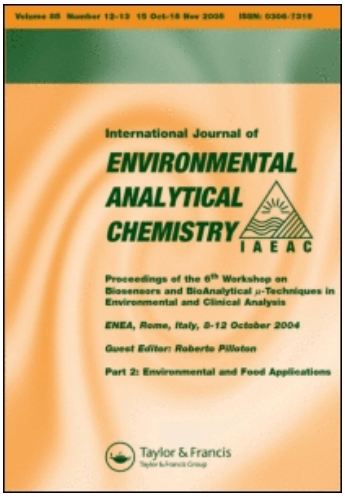

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Room Temperature Phosphorescence as a Liquid Chromatographic Detection Method for Polychlorinated Naphthalenes and Biphenyls in Complex Matrices†

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Quenched and sensitized room temperature phosphorescence techniques have been used for the detection of PCNs and PCBs after liquid chromatographic separation. The usefulness of these techniques to fingerprinting of commercial Aroclor and Halowax mixtures in complex matrices has been shown. The complementary nature of these detection modes yield valuable information in addition to UV detection. A signal inverter is proposed for linearization of the quenched RTPL signals. In this way linear calibration plots over more than two orders can be obtained. Detection limits are generally in the low nanogram or subnanogram concentration region. The application of RTPL detection techniques to the analysis of commercial PCN and PCB mixtures in surface water and urine is demonstrated. Pre-columns can be used to advantage for pre-concentration and clean-up of this type of samples.

KEY WORDS: PCBs, PCNs, Room temperature phosphorescence, HPLC, fingerprinting, pre-concentration, water, urine.

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INTRODUCTION

In earlier papers[1,2,3] we have introduced the use of room temperature phosphorescence in liquid solutions (RTPL) as a detection method in dynamic flow systems, particularly in liquid chromatography. Although RTPL has appeared to be a rare phenomenon, the few compounds exhibiting strong RTPL signals in various solvents can be utilized successfully to measure a variety of analytes in an indirect way, using energy transfer phenomena. For this purpose biacetyl has proven to be a very suitable compound.

In the liquid chromatographic experiments, biacetyl was added to the eluent (concentration around 10^{-4} M) and the analytes were detected either by measuring the sensitized or the partially quenched RTPL of biacetyl (see Figure 1). The former mode is, generally, suitable for analytes with triplet state energies higher than that of biacetyl. The latter mode can be used for compounds which are able

SCHEME I: SENSITIZED RTPL

(a) $^1A + h\nu_{ex}^A \longrightarrow {}^1A^*$

(b) $^1A^* \xrightarrow{k_{isc}^A} {}^3A^*$

(c) $^3A^* + {}^1B \xrightarrow{k_t} {}^1A + {}^3B^*$

(d) $^3B^* \xrightarrow{k_p^B} {}^1B + h\nu_p^B$

SCHEME II: QUENCHED RTPL

(e) $^1B + h\nu_{ex}^B \longrightarrow {}^1B^*$

(f) $^1B^* \xrightarrow{k_{isc}^B} {}^3B^*$

(g_1) $^3B^* + {}^1A \xrightarrow{k_Q} {}^1B + {}^1A + \text{heat}$

(g_2) $^3B^* \xrightarrow{k_p^B} {}^1B + h\nu_p^B$

FIGURE 1 Sequence of processes leading to sensitized RTPL (scheme I) and quenched RTPL (scheme II).

to react rapidly with biacetyl in the excited state via electron transfer, hydrogen abstraction or energy transfer. In view of these possibilities, in our opinion, detection based on RTPL can be considered as a method with a good potential in analytical chemistry. However, the general opinion about phosphorescence in liquid samples as a phenomenon which can only occur under stringent experimental conditions still forms a considerable barrier to a wider acceptance of RTPL as a detection technique.

In the present paper the attention is focused on the detection of polychlorinated naphthalenes (PCNs) and polychlorobiphenyls (PCBs). These compounds are also used to demonstrate the selectivity of the two RTPL detection modes by applying RTPL to their determination in complex environmental and bioanalytical samples.

Both PCNs and PCBs give good response by sensitized RTPL of biacetyl. The PCNs can also be studied by quenched RTPL of biacetyl. For these latter compounds, depending on the degree of chlorination and the position of the substituents, the triplet state energies do not differ much from the triplet state energy of biacetyl. A particular PCN is hence able to sensitize or to quench the biacetyl phosphorescence. It has been shown[4] earlier that for small energy differences between analyte and biacetyl the reversed energy transfer is taking place. In the present paper the effect of a reversed energy transfer in the quenched RTPL mode is discussed. It is demonstrated that a linear response can be obtained by recording an inverted phosphorescence signal.

THEORETICAL ASPECTS

The PCBs can be detected by sensitized but not by quenched RTPL, since their triplet state energies are distinctly higher than for biacetyl. The sequence of processes leading to sensitized RTPL is given in Scheme I, Figure 1. (a) A proper excitation wavelength λ_{ex} is chosen to bring the PCB (denoted as analyte A) into the excited state. (b) Subsequently, the excited PCB goes via intersystem crossing to its lowest triplet state ($^3A^*$). (c) Thereafter the crucial step takes place. Before being deactivated, upon collision of $^3A^*$ and a biacetyl molecule present as a solute in the eluent, energy transfer takes place. As a result biacetyl in its lowest triplet state (denoted as $^3B^*$)

is produced. (d) The phosphorescence of biacetyl is recorded at an emission wavelength around 520 nm. It is noted that reaction (c) can be considered as unidirectional if the energy difference between $^3A^*$ and $^3B^*$ is larger than 3 to 5 kcal mole^{-1}.[5]

The intensity of the sensitized RTPL signal, $I(sens)$, obtained in this way, is determined by the efficiencies of the 4 steps in Scheme I, i.e.,

$$I(sens) = I^A_{abs} \cdot \theta^A_{isc} \cdot \theta^{AB}_t \cdot \theta^B_p \tag{1}$$

where I^A_{abs} is the rate of light absorption by the analyte, step (a), and the other terms are the efficiencies of the reactions (b), (c) and (d) respectively. Since I^A_{abs} is the only term in Eq. (1) that depends on the analyte concentration, $[A]$, $I(sens)$ is a linear function of $[A]$. The signal obtained for a particular PCB depends strongly on θ^{AB}_t, the energy transfer efficiency. PCBs substituted at the 2-position for example are deactivated so rapidly that the chance for an energy transfer to biacetyl is very low: hence they give only a weak HPLC signal. This provides an interesting selectivity aspect when analyzing complex mixtures.

For many PCNs, the energy difference between $^3A^*$ and $^3B^*$ is so small that instead of reaction (c) in Scheme I (Figure 1) the following equilibrium has to be considered, i.e.

$$^3A^* + {}^1B \underset{k_{-t}}{\overset{k_t}{\rightleftharpoons}} {}^1A + {}^3B^* \tag{2}$$

where k_t and k_{-t} are bimolecular rate constants. Of course, the reversed energy transfer influences the sensitized RTPL signal. It has been shown[4] that instead of θ^{AB}_t an effective energy transfer efficiency $\theta^{AB}_{t,\,\mathrm{eff}}$ must be used in Eq. (1) where

$$\theta^{AB}_{t,\,\mathrm{eff}} = \frac{k_t \tau^A_0 [B]}{1 + k_{-t} \tau^B_0 [A] + k_t \tau^A_0 [B]} \tag{3}$$

τ^A_0 and τ^B_0 are the respective triplet lifetimes in absence of energy transfer between the analyte and biacetyl and $[B]$ is the biacetyl concentration. The term $k_{-t}\tau^B_0[A]$, reflecting the influence of the energy back transfer, makes the transfer efficiency dependent on the

analyte concentration so that $I(sens)$ is no longer a linear function of $[A]$. This has been confirmed experimentally.[4]

Because of the small energy gap between $^3A^*$ and $^3B^*$, mixtures of PCNs can also be successfully studied by quenched RTPL. In this mode, following scheme II in Figure 1, biacetyl is excited directly with an excitation wavelength around 415 nm (e). Subsequently it goes to its triplet state $^3B^*$ (f) and its phosphorescence is recorded (g_2). The presence of analytes able to react rapidly with $^3B^*$ according to (g_1) is established by recording the decrease of the biacetyl phosphorescence signal. The reversed energy transfer in Eq. (2) can be considered as a special case of (g_1); k_Q will be equal to k_{-t} provided that k_t is negligible.

The relationship between the phosphorescence signal intensity and the concentration of quenching analytes is readily formulated. In absence of quencher, the direct phosphorescence intensity of biacetyl, $I(dir)$, can be expressed as

$$I(dir) = I^B_{abs} \cdot \theta^B_{isc} \cdot \theta^B_p \tag{4}$$

where I^B_{abs} is the rate of light absorption by biacetyl and the other terms represent the efficiencies of (f) and (g_2).

If quenching analytes are present, $I(dir)$ reduces to $I'(dir)$ where

$$\frac{I(dir)}{I'(dir)} = 1 + k_Q \tau^B_0 [A] \tag{5}$$

similar to the well-known Stern–Volmer expression for fluorescence. According to Eq. (5), $I'(dir)^{-1}$ is linearly dependent on $[A]$. This implies that the quenched RTPL detector is linear provided that the inverted (partially) quenched signal is recorded.

The question arises whether the quenched RTPL detection method is also linear if the reversible reaction as given in Eq. (2) is responsible for the partial quenching of the biacetyl phosphorescence. In general, if the influence of k_t must also be taken into account, as applied for PCNs, k_Q is given by

$$k_Q = k_{-t,\,\mathrm{eff}} = \frac{k_{-t}}{1 + k_t \tau^A_0 [B]} \tag{6}$$

where $k_{-t,\,\mathrm{eff}}$ is considered as an effective rate constant.

Eq. (6) shows that k_Q is independent from $[A]$. This means that the quenched RTPL detector, based on the measurement of $I'(dir)^{-1}$, is also linear under conditions where reversible energy transfer occurs. However, whereas the linearity is unaffected, the sensitivity of the detector decreases with increasing k_t.

EXPERIMENTAL

Apparatus

The chromatographic system used for the on-line pre-concentration experiments is depicted in Figure 2. As published before,[1] the separation module forms a closed system. It consists of an eluent vessel, described in ref. 2, a LC pump (pump A), type 410 (Kontron, Zürich, Switzerland), an injection valve, type 70–10 (Rheodyne, Berkeley, CA, USA), and a valve (1) to remove the injected samples. The detector was a fluorimeter type SFM 22 (Kontron), provided with a 20 μl flow-cell and a red-sensitive photomultiplier, type R446-F (Hamamatsu, Berkeley, CA, USA). Maximum voltage (825 V) is applied to this photomultiplier during the measurements.

For the UV detected chromatograms the fluorimeter was replaced by a UV detector type LC 3 UV (Pye Unicam, Cambridge, UK).

The separations were carried out on a RP-8 column, length 10 cm, $d_p = 5\,\mu$, I.D. = 4.6 mm. The deoxygenation of the eluent was performed by means of nitrogen gas with oxygen content < 0.1 ppm, cleaned over pyrophorous copper. In Figure 2A the preconcentration part is depicted. The sample vessel is a 250 ml washing bottle equipped with a nitrogen gas deoxygenation unit. The sample solution is pumped with a pump, type TW-1515 (Orlita, Giessen, GFR) through a precolumn of 2 mm length and 4.6 mm I.D. designed in our laboratory[6] and filled with 5 μm RP-8 reversed phase material (Merck, Darmstadt, GFR). The precolumn is installed in the injection loop position. After loading the precolumn, the sample is directly transferred onto the analysis column by switching the Rheodyne valve and pumping eluent through pump A.

The signal inverter, placed between the spectrometer and the recorder, type BD-40 (Kipp, Delft, the Netherlands) is a multiplier/divider (M/D) system (Elliott) which transforms the spectrometer output E_{sp} in the range from 100 to 30 mV to E_{out}

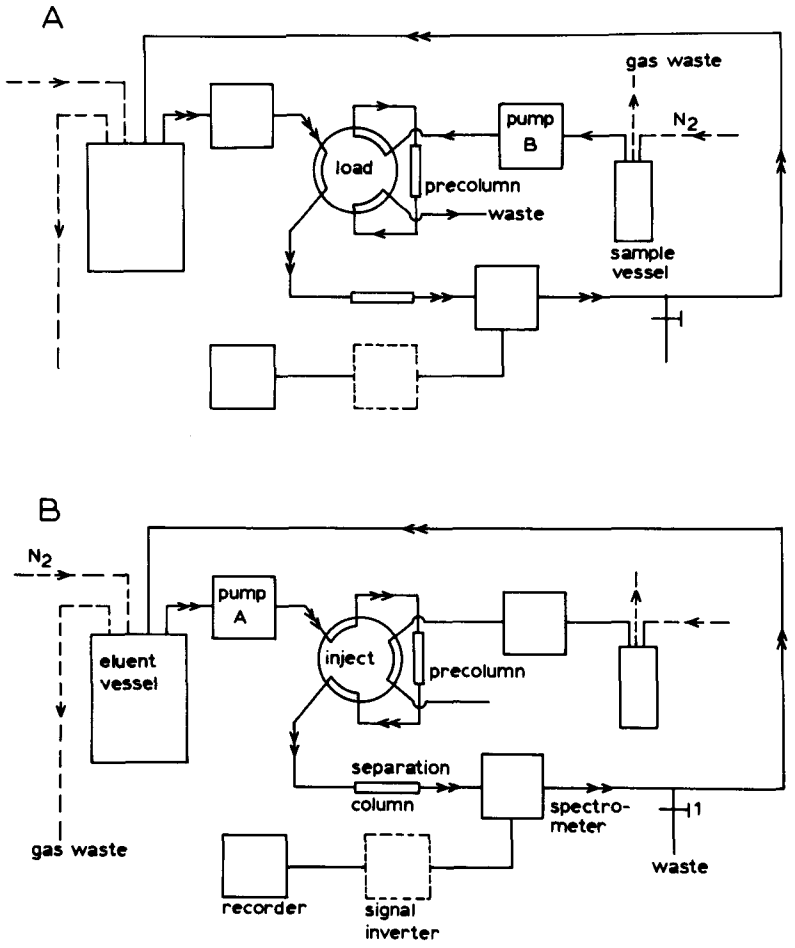

FIGURE 2 Chromatographic system with RTPL detection for on-line preconcentration experiments. Dashed lines indicate (brass) nitrogen gas capillaries (I.D. 1/16″) and solid lines correspond to stainless steel liquid capillaries (I.D. 1/8″ before pump A and 1/16″ after pump A).

A: preconcentration step; B: separation step. Valve 1 belonged to a model 332 Programmable Gradient System (Beckman, Mijdrecht, The Netherlands). The other parts are mentioned in the text.

according to

$$E_{out}(\text{mV}) = 5 \times 10^4 E_{sp}^{-1} \quad (7)$$

where E_{sp} is expressed in mV. For E_{sp} values smaller than 30 mV, E_{out} is no longer linearly dependent on E_{sp}^{-1} since a threshold potential is applied to protect the M/D system against division by zero. A larger linear range will be obtained if the threshold potential is decreased.

Finally it should be noted that the only difference between sensitized and quenched RTPL detection is the choice of the excitation wavelength. Of course our set-up can also be applied for fluorescence detection.

Chemicals and reagents

1,4-dichloronaphthalene (1,4-Cl_2N) was prepared from 1,4-dinitronaphthalene as described by Brinkman *et al.*,[7] 1-chloronaphthalene (1-ClN) (Fluka AG, Buchs, Switzerland) and the other PCNs, which were gifts from B.D. Geer (Montana State University, Bozeman, MO, USA) to U.A.Th. Brinkman were used as supplied. The pure PCBs, gifts from O. Hutzinger (University of Amsterdam, Amsterdam, The Netherlands), were also tested without further purification. The same holds for the Halowax mixtures (Koppers, Pittsburgh, PA, USA) and the Aroclor mixtures (Monsanto, St. Louis, MO, USA).

As described,[1] the azeotropic acetonitrile/water mixture consisted of acetonitrile p.a. (Baker, Deventer, The Netherlands) and distilled water in the ratio 83.7/16.3 (v/v). After distillation of this mixture biacetyl (Merck, Darmstadt, GFR) was added (1.0×10^{-4} M). Pure water was obtained by distillation of demineralized water. Tapwater and urine were filtered through a Millipore filter before use.

The urine samples were prepared as follows: In a 10 ml sample tube 3 ml of fresh urine was diluted with 2.5 ml water and 0.5 ml of alcohol (the latter to increase the solubility of the aromatics). These solutions were subsequently spiked with PCNs or PCBs.

The analysis of tapwater samples

First, the sample vessel is filled with pure water, which is deoxygenated by means of nitrogen gas. This is pumped with pump

B through the precolumn to remove traces of organic solvents and oxygen (Rheodyne valve in load position). Subsequently the sample vessel is filled with the sample solution (tapwater, spiked with PCNs), which in turn is deoxygenated. During 10 minutes, the deoxygenated sample solution is pumped through the precolumn (2.5 ml/min).

After this procedure the preconcentrated PCNs are transferred on-line onto the analysis column. While the chromatographic zones are passing through the detector, valve 1 is opened for the removal of these zones. In this way contamination of the eluent is prevented. This same function can of course be automated.

Analysis of the urine samples

For these experiments the construction of the dynamic system with the injection part as described before[1] is used. Here the gas waste as depicted in Figure 2B is used to deoxygenate the sample present in a 10 ml sample tube. This is subsequently loaded into the injection loop (20 μl) by means of a hypodermic syringe and injected.

Precolumn technology to protect the analysis column and for preconcentration and clean-up was not applied in this study but is well possible. The chromatographic conditions utilized for the investigation of the Halowaxes 1001 and 1099 and the Aroclor 1242 are described under Legends of Figure 3.

RESULTS AND DISCUSSION

Chromatography of PCN mixtures

In Figures 3A to F chromatograms of the commercial polychlorinated naphthalene mixtures Halowax 1001 and 1099 as detected by UV and RTPL modes are shown. Whereas with UV detection (Figures 3A and B) a distinction of the two mixtures is hardly possible, such a differentiation can be done more easily with both the sensitized and the quenched RTPL mode, as can be seen from Figures 3C, D and 3E, F respectively.

—The 1001 mixture contains traces of 1-ClN (peak 1) only detectable by sensitized RTPL; this peak is absent for the 1099 mixture.

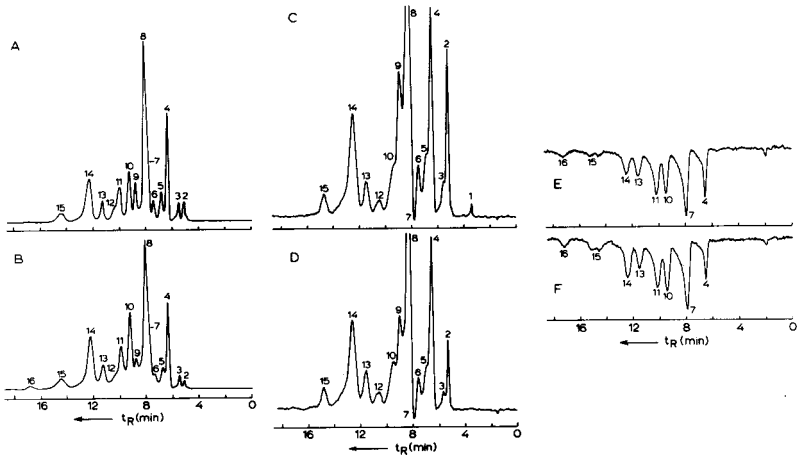

FIGURE 3 Chromatograms of Halowax 1001 (A, C and E) and Halowax 1099 (B, D and F). Eluent: acetonitrile/water (83.7/16.3 v/v) + 1.0×10^{-4} M biacetyl; flowrate 1 ml/min; column: RP-18, length 11 cm, I.D. = 4.6 mm; $d_p = 5\,\mu$m.

A and B: detected chromatograms, 0.32 aufs, $\lambda_{max} = 233$ nm; C and D: by sensitized RTPL detection $\lambda_{ex} = 300$ nm; E and F: by quenched RTPL detection $\lambda_{ex} = 420$ nm, $\lambda_{em} = 520$ nm. Identified peaks are given in Table I. Injected amount of sample of all chromatograms: 1 μg.

—In the sensitized RTPL mode peak 2, which can be attributed to a mixture of 1,3- 1,4- and 1,5-Cl_2N, is distinctly more intense for Halowax 1001 than for 1099 (Figures 3C, D).

—A comparison of the chromatograms detected in the quenched mode (Figures 3E, F) shows that Halowax 1099 contains a higher fraction of highly chlorinated PCNs since peaks 10–16 are more intense than for Halowax 1001.

Another interesting aspect is the complementary nature of the information which can be gathered from the UV and the sensitized and quenched RTPL detection modes respectively. Some of this is exemplified below:

—The UV chromatogram shows comparable intensities for peaks 2 and 3. However, in the sensitized RTPL chromatograms peak 3 is much weaker, so that we can conclude that this unknown PCN has poor sensitizing properties. In the quenched RTPL mode these PCNs are not detectable at all.

—An assignment of the hardly resolved peaks 7 and 8 in Figure 3A/B can be done simply on account of Figures 3C to F. Peak 7 is pronounced in the quenched RTPL chromatograms; it can be attributed to 1,4,5,8-Cl_4N,[2,7] a compound with strong quenching properties. Peak 8 is very strong in the sensitized RTPL chromatograms; it must be ascribed to 1,4,6-Cl_3N.

—Also the peak intensities of 9, 10, 11 and 12 (the last one being hardly detectable in the UV chromatogram) have different sensitivities in sensitized and quenched RTPL detection: peaks 10 and 11 represent quenchers but 9 and 12 sensitizers.

—Figures 3E and F show that the broad peak 15 in UV is composed of at least two compounds. In sensitized RTPL only one component is detectable.

In the chromatograms of Figure 3 not all peaks could be identified due to the lack of definite standards. Those which can be assigned with reasonable certainty are listed in Table I. Other data for PCNs, separated on RP-8 reversed phase material are also given in Table II. The two tables also give detection limits (LOD values) under these chromatographic conditions and in Table II a comparison of the detection limits for the quenched and sensitized mode are shown. The quenched mode is, with few exceptions, more than one order of magnitude less sensitive, which is not surprising when looking at $k_{-t,\,\mathrm{eff}}$ values in Table II. These are at least one order below the value expected for a diffusion controlled process.

LC of PCB mixtures

The UV- and sensitized RTPL-detected chromatograms of the PCB mixtures Aroclor 1242, separated on a RP-18 column are depicted in Figure 4; relevant chromatographic data are assembled in Table I. As mentioned under theoretical for PCBs quenched RTPL detection cannot be applied.

The selectivity of the sensitized RTPL detection method for PCBs is clearly seen. The number of peaks of Figure 4B is far smaller than in Figure 4A. Furthermore, peaks hardly detectable by UV at the chosen wavelength (e.g. peak 2) are strong in the sensitized RTPL detected chromatogram, and vice versa (e.g. peak 4).

This interesting selectivity has been mentioned under "theoretical" and is specific for PCBs substituted at the 2 position due to their

TABLE I

Chromatographic data of the sensitized RTPL method for some PCBs and PCNs[a], recorded on a RP-18 column: length 11 cm; I.D. = 4.6 mm; $d_p = 5\,\mu$m. Eluent: acetonitrile/water azeotropic mixture (83.7/16.3 v/v) + 1.0×10^{-4} M biacetyl.

Probable peak number	analyte	t_R(min)	λ_{ex}(nm)	LOD(ng)
Figure 3:1	1-ClN	3.4	285	0.28
2	1,3-Cl_2N	5.1	290	1.20
2	1,4-Cl_2N	5.1	295	0.41
2	1,5-Cl_2N	5.2	290	0.37
4	1,3,8-Cl_3N	6.4	300	0.60
8	1,4,6-Cl_3N	8.1	300	1.20
Figure 4:1	2-ClB	3.0	255	9.4
2	3-ClB	3.5	260	0.42
2	4-ClB	3.6	265	0.31
4	2,2′-Cl_2B	4.0	265	—
6	3,4-Cl_2B	4.55	265	5.6
8	3,5-Cl_2B	5.1	265	0.62
6	4,4′-Cl_2B	4.4	270	0.21
6	3,3′-Cl_2B	4.5	260	0.80

[a]Published earlier in ref. 2.

TABLE II

Quenching rate constants and chromatographic data for some tri- and tetrachloronaphthalenes. Eluent: acetonitrile/water azeotropic mixture + 1.0×10^{-4} M biacetyl. Column: RP-8; length 10 cm; I.D. = 4.6 mm; $d_p = 5\,\mu$m.

PCN	k_{-t}($M^{-1}s^{-1}$)[a]	$k_{-t,\mathrm{eff}}$[a] ($M^{-1}s^{-1}$) [B] = 10^{-4} M	t_R(min)	Quenched mode LOD(ng)[b]	Sensitized mode λ_{ex}[c]	Sensitized mode LOD(ng)
1,3,8-Cl_3N	7.6×10^8	2.7×10^8	3.60	8	300	0.45
1,4,6-Cl_3N	5.8×10^7	2.8×10^7	4.15	±230	300	1.5
1,2,3,4-Cl_4N	5.5×10^8	2.4×10^8	4.90	36	—	—
1,3,5,7-Cl_4N	4.3×10^8	2.0×10^8	5.80	66	300	1.2
1,4,6,7-Cl_4N	1.1×10^9	4.7×10^8	5.40	21	305	0.8
1,3,5,8-Cl_4N	2×10^9	2×10^9	4.95	2.0	310	1.8

[a]Data from ref. 4.
[b]These LODs are based on a three to one signal to noise ratio; injection volume 20 µl.
[c]Data from ref. 1.

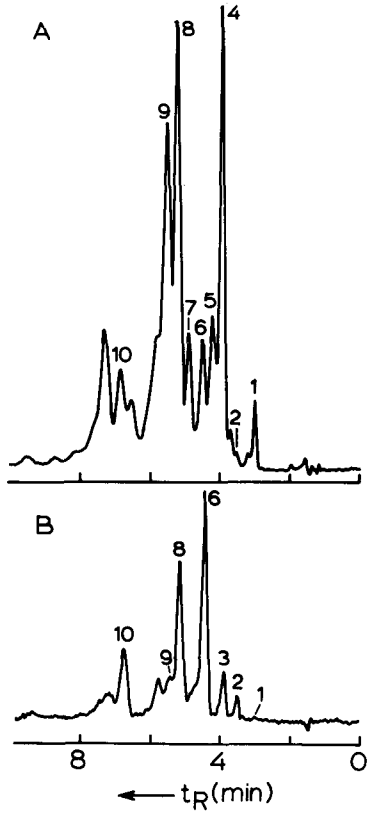

FIGURE 4 Chromatograms of Aroclor 1242. Chromatographic conditions as in Figure 3. A: by UV detection; 0.08 aufs, $\lambda_{max} = 244$ nm, concentration of the sample solution 50 ppm; B: by sensitized RTPL detection, $\lambda_{ex} = 260$ nm, concentration of the sample solution 10 ppm. Identified peaks are given in Table I.

short triplet lifetimes, hence energy transfer to biacetyl is very inefficient. As a consequence these PCBs have poor sensitivities. This explains why peak 1 (2-ClB) and peak 4 (2,2′-Cl_2B) are hardly or not observable in Figure 4B. On the other hand peak 6 (probably to be attributed to 4,4′-Cl_2B), which is relatively weak in the UV chromatogram, is sensitively detected by sensitized RTPL.

We can conclude that a combination of UV detection with sensitized RTPL detection provides an interesting element of selectivity especially with respect to fingerprinting techniques.

The linearity of the quenched RTPL method

The quenched RTPL detector as applied in Figure 3E and 3F, where the decrease of the biacetyl phosphorescence is a measure for the amount of analyte has no linear response. On theoretical grounds, such a non-linearity must be expected irrespective of the mechanism responsible for the quenching.

It was of interest to us to develop a quenched RTPL detector with a linear response, for instance for the determination of the total amount of quenching PCNs in a sample via integration procedures.

Equation (5) shows that a linear response can be expected if the inverted partially quenched signal is recorded because $I'(dir)^{-1}$ is proportional to the quenching analyte concentration. Furthermore the theoretical considerations imply that the presence of a reversible energy transfer, as is involved by the PCNs does not disturb this linearity.

The experimental results confirm these expectations. This is demonstrated for 1,4,6,7-Cl_4N in Figure 5. The dashed line

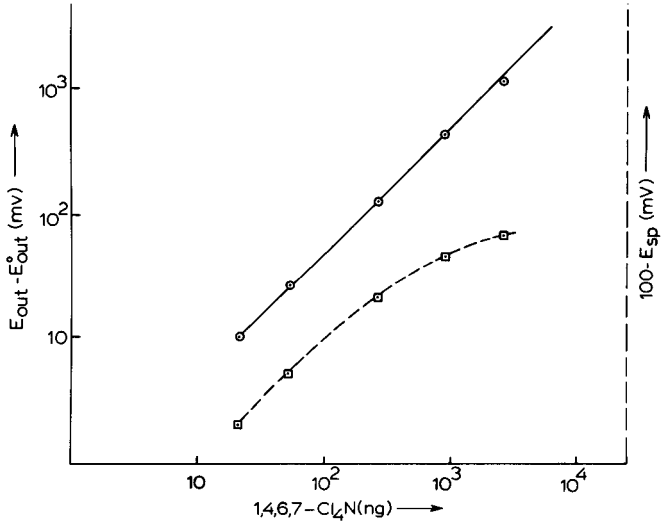

FIGURE 5 The linearity of the quenched RTPL method with signal inversion (solid line) and without signal inversion (dashed line). The peak intensity (peak height) of the non-inverted signal corresponds to $E^0_{sp}-E_{sp}$ (in mV). E^0_{sp}, the unquenched phosphorescence intensity corrected for background luminescence, is set equal to 100.0 mV.

represents the decrease of the spectrometer output versus the amount of analyte on a log-log scale. The solid line, showing the inverted signal coming from the M/D system, expressed as $E_{out} - E^0_{out}$, as a function of analyte concentration on a log-log scale, is linear with slope 1.00. The plots correspond to injected amounts retarded on a RP-8 column and the E-values were calculated from peak heights. The linear range obtained amounts to at least two decades. A further extension of this range can be achieved by improving the quality of the M/D system. A similar performance was observed for the other PCNs under study.

RTPL-detection applied to complex matrices

Equations (1) and (5) imply that both $I(sens)$, the intensity of the sensitized RTPL signal, and $I'(dir)^{-1}$, the intensity of the quenched RTPL signal after inversion, are proportional to τ_0^B, the triplet lifetime of biacetyl *in absence of analyte*. Hence, the sensitivities attainable for both modes of phosphorescence detection depend strongly on the amount of oxygen and impurities present in the solution since

$$\tau_0^B = 1/\left(k_p^B + k_{np}^B + \sum_{Q'} k_{Q'}[Q']\right) \tag{8}$$

The summation over Q' is over all quenching impurities, exclusive of the analyte; $k_{Q'}$ is the associated bimolecular rate constant. k_p^B and k_{np}^B are the respective intramolecular rate constants of radiative and nonradiative deactivation of the biacetyl triplet state.

Of course generally complex matrices contain a lot of ionic and/or organic substances quenching the biacetyl phosphorescence. Therefore, in order to apply sensitized and particularly quenched RTPL detection, the simultaneous presence of impurities and analytes in the flow-cell of the detector must be avoided, which is in essence, a chromatographic problem. With sensitized RTPL this problem is somewhat less pronounced, since similar to fluorescence the excitation wavelength is an extra selectivity parameter in this mode.

To exemplify the above mentioned aspect we have examined the applicability of RTPL detection for two systems, i.e., (i) tapwater, spiked with traces of PCNs, after preconcentration on a RP-8

precolumn (length 2 mm) and (ii) urine samples, spiked with PCNs and PCBs. The specific problems encountered will be discussed successively.

Detection of PCNs in tapwater. The role of the matrix is most simply seen by measuring its effect on the direct phosphorescence signal of biacetyl ($\lambda_{ex} = 415$ nm, $\lambda_{em} = 520$ nm), see Eqs. (4) and (8). For this purpose the tapwater was pumped through the precolumn (with a defined velocity during a defined time) and subsequently the analytes were transferred on-line onto the RP-8 column (eluent: azeotropic acetonitrile/water mixture with 10^{-4} M biacetyl).

It was found that under neutral pH conditions crystallization takes place, causing clogging of the capillaries. This difficulty was overcome by acidification of the tapwater to pH = 1.3 with $HClO_4$.

Figure 6 shows the chromatogram of 2 μg Halowax 1014 separated on a RP-8 column, and preconcentrated from 25 ml acidified tapwater. This mixture, mainly consisting of highly

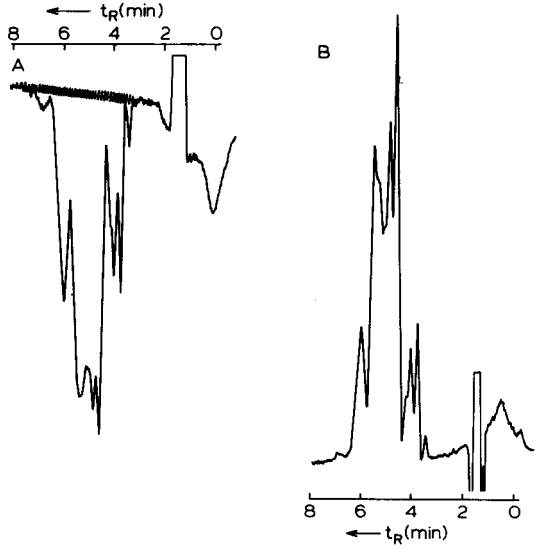

FIGURE 6 Quenched RTPL chromatograms of 2 μg Halowax 1014, preconcentrated from 25 ml tapwater and separated on a RP-8 column, length 10 cm; $d_p = 5$ μm, I.D. = 4.6 mm. Other chromatographic conditions as in Figure 3. A: without signal inversion, B: with signal inversion. The dashed line in A indicates a blank run.

chlorinated naphthalenes, can be appropriately detected by quenched RTPL.[2] In Figure 6A the direct signal is recorded without inversion. The positive signal with maximum intensity at $t_r = 1.35$ minutes must be ascribed to impurities emitting radiation (probably fluorescence) upon excitation at 415 nm. The important point is, that after $t_r = 2.0$ minutes the original $I(dir)$ value is reached again, as can be seen from a blank run in Figure 6A, demonstrating that in this part of the chromatogram impurities do not play any role. This implies that analytes present in tapwater with retention times longer than 2 minutes (under the chromatographic conditions at hand) can be detected with the same sensitivities as would be obtained in pure water. A comparison with Figure 6A reveals that, although the chromatographic patterns are similar, the most intense peaks are more pronounced in Figure 6B. This is not surprising since, in contrast to $I'(dir)^{-1}$, the decrease of $I(dir)$ to $I'(dir)$ is not linearly dependent on the analyte concentration.[3]

The relatively low resolution in Figure 6 may have to be improved for fingerprinting purposes, but can be favourable if one intends to determine the total amount of PCNs via integration of the total peak area.

Detection of PCNs and PCBs in urine. Figure 7 shows the chromatogram for 20 μl urine. Separation took place on a RP-8 column with pure water containing 10^{-4} M biacetyl as an eluent. The direct phosphorescence of biacetyl was recorded.

It can be seen from Figure 7 that urine contains a lot of compounds which are able to quench the biacetyl phosphorescence. Furthermore, a relatively weak positive signal is observed for small retention times, presumably originating from interfering compounds emitting fluorescence at 520 nm upon excitation at 415 nm. It is clear that under these conditions the phosphorescence detection methods are not appropriate.

Fortunately, it is found that far better results are obtained if instead of pure water the azeotropic acetonitrile/water mixture (containing 10^{-4} M biacetyl) is used. In this solvent interferences with native fluorescence or with quenching properties toward the biacetyl phosphorescence are rapidly eluted (see Figure 8A). Analytes with retention times larger than 2 minutes can therefore be detected by the sensitized or quenched RTPL mode.

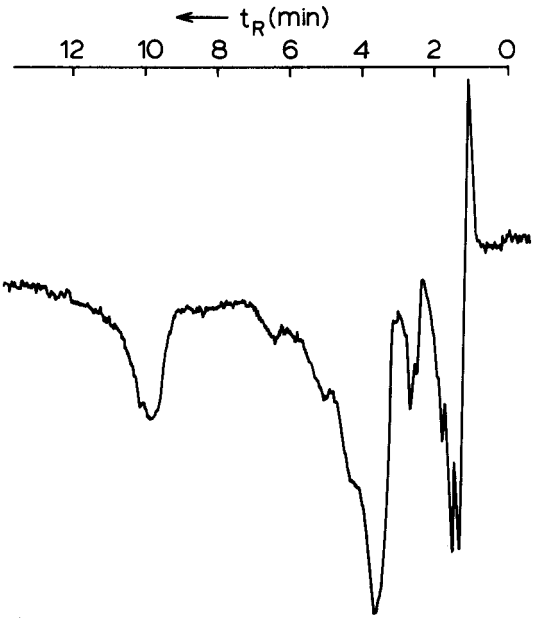

FIGURE 7 Chromatogram of 20 μl of urine sample separated on the RP-8 column. Eluent: water $+1.0 \times 10^{-4}$ M biacetyl. Other chromatographic conditions as in Figure 3. Detection by quenched RTPL.

In Figure 8B and C the chromatograms of 20 μl urine, spiked with 100 ng and 10 ng of Halowax 1001 respectively are depicted. In these chromatograms sensitized RTPL detection was applied giving rise to detection limits (see Figure 8C) in the order of 1 ng, in agreement with the data in Table II. However, obviously the peak heights in Figure 8B are not 10 times higher than those in Figure 8C, in other words the sensitized RTPL signals are not linearly dependent on the analyte concentration.

There can be two causes for this non-linearity. In the first place a number of PCNs in Halowax 1001 are also able to quench the biacetyl phosphorescence because of the reversed energy transfer, see Eq. (2). Under this condition Eq. (3) applies so that the sensitized signal intensity is not linear. In the second place PCNs may be present in the mixture which are not producing sensitized RTPL at all but only quenched RTPL. Their contribution will only be

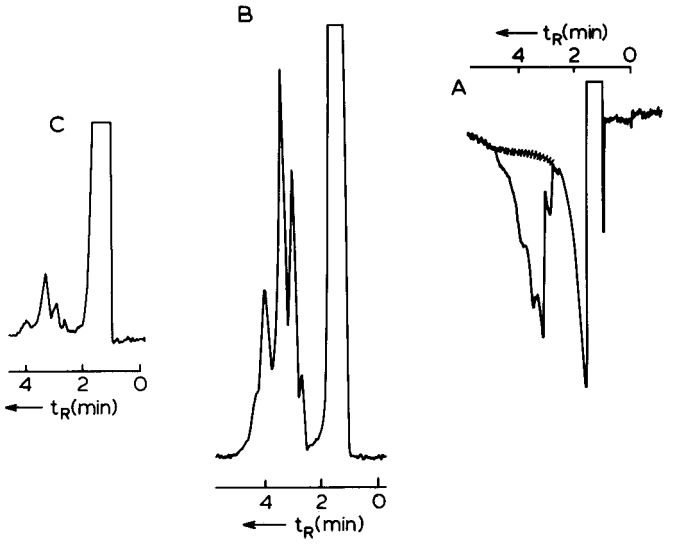

FIGURE 8 Chromatograms of 20 μl urine samples containing Halowax 1001. A: 100 ng Halowax detected by quenched RTPL without signal inversion. The strongest peak corresponds to O_2. B: 100 ng Halowax detected by sensitized RTPL. $\lambda_{ex} = 300$ nm. C: 10 ng Halowax detected by sensitized RTPL. $\lambda_{ex} = 300$ nm. Chromatographic conditions as in Figure 6.

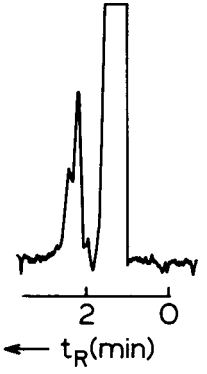

FIGURE 9 Sensitized RTPL chromatograms of 20 μl urine containing 2 ppm of Aroclor 1221. $\lambda_{ex} = 265$ nm. Chromatographic conditions as in Figure 6.

important for higher Halowax amounts because of the difference in sensitivities between sensitized and quenched RTPL for the PCNs (see Table II). For illustration of this point Fig. 8A shows the chromatogram recorded by quenched RTPL detection in the non-inverted mode as opposed to Figure 8B for the same chromatogram detected in the sensitized RTPL mode.

Finally in Figure 9, the chromatogram of 2 ppm of the PCB mixture Aroclor 1221 in urine is presented (the eluent is the same as used in Figure 7). Though the separation achieved is not optimal, at least three individual PCBs can be assigned, i.e. biphenyl, 4-chlorobiphenyl and 4,4′-dichlorobiphenyl.[2]

CONCLUSIONS

The potential for using sensitized and quenched RTPL for analysis of major groups of pollutants in complex matrices has been demonstrated. The inherent selectivity and sensitivity of the sensitized RTPL mode lends itself somewhat better to this end but its range of applicability is more limited. Information obtained from chromatograms recorded with RTPL techniques are often complementary to UV detection and lend themselves for finger-printing purposes to identify for example commercial PCN and PCB mixtures. Detection limits are up to one order better for sensitized RTPL than for UV detection and quenched RTPL shows comparable detection power. The RTPL methods can also be used for quantitative purposes. In quenched RTPL the signal vs. concentration relationship (calibration curves) can be linearized by recording the inversed signal. The power of the technique is enhanced by coupling it to on-line pre-concentration techniques on suitable precolumns. The information content of RTPL detection techniques and their broad applicability, particularly of the quenched mode, and their complementary nature to other detection modes such as UV, fluorescence or electrochemical, should render them of considerable interest in pollutants analysis.

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