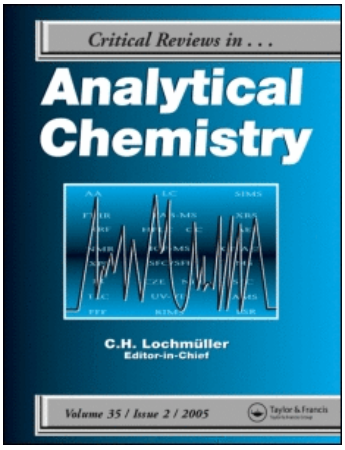

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Room-Temperature Phosphorescent Complexes with Macromolecular Assemblies and Their (Bio)chemical Applications

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ABSTRACT: A number of metal complexes, including hydroxyquinolines, phthalocyanines, porphyrins, chlorophyls, and others, phosphoresce at room temperature in the presence of macromolecular assemblies such as micelles, vesicles, proteins, and nucleic acids. The aim of this review is to illustrate the applicability of these systems to a variety of analytical problems through the discussion of representative examples. Finally, recent developments and likely prospects in the field of room temperature phosphorescent complexes, such as probes of vascular disorders, are also considered.

KEY WORDS: room temperature phosphorescence, metal complexes, macromolecular systems.

I. INTRODUCTION

A variety of metal complexes, including porphyrins, chlorophylls, hydroxyquinolines, phthalocyanines, and others, fluoresce and/or phosphoresce.[1] Phosphorescence is normally observed for metal complexes at cryogenic temperatures (77°K), at room temperature using adequate solid supports, and, in many instances, metal complexes can be induced to phosphoresce in solution at room temperature when the radiative deactivational pathways are minimized by isolating the excited triplet state in an organized media.

In a broad sense, organized media is a term that is applied to those systems in which one or more of its components spontaneously aggregate, or to those cases where solutes (molecular or ionic species) are compartmentalized or accepted inside a host system, in difference to its solvent (see Figure 1). Into the first type of organized media, with analytical relevance in liquid room temperature phosphorescence (LRTP), are included aqueous micelles and vesicles. We could also include into this group those systems that adopt a micelle-like microphase structure in aqueous solution such as proteins. On the other hand, host systems for analytical LRTP include most commonly cyclodextrins and cryptates. The vast majority of analytical applications of LRTP metal complexes have been demonstrated using normal micelles, while relatively few have been examined with other macromolecular assemblies.

In the present report we review the room temperature phosphorescence behavior of several metal complexes and comment on the experimental conditions that affect the triplet emission in solution. Attempts have been made to select representative examples and attention has also been directed toward the most recent fields of application.

A. Chemical Organized Media: Micelles and Vesicles

Application of micelles and vesicles to a particular analytical situation requires some knowledge about their structure as well as the nature of solubilization sites.

1. Micellar Characteristics

Surfactants are amphiphilic molecules possessing a polar group bound to a hydrophobic

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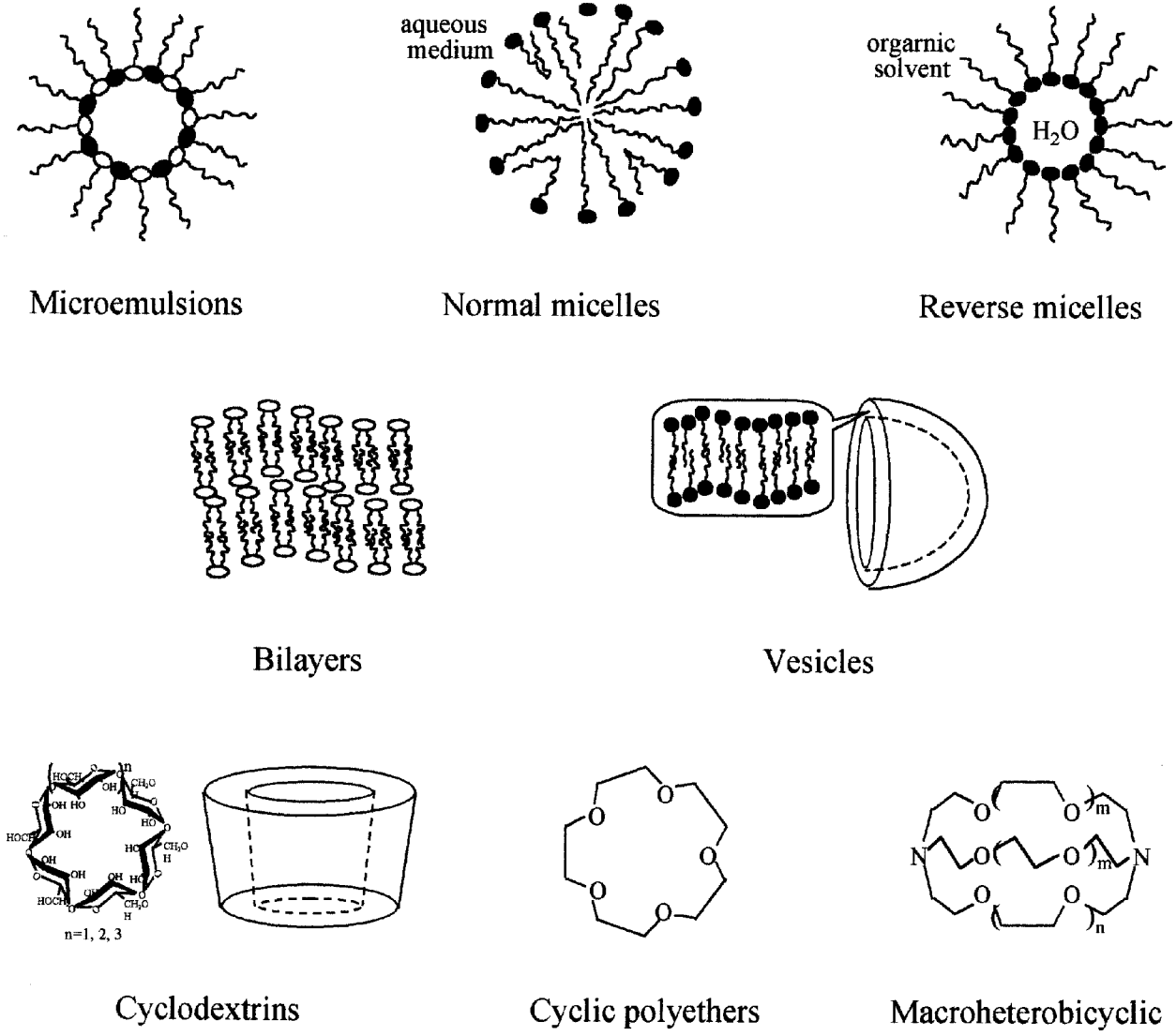

FIGURE 1. Simplified representation of some ordered macromolecular systems.

moiety, commonly a long chain. Depending on the nature of the polar head groups, surfactants can be classified as anionic, cationic, nonionic, and zwitterionic. Normal micelles are colloid-sized globular structures of surfactant species in aqueous solution. The most simple picture is the Hartley model.[2] As shown in Figure 1, these assemblies of amphiphiles have a hydrocarbon core surrounded by a highly hydrophilic region formed by the surfactant head groups, counterions, and water molecules. Micelle formation starts at a characteristic and narrow range of surfactant concentration known as the critical micelle concentration (cmc). The cmc values, the number of monomers forming the micelle, and the micellar size are dependent on the nature of the amphiphile as well as on the composition of the solution.[3-5] A list of surfactants commonly used in analytical chemistry, along with their characteristic parameters, is presented in Table 1. Surfactants can associate also in nonaqueous media, forming reverse micelles. The monomers are oriented in the opposite sense to those in normal micelles, with the hydrophobic tails extended into the bulk nonpolar solvent and the head groups drawn together to form the hydrophilic core. Most of analytical applications of micelles have been developed using normal micelles, while only a few have been examined with inverse micelles.

One of the key aspects on all successful applications of normal micelles consists in the capability of solutes to associate and bind with the micellar aggregate. Normal micelles provide regions of different polarity, acidity, and viscosity. So, the nature and strength of interaction of a solute with a micelle depends on both the solute and surfactant characteristics. For example, nonpolar species may be located (1) completely inside the hydrophobic core of the micelle, (2) adsorbed on the micellar surface, or (3) may penetrate to a certain depth into the surface layer. Substrates having amphiphilic character may align themselves with its polar position directed outward toward the micellar surface and its nonpolar portion directed inward toward the micelle core.

It is generally accepted that normal micelles modify the luminescence properties of molecules due to microenvironmental changes experienced by the lumiphor molecules in the oraganized medium.[6,7] So, differences in quantum yield, lifetime, excitation, and emission spectra can be found. Furthermore, chemical and photophysical pathways and rates can be altered and the luminescence intensity of an excited probe can be drastically enhanced when bound to a micelle. Micelles have also been shown to stabilize the triplet state of some molecules, thereby allowing room temperature liquid phosphorescence.[8,9] All these phenomena can be very useful from an analytical point of view, as it is possible to lower the detection limits of the assay, to reduce quenching interferences from impurities, to catalyze slow luminescence reactions, and, in some instances, to avoid analytical separation steps.[10,11]

TABLE 1
Characteristics of Commonly Used Surfactants

Surfactant	Abbreviation	cmc[a] (m*M*)	N[b]	KP[c] (°C)
Sodium dodecylsulfate	*SDS*	*8.1*	*62*	*16*
Cetyltrimethylammonium bromide	*CTAB*	*0.92*	*60*	*22*
N-Cethylpyridinium bromide	*CPyB*	*0.9*	—	—
Hexadecyltrimethylammonium bromide	*HTAB*	*0.026*	*169*	—
Brij-series (Brij 35, 56, etc.)		*0.06 (Brij-35)*	*40*	*100*
Triton-series (Triton X-100, X-400,etc.)		*0.24* (Triton X-100)	140	—

[a] cmc: critical micelle concentration.[5]
[b] N: aggregation number.[5]
[c] KP: Krafft Point, the temperature below which the solubility of the monomeric amphiphilic is less than the value of the cmc.[4]

2. Vesicle Characteristics

Surfactant vesicles are closed bilayer structures (see Figure 1) formed by ultrasonic dispersal of simple anionic or cationic long-chain di- or tri-alkyl surfactants in water or by gently injection of a nonaqueous dialkylsurfactant solution into pure water. When the amphiphilic molecule is a natural membrane-forming lipid, the aggregates are termed liposomes. As with micelles, there are different sites for the substrate localization in vesicles.[12] Hydrophilic substrates can be located inside the core of vesicle, whereas hydrophobic molecules may be embedded inside the hydrophobic bilayer. Molecules with more complex chemical characteristics can be wholly or partly intercalated among the hydrocarbon tails in the bilayer. Molecules can also be anchored to the exterior or interior surface of the vesicle.

The relative simple structure of vesicles, their kinetic stability, and their solubility power for many molecules made them a useful medium for developing analytically useful luminescence methodologies. There are many examples that demonstrated that vesicles influence the photochemical properties of guest molecules.[13]

II. ROOM TEMPERATURE PHOSPHORESCENCE IN SOLUTION

One important consequence of the slow radiative decay of phosphorescence (typically on the msec-sec time scale, compared with the nsec time scale of fluorescence decay) is that a variety of short- and long-range interactions, along with possible intramolecular vibrational-rotational relaxations, can take place in fluid solutions to bring about radiationless decay of triplet states. Quenching of the triplet state by small amounts of dissolved oxygen is particularly efficient in preventing phosphorescence in fluid solutions. These facts explain why it was believed that hindering bimolecular quenching and intramolecular relaxations by working at cryogenic conditions (liquid nitrogen at 77 K) or by immobilizing the molecule on different solid supports such as filter paper, alumina, sodium acetate, silica gel, etc.[14] were the only suitable ways for analytical phosphorescence.

In recent years this misconception has been abandoned and phosphorescence from many molecules may be conveniently and readily observed in liquid aqueous solution at room temperature by isolating the excited molecules in micellar interior.[8, 9,14] This approach, generally referred it as micelle-stabilized room temperature phosphorescence (MS-RTP), has attracted considerable attention as a companion tool to analytical fluorescence. RTP can also be observed from molecules incorporated into a vesicular or a cyclodextrin cavity. Among the different possibilities, normal micelles have been the organized media more commonly used to obtain analytically useful RTP in fluid solution.

Cline Love et al.[9] showed that there are three fundamental requirements to observe MS-RTP for most lumiphors: (1) securing the presence of micellar assemblies (i.e., using a surfactant concentration above its cmc); (2) the presence of a heavy atom or a heavy species, and (3) ensuring oxygen removal.

A. Presence of Micellar Aggregates

Micellar media provide a number of features that can promote RTP of phosphors buried inside the micellar structure. First, the structured nature of the inner micro-environment provides the local rigidity necessary to minimize collisional deactivation of excited molecules by restricting their diffusive motions. Only at surfactant concentrations well above the cmc becomes that protective effect apparent (micellar effect). Second, exclusion of quenchers from the micellar assembly through polarity or electrostatic considerations decrease the probability of other quenching pathways. An additional factor that influences the RTP observed is the relative phosphor/micelle polarity and charge. In fact, no MS-RTP is observed for negatively charged molecules in anionic micellar media. However, a negatively charged lumiphor may interact with a cationic micellar phase through coulombic attraction, which then act concurrently with adsorption, coassembly with surfactant monomers, hydrophobic interaction, etc. The array of possible interaction mechanisms provide the rigidity needed for the strong RTP signal observed.

B. Heavy Atoms

It is well know that heavy atoms impact spin-orbit coupling and reduce both the radiative and observed lifetimes of the phosphor. This results in an enhanced emission intensity because most nonradiative competing processes are not similarly affected by the heavy atom.

By adequate choice of heavy atoms and surfactant assemblies, it is possible to induce MS-RTP in a variety of metal complexes and/or organic molecules. As an example, analytically useful MS-RTP has been observed from the Nb(V)-ferron complex solubilized in CTAB cationic micelles and in the presence of external heavy atom perturbers such as Br_3CH.[15] In the case of anionic surfactants such as SDS the counter cation can be easily replaced by a heavy element such as thallium, silver, or lead. Nugara and King[16] demonstrated that problems arising from precipitation of Tl^+-alkylsulfate salts at high Tl^+ concentration in MS-RTP solutions can be avoided by using mixed surfactant systems that include a short chain alkylsulfate.

C. Oxygen Removal

At room temperature, oxygen is the most persistent quencher of phosphorescence, and care must be taken to remove it from the solution. Micelles have been observed to diminish quenching by molecular oxygen;[17] however, deoxygenation of the micellar media is crucial to achieve consistent RTP measurements. Removal of oxygen can be achieved through a variety of means. Deoxygenation is commonly done by purging the sample with an inert gas, usually nitrogen or argon. In micellar media this method is time consuming and cumbersome due to the foam formed.

A method of sample deoxygenation, based on the principle of oxygen diffusion through a permeable membrane in conjunction with chemical scavenging of oxygen, has been also described.[18] A solution of the analyte is pumped through an oxygen-permeable tubing that is stored in a strongly reducing chromous sulfate, amalgamated zinc solution. The reactions produced were

$$Zn(Hg) + 2Cr^{3+} \leftrightarrow Zn^{2+} + Hg + 2Cr^{2+}$$
$$4Cr^{2+} + O_2 + 4H_3O^+ \leftrightarrow 4Cr^{3+} + 6H_2O$$

The method was fluorimetrically monitored using pyrene as the probe.

Chai and Danielsson[19] described two methods for the removal of oxygen in analytical flow systems. Deoxygenation was realized (1) by introducing N_2 into the stream to form a two-phase flow combined with a membrane gas-liquid separator, or (2) by passing the flow through a length of Poreflon membrane tubing placed in a water bath at 100ºC. These methods have not yet been applied in luminescence analysis.

Recently, new deoxygenation methods were proposed for cyclodextrin-induced room-temperature phosphorescence (CD-RTP) by using Zn(s)/HCl or Na_2CO_3/HCl based on the following reactions:[20]

$$Zn(s) + 2HCl \leftrightarrow ZnCl_2 + H_2\,(g)$$
$$Na_2CO_3 + 2HCl \leftrightarrow CO_2\,(g) + H_2O + 2NaCl$$

The gases (H_2 or CO_2) produced can remove the oxygen dissolved. The methods have been proven for deoxygenation of cyclodextrin solutions containing polycyclic aromatic hydrocarbons as well as their subtituents and azacycle compounds.

An additional deoxygenation technique that has been described is based on the use of sodium sulfite.[21] The method, based on the redox reaction $2SO_3^{2-} + O_2 \leftrightarrow 2SO_4^{2-}$, is simple and reliable. The analytical performance of MS- and vesicle-stabilized RTP was tested for the determination of metal ions[22-26] as well as for organic compounds,[21, 27] and the method can be extended to chemical deoxygenation of other organized media such as cyclodextrins[21] to develop RTP in solution. Nugara and King[16] reported that in cases where the sulfite ion is used as a chemical deoxygenating agent, light absorption by a Tl^+-SO_3^{2-} complex can severely attenuate the exciting radiation.

III. ANALYTICAL APPLICATIONS OF RTP METAL COMPLEXES IN ORDERED MEDIA

Metal complexes, including porphyrins, hydroxyquinolines, and phtalocyanines, phos-

phoresce in ordered media, providing opportunities for analysis of different species.

A. METAL IONS DETERMINATIONS

Ferron (7-iodo-8-hydroxyquinoline-5-sulfonic acid) has been exploited as a complexing agent in order to determine metal concentrations by micelle-stabilized room-temperature phosphorimetry.[22-25] Chemical deoxygentation by sodium sulfite was adopted.

Niobium (V) complexes of 8-hydroxyquinoline derivatives were studied in different micellar media in the presence of heavy atoms.[22] Phosphorescent signals were observed from those complexes where the reagent bears a sulfonic group in a cationic micellar media (CTAB) and using bromoform as external heavy atom source (see Figure 2). Strong and stable phosphorescent signals were attained by preirradiation of the samples with sunlight. Although two complexes of 1:1 and 1:3 stoichiometries were formed, the last constitutes the basis of the MS-RTP method for the determination of niobium.

$$Nb(V) + Ferron \xrightarrow{pH6} [Nb(V) - Ferron]_W \xrightarrow{CTAB}$$

Weakly fluorescent

$$[Nb(V) - Ferron]_{mic} \xrightarrow{Br_3CH,\ Na_2SO_3 + h\nu} [Nb(V) - Ferron]_{mic}$$

non – fluorescent *Phosphorescent*

Micellar room-temperature phosphorescence of aluminium-Ferron complex in CTAB micellar medium was also observed,[23] and the same reaction scheme proposed for the Nb-Ferron complex could apply to the aluminium-Ferron complex without the addition of bromoform. The iodide substituent, present in the Ferron molecule, acts as internal heavy atom.

When sulfite was used as the chemical oxygen scavenger for developing the MS-RTP of the niobium-Ferron complex, preirradiation with sunlight was necessary in order to obtain a high and stable signal. This affect was not observed for the aluminium complex. Such behavior was attributed to the presence of tartaric acid in niobium solutions.[22]

Room-temperature decay measurements offer a useful complementary information to emission data of phosphorescent metal chelates in micellar media.[24] The influence of about 30 metal ions on the phosphorescence and triplet state lifetime parameters of the aluminium-Ferron complex in CTAB media was reported. Metallic ions may be potential quenchers of the Al-chelate triplet state and the quenching mechanism seemed to be directly related to the particular chemical characteristics of the ions.

Cationic (CTAB) and nonionic (Brij-35) micellar media have been proven to be very efficient enhancing agents to the MS-RTP of the gallium- and indium-Ferron complexes.[25] Kalman filtering was used to deconvolute the phosphorescence spectra of mixtures of aluminium and gallium allowing the simultaneous determination of both metals.[25] Simultaneous time-resolved RTP determination of Ga (III) and In(III) with Ferron in presence of CTAB has been also reported.[27]

Apparently, in the above examples, the negatively charged metal is bound electrostatically to the cationic micelle and, probably by twisting of its long hydrocarbon-chains toward the bound complex, an effective micro-environment favorable to the existence of excited triplet states is created, so that the radiative phosphorescence process can compete with quenching and nonradiative processes. With nonionic micellar systems the electrostatic forces are absent so that hydrophobic and, perhaps, dipole-dipole interactions would account for the solubilization and stabilization of the excited state complexes in such media.

Anionic surfactants showed the smallest enhancing effect probably due to the electrostatic repulsion between anionic micelles and the negatively charged metal-Ferron complex (resulting from the $^{-}SO_3^{-}$ groups in the Ferron molecules of the chelate).

Analytical characteristics for the determination of metals in the cited systems are given in Table 2.

B. Cloud-Point Separation-RTP Determination

When an aqueous micellar solution of nonionic surfactant is heated above a certain tempera-

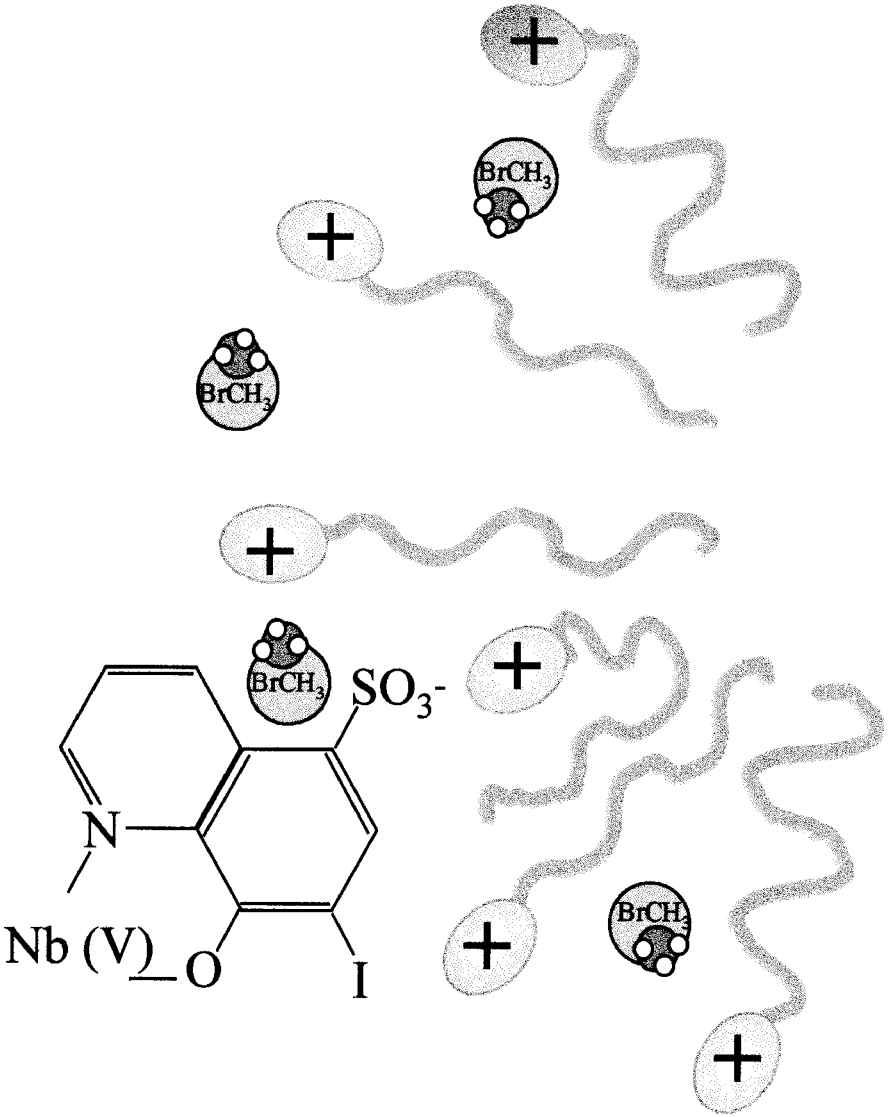

FIGURE 2. Nb(V)-Ferron complex in a cationic micellar medium.

TABLE 2
Analytical Features of Merit for RTP Metal Determination in the Metal-Ferron-CTAB System

Metal	λ_{ex} /nm	λ_{em} /nm	pH	Detection limit	RSD (%)	Ref.
Nb (V)	363	572	5.7	4 ppb[a]	2 (500 ppb)	22
Al (III)	386	586	5.5	5.4 ppb[a]	4.5 (20 ppb)	23
Al (III) (Vesicles)	386	586	5.5	19 ppb[b]	4.1 (400 ppb)	26
Ga (III)	396	615	5.5	5 ppb[b]	4 (50 ppb)	25

[a] $2s_B$ criterium (s_B, standard deviation of the blank).
[b] $3s_B$ criterium.

ture, the solution suddenly becomes turbid (cloud point). After a suitable equilibration time (centrifugation can speed up this step), the solution separates into two transparent liquid phases: a surfactant-rich phase and an aqueous solution phase. The cloud point methodology was used to concentrate the palladium (II) complex of Coproporphyrin III in a 6.4 wt. % Triton X-100 phase and RTP determination of 10^{-9} *M* levels of Pd (II) was obtained.[28] A long-lived fluorescence component, λ_{em} = 624 nm, attributed to delayed fluorescence was observed besides the normal fluorescence at λ_{em} = 622 nm. The palladium complex (Figure 3) showed strong RTP (λ_{em} = 666 nm) when the temperature was decreased. The room temperature phosphorescence measurements of the Pd (II)-Coproporphyrine III were achieved at 1ºC with λ_{ex} = 395 nm. The detection limit (signal to noise ratio 3) was 2 x 10^{-9} *M* (2.7 mg Pd^{2+}/ 15 ml) and the relative standard deviation was 2.1% for 1.6 x 10^{-6} *M* (1.6 μg Pd (II)/ 15 ml) sample solution.

The use of the cloud-point event is, in this case, advantageous because of the possibility of concentrate the metal ion and, simultaneously, allowed enhanced RTP detection in the surfactant rich phase. Future work in this area should focus on further development of extraction-RTP detection schemes that exploit the advantages of the methodology for the determination of a variety of substances, including metal ions, biological substances, and environmentally important compounds.

C. RTP Metal Chelates as Probes in Biological Systems

As chemical-ordered media, biological supramolecular assemblies such as proteins, nucleic acids, membranes, etc. provide a particularly useful microenvironment where analytes (molecules, metal complexes, metal ions) can be isolated or protected from the aqueous phase and placed in a hydrophobic environment. In these microdomains the molecular motions are highly restricted so that luminescence properties of incorporated species in those systems may be drastically modified.[29, 30] So, for example, it was reported that the binding of the Pd-meso-4-tetracarboxyphenyl)porphyrin complex to bovine serum albumin decreases k_q (second-order rate

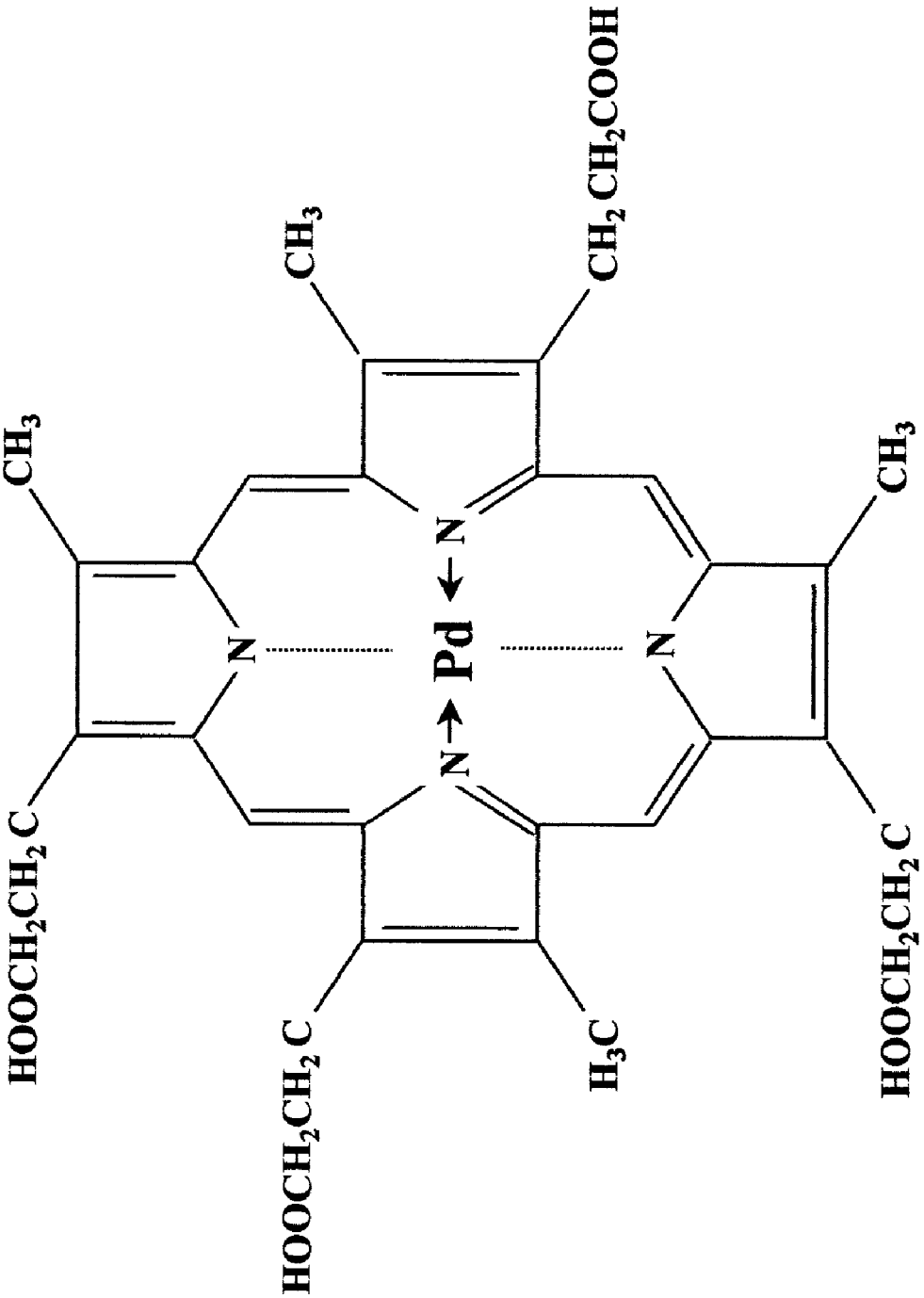

FIGURE 3. Coproporphirin III-Pd Complex.

quenching constant, related to the collision frequency between triplet-state probe molecules and the quencher) from about 3 x 10^9 dm^3 $mol^{-1}s^{-1}$ to about 3 x 10^8 dm^3 $mol^{-1}s^{-1}$.[31] This effect can be explained as due to the protection that the protein gives to the complex when the latter is placed inside a hydrophobic microdomain (Figure 4).

In order to observe RTP, chemical deoxygenation of biological macromolecular systems can be carried out using dithionite.[33] However, this chemical frequently damages proteins. Additional deoxygenation techniques that have been described for biological systems include the use of enzymatic systems such a mixture of glucose and catalytic amounts of glucose oxidase and catalase,[33, 34] according to the reactions:

$$Glu\cos e + O_2 + H_2O \xrightarrow{glu\cos eoxidase} GluconicAcid + H_2O$$

$$2H_2O_2 \xrightarrow{catalase} O_2 + H_2O$$

Another described method[33] to deplete oxygen in biological media is based on the ascorbate/ascorbate oxidase system that reduces the oxygen to water without generation of reactive oxygen species such as hydrogen peroxide: 2 ascorbate + $O_2 \rightarrow 2H_2O + 2 - dehydroascorbate.$ Affinities of the enzyme for both ascorbate and oxygen are enough high that oxygen can be reduced to very low pressures.[35]

Most of the RTP metal complexes used in biological studies at the present time are based on Pt- or Pd-porphyrin complexes, which have strong phosphorescence in the near infrared region (760 to 800 nm) and can be excited either at Soret (380 to 430 nm) or Q bands (500 to 560 nm). These complexes show a very high phosphorescence quantum yield even in an aqueous environment at room temperature. All these properties make the Pt- and Pd-porphyrin complexes very interesting room temperature probes for biochemical and/or medical studies (see Table 3).

So, the O_2-dependent quenching of the Pd-meso-tetra(4-carboxyphenyl)porphine complex has been used to measure the O_2 dependence of respiration by suspensions of cells and mitochondria[36,37] and to obtain two-dimensional maps of O_2 pressure in tissues.[38-40]

The basis of the measurement is the well known reaction of oxygen with excited states. It can be quantitatively described by the Stern Volmer relationship,

$$\frac{I^\circ}{I} = \frac{t^\circ}{t} = 1 + k_q t^\circ [O_2]$$

where t^o is the lifetime in the absence of oxygen and t is the lifetime at an oxygen concentration $[O_2]$. I^o and I are room temperature phosphorescence intensities in the absence and presence of oxygen. The quenching constant k_q is a diffusion-limited second-order rate constant. RTP lifetime is a more reliable measure of oxygen pressure than is phosphorescence intensity.

The phosphorescent probe was used as a 2:1 molecular complex with albumin during its calibration both *in vitro* and *in vivo* experiments. The binding site on albumin determines the microenvironment of the probe, and the lifetime measurements are independent of the concentration of the probe and of the intensity of the excitation light.

O_2 is the only quenching agent present in significant concentrations in blood, and the result is an optical method that provides an unambiguous measurement of O_2 pressure, as indicated by the fact that the lifetime of the probe in the absence of O_2 was the same in intact animal tissues as it was *in vitro* for the same temperature and pH.[40] Thus, the values of k_q and t^o determined *in vitro* hold for measurements *in vivo*.[34, 35, 41] RTP lifetime measurements also have the advantage that they are practically unaffected by changes in the absorbance of pigments present in the sample. Thus, for example, the measurements are independent of the oxygenation degree of myoglobin and haemoglobin.[42]

The analytical potential of RTP-metal porphyrin complexes for direct measurement of O_2 pressure in biological systems has been clearly demonstrated, but other biological and/or clinically important species can be also be determined. A selective determination of DNA in the presence of RNA was achieved using the RTP enhancement of a Pd-TMPyP complex bound to DNA.[43] The "switch-on" of RTP for Pd-TMPyP after binding to double-stranded DNA is concluded to be a result of its limited freedom of motion (Figure 5). This is inferred from a similar behavior in the microheterogeneous environment provided by

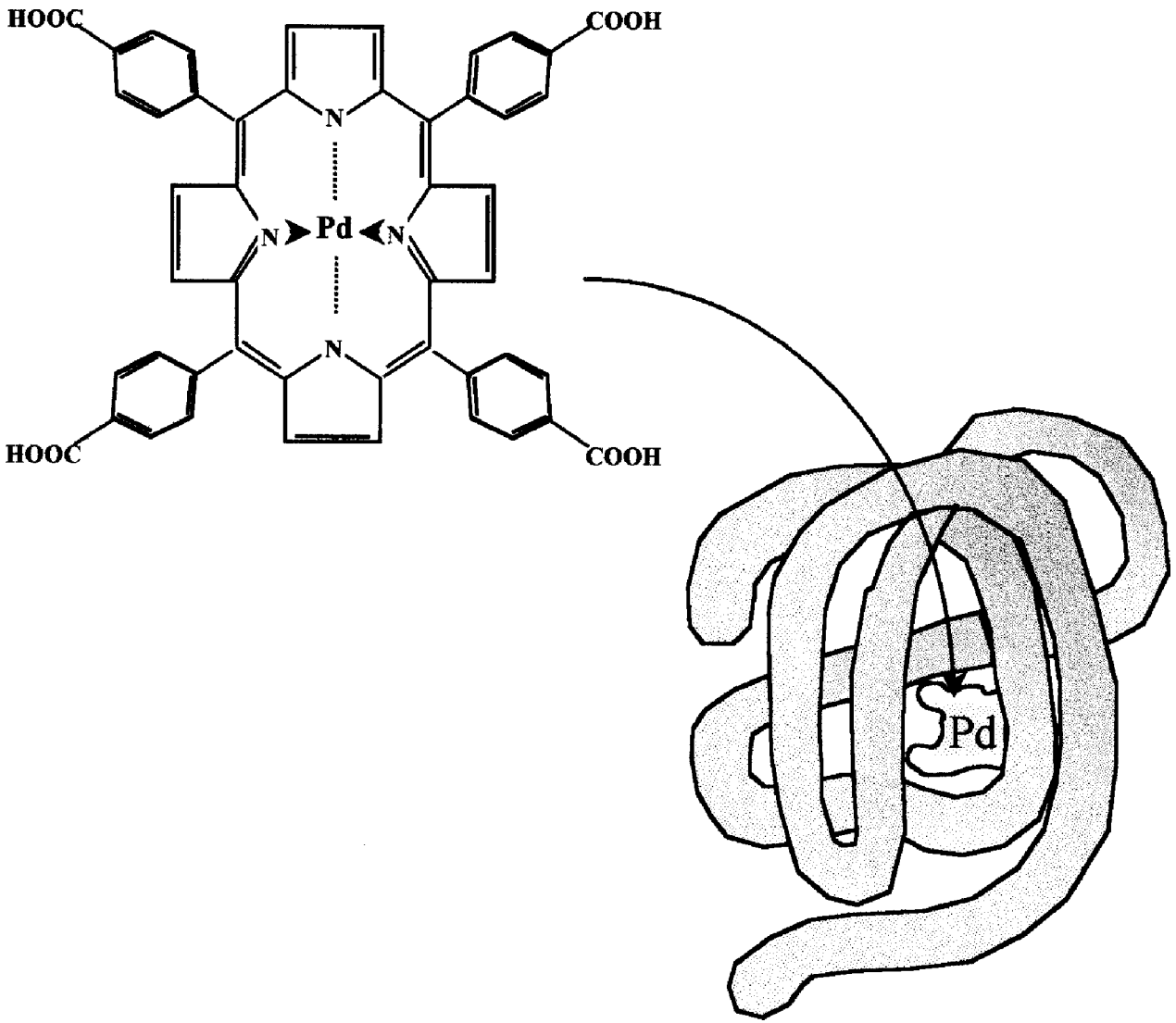

FIGURE 4. Pd-meso-tetra(4carboxyphenyl)porphine (PTP) in a protein domain.

Table 3
Phosphorescence Properties of Metalloporphyrines and Oxygen-Quenching Constants

Compound	*Medium*	λ_{ex} /nm	λ_{em} /nm	t^o /ms	k_q /$M^{-1}s^{-1}$	ϕ_p	*ref.*
Pd coproporphyrin	Aqueous, pH 7	380	667	0.53	3.8×10^9		34
	Aqueous, pH 7, 2% BSA	380	667	1.2	1.0×10^8		
Pd TSPP	Aqueous, pH 7	412	698	0.5	2.9×10^9		34
	Aqueous, pH 7, 2% BSA	412	698	1.0	4.1×10^8		
	Aqueous	412	695	0.35		0.007	32
Pd TMPP	Aqueous, pH 7	420	680	0.1			34
	Aqueous, pH 7, 2% ASB	420	680	0.79			34
	Aqueous solution	419	685	0.144		0.006	32
Pd TCPP	Aqueous			0.22 (DMF)	3×10^9		31
	Aqueous solution (10% DMF)	415	700	0.285			32
Lu coproporphyrin	Aqueous, pH 7	397	704	0.22	1.3×10^9		34
	Aqueous, pH 7, 2% BSA	397	704	1.5	3.1×10^8		
Lu TSPP	Aqueous, pH 7	416	760	0.73			
	Aqueous, pH 7, 2% BSA	416	760	1.0			34
Zn TSPP	Aqueous		769	1.4			34
	Aqueous, pH 7, 2% BSA	421	795	12.5			
	Aqueous		769	1.4		$< 10^{-4}$	32
Y TSPP	Aqueous, pH 7	424	775	0.9			34
	Aqueous, pH 7, 2% BSA	424	775	8.5			34
Pd $Ph_4(SO_3Na)_4TBP$	Aqueous, pH 7.2	631	790	0.31	2.6×10^9		31
	Aqueous, pH 7.2, 2% BSA	631	790	0.32	2.1×10^8		
Pd $Ph_4(PEG)_4TBP$	Aqueous, pH 7.2	634	793	0.26			31
	Aqueous, pH 7.2, 2% BSA	634	793	0.28			

TSPP: meso-tetra (4-sulfonato-phenyl)-porphine, TMPP: meso-tetra (N-methyl-4-pyridil)-porphine, TBP: tetra-benzo-porphyrin

TCPP: meso-tetra (4-carboxyphenyl)-porphyrin, BSA: bovine serum albumin, DMF: dimethylformamide

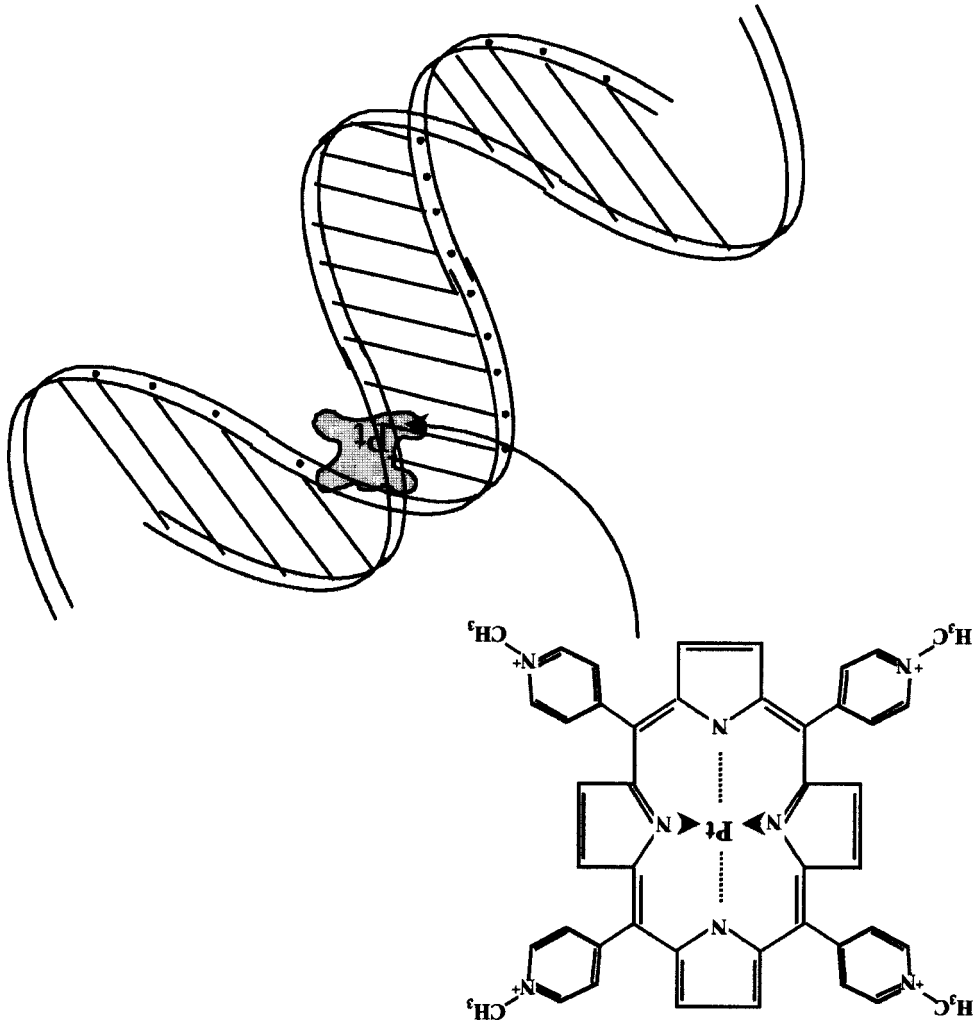

FIGURE 5. Pt-5,10,15,20-Tetrakis(1methyl-4-pyridyl)-21H,23H-porphine in a nucleic acid environment.

sodium lauryl sulfate micelles, which is both hydrophobic and negatively charged. The Pd-TMPyP complex bound to DNA exhibit maximum RTP at pH 7 with maximum excitation and emission wavelengths at 435 and 680 nm, respectively. Oxygen scavenging was carried out chemically using sodium sulfite. The calibration graphs were linear up to 6 x 10^{-5} μ*M* DNA. The detection limit was 5 x 10^{-8} μ*M* ($3\sigma_B$), and the relative standard deviation was 2.6% (3.4 x 10^{-5} μ*M* DNA). The method has been applied to DNA determination in tissues.

Papkovskii et al.[44] have described a flow injection method for the determination of glucose using either Pt^{2+}- or Pd^{2+}-coproporphyrin as internal room temperature phosphorescent oxygen sensor. The RTP is developed in the flow system using either non-ionic micelles of Tween-20 or fetal calf serum as oraganized medium. The calibration graphs were linear up to 50 m*M* glucose using Pt-coproporphyrin and up to 500 m*M* glucose when using Pd-coproporphyrin. The detection limit was 2 m*M* glucose, and the new method allowed the determination of glucose in biological fluids (e.g., serum).

In the same line, Papkovskii et al.[45] have recently demonstrated the use of a Pt-coproporphyrin RTP probe for optical sensing of sulfite, in the presence of bovine serum albumin as oraganized media. The analytical system is based on the RTP quenching by the sulfite and was adapted to a flow-through sensing system. The method allowed rapid on-line detection of sulfite with a detection limit of about 10 μ*M* and analytical linear range up to 10 m*M* sulfite. The new flow-through sensor was tested with real samples such as wines, juices, dried fruit, vinegar, etc. with excellent results.

Recently, the RTP complex Pd-coproporphyrin has been used as probe to develop a "universal phosphorescence immunoassay" format. The universal character of the amplified enzyme-linked immunosorbent assay (a-ELISA) is combined here with the high sensitivity and simplicity of a time-resolved RTP immunossay. The assay allows convenient determination of insulin.[46] Phosphorescence of the probe is enhanced in a micellar medium (Triton X-405) and chemical deoxygenation using Na_2SO_3 was carried out. The minimum concentration of insulin determined by the proposed assay was 6.67 x 10^{-10} *M*. The universal RTP immunoassay appeared to have higher sensitivity than other conventional insulin detection formats and can be extended to the determination of other antigens than insulin.[47] The technique allows very efficient time-discrimination of the background luminescence of biological samples.

Pt- and Pd-coproporphyrin complexes may also be used as labels for antibodies and (strep)avidin for application in time-resolved microcopy.[48] The metalloporphyrins were converted into an active *N*-hydroxysuccinimide ester by a 1,3-dicyclohexylcarbodiimide-mediated reaction in the presence of a catalyst (1-hydroxybenzotriazol). Then, the probes were coupled to the antibodies and (strep)avidin and subsequently tested for various immunocytochemical applications, including detection of DNA and RNA sequences by fluorescence *in situ* hybridization (FISH).

Many other potential uses of RTP-metal porphyrin complexes include applications such as image oxygen distribution in cancerous tissues (tumors, for example, are known to concentrate porphyrins) and in diagnosis of vascular disorders. The technique could be miniaturized and used for oxygen measurements within cells. However, and despite its great potential and its noninvasive nature, it is premature to suggest that such a method can be used clinically in its present state of knowledge.

IV. CONCLUSIONS

Ordered media as well as biological macromolecular systems provide an ideal media in which luminescent metal complexes are protected against collisional quenching, allowing them to develop RTP. Increasingly numerous examples have firmly demonstrated that the physical and chemical properties of these RTP metal complex/ordered media systems offer a great analytical potential in the signaling of a variety of analytes. The examples given illustrate that the subject is still in its infancy and is currently receiving a significant amount of attention, especially in medical and analytical fields. However, in its present state of

knowledge it is somewhat premature to predict that such a systems could be used for clinical purposes. It must be remembered that the success of the RTP technique among the consumers will depend on how it performs relative to others, principally fluorescence. The majority of the effort in the future is likely to be in transforming the demonstrated concepts into practical approaches. These include imaging *in vivo* oxygen pressure in tissues, immunological assays where the time discrimination of RTP provides a large technical advantage, studies of conformational dynamics in proteins yielding new information on proteins in solution and miniaturization of the technique. We hope that this overview of RTP metal complexes will stimulate researchers and students to consider this frontier field as a potential solution for the design of useful RTP systems, generating new ideas, especially for analytical, biophysical, and biochemical applications.

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REFERENCES

1. Schulman, S.G.; (Ed). *Molecular Luminescence Spectroscopy: Methods and Applications*. Part 1. Wiley & Sons. Inc., **1985**.
2. Hartley, G.S.; *Trans. Faraday Soc.*, **1935**, 31, 31.
3. Pelizzetti, E.; Pramauro, E.; *Anal. Chim. Acta*, **1985**, 169, 1.
4. Mittall, K.L.*; "Use of Surfactant and Micellar Systems in Analytical Chemistry"* in "Solution Chemistry Surfactants", K.L. Mittall (Ed), **1979**, Chap. 1, 79–127.
5. Fendler, J.H.; Fendler, E.J.; *"Catalysis in Micellar and Macromolecular Systems"*, Academic Press, New York, **1975**.
6. Georges, J. ; *Analusis*, **1985**, 17, 231.
7. Gehlen, M.H.; de Schryver, F.C.; *Chem. Rev.*, **1993**, 93, 199–221.
8. Cline Love, L.J.; Skrilec, M.; Habarta, J.G.; *Anal. Chem.*, **1980**, 52, 754.
9. Cline Love, L.J.; Skrilec, M.; *Am. Lab.*, **1981**, 13, 103.
10. McIntire, G.L; *Crit. Rev. in Anal. Chem.*, **1990**, 21, 257.
11. Von. Wandruszka, R.*; Crit. Rev. in Anal. Chem.*, **1992**, 23, 187.
12. Fendler, J.H.; *J. Phys. Chem.*, **1980**, 84, 1485.
13. Neal, S.L.; Villegas, M.M.; *Anal. Chem.*, **1995**, 67, 2659.
14. Hurtubise, R.J.; *"Phosphorimetry: Theory, Instrumentation and Applications"*, VCH, Verlagsgesellschaft, **1990**.
15. Sanz Medel, A.; Martínez García, P.L.; Díaz-García, M.E.; *Anal. Chem.*, **1987**, 59, 774.
16. Nugara, N.E.; King, A.D.; *Anal. Chem.*, **1989**, 61, 1431.
17. Hinze, W.L.; Singh, H.N.; Baba, Y.; Harvey, N.G.; *Trends in Anal. Chem.*, **1984**, 3, 193.
18. Rollie, M.E.; Ho, C.-N.; Warner, I.M.; *Anal. Chem.*, **1983**, 55, 2445.
19. Chai, X.-S.; Danielsson, G.; *Anal. Chim. Acta*, **1996**, 332, 31.
20. Zhang, H.R.; Zhang, J.; Wei, Y.S.; Jin, W.J.; Liu, C.S. ; *Anal. Chim. Acta*, **1997**, 357, 119.
21. Díaz-García, M.E.; Sanz Medel, A.; *Anal. Chem.*, **1986**, 58, 1436.
22. Sanz Medel, A.; Martínez García, P.L.; Díaz-García, M.E.; *Anal. Chem.*, **1987**, 59, 774.
23. Fernández de la Campa, MR.; Díaz-García, M.E.; Sanz-Medel, A.; *Anal. Chim. Acta*, **1988**, 212, 235.
24. Díaz-García, M.E.; Fernández de la Campa, M.R.; Hinze, W.L.; Sanz Medel, A.; *Mikrochim. Acta*, **1988**, III, 269.
25. Liu, Y.-M.; Fernández de la Campa, M.R.; Díaz-García, M.E.; Sanz Medel, A.; *Mikrochim. Acta*, **1991**, I, 53.
26. Fernández de la Campa, M.R.; Liu, Y.-M.; Díaz García, M.E.; Sanz Medel, A.; *Anal. Chim. Acta*, **1990**, 238, 297.
27. Liu, Y.-M.; García Alonso, J.I.; Fernández de la Campa, M.R.; Díaz-García, M.E.; Sanz Medel, A.; *Mikrochim. Acta*, **1991**, I, 199.
28. Igarashi, S.; Endo, K.; *Anal. Chim. Acta*, **1996**, 320, 133.
29. Latt, S.A.; Langlois, R.G.; *"Fluorescent probes of DNA microstructure and DNA synthesis" in "Flow Cytometry and Sorting"*. Wiley-Liss, Inc., **1990**. Chap. 14, 249-290.
30. Vanderkooi, J.M.; Berger, J.W.; *Biochim. Biophys. Acta*, **1989**, 976, 1.
31. Vinogradov, S.A.; Wilson, D.F.; *J. Chem. Soc. Perkin Trans.2*; **1995**, 103.
32. Kalyanasundaram, K.; Newman-Spallart, M.; *J. Phys. Chem.*, **1982**, 86, 5163-5169.
33. Englander, S.W.; Calhoun, D.B.; Englander, J.J.; *Anal. Biochem.*, **1987**, 161, 300.
34. Vanderkooi, J.M.; Maniara, G.; Green, T.J., Wilson, D.F.; *J. Biol. Chem.*, **1987**, 262, 5476.
35. Lo, L.-W.; Koch, C.J.; Wilson, D.F.; *Anal. Biochem.*, **1996**, 236, 153.

36. Robiolio, M.; Rumsey, W.L.; Wilson, D.F.; *Am. J. Physiol.*, **1989**, 256, C1207.
37. Wilson, D.F.; Rumsey, W.L.; Green, T.J.; Vanderkooi, J.M.; *J. Biol. Chem*., **1988**, 263, 2712.
38. Wilson, D.F.; Cerniglia, G.J.; *Cancer Res.*, **1992**, 52, 3988.
39. Rumsey, W.L.; Vanderkooi, J.M.; Wilson, D.F.; *Science*, **1988**, 241, 1649.
40. Wilson, D.F.; Pastuszko, A.; Di Giacomo, J.E.; Pawlowski, M.; Schneiderman, R.; Delivoria-Papadopoulos, A.M.; *J. Appl. Physiol*., **1991**, 70, 2691.
41. Sinaasappel, M.; Ince, C.; *J. Appl. Physiol*., **1996**, 81, 2297.
42. Rumsey, W.L.; Pawlowski, M.; Lejarvardi, N.; Wilson, D.F.*; Am. J. Physiol. (Hearth Circ.Physiol.35)*, **1980**, 266, H1676-1680.
43. Roza-Fernández, M.; Valencia González, M.J.; Díaz-García, M.E.; *Anal. Chem*., **1997**, 69, 2406.
44. Papkovskii, D.B.; Savitskii, A.P.; Yaropolov, A.I.; Ponomarev, G.V.; Rumyantseva, V.D.; Mironov, A.F.; *Biomedical Science*, **1991**, 2, 63.
45. Papkovskii, D.B.; Uskova, M.A.; Ponomarev, G.V.; Korpela, T.; Kulmala, S.; Guilbault, G.G.; *Anal. Chim. Acta*, **1998**, 374, 1.
46. Mantrova, E.Y.; Demcheva, M.V.; Savitsky, A.P.; *Anal. Biochem*., **1994**, 219, 109.
47. Demcheva, M.V.; Mantrova, E.Y.; Savitsky, A.P.; Behrsing, O.; Micheel, B.; Hemmila, I.; *Anal. Lett.*, **1995**, 28, 249.
48. De Haas, R.R.; Van Gijlswijk, R.P.M.; Van der Tol, E.B.; Zijlmans, H.J.M.A.A.; Bakker-Schut, T.; Bonnet, J.; Verwoerd, N.P.; Tanke, H.J.; *J. of Histochem. & Cytochem.*, **1997**, 45, 1279.