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Analytical survey

Chemiluminescence in drug assay

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Abstract

The phenomenon of chemiluminescence allows the development of simple analytical procedures using low cost instrumentation which can be easily controlled by computers. The sensitivity of the methods is excellent, and selectivity is improved by high-performance liquid chromatographic separation. A broad area of chemiluminometric applications is drug analysis, which is reviewed in this paper.

Keywords: Chemiluminescence; Drug assay

1. Introduction

Chemiluminescence (CL) is defined as the production of electromagnetic radiation (ultraviolet, visible or infrared) by a chemical reaction [1]. The phenomenon can be observed when a chemical reaction yields an electronically excited intermediate or product which either luminesces or donates its energy to another molecule which then luminesces. If radiation is emitted by energy-transfer, then the process is usually called chemi-excitation. When the chemiluminogenic reaction is enzymatic and/or occurs within a living organism, such as the firefly, the phenomenon is called bioluminescence (BL).

The sufficiently low limits of detection, the excellent sensitivity and the versatility of the chemiluminometric methods of analysis are the main reasons for the recent surge of interest in CL and BL. Historically, the first observation of CL was made in 1877 by Radziszewski, but the introduction of flow injection analysis and liquid chromatography with chemiluminometric detectors was responsible for the plethora of original research work devoted to this area of chemical analysis.

2. Mechanism of chemiluminescence

A chemical reaction may generate radiation by direct CL or by chemi-excitation.

2.1. Direct chemiluminescence

A direct chemiluminogenic reaction takes place in two steps, which can be simplified as follows:

excitation reaction: $A + B \rightarrow C^*$

de-excitation reaction: $C^* \rightarrow C + h\nu$

The excited molecule C* can be either the final or an intermediate product of the reaction. The total efficiency, Φ_{CL}, of a chemiluminogenic reaction lies in the range 1–20% but very often can be much less than 1%, while bioluminogenic reactions may have efficiencies of up to 100%.

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2.2. Chemi-excitation

A great variety of applications is based on chemiluminogenic reactions which take place by chemi-excitation. In this case, the energy of the excited state molecule, C*, is transferred to a compound, F, which is subsequently excited via an energy transfer process:

energy transfer step: $C^* + F \rightarrow C + F^*$

de-excitation reaction: $F^* \rightarrow F + h\nu$

The molecule F is a fluorophore which can also be excited by absorption of radiation (photoluminescence).

If a molecule does not have the ability to participate in a chemiluminogenic reaction, it may still be converted into another molecule which does have this property. Alternatively, the molecule can be converted into a fluorophore by derivatization. The fluorophore can then be chemi-excited.

The general requirements for CL [2] are as follows.

(1) the molecule C should be capable of receiving the energy released from the reaction to form C* and the efficiency of this process should be sufficiently high.

(2) C* should be capable of luminescing under the conditions of the reaction and the intensity of the radiation should be sufficiently high. Alternatively, a suitable acceptor molecule, F, capable of accepting energy should be available for chemi-excitation and subsequently emission of radiation.

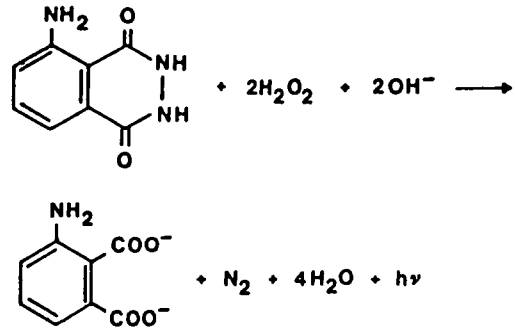
(3) the energy required for excitation must be supplied by the reaction in one step, if possible. In a multi-step reaction, the necessary energy must be released in a single step since the excitation step should occur instantaneously.

The limiting factor for the occurrence of CL is that the energy required for luminescence in the visible region lies between 44 and 71 kcal mol^{-1}. Hence, a minimum requirement for CL is that the reaction produces 44 kcal mol^{-1} of energy [3]. A variety of organic compounds meet this requirement and, in some instances, their chemiluminogenic properties have been thoroughly studied during redox reactions [4]. CL can be employed analytically using batch or flow chemiluminometers. Flow chemiluminometers can operate either by continuous flow or flow injection of the analyte, and various simple modifications allow the use of the design as a detector for HPLC.

3. Direct CL reactions

3.1. Luminol and derivatives

The most well-known chemiluminescent reaction in the liquid phase is the oxidation of luminol (5-amino-2,3-phthalazinedione) by hydrogen peroxide in alkaline media [5]:



The emission spectrum coincides with the fluorescent spectrum of the dianion of 3-aminophthalic acid, which is the CL reaction product [6]. This reaction may be used for the determination of all compounds or ions which catalyze the reaction or alter the action of catalysts or produce hydrogen peroxide during a reaction. Luminol derivatives, such as *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI), are very often used as labels to convert non-chemiluminogenic compounds into compounds which can generate CL by the action of hydrogen peroxide. Table 1 summarizes typical applications of the reactions of luminol and its derivatives in drug analysis.

3.2. Other redox reactions

A wide variety of chemiluminescent reactions of compounds of pharmaceutical interest giving very good results in the analysis of real samples have been discovered and studied thoroughly. Table 2 summarizes some illustrative applications of direct CL reactions in drug analysis. Direct CL reactions are not widely used for detection after liquid chromatographic separation as shown in Table 3.

4. Energy transfer reactions

4.1. Chemi-excitation by the reaction of oxalate esters with hydrogen peroxide

The reaction of oxalate esters with hydrogen peroxide is the common reaction for the chemi-

Table 1
Tyical applications of CL by luminol and derivatives in drug assay

Analyte	Procedure	Detection limit	Reference
Acetylcholine, choline	Hydrolysis to choline (acetylcholinesterase) + choline oxidase to H_2O_2, luminometer	1×10^{-9} mol (choline)	[24]
Amino acids	Suppression of Co(II)–luminol–H_2O_2 reaction, HPLC	4×10^{-12} mol (histidine)	[25]
α-Amylase	Peroxidase-catalyzed luminol–H_2O_2–*p*-iodophenol, luminometer	1×10^{-8} M	[26]
Ascorbic acid	Suppression of emission from the luminol–H_2O_2 peroxidase reaction, stopped flow	1×10^{-7} M	[27]
Ascrobic acid	Suppression of the Fe(III)–luminol-H_2O_2 reaction, FIA	1×10^{-12} M	[28]
Ascrobic acid, uric acid	Competitive reaction, CL-delay technique	1×10^{-6} M	[29]
Bile acids	ABEI derivatives, HPLC	2×10^{-14} mol	[30]
Biotin	Isoluminol derivative, luminometer	5×10^{-8} M	[31]
Cannabinoids	CL enzyme immunoassay, luminol–H_2O_2, luminometer	1×10^{-7} g l^{-1}	[32]
Captopril	Complexation of Cu(II) and reduction of CL from luminol–H_2O_2 reaction, luminometer	1×10^{-6} M	[33]
Cephalothin	Enhancement of CL from Co(II)–luminol–H_2O_2 reaction, luminometer	1×10^{-6} M	[34]
Cholesterol	Addition of cholesterol oxidase to H_2O_2, FIA	5×10^{-6} M	[35]
Cyanocobalamin (Vit. B_{12})	Catalysis of luminol–H_2O_2, FIA	7×10^{-4} mol	[36]
Estriol	Aminopentylethylisoluminol, CL immunoassay	3×10^{-10} M	[37]
Methamphetamine	ABEI derivatives, HPLC	2×10^{-8} M	[38]
Oxazepam	ABEI derivative, HPLC	3×10^{-15} mol	[39]
Penicillamine	Suppression of the Cu(II)–luminol–H_2O_2 reaction, FIA	1×10^{-4} M	[40]
Penicillins	Inhibition of luminol–iodine reaction, stopped flow (amoxycillin)	3×10^{-7} M [41]	
Protein	Suppression of the Cu(II)–luminol–H_2O_2 reaction, FIA	4×10^{-8} g	[42]
Promethazine	Inhibition of luminol–H_2O_2–Cr(III) reaction, FIA	3×10^{-9} M	[43]
Riboflavin	Photoreduction with EDTA monitored by the luminol–H_2O_2 reaction, FIA	1×10^{-7} M	[44]
Tartaric acid	Oxidation with periodate then measured by luminol–Mn(II) reaction, stopped flow	1.5×10^{-6} M	[45]
Thyroxine	Isoluminol derivative-microperoxidase–H_2O_2, CL immunoassay	6×10^{-9} M	[46]

excitation of fluorophores. The chemical system is widely known as the peroxyoxalate chemiluminescence system. The esters are mostly insoluble and rather unstable in aqueous solutions, and must be used in organic solvents. This property makes them well-suited to liquid chromatographic applications. Problems may occur, however, when using the original hydrophobic esters in combination with the mainly hydrophylic reversed-phase eluents, since ester precipitation may occur at various pump flows. The effects of various organic solvents and other experimental parameters have been extensively studied [7]. Commonly used esters are bis(2,4,6-trichlorophenyl) oxalate (TCPO) and bis(2,4-dinitrophenyl) oxalate (DNPO) (Table 4). Derivatization to give fluorophoric compounds is very often used for peroxyoxalate chemiluminometric detection (Table 5).

4.2. Chemi-excitation from sulphite oxidation

Excited sulphur dioxide has been proposed as the emitting species during the oxidation of sulphite by potassium permanganate [8] and cerium (IV) [9]. The mechanism proposed by Meixner and Jaeschke [10] for the CL reaction with permanganate is

$$HSO_3^- + MnO_4^- \rightarrow HSO_3 + MnO_4^{2-}$$

$$2\,HSO_3 \rightarrow S_2O_6^{2-} + 2\,H^+$$

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2^*$$

Table 2
Applications of redox CL reactions in drug analysis

Analyte	Oxidant system	Detection limit	Reference
Adrenaline	H_2O_2–OH^-	6×10^{-7} M	[47]
Adrenaline	Fe^{2+}–H_2O_2	3×10^{-8} M	[48]
Amiloride, streptomycin	*N*-Bromosuccinimide–OH^-	7×10^{-7} M (amiloride)	[49]
Anaesthetics	$KMnO_4$–H^+	30 ng ml^{-1} (benzocaine)	[50]
Ascorbic acid	Lucigenin–OH^-	1×10^{-6} M	[51]
Ascorbic acid	Action of ascorbate oxidase, then lucigenin (pH 5.7)	6×10^{-7} M	[52]
Bilirubin	NaClO–OH^-	0.05 μg ml^{-1}	[53]
Buprenorphine	$KMnO_4$–H^+	1×10^{-8} M	[54]
Captopril	Ce(IV)–H^+ sensitized by rhodamine-B	3.7×10^{-8} M	[55]
Catecholamines	Lucigenin–cetyltrimethylammonium hydroxide	1×10^{-8} M (adrenaline)	[56]
Catecholamines	$KMnO_4$–H^+	1×10^{-7} M (adrenaline)	[57]
Isoniazid	N-Bromosuccinimide–OH^-	1×10^{-7} M	[58]
Heparin	Lucigenin–OH^-	90 μg	[59]
Loprazolam	$KMnO_4$–H^+	7×10^{-6} M	[60]
Morphine	$KMnO_4$–H^+	1×10^{-10} M	[61]
Morphine	$KMnO_4$–H^+	5×10^{-8} M	[62]
Paracetamol	Ce(IV)–H^+	4×10^{-7} M	[63]
Rifampicin, rifamycin SV	*N*-Bromosuccinimide–OH^-	7×10^{-9} M (rifamycin SV)	[64]
Tetracycline	Br_2–H^+–dibromodimethylhydantoin	4×10^{-5} M	[65]
Tetracycline	Dibromodimethylhydantoin after oxidation by $S_2O_8^{2-}$	8×10^{-7} M	[66]
Tetracyclines	$[Fe(CN)_6]^{3-}$–OH^-	9×10^{-8} M (tetracycline)	[67]
Tetracyclines	*N*-Bromosuccinimide–OH^-	1×10^{-8} M (tetracycline)	[68]
Tetracyclines	$H_2O_2 + S_2O_8^{2-}$ (co-oxidant) catalyzed by Cu(II)	2×10^{-9} M (chlortetracycline)	[69]
Tetrahydroaldosterone	Quinoxaline derivative + H_2O_2 [a]	6×10^{-10} M	[70]
Thiamine (Vit. B_1)	$[Fe(CN)_6]^{3-}$–OH^-	9×10^{-6} M	[71]
Thiazide compounds [b]	Tris(2,2′-bipyridine)–Ru(III)	6×10^{-14} mol (hydroflumethiazide)	[72]
Urea	NaBrO–OH^-	9×10^{-8} M	[73]

[a] The analyte is converted to the corresponding glyoxal compound by oxidation with copper(II) acetate which then is derivatized with 4,5-diaminophthalhydrazide into the chemiluminescent quinoxaline derivative.
[b] Hydroflumenthiazide, hydrochlorothiazide, cyclothiazide, chlorothiazide, trichlormethiazide.

$$SO_2^* \rightarrow SO_2 + h\nu$$

Thus, sulphite acts as the reductant and the energy released from the chemical reaction chemi-excites sulphur dioxide, which emits radiation in the range 310–370 nm [11].

The oxidation of sulphite by Ce(IV) has been used for the measurement of many compounds of pharmaceutical interest, as shown in Table 6.

5. Immobilization in chemiluminescence

The rapid development of immobilization techniques has improved tremendously the area of applications in chemiluminometry, especially with FIA and liquid chromatographic systems.

The immobilization of enzymes can be used in CL in one of the following approaches.

(1) Immobilization of luciferase in a flow cell in front of the PMT, so that light is emitted as the substrates flow over the enzyme [12]. The technique is very sensitive and selective. Some typical applications are presented in Table 7.

(2) Immobilization of the enzyme within the following stream in order to generate a product which participates in a chemiluminogenic reaction. The most well-known example is the action of enzymes on various analytes to generate hydrogen peroxide, which then is determined by the luminol reaction. Other reagents which can

Table 3
Direct CL redox reactions for detection after HPLC separation

Analyte	Chemiluminogenic reactant	Detection limit	Reference
Corticosteroids	Lucigenin in Triton + OH	5×10^{-11} mol	[74]
Amino acids, etc. [a]	Suppression of the Cu(II)–luminol–H_2O_2 reaction	1×10^{-12} mol	[75]
Ascorbic acid	Lucigenin–OH^-	2×10^{-6} M	[76]
Bile acids	Post column generation of NADH–isoluminol–microperoxidase	2×10^{-12} mol	[77]
Corticosteroids	Lucigenin–OH^-	5×10^{-13} mol	[78]
Methamphetamine	ABEI derivative, $[Fe(CN)_6]^{3-}$–OH^-–H_2O_2	2×10^{-11} M	[79]
Morphine	$KMnO_4 - H^+$	4×10^{-8} M	[80]

[a] Catecholamines, streptomycin, gentamicin.

Table 4
Applications of peroxyoxalate CL detection without prior derivatization

Analyte	Peroxyoxalate [a], mode	Detection limit	Reference
Ascorbic acid, etc. [b]	Formation of H_2O_2 + TCPO, HPLC	1×10^{-6} M	[81]
Bilirubin	TCPO, luminometer	8×10^{-9} M	[82]
Dansyl-alanine	TCPO, HPLC	5×10^{-14} mol	[83]
	DNPO, FIA	5×10^{-15} mol	[84]
Dansyl amino acids	TCPO, TLC	7×10^{-9} g DNS-Gly	[85]
	TCPO, HPLC	1×10^{-12} mol	[86]
	TCPO, DNPO, HPLC	2×10^{-13} mol	[87]
	TCPO, HPLC	2×10^{-15} mol	[88]
	TCPO, HPLC	2×10^{-16} mol	[89]
Dipyridamole, benzydamine	TDPO and/or DNPO, HPLC	3×10^{-10} M (dipyridamole)	[90,91]
Thiourea	Quenched CL, DNPO, HPLC	1×0^{-11} mol	[92]

[a] TCPO: bis(2,4,6-trichlorophenyl)oxalate. DNPO: bis(2,4-dinitrophenyl)oxalate. TDPO: bis[4-nitro-2-(3,6,9-trioxadecylcarbonyl)phenyl]oxalate.
[b] Catecholamines, caffeine, theophylline.

Table 5
Derivatization for peroxyoxalate CL detection

Anlayte	Label	PO [a]	Mode	Detection limit	Reference
Amines	Luminarine I	TCPO	HPLC	1×10^{-14} mol (tyramine)	[93]
Bile acids steroids	Dansyl hydrazine	TDPO	HPLC	3×10^{-15}–8×10^{-15} mol	[94]
Bradykinin	Dansyl-Cl	TCPO	HPLC	1×10^{-12} mol	[95]
Catecholamines	Ethylenediamine	TDPO	HPLC	1×10^{-15} mol	[96]
Corticosteroids	Dansyl hydrazine	TDPO	HPLC	1×10^{-10} M (testosterone)	[97]
Estradiol	Dansyl-Cl	TCPO	HPLC	1×10^{-13} mol	[98]
Fluocortin butyl	Dansyl hydrazine	TCPO	LC	2×10^{-13} M	[99]
Fluoropyrimidine compounds	DCIA [b]	TCPO	HPLC	2×10^{-14} mol	[100]
Methamphetamine	DBD-F [c]	TDPO	HPLC	3×10^{-14} mol	[101]
Metoprolol	DBD-F	TDPO	HPLC	1×10^{-15} mol	[102]
Mexiletine	Dansyl-Cl	TDPO	HPLC	1×10^{-15} mol	[103]
Thyroxine, tri-iodothyronine	Fluorescamine	TCPO	FIA	1×10^{-11} M	[104]

[a] PO: peroxyoxalate ester.
[b] DCIA: 7-(diethylamino)-3[4-iodoacetyl)amino)phenyl]-4-methylcoumarin.
[c] DBD-F: 4(*N*,*N*-dimethylaminosulphonyl)-7-fluoro-2,1,3-benzoxadiazole.

Table 6
Chemiluminogenic reactions by energy-transfer from the oxidation of sulphite

Analyte	Oxidant + SO_3^{2-}	Detection limit (M)	Reference
Bile acids	Ce(IV) or $KMnO_4$ or $K_2Cr_2O_7$ or $KBrO_3$–H^+	1×10^{-6} (cholic acid)	[105]
Corticosteroids	Ce(IV)–H^+	1×10^{-7} (cortisone)	[106]
Cyclamate	Ce(IV)–H^+	2×10^{-6}	[107]
Quinine, quinidine	Ce(IV)–H^+	2×10^{-6} (quinidine)	[108]
Steroids	BrO_3^-–H^+	8×10^{-7} (cortisone)	[109]
Steroids	Ce(IV)–H^+ + organic solvents	3×10^{-8} (cortisone)	[110]

Table 7
Applications of immobilized enzymes and reagents in flow injection chemiluminometry and bioluminometry

Analyte	Immobilized enzyme or reagent	Detection limit	Reference
ATP	Firefly luciferase	1×10^{-14} mol	[111]
Creatine kinase	Firefly luciferase	0.1 $U\,l^{-1}$	[112]
Ethanol	Bacterial luciferase – oxireductase	3×10^{-11} mol	[113]
Hydroxy bile acids	Bacterial luciferase – oxireductase	1×10^{-13} mol	[114]
Lactate dehydrogenase, L-lactate	Bacterial luciferase	1×10^{-6} M	[115]
NADH	Bacterial luciferase – oxireductase	2×10^{-12} mol	[116]
NADH, ATP	Firefly + bacterial luciferase	2.5×10^{-12} mol ATP	[117]
Acetylcholine, choline	Acetylcholine esterase	1×10^{-12} mol	[118]
Hydrogen peroxide	3-Aminofluoranthrene [a]	1×10^{-8} M	[119]
Hydrogen peroxide	Luminol	1×10^{-10} mol	[120]
Hydrogen peroxide	TCPO	6×10^{-9} M	[121]
Glucose [b]	TCPO + 3-aminofluoranthrene	8×10^{-7} M	[122]

[a] Fluorophore.
[b] Via glucose oxidase to H_2O_2.

be immobilized within flowing systems for chemiluminometric measurement are presented in Table 7.

6. Recent developments in chemiluminescence

6.1. Biosensors

All applications of immobilized reagents in CL can easily be adapted in biosensing systems. This area of applications increased tremendously after the introduction of fibre-optics in analytical research. Research is focussed on the immobilization of enzymes on the fibre, since immobilization of chemiluminogenic reagents, such as luminol, would cause functioning problems owing to the fast consumption of the reagent.

Freeman and Seitz [13] developed a fibre-optic biosensor by immobilizing peroxidase in polyacrylamide gel and inserting the end of the fibre-optic into the enzyme phase. When the probe was immersed in a solution of hydrogen peroxide in the presence of luminol, CL was generated as peroxide diffused into the peroxidase phase. The radiation emitted from the enzymatically catalyzed luminol–hydrogen peroxide reaction was transmitted through the fibre-optic to the PMT.

A biophotodiode has also been reported for the measurement of hydrogen peroxide and glucose [14]. Horseradish peroxidase was entrapped in polyacrylamide gel and thin slices of the gel were attached to a silicon photodiode. The photocurrent was proportional to the concentration of hydrogen peroxide using luminol as the chemiluminogenic reagent. Co-immobilization of glucose oxidase with peroxidase allowed the use of the biosensor for the determination of glucose.

A fibre-optic biosensor for monitoring NADH has been constructed by co-immobilizing bacterial luciferase and flavin mononu-

cleotide oxidoreductase on collagen strips [15]. Enzyme immobilization on pre-activated membranes [16] and nylon membranes [17] has also been used by Blum and co-workers for the construction of chemiluminescent biosensors.

Fibre-optic biosensors for continuous flowing systems have been designed recently by the same group. A characteristic example involves the use of a flow-through cell which is continuously stirred. The enzymatic membrane is kept in close contact with the sensing tip of the fibre bundle by a screw-cap. The emitted light is transmitted to the photomultiplier through the optical fibres [18]. The design has been thoroughly studied [19] and can also be used in a batch device [20]. Immobilization of peroxidase on a membrane inside a flow-through cell has been evaluated for the flow injection analysis of glutamate and glutamine [21]. Hydrogen peroxide is generated from the analyte by the action of L-glutamate oxidase and glutaminase, and is measured by the action of luminol in the presence of peroxidase from the membrane sensor. Similarly, L-lysine has been measured in the range 10–1000 μM via the hydrogen peroxide generated by the action of lysine oxidase on the analyte [22].

6.2. *Capillary electrophoresis*

Research on chemiluminometric detection after capillary electrophoretic separation is progressing very fast, although difficulties in coupling the separation technique with the CL detector still exist [23]. Academic and industrial research laboratories are focusing on the design of suitable, safe and minimum-band broadening capillary devices for adding the CL reagents after the high-voltage driven separation. Micromachining techniques, as taken from computer hardware developments, are bound to miniaturize electrophoresis to square inch-channelled devices, i.e. to ultrafast droplet analysis.

7. Conclusions

CL is a powerful tool for drug analysis since its detection limits are extremely low and its instrumentation very simple and of low cost. In combination with derivatization techniques in order to increase sensitivity, it has a wide range of applications.

References

[1] K. Robards and P.J. Worsfold, Anal. Chim. Acta, 266 (1992) 147–173.
[2] G.B. Schuster and S.P. Schmidt, Adv. Phys. Org. Chem., 18 (1982) 187–238.
[3] L.J. Kricka and G.H.G. Thorpe, Analyst, 108 (1983) 1274–1296.
[4] F. McCapra, Prog. Org. Chem., 8 (1973) 231–277.
[5] F. McCapra and I. Behesti, in K. Van Dyke (Ed.), Bioluminescence and Chemiluminescence: Instruments and Applications, Vol. I, CRC Press, Boca Raton, FL, 1985, pp. 9–42.
[6] E.H. White and M.M. Bursey, J. Am. Chem. Soc., 86 (1964) 941–942.
[7] N. Imaizumi, K. Hayakawa, M. Miyazaki and K. Imai, Analyst, 114 (1989) 161–164.
[8] J. Stauff and W. Jaeschke, Atmos. Environ., 9 (1975) 1038–1039.
[9] K. Takeuchi and T. Ibusuki, Anal. Chim. Acta, 174 (1985) 359–363.
[10] F. Meixner and W. Jaeschke, Fresenius' Z. Anal. Chem., 317 (1984) 343–344.
[11] R.W.B. Pearse and A.G. Gaydon, The Identification of Molecular Spectra, 4th ed., Chapman and Hall, London, 1976.
[12] T.A. Nieman, Analytical Chemiluminescnece in Flowing Steams: FIA and HPLC, in Luminescence Techniques in Chemical and Biochemical Analysis, W.R.G. Baeynes, D. De Keukeleire and K. Korkidis (Eds.), Marcel Dekker, New York, 1991, pp. 523–565.
[13] T.M. Freeman and W.R. Seitz, Anal. Chem., 50 (1978) 1242–1246.
[14] M. Aizawa, Y. Ikariyama and H. Kuno, Anal. Lett., 17 (1984) 555–564.
[15] L.J. Blum and P.R. Coulet, Anal. Chim. Acta, 161 (1984) 355–358.
[16] L.J. Blum, J.M. Plaza and P.R. Coulet, Anal. Lett., 20 (1987) 317–326.
[17] L.J. Blum, S.M. Gautier and P.R. Coulet, Anal. Lett., 21 (1988) 717–726.
[18] L.J. Blum, S.M. Gautier and P.R. Coulet, Anal. Lett., 22 (1989) 2211–2222.
[19] S.M. Gautier, L.J. Blum and P.R. Coulet, Anal. Chim. Acta., 243 (1991) 149–156.
[20] S.M. Gautier, L.J. Blum and P.R. Coulet, Biosensors, 4 (1989) 181–194.
[21] G. Blankenstein, F. Preuschoff, U. Spohn, K.H. Mohr and M.R. Kula, Anal. Chim. Acta, 271 (1993) 231–237.
[22] F. Preuschoff, U. Spohn, E. Weber, K. Unverhau and K.H. Mohr, Anal. Chim. Acta, 280 (1993) 185–189.
[23] W.R.G. Baeyens, B. Lin Ling, K. Imai, A.C. Calokerinos and S.G. Schulman, J. Microcol. Sep., 6 (1994) 195–206.
[24] J.R. Wetherell and M.C. French, Biochem. Soc. Trans., 14 (1986) 1148–1149.
[25] A. MacDonald and T.A. Nieman, Anal. Chem., 57 (1985) 936–940.
[26] L.J. Kricka, J.M. Marcinkowski, P. Wilding and S. Lekhakula, Talanta, 37 (1990) 971–974.
[27] J. Min Kim, Y. Huang and R.D. Schmid, Anal. Lett., 23 (1990) 2273–2282.
[28] A.A. Alwarthan, Analyst, 118 (1993) 639–642.

[29] J.E. Frew and P. Jones, Anal. Lett., 18 (1985) 1579–1592.
[30] T. Kawasaki, M. Maeda and A. Tsuji, J. Chromatogr., 328 (1985) 121–126.
[31] H.R. Schroeder, P.O. Vogelhut, R.J. Carrico, R.C. Boguslaski and R.T. Buckler, Anal. Chem., 48 (1976) 1933–1937.
[32] J.D. Sharma, G.W. Aherne and V. Marks, Analyst, 114 (1989) 1279–1282.
[33] Z. Xinrong, W.R.G. Baeyens, G. Van Der Weken, A.C. Calokerinos and K. Nakashima, J. Pharm. Biomed. Anal., 13 (1995) 425–429.
[34] S.G. Schulman, J.H. Perrin, Guo Fan Yan and Shagxian Chen, Anal. Chim. Acta, 255 (1991) 383–385.
[35] N.L. Malavolti, D. Pilosof and T.A. Nieman, Anal. Chim. Acta, 170 (1985) 199–207.
[36] Y.K. Zhou, H. Li, Y. Liu and G.Y. Liang, Anal. Chim. Acta, 243 (1991) 127–130.
[37] J. de Boever, W. Ulrix, D. Vandekerckhove and F. Kohen, Anal. Chim. Acta, 205 (1988) 215–222.
[38] K. Nakashima, K. Suetsugu, K. Yoshida, K. Imai and S. Akiyama Anal. Sci., 7 (1991) 815–816.
[39] G. Zomer, R.H. van den Berg, E.H.J.M. Jansen and T. Roos, Anal. Chim. Acta, 205 (1988) 249–254.
[40] P. Vinas, I. Lopez Garcia and J.A. Martinez Gil, J. Pharm. Biomed Anal., 11 (1993) 15–20.
[41] S. Ventura, M. Silva and D. Perez-Bendito, Anal. Chim. Acta, 266 (1992) 301–307.
[42] T. Hara, M. Toriyama and K. Tsukagoshi, Bull. Chem. Soc. Jpn., 57 (1984) 1551–1555.
[43] A.A. Alwarthan, S.A. Al-Tamrah and A.A. Akel, Anal. Chim. Acta, 282 (1993) 169–174.
[44] T. Perez-Ruiz, C. Martinez-Lozano, A. Sanz and V. Thomas, Analyst, 119 (1994) 1825–1828.
[45] A. Gaikwad, M. Silva and D. Perez-Bendito, Analyst, 119 (1994) 1819–1824.
[46] D.A. Weerasekera, J.B. Kim G.J. Barnard and W.P. Collins, Ann, Clin. Biochem., 20 (1983) 100–104.
[47] T. Nakagama, M. Yamada and S. Suzuki, Anal. Chim. Acta, 217 (1989) 371–376.
[48] Y. Katsuoka, J. Hayashi, M. Yamada and T. Hobo, Bunseki Kagaku, 40 (1991) 525–529.
[49] S.M. Halvatzis, A.M. Mihalatos, L.P. Palilis and A.C. Calokerinos, Anal. Chim. Acta., 290 (1994) 172–178.
[50] X.R. Zhang, W.R.G. Baeyens, G. Van Der Weken, A.C. Calokerinos and K. Imai, Anal. Chim. Acta, 303 (1995) 137–142.
[51] R.L. Veazey and T.A. Nieman, Anal. Chem., 51 (1979) 2092–2096.
[52] K. Sato, Y. Chiba and S. Tanaka, Anal. Chim. Acta, 277 (1993) 61–65.
[53] L.P. Palilis, N. Grekas and A.C. Calokerinos, unpublished work, 1994.
[54] A.A. Al-Warthan and A. Townshend, Anal. Chim. Acta., 185 (1986) 329–333.
[55] X.R. Zhang, W.R.G. Baeyens, G. Van Der Weken, A.C. Calokerinos and K. Nakashima, Anal. Chim. Acta, 303 (1995) 121–125.
[56] T. Kamidate, K. Yoshida, T. Kaneyasu, T. Segawa and H. Watanabe, Anal. Sci., 6 (1990) 645–649.
[57] N.T. Deftereos, A.C. Calokerinos and C.E. Efstathiou, Analyst, 18 (1993) 627–632.
[58] S.A. Halvatzis, M.M. Timotheou-Potamia and A.C. Calokerinos, Analyst, 115 (1990) 1229–1234.
[59] R.A. Steen and T.A. Nieman, Anal. Chim. Acta, 155 (1983) 123–129.
[60] A.R.J. Andrews and A. Townshend, Anal. Chim. Acta, 227 (1989) 65–71.
[61] R.W. Abbott, A. Townshend and R. Gill, Analyst, 111 (1986) 635–640.
[62] N.W. Barnett and D.G. Rolfe, Anal. Chim. Acta, 282 (1993) 551–557.
[63] I.I. Koukli, A.C. Calokerinos and T.P. Hadjiioannou, Analyst, 114 (1989) 711–714.
[64] S.A. Halvatzis, M.M. Timotheou-Potamia and T.P. Hadjiioannou, Anal. Chim. Acta, 272 (1993) 251–263.
[65] A.A. Al-Warthan and A. Townshend, Anal. Chim. Acta, 205 (1988) 261–265.
[66] T. Owa, T. Masujima, H. Yoshida and H. Imai, Bunseki Kagaku, 33 (1984) 568–570.
[67] A.B. Syropoulos and A.C. Calokerinos, Anal. Chim. Acta, 255 (1991) 403–411.
[68] S.A. Halvatzis, M.M. Timotheou-Potamia and A.C. Calokerinos, Analyst, 118 (1993) 633–637.
[69] X.R. Zhang, W.R.G. Baeyens, A. Van Den Borre, G. Van Der Weken, A.C. Calokerinos and S.G. Schulman, Analyst, 120 (1995) 463–466.
[70] J. Ishida, S. Sonezaki, M. Yamaguchi and T. Yoshitake, Analyst, 117 (1992) 1719–1724.
[71] N. Grekas and A.C. Calokerinos, Talanta, 37 (1990) 1043–1048.
[72] J.A. Holeman and N.D. Danielson, Anal. Chim. Acta, 277 (1993) 55–60.
[73] X. Hu, N. Takenada, M. Kitano, H. Bandow, Y. Maeda and M. Hattori, Analyst, 119 (1994) 1829–1833.
[74] M. Takeda, M. Maeda and A. Tsuji, Biomed. Chromatogr., 4 (1990) 119–122.
[75] P.J. Koerner and T.A. Nieman, Mikrochim. Acta, II (1987) 79–90.
[76] R.L. Veazey and T.A. Nieman, J. Chromatogr., 200 (1980) 153–162.
[77] M. Maeda, S. Shimada and A. Tsuji, J. Chromatogr., 515 (1990) 329–335.
[78] M. Maeda and A. Tsuji, J. Chromatogr., 352 (1986) 213–220.
[79] K. Nakashima, K. Suetsugu, S. Akiyama and M. Yoshida, J. Chromatogr., 530 (1990) 154–159.
[80] R.W. Abbott, A. Townshend and R. Gill, Analyst, 112 (1987) 397–406.
[81] I. Aichinger G. Gubitz and J.W. Birks, J. Chromatogr., 523 (1990) 163–172.
[82] N. Wu, W.J. Horvath and C.W. Huie, Anal. Chim. Acta, 269 (1992) 99–107.
[83] S. Kobayashi and K. Imai, Anal. Chem., 52 (1980) 424–427.
[84] K. Honda, J. Sekino and K. Imai, Anal. Chem., 55 (1983) 940–943.
[85] T.G. Curtis and W.R. Seitz, J. Chromatogr., 134 (1977) 343–350.
[86] W. Baeyens, J. Bruggeman and B. Lin, Chromatographia, 27 (1989) 191–193.
[87] W. Baeyens, J. Bruggeman, C. Dewaele, B. Lin and K. Imai, J. Biolumin. Chemilumin., 5 (1990) 13–23.
[88] K. Miyaguchi, K. Honda and K. Imai, J. Chromatogr., 303 (1984) 173–176.
[89] K. Miyaguchi, K. Honda and K. Imai, J. Chromatogr., 316 (1984) 501–505.

[90] K. Imai, A. Nishitani, Y. Tsukamoto, Wei-Hong Wang, S. Kanda, K. Hayakawa and M. Miyazaki, Biomed. Chromatogr., 4 (1990) 100–104.
[91] A. Nishitani, Y. Tsukamoto, S. Kanda and K. Imai, Anal. Chim. Acta, 251 (1991) 247–253.
[92] P. Van Zoonen, H. Bock, C. Gooijer, N.H. Velthorst and R.W. Frei, Anal. Chim. Acta, 200 (1987) 131–141.
[93] H. Kouwatli, J. Chalom, M. Tod, R. Farinotti and G. Mahuzier, Anal. Chim. Acta, 266 (1992) 243–249.
[94] S. Higashidate, K. Hibi, M. Senda, S. Kanda and K. Imai, J. Chromatogr., 515 (1990) 577–584.
[95] K. Miyaguchi, K. Honda, T. Toyo'oka and K. Imai, J. Chromatogr., 352 (1986) 255–260.
[96] S. Higashidate and K. Imai, Analyst, 117 (1992) 1863–1868.
[97] K. Imai, S. Higashidate, A. Nishitani, Y. Tsukamoto, M. Ishibashi, J. Shoda and T. Osuga, Anal. Chim. Acta, 227 (1989) 21–27.
[98] O. Nozaki, Y. Ohba and K. Imai, Anal. Chim. Acta, 205 (1988) 255–260.
[99] T. Koziol, M.L. Grayeski and R. Weinberger, J. Chromatogr., 317 (1984) 355–366.
[100] S. Yoshida, K. Urakami, M. Kito, S. Takeshima and S. Hirose, J. Chromatogr., 530 (1990) 57–64.
[101] K. Nakashima, K. Suetsugu, K. Yoshida, S. Akiyama, S. Uzu and K. Imai, Biomed. Chromatogr., 6 (1992) 149–154.
[102] S. Uzu, K. Imai, K. Nakashima and S. Akiyama, Analyst, 116 (1991) 1353–1357.
[103] A. Nishitani, S. Kanda and K. Imai, Biomed. Chromatogr., 6 (1992) 124–127.
[104] V.K. Mahant, J.N. Miller and H. Thakrar, Anal. Chim. Acta, 145 (1983) 203–206.
[105] I.M. Psarelis, N.T. Deftereos, E.G. Sarantonis and A.C. Calokerinos, Anal Chim. Acta, 294 (1994) 27–34.
[106] I.I. Koukli and A.C. Calokerinos, Analyst, 115 (1990) 1553–1557.
[107] I.M. Psarellis, E.G. Sarantonis and A.C. Calokerinos, Anal. Chim. Acta, 272 (1993) 265–270.
[108] I.I. Koukli and A.C. Calokerinos, Anal. Chim. Acta, 236 (1990) 463–468.
[109] A.B. Syropoulos, E.G. Sarantonis and A.C. Calokerinos, Anal. Chim. Acta, 239 (1990) 195–202.
[110] N.T. Deftereos and A.C. Calokerinos, Anal. Chim. Acta, 290 (1994) 190–200.
[111] P.J. Worsfold and A. Nabi, Anal. Chim. Acta, 171 (1985) 333–336.
[112] S. Girotti, M.L. Cascione, S. Ghini, G. Carrea, R. Bovara, A. Roda, R. Motta and R. Petilino, Anal. Chim. Acta, 227 (1989) 29–36.
[113] A. Nabi and P.J. Worsfold, Analyst, 112 (1987) 531–533.
[114] K. Oda, S. Yoshida, S. Hirose and T. Takeda, Anal. Chim. Acta, 225 (1989) 273–282.
[115] A. Nabi and P.J. Worsfold, Anal. Proc., 24 (1987) 139–140.
[116] L.J. Blum, S.M. Gautier and P.R. Coulet, Anal. Clim. Acta, 226 (1989) 331–336.
[117] S.M. Gautier, L.J. Blum and P.R. Coulet, Anal. Chim. Acta, 235 (1990) 243–253.
[118] K. Honda, K. Miyaguchi, H. Nishino, H. Tanaka, T. Yao and K. Imai, Anal. Biochem., 153 (1986) 50–53.
[119] G. Gubitz, P. van Zoonen, C. Gooijer, N.H. Velthorst and R.W. Frei, Anal. Chem., 57 (1985) 2071–2074.
[120] K. Hool and T.A. Nieman, Anal. Chem., 60 (1988) 834–837.
[121] P. van Zoonen, D.A. Kamminga, C. Gooijer, N.H. Velthorst and R.W. Frei, Anal. Chim. Acta, 167 (1985) 249–256.
[122] P. van Zoonen, I. de Herder, C. Gooijer, N.H. Velthorst, R.W. Frei, E. Kuntzberg and G. Gubitz, Anal. Lett., 19 (1986) 1949–1961.