]. An upper limit of fluoride ion concentration in drinking water of the World Health Organization (WHO) is 1.5 mg L^{-1}, and that of drinking water quality standards in Japan should be less than 0.8 mg L^{-1}.

Several analytical techniques for fluoride ion have been reported: potentiometric method with fluoride-ion selective electrode (ISE) [3–5], ion chromatography (IC) [6–8], spectrophotometry [9–11], fluorimetry [12–14], and capillary zone electrophoresis (CZE) [15–17]. ISE and IC are widely used because of their simplicity and convenience, but their sensitivity and selectivity are limited. Various chemical reactions are adopted to spectrophotometric and fluorimetric methods because they have advantages of selectivity and higher sensitivity. A number of spectrophotometric methods have been developed with chelate complexes, such as La(III), Ce(III), Zr(IV), Th(IV) and Al(III) complexes [9–14]. However, these method are interfered by Al(III), Fe(III) and Cu(II) ions, and generate large amounts of heavy metal waste. Recently, spectrophotometric or spectrofluorimetric methods using specific reaction between fluoride ion and silyl group introduced in an organic reagent have been reported. Descalzo et al. used mesoporous solid silica as a base material for immobilizing a dye [18], Zhu et al. synthesized a new colorimetric probe, 1-ethyl-4-(*p*-*tert*-butyldimethylsilane ether styryl) quinolinium iodide [19] and Kim and Hong synthesized a reagent by coupling 4-(*tert*-butyldiphenylsilyloxyl)benzyl bromide to resorufin [20]. Yang et al. developed a fluorescein-based fluorogenic probe, fluorescein di-*tert*-butyldimethylsilyl ether (FTBS), to use in aqueous media [21]. In this method, non-fluorescent FTBS was selectively cleaved their Si–O bond by fluoride ion because of high affinity of fluoride ion to silicon, and fluorescein was formed. As a result, the fluorescence intensity increased proportionally with the concentration of fluoride ion. Though this method is simple, sensitive and highly selective, it required longer reaction time (30 min/1 sample), organic solvent for dissolving FTBS. Moreover, blank fluorescence intensity was increased with time due to the decomposition of FTBS by attacking from hydroxide ion in a sample aqueous solution.

To overcome these problems, we applied this detection method with FTBS to flow injection analysis (FIA), which could easily control the reaction time. In this paper, a novel fluorescence reagent, FCl_2TBS, was synthesized to increase reactivity by decreasing the electron density on oxygen at Si–O bond and applied to a fluorescence detection/FIA. The synthesis was simple one step. The background signals could be decreased because of the reagent sta-

---

* Corresponding author. Tel.: +86 2518511; fax: +86 2844536.
E-mail address: oshimam@cc.okayama-u.ac.jp (M. Oshima).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.03.081

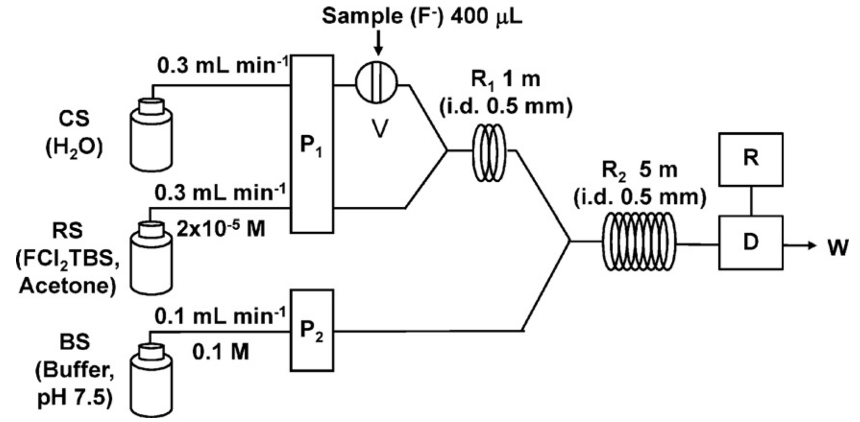


Fig. 1. Flow diagram for fluoride determination P_1 and P_2: double plunger pump; *V*: injection valve; R_1 and R_2: reaction coil; *D*: spectrofluorometer (λ_{ex}, 503 nm, λ_{em}, 527 nm); *R*: recorder; *W*: waste.

bility in aqueous solution, and sample throughput was improved compared with FTBA method. The procedure is simple, rapid and harmless with a good reproducibility, sensitivity and accuracy. The proposed method could be successfully applied natural water samples without any pretreatments.

2. Experimental

2.1. Apparatus

The schematic diagram of FIA system is shown in Fig. 1. The carrier solution (CS), reagent solution (RS) and buffer solution (BS) were propelled with two double plunger pumps (Sanuki Kogyo SR-3300T and SRX-3400T, Japan). The sample or standard solutions were injected into the carrier stream using a six-way valve with a 400 μL sample loop. Flow lines were made of PTFE tubing (0.5 mm i.d.). A water bath (MTU-1, ADVANTEC, Japan) was used for control the reaction coil temperature. Fluorescence intensity was measured at λ_{ex} 503 nm and λ_{em} 527 nm by a spectrofluorometer (Shimadzu, RF-10A XL, Japan) with a flow cell (16 μL volume). Flow signals were recorded with a FIA monitor (F I A Instruments, Japan).

The fluorescence spectra were measured with a spectrofluorophotometer (RF-5300PC, Shimadzu, Japan) and pH was measured with a MP 220 pH meter (Mettler Toledo, Swizerland). ^{1}H NMR and Mass spectra were obtained with JNM-AL400 (JEOL, Japan) and LCT (ESI, TOF-MS, Micromass, UK), respectively.

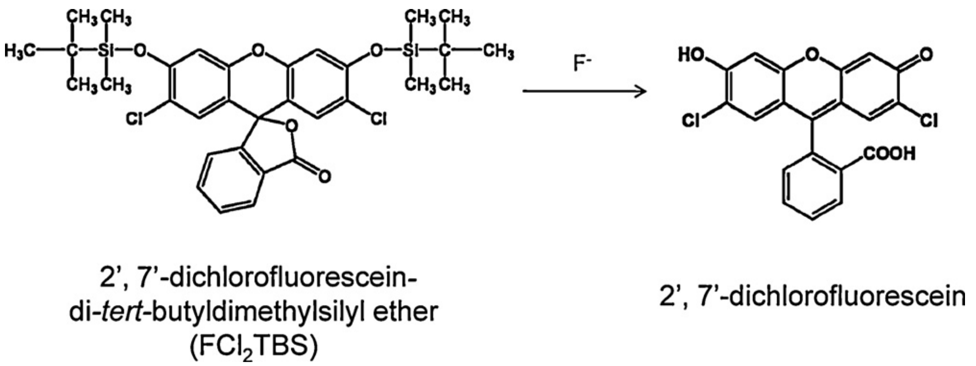
2.2. Reagents

FCl_2TBS and 4′,5′-dibromofluorescein di-*tert*-butyldimethylsilyl ether (FBr_2TBS) were synthesized with each fluorescein dihalogenoderivative according to the previous paper (Yang et al. [21]): for FCl_2TBS, 2,7-dichlorofluorescein was silanated with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide (DMF, 66.5% yield). The purity of FCl_2TBS was confirmed by ^{1}H NMR and MS spectra: ^{1}H NMR ($CDCl_3$, 400 MHz): δ of the typical peaks were nearly the same as those of FTBS, whereas δ of 2 hydrogens of adjacent Cl were moved lower magnetic field by 0.5; ESI MS: calculated for $[M+Na]^+$, 651.15; found, 650.49. Fluorescein diisopropylsilyl ether (FTIPS) and fluorescein *tert*-butyldiphenylsilyl ether (FTBDPS) were synthesized with each silyl chloride as a similar manner as FTBS. FI_4TBS (2′,4′,5′,7′-tetraiodofluorescein di-*tert*-butyldimethylsilyl ether) could not be synthesized. FCl_2TBS and reaction scheme were shown in the Scheme 1.

A fluoride ion stock solution (0.1 M) was prepared by dissolving 0.1050 g of sodium fluoride (Yoneyama Chemicals, Japan) in water and diluting to 25 mL with ultrapurified water. Standard solutions were accurately diluted before use. A 1.0×10^{-3} M FCl_2TBS solution was prepared by dissolving 0.0063 g of FCl_2TBS in tetrahydrofuran (THF: Wako Pure Chemicals, Japan) and diluting to 10 mL with DMF. RS was prepared by diluting 1.0×10^{-3} M FCl_2TBS DMF solution to be 2.0×10^{-5} M with acetone (Kanto Chemical, Japan). BS was 0.1 M phosphate buffer solution (pH 7.5). Ultrapurified water prepared by Elix 3/Milli-Q element system (Nihon Millipore, Japan) and analytical reagent grade chemicals were used throughout.

2.3. Batchwise procedure for fundamental conditions

To a 25 mL polypropylene volumetric flask, 12.5 mL of acetone, 500 μl of 1×10^{-4} M reagent solution, appropriate volume of sample solution and 2.5 mL of 0.1 M phosphate buffer solution (pH 7.5) were transferred. The mixture was diluted to the mark with purified



Scheme 1. The structure of FCl_2TBS and the reaction scheme with F^-.

water and mixed thoroughly. Fluorescence intensity was measured at appropriate wavelength of each reagent.

3. Results and discussion

3.1. Comparison of the synthesized reagents

Fluorescein di-isopropylsilyl ether (FTIPS) and fluorescein *tert*-butyldiphenylsilyl ether (FTBDPS) which *tert*-butyldimethylsilyl (TBS) group of FTBS was changed to more hydrophobic and large ones, were expected to reduce the background signal by preventing from attack of hydroxide ion in aqueous medium. As a result, the decomposition of the reagents could be reduced, but the reactivity with F^- was also decreased. Therefore, TBS was selected because of a cost and easy treatment. To enhance the reactivity between Si at the reagent and F^-, halogens were introduced to reduce electron density on oxygen of Si–O bond by their electron withdrawn effect: FCl_2TBS and FBr_2TBS were tested. Recovered fluorescence intensity of FCl_2TBS after reacting with F^- was 5 times stronger than that of FBr_2TBS, depending on the original halogenofluoresceins (relative fluorescence quantum yields are 0.76 and 0.44, respectively [22]). We selected FCl_2TBS.

3.2. Solvent for FCl_2TBS

For the determination of fluoride ion in natural water samples, FCl_2TBS solution must be stable when mixed with aqueous media. These silylated fluorescein analogues were slightly soluble in water and gradually decomposed to original fluoresceins by hydroxide ion in aqueous media. Therefore, water soluble organic solvents, such as DMF, acetone, ethanol, acetonitrile, 1,4-dioxane and tetrahydrofuran, were tested to keep stability of FCl_2TBS in aqueous solution. As a result, FCl_2TBS was stable in organic solvent tested, except ethanol, as shown in Table 1. Of these, acetone reduced the blank signals and led comparatively higher sensitivity. Therefore, acetone was selected to prepare the reagent solution (RS). The concentration of acetone optimized for FIA was around 50% at a flow cell: the reagent solution was almost 100% acetone solution of FCl_2TBS.

3.3. Optimization for FIA conditions

To simplify the procedure and control the reaction time for minimizing the decomposition of the reagent, we applied this reaction to FIA: three-flow lines system as shown in Fig. 1 was selected because of stable baseline.

The concentration of FCl_2TBS solution was studied from 1.0×10^{-5} M to 6.0×10^{-5} M and fixed at 2.0×10^{-5} M in the further study because of better baseline and enough sensitivity.

Table 1
Effect of organic solvent mixing with FCl_2TBS solution in the absence and the presence of fluoride ion on the fluorescence intensities by batchwise method.

Solvent[a]	Fluorescence intensity		
	F_0	$F–F_0$	F/F_0
None	178	306	2.7
1,4-dioxane[b]	252	100	1.4
Ethanol[c]	246	169	1.7
THF[d]	341	331	2.0
Acetone[d]	228	590	3.6

[a] solvent concentration: 10% v/v. [FCl_2TBS]: 1.0×10^{-5} M; F_0: [F^-] = 0 M; F: [F^-] = 5.0×10^{-6} M; reaction time: 1 min; $n = 3$; $\lambda_{ex} = 503$ nm; $\lambda_{em} = 523$ nm.
[b] $\lambda_{em\ max} = 526$ nm.
[c] $\lambda_{em\ max} = 525$ nm.
[d] $\lambda_{em\ max} = 527$ nm.

The sensitivity depended on the reaction time. The effect of the flow rate was examined by varying it from 0.4 to 0.8 mL min^{-1} for the pump 1. When the flow rate of the pump 1 was examined, that of the pump 2 was fixed at 0.1 mL min^{-1}. The maximum intensity was obtained around 0.5 mL min^{-1} and 0.6 mL min^{-1} was selected for pump 1 (total flow rate) considering enough reaction time and analysis time. The flow rate of the pump 2 was varied in the range from 0.1 to 0.4 mL min^{-1} while that of the pump 1 was fixed at 0.6 mL min^{-1}. When the flow rate increased, the sensitivity slightly decreased because of shorter reaction time and relative increase in buffer concentration. Therefore, the flow rate of 0.1 mL min^{-1} for the pump 2 was selected.

The longer reaction coil gave higher reaction efficiency, but the dispersion of the sample zone became larger and the peak profiles were broadened. The effect of reaction coil lengths for R1 and R2 were studied over the range of 0.5–2 m and 1–7 m, respectively: the best lengths were 1 m and 5 m, respectively.

The effect of the reaction temperature was examined in the range from 25 to 40 °C using a water bath. The results were shown in Fig. 2(a) with signal to noise ratio (S/N) of the baseline. As the

Table 2
Optimum conditions of FIA for the determination of fluoride ion.

Variables	Range examined	Optimized conditions
Temperature (°C)	25–40	25 (r.t.)
RC_1 length (m)	0.5–2	1
RC_2 length (m)	1–7	5
Flow rate of P_1 (mL min^{-1})	0.4–0.8	0.6
Flow rate of P_2 (mL min^{-1})	0.1–0.4	0.1
Sample volume (μL)	100–500	400
FCl_2TBS concentration (M)	1.0×10^{-5}–6.0×10^{-5}	2.0×10^{-5}
pH	6.0–9.0	7.5
Buffer concentration (M)	0.05–0.3	0.1

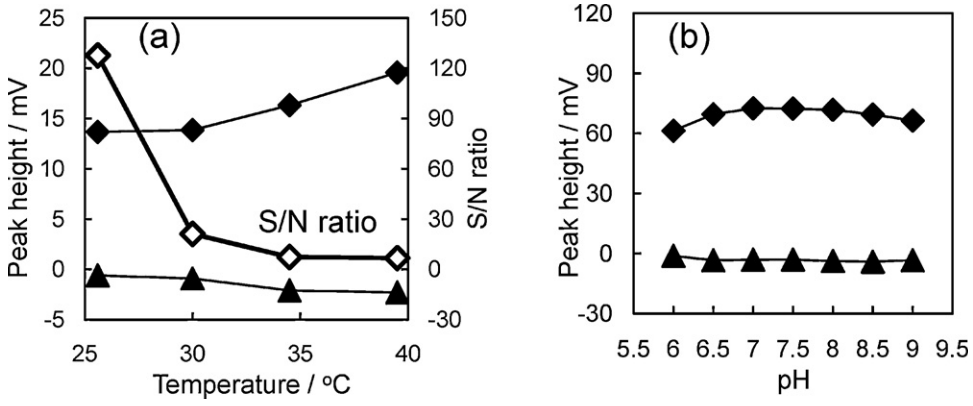


Fig. 2. Effect of temperature (a) and pH (b) ◇:S/N ratio;◆:Peak height ([F^-] = 1.0×10^{-5} M);▲:Blank.

Table 3
Tolerable concentrations of foreign ions on the determination of 5.0×10^{-6} M fluoride ion.

Foreign ions	Added as	Concentration (M)	Molar ratio to fluoride ion	Relative error (%)
Na^+	NaCl	1×10^{-2}	2000	−0.3
K^+	KCl	1×10^{-2}	2000	−3.2
Cl^-	NaCl	1×10^{-2}	2000	−0.3
Br^-	NaBr	1×10^{-2}	2000	−4.6
I^-	KI	1×10^{-2}	2000	−2.7
$NO_3{}^-$	$NaNO_3$	5×10^{-3}	1000	+0.5
$SiO_3{}^{2-}$	Na_2SiO_3	1×10^{-3}	200	+3.2
$PO_4{}^{3-}$	$Na_3PO_4 \cdot 12H_2O$	1×10^{-3}	200	+0.5
Mg^{2+}	$MgSO_4$	5×10^{-4}	100	−3.4
$SO_4{}^{2-}$	Na_2SO_4	5×10^{-4}	100	+2.0
Ca^{2+}	$CaCl_2 \cdot 12H_2O$	2×10^{-4}	40	−5.0
Al^{3+}	$Al(NO_3)_3 \cdot 9H_2O$	1×10^{-4}	20	+4.2
Fe^{3+}	$FeNH_4(SO_4)_2 \cdot 12H_2O$	1×10^{-4}	20	+4.5
$NH_4{}^+$	NH_4Cl	1×10^{-4}	20	−3.2

temperature raise the peak height increased by 1.5 times, but the baseline noise also increased by the mixed solvent. As a result from S/N ratio, 25 °C (room temperature) was chosen.

The effect of pH of BS was examined from 6.0 to 9.0 as shown in Fig. 2(b). The highest peak was obtained at pH 7.0–8.0: at lower pH than 7.0, the reagent was gradually decompose by H^+ and at higher pH than 8, Si–O bond was attacked by OH^-. Considering S/N ratio, pH 7.5 was selected.

The effect of buffer concentration was studied in the range from 0.05 to 0.3 M. The buffer concentration did not affect on the sensitivity. In consideration of the buffer capacity, 0.1 M was chosen.

The sample injection volumes of 100, 200, 300, 400 and 500 μL were examined by changing the length of the sample loop. The larger volumes were preferable to obtain higher peak height, and the volumes above 400 μL gave only a small increase: the sample volume of 400 μL was selected as a compromise of the sensitivity and the dispersion of the sample zone.

The optimum conditions were summarized in Table 2.

3.4. Calibration graph and analytical characteristics

Under the optimum conditions, the calibration graph was linear over the range of 1.0–50.0 × 10^{-6} M. The equation of the calibration graph is $Y = 7.98X - 5.05$ and correlation coefficient is $r = 0.9999$, where Y and X are peak height in mV and fluoride ion concentration in 10^{-6} M, respectively. The determinable limit was 1.0×10^{-6} M. The relative standard deviation (RSD) of 12 measurements with 1.0×10^{-5} M fluoride ion was 1.0%. Fig. 3 shows typical flow signals for a calibration graph and RSD. Sample throughput was 13 h^{-1}.

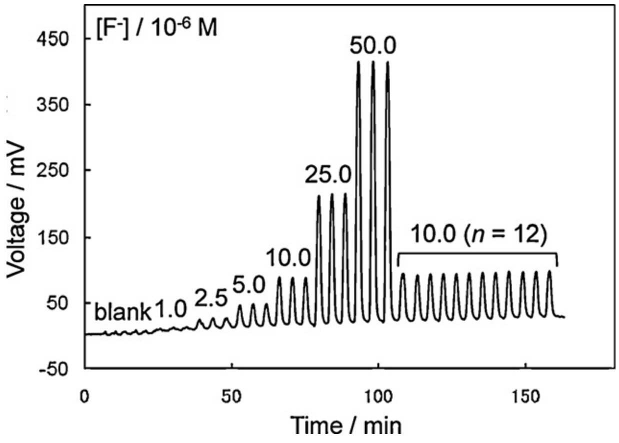


Fig. 3. Flow signals for calibration graph and reproducibility test.

Table 4
Analytical results for the determination of fluoride ion in water samples.

Samples	F^- added/10^{-6} M	F^- found/10^{-6} M	Recovery (%)
Tap water	0	7.7 ± 0.1	–
	5.0	12.7 ± 0.2	100.0
Asahi River	0	8.4 ± 0.2	–
	5.0	13.3 ± 0.2	98.8
Zasu River	0	8.6 ± 0.1	–
	5.0	13.4 ± 0.2	97.5

Means ($n = 5$) ± σ.
Sampling date: 2nd September, 2009; sampling place: Okayama-shi, Japan.

3.5. Effect of coexisting ions

For application to water samples, the effect of foreign ions was examined with 5.0×10^{-6} M fluoride ion solution. The tolerable concentrations of typical diverse ions within ±5% error were summarized in Table 3. Most of ions at usual concentration level in environmental waters showed little interferences, so that the proposed method can be applied without any pretreatments except high salinity water sample.

3.6. Analytical applications to water samples

The proposed method was applied to tap and river water samples: the sample solution was filtered with 0.45 μm pore size membrane filter when sampling and with 0.2 μm pore size membrane filter before injection. The analytical results with recovery test obtained were shown in Table 4. The results showed good recoveries from 97.5% to 100%. Fluoride ion concentrations in practical samples could be determined at around 8×10^{-6} M with enough sensitivity and were satisfied Japanese water quality standard (0.8 mg L^{-1}, 4×10^{-5} M).

4. Conclusions

A fluorescence detection/FIA with a novel reagent, FCl_2TBS, for the determination of fluoride ion was developed. The synthesis of the reagent was simple, the detection reaction was specific for fluoride ion contained in aqueous media and the procedure was automated. The proposed method had a good reproducibility, accuracy and sensitivity, and could be directly applied to the determination of fluoride ion in natural water samples.

Acknowledgement

We appreciate to our colleagues working at organic chemistry department for helpful advice to synthesis and measurements by NMR and MS.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.03.081

References

[1] Japan water works association, Standard Methods for Examination of Water (2001).
[2] The Impact of Fluoride on Health J. Am. Diet. Assoc. 105 (2005) 1620-1628.
[3] J.R. Santos, R.A.S. Lapa, J.L.F.C. Lima, Anal. Chim. Acta 583 (2007) 429.
[4] V. Capka, C.P. Bowers, J.N. Narvesen, R.F. Rossi, Talanta 64 (2004) 869.
[5] H. Hara, K. Yabuuchi, M. Higashida, M. Ogawa, Anal. Chim. Acta 364 (1998) 117.
[6] M.T.S.D. Vasconcelos, C.A.R. Gomes, A.A.S.C. Machado, J. Chromatogr. A 685 (1994) 53.
[7] S. Thangavel, K. Dash, S.M. Dhavile, S.C. Chaurasia, T. Mukherjee, J. Chromatogr. A 1074 (2005) 229.
[8] E.N. Kapinus, I.A. Revelsky, V.O. Ulogov, Y.A. Lyalikov, J. Chromatogr. B 800 (2004) 321.
[9] X. Gao, H. Zheng, G. Shang, J.G. Xu, Talanta 73 (2007) 770.
[10] O.A. Zaporozhets, L.Y. Tsyukalo, Anal. Chim. Acta 597 (2007) 171.
[11] M.E. Khalifa, M.A.H. Hafez, Talanta 47 (1998) 547.
[12] H. Matsunaga, C. Kanno, H. Yamada, Y. Takahashi, T.M. Suzuki, Talanta 68 (2006) 1000.
[13] R.S. Sathish, M.R. Kumar, G.N. Rao, K.A. Kumar, C. Janardhana, Spectrochim. Acta A 66 (2007) 457.
[14] R.S. Sathish, U. Sujith, G.N. Rao, C. Janardhana, Spectrochim. Acta A 65 (2006) 565.
[15] R. Bodor, V. Madajova, D. Kaniansky, M. Masar, M. Johnck, B. Stanislawski, J. Chromatogr. A 916 (2001) 155.
[16] D. Kaniansky, I. Zelensky, A. Hybenova, F.I. Onuska, Anal. Chem. 66 (1994) 4258.
[17] T. Meissner, F. Eisenbeiss, B. Jastorff, J. Chromatogr. A 829 (1998) 351.
[18] A.B. Descalzo, D. Jimenez, J.E. Haskouri, D. Beltran, P. Amoros, M.D. Marcos, R. Martinez-Manez, J. Soto, Chem. Commun. (2002) 562.
[19] C.Q. Zhu, J.L. Chen, H. Zheng, Y.Q. Wu, J.G. Xu, Anal. Chim. Acta 539 (2005) 311.
[20] S.Y. Kim, J.I. Hong, Org. Lett. 9 (2007) 3109.
[21] X.F. Yang, S.J. Ye, Q. Bai, X.Q. Wang, J. Fluoresc. 17 (2007) 81.
[22] M.A. Matveets, S.D. Akhmetova, J. Appl. Spectrosc. (1991) 1160.