US005942189A

United States Patent [19]

Wolfbeis et al.

[11] **Patent Number:** 5,942,189

[45] **Date of Patent:** Aug. 24, 1999

[54] **LUMINESCENCE-OPTICAL METHOD AND SENSOR LAYER FOR QUANTITATIVE DETERMINATION OF AT LEAST ONE CHEMICAL COMPONENT OF A GASEOUS OR LIQUID SAMPLE**

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[21] Appl. No.: **08/950,373**

[22] Filed: **Oct. 14, 1997**

[30] **Foreign Application Priority Data**

Oct. 16, 1996 [AT] Austria 1818/96

[51] **Int. Cl.^{6}** **G01N 21/64**

[52] **U.S. Cl.** **422/82.08**; 422/82.07; 422/58

[58] **Field of Search** 422/82.08, 82.07, 422/58; 436/172

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,645,744	2/1987	Charlton et al. .	
4,762,799	8/1988	Seitz et al.	436/79
5,037,615	8/1991	Kane	422/82.08
5,232,858	8/1993	Wolfbeis et al.	436/77
5,565,328	10/1996	Bascomb et al.	435/25
5,631,169	5/1997	Lakowicz et al.	436/537
5,776,782	7/1998	Tsuji	436/171
5,849,489	12/1998	Heller	435/6

FOREIGN PATENT DOCUMENTS

393035	7/1991	Austria .
205870	12/1986	European Pat. Off. .
214768	3/1987	European Pat. Off. .
461392	12/1991	European Pat. Off. .
358991	2/1994	European Pat. Off. .

OTHER PUBLICATIONS

Jordan et al., Analytical Chemicstry 59, 437–439, 1987.

Yuan et al., Journal of Fluorescence, vol. 2, No. 4, pp. 231–235, 1992.

Silva et al., New J. Chemistry, 20, 871–880, 1996.

Stufkens et al., Coordination Chemistry Reviews, 177, 127–179, 1998.

Kosch et al., Analytical Chemistry, 70, 3892–3897, 1998.

Preininger, Journal of Fluorescence, vol. 8, No. 3, pp. 199–205, 1998.

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[57] **ABSTRACT**

In a sensor layer for quantitative determination of at least one chemical component in a gaseous or liquid sample medium containing a chromophore which is directly or indirectly responsive to the component being determined by changing its absorption spectrum, and a luminophore which is not responsive to the component being determined, where there is an at least partial overlap between the emission spectrum of the luminophore and the absorption spectrum of the chromophore, and where the energy transfer between luminophore and chromophore produces a measurable change in at least one luminescence characteristic of the luminophore, the luminophore L and the chromophore Γ are ionic substances with differing electrical charges, which are incorporated in a matrix material that is permeable to the chemical component being determined.
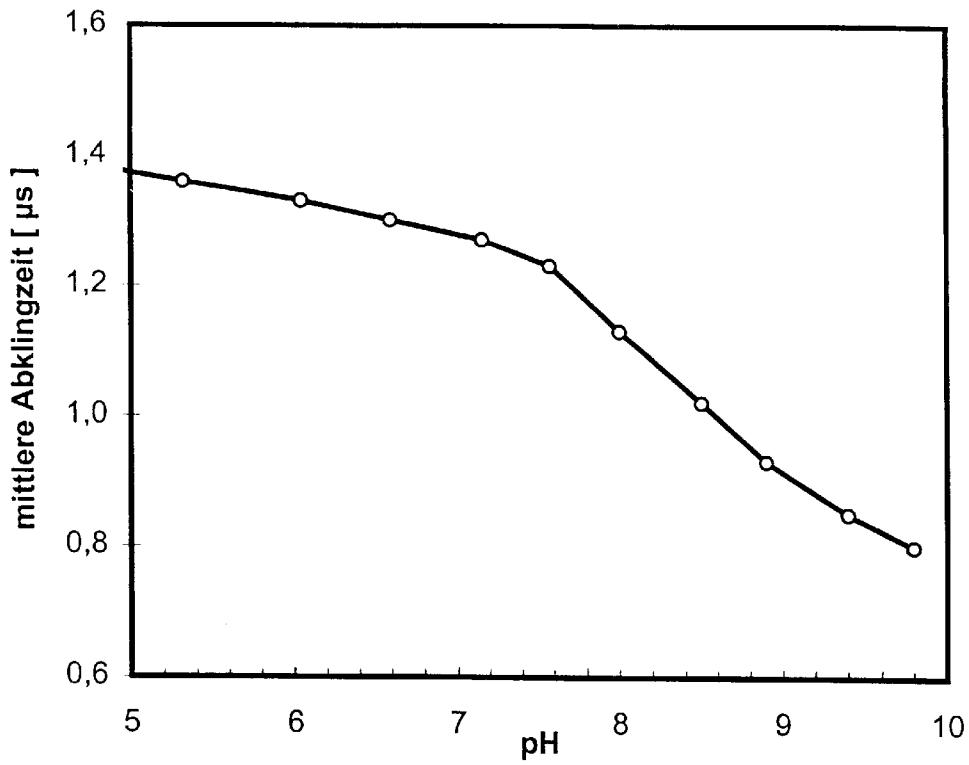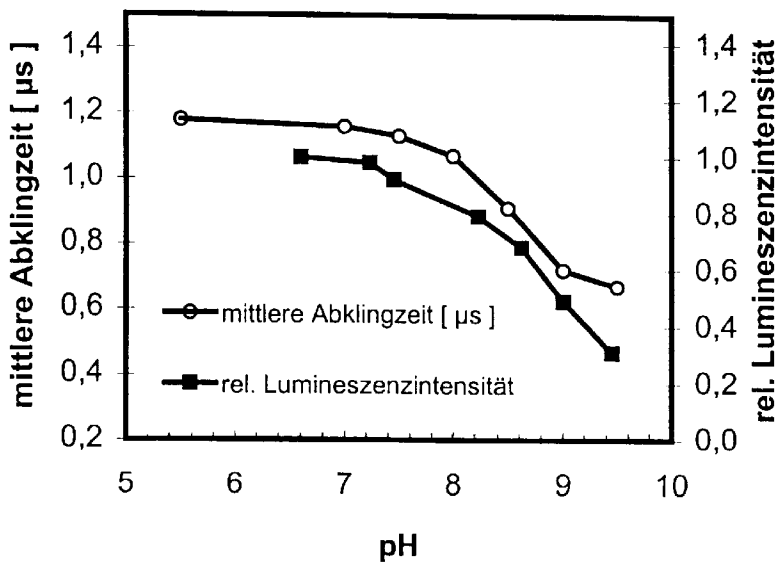
21 Claims, 2 Drawing Sheets

Fig.1

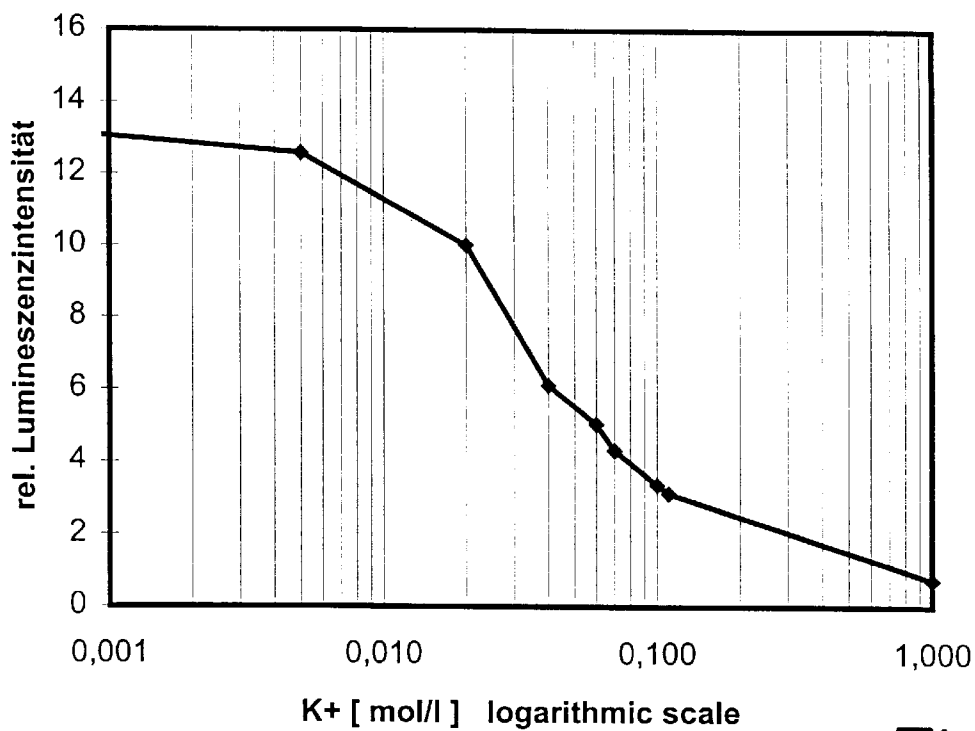

Fig. 2

K+ [mol/l]   logarithmic scale
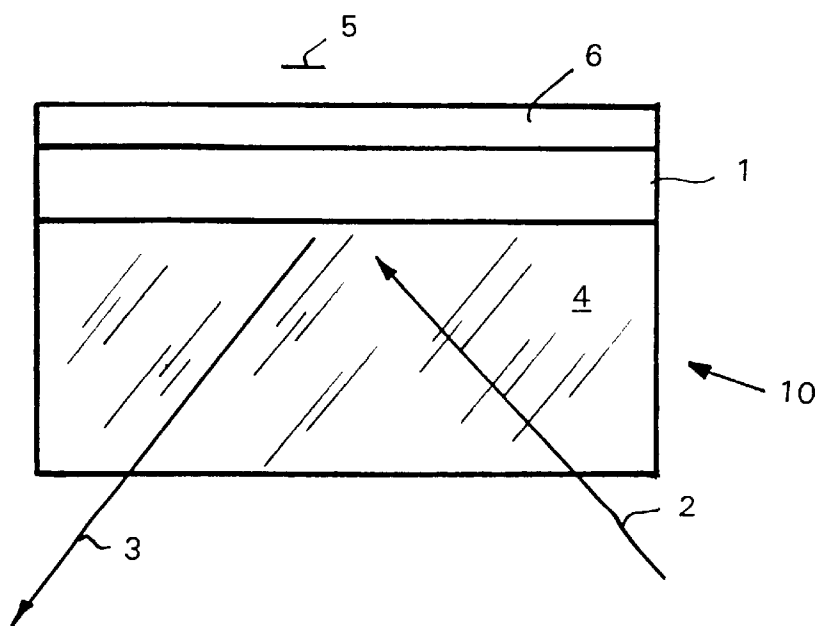
Fig. 3

Fig. 4

LUMINESCENCE-OPTICAL METHOD AND SENSOR LAYER FOR QUANTITATIVE DETERMINATION OF AT LEAST ONE CHEMICAL COMPONENT OF A GASEOUS OR LIQUID SAMPLE

BACKGROUND OF THE INVENTION

The invention relates to a sensor layer for quantitative determination of at least one chemical component of a gaseous or liquid sample containing a chromophore which is directly or indirectly responsive to the component being determined by changing its absorption spectrum and a luminophore which is not responsive to the component being determined, where there is an at least partial overlap of the emission spectrum of the luminophore and the absorption spectrum of the chromophore, and where the energy transfer between luminophore and chromophore effects a measurable change in at least one luminescence characteristic of the luminophore.

In the following luminophores are understood as dyes which emit phosphorescence or fluorescence radiation upon suitable excitation. The absorption spectrum of the chromophore is influenced either directly by the component being measured or indirectly by a chemical reaction product of the component being measured. The term "quantitative determination of a chemical component" refers to the determination of concentration and activity as well as gas partial pressure, the values of luminescence decay time or luminescence intensity of the luminophore being used to infer the measured quantity.

DESCRIPTION OF THE PRIOR ART

The above sensor layer is based on a method described in AT 393 035, in which pH- and cation-sensitive chromophores (acceptor) are attached, preferably covalently, to a luminophore (donor). The luminescence decay time of the luminophore is employed for determining the pH value and concentration of the cation being determined in the sample medium.

A similar method is known from EP-A 214 768, where the concentration of the parameter being determined is inferred from the luminescence intensity measured.

The rate of nonradiative energy transfer of donor and acceptor molecules depends on the spatial proximity of the molecules of the two substances. As a consequence, the macroscopically monitored values of the luminescence-optical parameters (luminescence quantum efficiency, luminescence decay time) will undergo a particularly efficient change if a substantial number of molecules of the two substances are brought into close contact with each other.

To obtain close spatial contact, AT 393 035 proposes a covalent bonding of donor and acceptor molecules. In EP 214 768 individual donor and acceptor molecules are covalently attached to the surface of a joint substrate, such as glass.

The main disadvantage of a covalent bond between donor and acceptor molecules is that considerable synthesis efforts are required to produce covalent bonds between desirable luminophores and suitable pH- or ion-sensitive chromophores.

Considerable efforts are further needed to covalently attach donor and acceptor molecules to the surface of a joint substrate, with the additional drawback of the negative effects boundary surface phenomena have on the quality of the measured results.

SUMMARY OF THE INVENTION

It is an object of the present invention to propose a sensor layer in which the chemical synthesis efforts needed to obtain the spatial proximity of a substantial number of donor and acceptor molecules are minimized and negative influences of boundary surface effects are avoided.

In the invention this object is achieved by providing that luminophore and chromophore be ionic substances of differing electrical charges, which are present as ion pairs in a matrix material that is permeable to the chemical component being determined.

It is provided in a first variant of the invention that the matrix material have an anionic chromophore and a cationic luminophore, where n chromophore molecules of the charge number −m and m luminophore molecules of the charge number +n form a n:m ionic pair, while it is provided in a second variant that the matrix material have a cationic chromophore and an anionic luminophore, where n chromophore molecules of the charge number +m and m luminophore molecules of the charge number −n form a n:m ionic pair.

As is seen in the following examples, it is much easier to produce ion pairs than complex molecules using multistep synthesis.

According to the invention, desirable luminophores (donor) are characterized by high luminescence quantum efficiency and long luminescence decay time (>100 ns). Preferred luminophores are cationic, metalorganic complexes of palladium, rhodium, platinum, ruthenium, osmium, rare earths (in particular, europium and lanthanum). The organic portion of these metalorganic complexes may consist of ligands from the group of porphyrins, bipyridyls, phenanthrolines or other heterocyclical compounds.

Preferred pH- and cation-sensitive chromophores (acceptor) are anionic substances whose light absorption will change upon direct or indirect chemical/physical interaction with the sample component to be determined, and whose absorption spectrum overlaps the emission spectrum of the luminophore, at least partially.

I) Determination of the pH value of a sample

State-of-the-art optical sensors for pH determination (cf M. J. P. Leiner and O. S. Wolfbeis, "Fiber Optic pH Sensors" in O. S. Wolfbeis, "Fiber Optic Chemical Sensors and Biosensors", CRC-Press, Boca Raton, 1991, Vol.I, Chapter 8) usually contain an absorption dye (chromophore) or fluorescent dye incorporated in an ion-permeable, preferably hydrophilic polymer matrix. In dependence of the pH value $(pH=-\log(aH+))$ of the sample medium a thermodynamic equilibrium is established between the protonated and deprotonated forms of the chromophore or fluorophore. From the concentration ratio of the two forms measured by optical methods the pH value of the sample medium may be obtained.

Reaction

$$\Gamma H \leftrightarrows \Gamma^- + H^+$$

ΓH is the protonated, and Γ^- the deprotonated form of the chromophore. H^+ denotes a proton.

In AT 393 035 referred to above, pH-sensitive chromophores are described, which are attached, preferably covalently, to a pH-insensitive luminophore (donor). From the luminescence decay time of the luminophore (L) the pH value of the test solution is obtained.

Reaction

$$L\Gamma H \leftrightarrows L\Gamma^- + H^+$$

For luminescence-optical pH-determination as proposed by the invention, n:m ion pairs $\{n(\Gamma H)^{-m}mL^{+n}\}$ consisting of an anionic, pH-sensitive chromophore (ΓH^{-m}) with the charge number −m and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number +n are used, which are incorporated in a hydrophilic polymer matrix.

Reaction

$$\{n(\Gamma H)^{-m}mL^{+n}\} \leftrightarrows \{n\Gamma^{-m}mL^{+n}\}^{-n} + nH^+$$
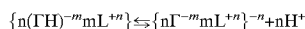
In the instance of low pH values (pH<<pKa of the chromophore) of the sample medium, the chromophore is present in fully protonated form. Due to the minimal spectral overlap of the absorption band of the deprotonated chromophore and the emission band of the luminophore the nonradiative energy transfer rate from luminophore to chromophore reaches a minimum. Correspondingly, the values of mean luminescence decay time and relative luminescence intensity of the luminophore reach a maximum.

In the instance of high pH values (pH>>pKa of the chromophore) of the sample medium, the chromophore is present in fully deprotonated form. Due to the maximal spectral overlap of the absorption band of the deprotonated chromophore and the emission band of the luminophore the nonradiative energy transfer rate from luminophore to chromophore reaches a maximum. Correspondingly, the values of mean luminescence decay time and relative luminescence intensity of the luminophore reach a minimum.

For pH values of the sample medium in the range of +/−1.5 pH units of the pKa value of the chromophore, the mean luminescence decay time or relative luminescence intensity of the luminophore will supply a sufficiently accurate pH value of the sample medium.

II) Determination of the concentrations and/or activities of cations and anions in a sample (Li$^+$, Na$^+$, K$^+$, Mg^{++}, Ca^{++}, Cl$^-$)

Previous optical sensors and processes for determining the concentrations and/or activities of cations in a sample medium employ different methods. For example, the above mentioned publication AT 393 035 is concerned with cation-sensitive chromophores (Γ) which are attached, preferably covalently, to a cation-sensitive luminophore (L).
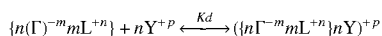
Reaction:

$$(\Gamma L) + Y^{+p} \leftrightarrows (L\Gamma Y)^{+p}$$
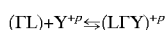
Y is the cation being determined, with the charge number +p.

According to the invention, cation-sensitive chromophores (Γ^{-m}) have at least one anionic group of the charge number −m, which will permit them to form a n:m ionic pair with a cationic luminophore (L^{+n}) of the charge number +n.

Reaction:

$$\{n(\Gamma)^{-m}mL^{+n}\} + nY^{+p} \xrightarrow{Kd} (\{n\Gamma^{-m}mL^{+n}\}nY)^{+p}$$

Y is the cation being determined, with the charge number +p, and Kd is the dissociation constant of the cation-sensitive chromophore.

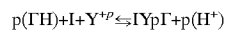In the instance of very high cation concentrations (cY$^+$$_p$>>Kd of the chromophore) of the sample medium the chromophore is present in fully complexed form. In the instance of very low cation concentrations (cY^{+p}<<Kd of the chromophore) of the sample medium the chromophore is present in free, noncomplexed form.

If the logarithmic concentration log(cY^{+p}) of the interesting cation of the sample medium is in the range of log(Kd) +/−1.5, the concentration of the respective cation in the sample medium may be inferred with sufficient accuracy from the mean luminescence decay time and/or relative luminescence intensity of the luminophore.

Further optical measuring methods and sensors for determining the concentrations and/or activities of cations are described in U.S. Pat. No. 4,645,744, EP 0 358 991 B1, and EP 0 461 392 A2, for instance, where a pH-sensitive chromophore (ΓH), or a pH-sensitive luminophore and a neutral ionophore (I) are provided in a substantially hydrophobic polymer matrix. According to the disclosed measuring method, cations (Y^{+p}) are exchanged with the sample medium (for example, K$^+$ for H$^+$ or Ca^{++} for 2H$^+$). As a consequence, the measured results are dependent on the pH value of the sample medium. Such measuring methods are suitable under conditions in which the pH value of the sample medium is known or may be adjusted to a known value by means of a pH buffering layer.
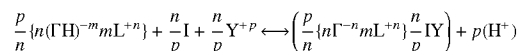
Reaction

$$p(\Gamma H) + I + Y^{+p} \leftrightarrows IY p\Gamma + p(H^+)$$

Y is the cation being determined, +p its charge number.

In further development of these methods the invention proposes that for determination of the cationic concentration of a sample provisions be made for n:m ion pairs $\{n(\Gamma H)^{-m}mL^{+n}\}$ consisting of an anionic, pH-sensitive chromophore $((\Gamma H)^{-m})$ with the charge number −m and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number +n, as well as for a neutral ionophore (I) that is selective for the cation being determined, which are incorporated in a hydrophobic polymer matrix.
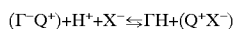
Reaction

$$\frac{p}{n}\{n(\Gamma H)^{-m}mL^{+n}\} + \frac{n}{p}I + \frac{n}{p}Y^{+p} \longleftrightarrow \left(\frac{p}{n}\{n\Gamma^{-n}mL^{+n}\}\frac{n}{p}IY\right) + p(H^+)$$

Y is the cation being determined, +p is its charge number.

Examples of neutral ionophores suitable for the purposes of the invention are listed in Table 2, examples of suitable chromophores in Table 1. Example 3 is a description of how a potassium-sensitive sensor is prepared. The respective measurement results are given in FIG. 3.

Publications EP 9 358 991 B1 and Anal.Chim.Acta 255 (1991), p.35–44 describe optical sensors for determining anions, such as Cl$^-$, where the anion to be determined is co-extracted from the sample medium together with a cation (for example, Cl$^-$ and H$^+$). In that instance a lipophilic, pH-sensitive chromophore (fluorescein derivative) and an optically inactive, cationic substance (Q$^+$) are incorporated in a substantially hydrophobic polymer matrix.

In dependence of the H$^+$ and Cl$^-$ concentration of the sample medium the pH-sensitive chromophore (ΓH) in the polymer matrix is present in protonated and/or deprotonated form. Absorption of the deprotonated form will rise with a growing degree of deprotonation. The degree of deprotonation (and hence, absorption) depends on the pH value and concentration of the anion being determined. The pH value of the sample medium must be known or adjusted to a known value, so as to indicate the concentration of the anion being determined.
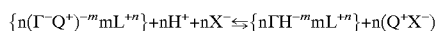
Reaction

$$(\Gamma^-Q^+)+H^++X^-\leftrightarrows\Gamma H+(Q^+X^-)$$

X^- is the anion being determined.

In further development of the method disclosed here the invention proposes, for example, when the chloride concentration of a sample is to be determined, that n:m ion pairs $\{n(\Gamma^-Q^+)^{-m}mL^{+n}\}$ be provided, consisting of an anionic, deprotonated, pH-sensitive chromophore $((\Gamma)^{-(m+1)})$ with the charge number $-(m+1)$ combined with a lipophilic, cationic substance (Q^+) with the charge number $+1$, hence an overall charge number $-m$, and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number $+n$, which are supplied in a hydrophobic polymer matrix that is permeable to chloride ions of the sample by coextraction. The lipophilic substance (Q^+) could be a quaternated ammonium compound, for instance.

Reaction

$$\{n(\Gamma^-Q^+)^{-m}mL^{+n}\}+nH^++nX^-\leftrightarrows\{n\Gamma H^{-m}mL^{+n}\}+n(Q^+X^-)$$

m being the charge number of the protonated, anionic chromophore, and n the charge number of the cationic luminophore.
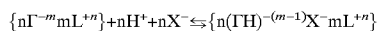
Examples of pH-sensitive chromophores according to the invention are listed in Table 1. In the instance of low pH values and high chloride concentrations of the sample medium the pH-sensitive chromophore preferably is supplied in protonated form; the optically inactive, cationic substance forms a Cl^- counterion in the matrix. Absorption of the deprotonated form of the chromophore reaches a minimum. The values of mean luminescence decay time and relative luminescence intensity of the luminophore reach a maximum.

In the instance of high pH values and low chloride concentrations of the sample medium the pH-sensitive chromophore preferably is present in deprotonated form, the optically inactive, cationic substance compensating the negative charge generated by dissociation of the proton. Absorption of the deprotonated form of the chromophore reaches a maximum. The values of mean luminescence decay time and relative luminescence intensity of the luminophore reach a minimum.

If the pH value of the sample medium is known, the values obtained for mean luminescence decay time and relative luminescence intensity of the luminophore may be used to indicate the chloride concentration of the sample medium.

According to the invention it will also be possible, for example, for determination of the chloride concentration of a sample, to provide n:m ion pairs $\{n\Gamma^{-m}mL^{+n}\}$ consisting of an anionic, deprotonated, pH-sensitive chromophore (Γ^{-m}) with the charge number $-m$ and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number $+n$, which are supplied in a hydrophobic polymer matrix that is permeable to chloride ions of the sample by coextraction. The optically inactive, cationic substance (Q^+) is replaced by the cationic luminophore:

Reaction

$$\{n\Gamma^{-m}mL^{+n}\}+nH^++nX^-\leftrightarrows\{n(\Gamma H)^{-(m-1)}X^-mL^{+n}\}$$

m being the charge number of the deprotonated, anionic chromophore, and n the charge number of the cationic luminophore.
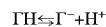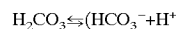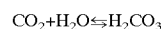
III) Determination of components of liquid or gaseous sample media, which exhibit weak acid or basic reactions in aqueous environments and are gaseous under normal conditions

Determination of CO_2

Optical sensors for determination of the CO_2 partial pressure of a liquid or gaseous sample medium usually comprise a reaction space which is separated from the specimen by an ion-impermeable, gas-permeable material. The reaction space is often identical with the indicator substrate material of an optical pH sensor. In addition, the reaction space usually includes one or several pH buffering substances, such as carbonates, phosphates, and/or organic compounds exhibiting acid or basic reactions in aqueous media. As a consequence, pCO_2 determination of the sample medium may be performed by optical pH determination.
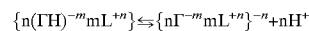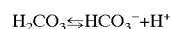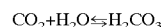
In a variant of this measuring principle described in EP-A 0 205 870, the reaction space is provided in the shape of "droplets" in ion-impermeable, gas-permeable, polymer material.

Reaction

$$CO_2+H_2O\leftrightarrows H_2CO_3$$

$$H_2CO_3\leftrightarrows(HCO_3^-+H^+$$

$$\Gamma H\leftrightarrows\Gamma^-+H^+$$

In further development of the luminescence-optical determination of CO_2 as proposed by the invention, n:m ion pairs $\{n(\Gamma H)^{-m}mL^{+n}\}$ are provided, consisting of an anionic, pH-sensitive chromophore (ΓH^{-m}) with the charge number $-m$ and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number $+n$, which are present in aqueous buffer solution, in a reaction space that is separated from the sample medium by an ion-impermeable, gas-permeable material.
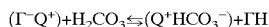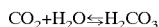
Reaction

$$CO_2+H_2O\leftrightarrows H_2CO_3$$

$$H_2CO_3\leftrightarrows HCO_3^-+H^+$$

$$\{n(\Gamma H)^{-m}mL^{+n}\}\leftrightarrows\{n\Gamma^{-m}mL^{+n}\}^{-n}+nH^+$$

Under the invention, CO_2 determination by means of the sensors described above, comprising a reaction space with an aqueous pH buffer and an ion-impermeable, gas-permeable material separating the reaction space from the sample medium, is performed by pH determination in the reaction space of the sensor (see Examples 1 and 2). High CO_2 values of the sample medium correspond to low pH values of the reaction space, and low CO_2 values of the sample medium correspond to high pH values of the reaction space.

An alternative method of optical CO_2 determination, for which only a single reaction space and no aqueous pH buffering substances are required, is described by Mills et al., Anal.Chem. 64, 1992, 1383–1389. In that case the deprotonated form of a pH-sensitive chromophore with an optically inactive, cationic substance (Q^+) is present in a reaction space consisting of essentially ion-impermeable, gas-permeable, polymer material. CO_2 from the sample diffuses into the polymer material, where it is hydrated and reacts in a chemical equilibrium reaction with the deprotonated form of the chromophore and the optically inactive substance. From the light absorption of the deprotonated
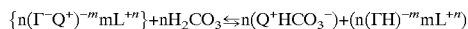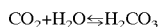
form of the chromophore the CO_2 partial pressure of the sample medium is inferred.

Reaction

$$CO_2+H_2O \leftrightarrows H_2CO_3$$

$$(\Gamma^-Q^+)+H_2CO_3 \leftrightarrows (Q^+HCO_3^-)+\Gamma H$$

In a variant of the invention concerned with luminescence-optical CO_2 determination n:m ion pairs $\{n(\Gamma^-Q^+)^{-m}mL^{+n}\}$ are provided, which consist of an anionic, deprotonated, pH-sensitive chromophore $((\Gamma)^{-(m+1)})$ with the charge number $-(m+1)$ combined with a lipophilic, cationic substance (Q^+) with the charge number $+1$, hence an overall charge number $-m$, and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number $+n$, which are supplied in a hydrophobic polymer matrix that is impermeable to ionic substances in the case of aqueous sample media.

Reaction

$$CO_2+H_2O \leftrightarrows H_2CO_3$$

$$\{n(\Gamma^-Q^+)^{-m}mL^{+n}\}+nH_2CO_3 \leftrightarrows n(Q^+HCO_3^-)+(n(\Gamma H)^{-m}mL^{+n})$$

m being the charge number of the protonated, anionic chromophore, and n the charge number of the cationic luminophore.
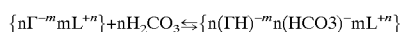
In accordance with the invention, CO_2 determination by means of a sensor comprising a reaction space without aqueous buffering substances, is based on the determination of the ratio of the protonated and deprotonated forms of a pH-sensitive chromophore.

In the instance of very low CO_2 values of the sample medium the chromophore preferably is provided in deprotonated form, forming a ionic bond with the optically inactive, cationic substance in the matrix. Absorption of the deprotonated form of the chromophore reaches a minimum. The values of mean luminescence decay time and relative luminescence intensity of the luminophore reach a maximum.

In the instance of high CO_2 values of the sample medium the chromophore is provided in protonated form. The optically inactive, cationic substance forms a ionic bond with hydrogen carbonate. Absorption of the deprotonated form of the chromophore reaches a minimum. The values of mean luminescence decay time and relative luminescence intensity of the luminophore reach a minimum.

According to the invention it will also be possible to provide n:m ion pairs $\{n\Gamma^{-m}mL^{+n}\}$, consisting of an anionic, deprotonated, pH-sensitive chromophore (Γ^{-m}) with the charge number $-m$ and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number $+n$, which are supplied in a hydrophobic polymer matrix that is impermeable to ionic substances in the instance of aqueous sample media. Unlike in the example referred to before, the optically inactive, cationic substance (Q^+) is replaced by the cationic luminophore in this case:

Reaction

$$\{n\Gamma^{-m}mL^{+n}\}+nH_2CO_3 \leftrightarrows \{n(\Gamma H)^{-m}n(HCO3)^-mL^{+n}\}$$
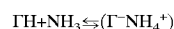
m being the charge number of the deprotonated, anionic chromophore, and n the charge number of the cationic luminophore.

Determination of NH_3

The determination of NH_3, as an example of a component exhibiting a basic reaction in aqueous environment, may be effected in a way similar to the determination of CO_2 according to Mills (T. Werner et al., Analyst 120,1995, 1627–1631). No optically inactive, cationic substance is required.

Reaction

$$\Gamma H+NH_3 \leftrightarrows (\Gamma^-NH_4^+)$$

According to the invention the determination of NH_3 is effected in the same way as the CO_2 determination described above.

Reaction

$$\{n(\Gamma H)^{-m}mL^{+n}\}+nNH_3 \leftrightarrows (n\{\Gamma^{-m}mL^{+n}\}^-nNH_4^+)$$
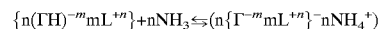
pH-sensitive chromophores suitable for use with the invention

Ion pairs in accordance with the invention, consisting of anionic, pH-sensitive chromophores from Table 1 and suitable cationic luminophores from Table 3 are obtained as described in Examples 1a and 2a. The preparation of pH sensors using these ion pairs in accordance with the invention is described in Examples 1b (ion-permeable sol-gel matrix) and 2b (ion-permeable hydrogel matrix). The respective measurement data (mean luminescence decay time, relative luminescence intensity) are displayed in FIGS. 1 and 2, in dependence of the pH value of the sample medium.

TABLE 1

pH-sensitive Chromophores		
Chromophore	Absorption wavelength [nm] protonated/deprotonated	pKa
Triphenylmethane dyes		
Bromophenol blue	430/617	3.8
Bromothymol blue	430–435/615–618	6.7
Dibromoxylenol blue	420/614	7.6
Azo dyes		
Calmagit	530/605	8.0
Nitrazine yellow	460/590	6.5
Others		
o-chlorophenol-indophenol	555/625	7.1
Naphthol-phthalein	428/661	6.7, 7.9

In addition, pH-sensitive triphenylmethane dyes of the general form
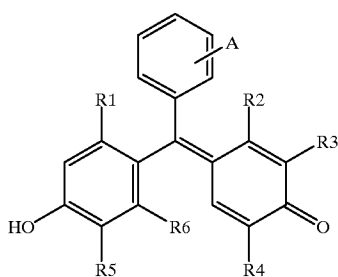
are used, A⁻ standing for the deprotonated form of a strong acid, such as SO_3^- or $—COO^-$, and R1–R6 for halogen atoms, nitro groups or alkyl groups; as well as pH-sensitive azo dyes of the general form
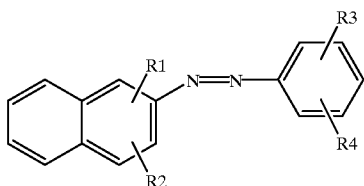
R1–R4 standing for substituents, such as halogen atoms, nitro groups or alkyl groups, while at least one —OH group and one $—SO_3^-$ or $—COO^-$ group must be provided.

Cation-sensitive chromophores suitable for use with the invention

TABLE 2

Neutral Ionophores	
Ionophore	Ion
PTM14C4 (14-crown-4)**)	Li+
Sodium Ionophore I–II*)	Na+
Valinomycin	K+
Magnesium Ionophore ETH 3832*)	Mg++
Calcium Ionophore I–IV*)	Ca++

*) Ionophores for Ion-Selective Electrodes and Optodes, Fluka Chemie AG, CH-9470 Buchs, Switzerland.

**) K. Watanabe et al. Anal. Chem. 65, 1993.

TABLE 3

Cationic Luminophores			
Luminophore (L)	Abbrev.	Absorption maximum (nm)	Luminescence maximum (nm)
[(Ru(II)-tris-(2,2'-bibyridyl)]²⁺	Ru(bpy)₃²⁺	452	628
[(Ru(II)-tris-(4,4'-diphenyl-2,2'-bipyridyl)]²⁺	Ru(dph-bpy)₃²⁺	474	632
[(Ru(II)-tris-(1,10-penanthroline)]²⁺	Ru(phen)₃²⁺	447	604
[(Os(II)-bis-(terpyridine)]²⁺		510	729
[(Os(II)-tris-(1,10-phenanthroline)]²⁺		650	690

Examples of cation-sensitive chromophores for determination of lithium, potassium, sodium, magnesium and calcium ions include anionic azo dyes, stilbene dyes and merocyanines, which contain at least one anionic group and an ion-selective crown ether system, and whose absorption band with the longest wavelengths overlaps the emission band of the luminophore at least partially, the interaction with the alkali or alkaline-earth ions being determined leading to a spectral shift of the longest-wave absorption band.

Ionic bonds between anionic, cation-sensitive chromophores and cationic luminophores are obtained in the same way as the ionic bonds between anionic, pH-sensitive chromophores and cationic luminophores described by the invention (see Examples 1 and 2).

Other central atoms used are Ir, Rh, Pd, Pt, Re.

In order to obtain ion pairs of the general form $\{n(\Gamma H)^{-m}mL^{+n}\}$ or $\{n(\Gamma)^{-m}mL^{+n}\}$ from anionic, pH- or ion-sensitive chromophores (Γ^{-m}) with cationic luminophores (L^{+n}), those chromophores or chromoionophores (ion-sensitive chromophores) may be used which contain an anionic group whose charge number does not change vis-a-vis the pH values of the sample material.

For example, the triphenylmethane dyes in Table 1 have a sulpho group which is present in the anionic form $(R—SO_3^-)$ with the charge number −1 for pH values >3.

According to the invention it would also be possible to attach at least one anionic group of the general form $—(CH_2)_x—A^{-n}$ (x=0–4;n=1,2) to those chromophores which do not contain this group.

Preferred anionic groups are derived from compounds exhibiting acid reactions in aqueous environment, such as sulphonic acids of the general form $(R—SO_3H)$ and carbonic acids of the general form R—COOH.

Ion pairs offering special advantages in determining CO_2 (and chloride) include 1:1 ion pairs consisting of an anionic chromophore with the charge number −2 (Table 1, triph-

enylmethane dyes or azo dyes with at least one pH-active group and an anionic group) and a cationic luminophore with the charge number +2 (Table 3).

As is seen from the above, the ion pairs which are especially preferred by the invention for determination of CO_2 and anions (chloride) are identical, and are supplied in hydrophobic polymer materials that are substantially impermeable to ionic sample constituents. In anion determination (e.g., chloride) the anion being determined is coextracted into the polymer material with a cation (H^+), whereas in the instance of CO_2 and NH_3 the charge-neutral molecules will diffuse into the material regardless of the presence of any counterions. For gaseous samples it is thus evident that CO_2 determination is not affected by any anionic sample components. This is not true in general for aqueous samples, however. Hence, in order to avoid interferences in CO_2 determination caused by anionic components of aqueous samples, it is provided that the sensor layer be covered by a gas-permeable layer which does not contain the ion pair. Suitable materials feature high permeability to the gaseous components to be determined, for example, teflon

Essentially hydrophobic, ion-impermeable matrices

To obtain sensors as proposed by the invention, which exchange the cation being determined (such as K^+) with a proton of the sample medium, or coextract the anion being determined (such as Cl^-) with a proton of the sample medium, polymer materials are suited that are substantially impermeable to ionic substances of the sample medium.

These materials are also suitable for preparing sensors according to the invention which are used for determination of gases or gaseous components in liquid sample media.

Preferred are all essentially hydrophobic polymers that are soluble in organic solvents, such as polyvinyl chloride, polystyrenes, polycarbonates, polyethylenes, polyurethanes, silicones, copolymers of polyvinyl alcohol and polyvinyl acetate, and copolymers of polyvinyl chloride, polyvinyl alcohol and polyvinyl acetate.

To these materials up to 80 percent by weight plasticizers may be added, such as dioctyl sebacate, tris-(2-ethylhexyl)-phosphate, 2-nitrophenyl-octyl-ether, 2-nitrophenyl-butyl-ether.

Essentially hydrophilic, ion-permeable matrices

To obtain sensors exhibiting pH- and ion-sensitive chromophores as proposed by the invention, hydrophilic, ion-permeable polymers are preferred.

Representatives are nafion, cellulose, and cellulose acetates. An important group of hydrophilic polymers are the so-called hydrogels. Relevant examples are polyurethanes with hydroxylic groups, polyhydroxyethylmethacrylates, crosslinked polyvinyl alcohols, and polyacrylamides.

Moreover, incorporation into sol-gels will yield pH- and ion-permeable materials. Sol-gels are porous, organically produced, vitreous materials based on silicon compounds or titanium-organic compounds. The most typical representative is a sol-gel made from tetraethoxyorthosilane.

The use of ion-permeable materials for gas sensors in accordance with the invention is possible if the gas-sensitive material is separated from a liquid sample medium by an additional material consisting of ion-impermeable, gas-permeable polymers, such as teflon or silicones.

EXAMPLE 1

(a) Preparation of a 2:1 ion pair of the general form $\{n(TH)^{-m}mL^{+n}\}$ using Bromothymol Blue and $Ru(phen)_3^{2+}$

129.2 mg bromothymol blue sodium salt (200 mmole) is dissolved in 3 ml 0.1 N NaOH and 20 ml water. After adding

4 ml of 0.1 N HCl and a solution of 82.1 mg [Ru(II)-tris(1, 10-phenanthroline)]Cl_2.6H_2O (100 mmole) in 3 ml water, the ion pair is obtained as orange precipitate. The latter is filtered off by suction, rinsed with water and dried over NaOH. Yield: 80 percent; melting point: 237° C. Data of elementary analysis (computed/found): C:54.14/55.68, H:4.54/4.64, N:4.21/4.28.

(b) Preparation of a pH-sensitive sensor layer

3 mg ketyltrimethyl ammonium chloride and 3 mg of the 2:1 ion pair $\{2(\text{bromothymol blue})^{1-}$ $Ru(phen)_3^{2+}\}$ is dissolved by ultrasound in 1 ml methanol. After adding 200 μl tetramethoxysilane and 100 μl of a 0.1 N hydrochloric acid, the mixture is allowed to gellate for 5 days in snap-cover jars. This material is used for sensor films which are applied on optically transmissive substrate material (polyester), or for coating optical fibers. The sensors must be left to dry for several days at 40° C.

FIG. 1 displays the dependence of mean decay time t_m on the pH value of the sample medium.

EXAMPLE 2

(a) Preparation of a 2:1 ion pair of the general form $\{n(TH)^{-m}mL^{+n}\}$ using $Ru(dph-bpy)_3^{2+}$ and Bromothymol Blue

129.2 mg bromothymol blue sodium salt (200 mmole) is dissolved in 3 ml of 0.1 N NaOH and 20 ml water. Diluted hydrochloric acid is added until the indicator changes to acid form. This solution is added dropwise to a saturated aqueous solution of the ruthenium complex [Ru(II)-tris(4,4'-diphenyl-2,2'-bipyridyl)]Cl_2 by continuous stirring. The ion pair is obtained as orange precipitate. The latter is filtered off by suction, rinsed with water and dried over NaOH. Yield: 80 percent. Data of elementary analysis (computed/found): C:61.99/60.38, H:4.64/4.61, N:3.61/3.87.

(b) Preparation of a pH-sensitive sensor layer

To obtain a 5 percent hydrogel solution, 4 g of hydrogel D4 produced by Tyndale-Plains Hunter Ltd., Ringoes, N.Y. 08551, is dissolved in 72 ml EtOH and 8 ml H_2O by stirring within 5 hours.

10 g of this solution is added to 5.82 mg of the 2:1 ion pair $\{2(\text{bromothymolblue})^{1-}$ $Ru(dph-bpy)_3^{2+}\}$ and stirred until a homogeneous, clear solution is obtained.

From this solution sensor films are prepared which are applied on optically transmissive substrate material (polyester), where they are left to dry for several hours at room temperature, until evaporation of the solvent is complete.

FIG. 2 displays the dependence of mean decay time t_m and relative luminescence intensity on the pH value of the sample medium.

EXAMPLE 3

Preparation of a potassium-sensitive sensor layer

2.7 mg of the 2:1 ion pair $\{(\text{bromothymol blue})^{1-}$ $Ru(dph-bpy)_3^{2+}\}$, and 3.8 mg valinomycin, together with 100 mg dioctyl sebacate and 50 mg PVC is dissolved in 3 ml tetrahydrofurane. This mixture is used for preparing sensor films or coating optical fibers. The sensors are stored in a dry place and activated in buffer solution for at least 8 hours before measurement.

FIG. 3 displays the dependence of relative luminescence intensity on the K^+-concentration of the sample medium.

In FIG. 4 the proposed sensor layer 1 of a sensor 10 shown schematically in sectional representation, is applied on a

substrate **4** that is transmissive to excitation and emission radiation **2,3**. In the sensor layer **1**, which consists of a matrix material that is permeable to the interesting chemical component of the sample **5**, the ionic luminophore and the ionic chromophore are immobilized. On the side of the sample the sensor layer **1** may have a cover layer **6**.

According to the invention, the sensor layer **1** could also be provided at the end of an optical fiber guide.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** displays the dependence of mean decay time t_m on the PH value of the sample medium.

FIG. **2** displays the dependence of mean decay time t_m and relative luminescence intensity of the pH value of the sample medium.

FIG. **3** displays the dependence of relative luminescence intensity on the K^+ concentration of the sample medium.

FIG. **4** shows schematically the proposed sensor layer of a sensor in sectional representation.

We claim:

1. A sensor layer for quantitative determination of at least one chemical component of a gaseous or liquid sample comprising a matrix material, permeable to the chemical component being determined, containing a chromophore which is directly or indirectly responsive to the component being determined by changing its absorption spectrum and a luminophore which is not responsive to the component being determined;

wherein there is at least partial overlap between the emission spectrum of the luminophore and the absorption spectrum of the chromophore, and the energy transfer between luminophore and chromophore effecting a measurable change in at least one luminescence characteristic of the luminophores; and

wherein the luminophore (L) and the chromophore (Γ) are ionic substances of differing electrical charges which are present as ion pairs in said matrix material.

2. Sensor layer as claimed in claim **1**, wherein the matrix material has an anionic chromophore (Γ) and a cationic luminophore (L), n chromophore molecules of the charge number –m and m luminophore molecules of the charge number +n forming a n:m ionic pair.

3. Sensor layer as claimed in claim **2**, wherein n:m ion pairs $\{n(\Gamma H)^{-m} mL^{+n}\}$ consisting of an anionic, pH-sensitive chromophore (ΓH^{-m}) with the charge number –m and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number +n are provided, which are present in a hydrophilic polymer matrix, wherein said sensor layer is used in the quantitative determination of the pH value of a sample.

4. Sensor layer as claimed in claim **1**, wherein the matrix material has a cationic chromophore (Γ) and an anionic luminophore (L), n chromophore molecules of the charge number +m and m luminophore molecules of the charge number –n forming a n:m ionic pair.

5. Sensor layer as claimed in claim **2**, wherein the luminophore (L) is a transition metal complex with a central atom of a group consisting of Ru, Os, Ir, Rh, Pd, Pt, and Re, the ligands provided being of a group consisting of 2,2'-bipyridine (bpy), 2,2'(4,4-diphenyl) bipyridyl (dph-bpy) and 1,10-phenanthroline (phen).

6. Sensor layer as claimed in claim **1**, wherein n:m ion pairs $\{n(\Gamma^- Q^+)^{-m} mL^{+n}\}$ are provided, which consist of an anionic, deprotonated, pH-sensitive chromophore (($\Gamma^{-(m+1)}$) with the charge number –(m+1) combined with a lipophilic,

cationic substance (Q^+) with the charge number +1, hence an overall charge number –m, and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number +n, which are supplied in a hydrophobic polymer matrix that is impermeable to ionic substances in the case of aqueous sample media, wherein said sensor layer is used in the quantitative determination of the CO_2 partial pressure of a gaseous or liquid sample.

7. Sensor layer as claimed in claim **6**, wherein the lipophilic substance is a quaternated ammonium compound.

8. Sensor layer as claimed in claim **1**, wherein n:m ion pairs $\{n\Gamma^{-m} mL^{+n}\}$ are provided, which consist of an anionic, deprotonated, pH-sensitive chromophore (Γ^{-m}) with the charge number –m and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number +n, which are supplied in a hydrophobic polymer matrix that is impermeable to ionic substances in the instance of aqueous sample media, wherein said sensor layer is used in the quantitative determination of the CO_2 partial pressure of a gaseous or liquid sample.

9. Sensor layer as claimed in claim **1**, wherein n:m ion pairs $\{n(\Gamma^- Q^+)^{-m} mL^{+n}\}$ are provided, which consist of an anionic, deprotonated, pH-sensitive chromophore ((Γ)$^{-(m+1)}$) with the charge number –(m+1) combined with a lipophilic, cationic substance (Q^+) with the charge number +1, hence an overall charge number –m, and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number +n, which are supplied in a hydrophobic polymer matrix that is permeable to chloride ions of the sample by coextraction, wherein said sensor layer is used in the quantitative determination of the chloride concentration of a sample.

10. Sensor layer as claimed in claim **9**, wherein the lipophilic substance is a quaternated ammonium compound.

11. Sensor layer as claimed in claim **1**, wherein n:m ion pairs $\{n\Gamma^{-m} mL^{+n}\}$ are provided, which consist of an anionic, deprotonated, pH-sensitive chromophore (Γ^{-m}) with the charge number –m and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number +n, which are supplied in a hydrophobic polymer matrix that is permeable to chloride ions of the sample by coextraction, wherein said sensor layer is used in the quantitative determination of the chloride concentration of a sample.

12. Senor layer as claimed in claim **1**, wherein n:m ion pairs $\{n(\Gamma H)^{-m} mL^{+n}\}$ are provided, which consist of an anionic, pH-sensitive chromophore (($\Gamma H)^{-m}$) with the charge number –m and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number +n, in addition to a neutral ionophore (**1**) that is selective for the cation being determined, which are present in a hydrophobic polymer matrix, wherein said sensor layer is used in the quantitative determination of the cationic concentration of a sample.

13. Sensor layer as claimed in claim **12**, wherein the neutral ionophore PTM14C4 (14-crown-4) is used for the ion Li+.

14. Sensor layer as claimed in claim **12**, wherein the neutral ionophore Sodium Ionophore I–II is used for the ion Na+.

15. Sensor layer as claimed in claim **12**, wherein the neutral ionophore Valinomycin is used for the ion K+.

16. Sensor layer as claimed in claim **12**, wherein the neutral ionophore Magnesium Ionophore ETH 3832 is used for the ion Mg++.

17. Senor layer as claimed in claim **12**, wherein the neutral ionophore Calcium Ionophore I–IV is used for the ion Ca++.

18. Sensor layer as claimed in claim **1**, wherein the luminophore (L) is a transition metal complex with a central

atom of a group consisting of Ru, Os, Ir, Rh, Pd, Pt, and Re, the ligands provided being of a group consisting of 2,2'-bipyridine (bpy), 2,2'(4,4-diphenyl) bipyridyl (dph-bpy) and 1,10-phenanthroline (phen).

19. Sensor layer as claimed in claim **1**, wherein the chromophore (Γ) is pH sensitive and is a triphenylmethane.

20. Sensor layer as claimed in claim **1**, wherein the chromophore (Γ) is pH sensitive and is an azo dye.

21. A sensor layer for quantitative determination of CO_2 partial pressure of a sample comprising an aqueous buffer solution separated from the sample by an ion-impermeable, gas-permeable material containing a chromophore which is directly or indirectly responsive to the component being determined by changing its absorption spectrum and a luminophore which is not responsive to the component being determined;

wherein there is at least partial overlap between the emission spectrum of the luminophore and the absorption spectrum of the chromophore, and the energy transfer between luminophore and chromophore effecting a measurable change in at least one luminescence characteristic of the luminophore;

wherein the luminophore (L) and the chromophore (Γ) are ionic substances of differing electrical charges; and $\{n(\Gamma H)^{-m}mL^{+n}\}$; and

wherein n:m ion pairs $\{n(\Gamma H)^{-m}mL^{+n}\}$ consisting of an anionic, pH-sensitive chromophore (ΓH^{-m}) with the charge $-m$ and a cationic, pH-insensitive luminophore (L^{+n}) with the charge number $+n$ are provided.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.   : 5,942,189
DATED   : August 24, 1999   Page 1 of 4
INVENTOR(S)   : Otto S. Wolfbeis, et. al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

The Title page should be deleted and substitute therefore the attached Title page.

Drawings:

Delete drawing sheets 1-2 and substitute therefore the Drawing sheets 1-2 as shown on the attached pages.

Signed and Sealed this

Fifteenth Day of August, 2000

Attest:

Q. TODD DICKINSON

Attesting Officer   Director of Patents and Trademarks

US005942189A

United States Patent [19]

Wolfbeis et al.

[11] Patent Number: 5,942,189

[45] Date of Patent: Aug. 24, 1999

[54] LUMINESCENCE-OPTICAL METHOD AND SENSOR LAYER FOR QUANTITATIVE DETERMINATION OF AT LEAST ONE CHEMICAL COMPONENT OF A GASEOUS OR LIQUID SAMPLE

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[73] Assignee: **AVL Medical Instruments AG**, Schaffhausen, Switzerland

[21] Appl. No.: **08/950,373**

[22] Filed: **Oct. 14, 1997**

[30] **Foreign Application Priority Data**

Oct. 16, 1996 [AT] Austria 1818/96

[51] Int. Cl.^{6} G01N 21/64

[52] U.S. Cl. **422/82.08**; 422/82.07; 422/58

[58] Field of Search 422/82.08, 82.07, 422/58; 436/172

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,645,744	2/1987	Charlton et al.	
4,762,799	8/1988	Seitz et al.	436/79
5,037,615	8/1991	Kane	422/82.08
5,232,858	8/1993	Wolfbeis et al.	436/77
5,565,328	10/1996	Bascomb et al.	435/25
5,631,169	5/1997	Lakowicz et al.	436/537
5,776,782	7/1998	Tsuji	436/171
5,849,489	12/1998	Heller	435/6

FOREIGN PATENT DOCUMENTS

393035	7/1991	Austria .

205870	12/1986	European Pat. Off. .
214768	3/1987	European Pat. Off. .
461392	12/1991	European Pat. Off. .
358991	2/1994	European Pat. Off. .

OTHER PUBLICATIONS

Jordan et al., Analytical Chemicstry 59, 437–439, 1987.

Yuan et al., Journal of Fluorescence, vol. 2, No. 4, pp. 231–235, 1992.

Silva et al., New J. Chemistry, 20, 871–880, 1996.

Stufkens et al., Coordination Chemistry Reviews, 177, 127–179, 1998.

Kosch et al., Analytical Chemistry, 70, 3892–3897, 1998.

Preininger, Journal of Fluorescence, vol. 8, No. 3, pp. 199–205, 1998.

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[57] **ABSTRACT**

In a sensor layer for quantitative determination of at least one chemical component in a gaseous or liquid sample medium containing a chromophore which is directly or indirectly responsive to the component being determined by changing its absorption spectrum, and a luminophore which is not responsive to the component being determined, where there is an at least partial overlap between the emission spectrum of the luminophore and the absorption spectrum of the chromophore, and where the energy transfer between luminophore and chromophore produces a measurable change in at least one luminescence characteristic of the luminophore, the luminophore L and the chromophore Γ are ionic substances with differing electrical charges, which are incorporated in a matrix material that is permeable to the chemical component being determined.
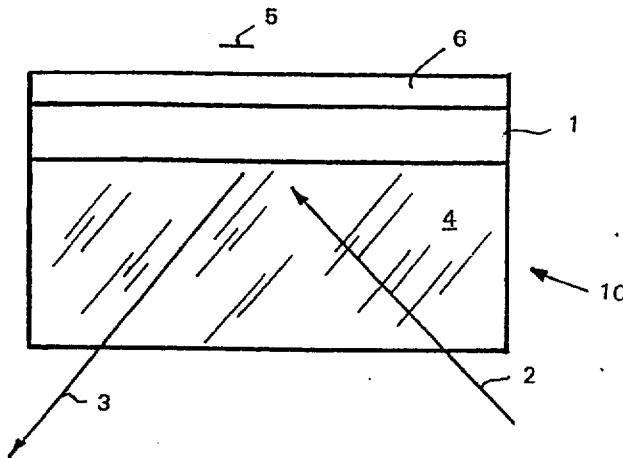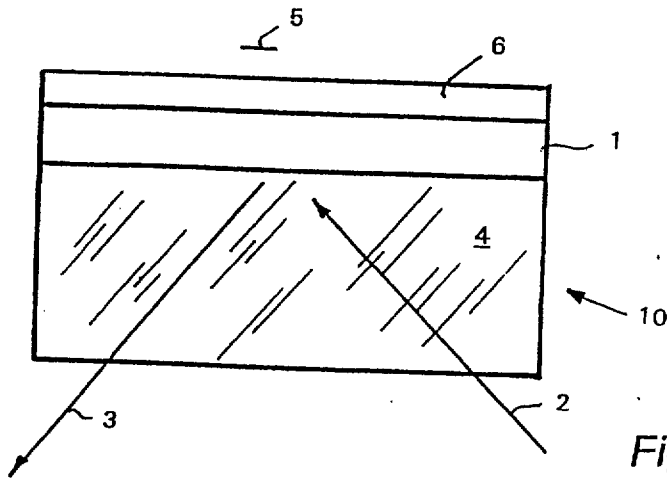
21 Claims, 2 Drawing Sheets

Fig. 1

Fig. 2

Fig. 3

Fig. 4