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PHOSPHORESCENCE DETECTION OF POLYCHLORONAPHTHALENES AND POLYCHLOROBIPHENYLS IN LIQUID CHROMATOGRAPHY

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SUMMARY

Room temperature phosphorescence in liquid solutions (RTPL) is applied as a detection method in the chromatographic analysis of technical mixtures of polychloronaphthalenes (PCNs) and polychlorobiphenyls (PCBs). The identification of PCBs is strongly facilitated by combining UV detection and detection based on sensitized RTPL of biacetyl, since the compounds substituted at position 2 are not detected by the latter method. For PCNs, in addition to sensitized RTPL, quenched RTPL detection can be successfully applied. This method is based on the partial quenching of the direct RTPL of biacetyl by the analyte. Combination of UV detection with the two phosphorescence detection methods provides interesting possibilities for identification.

INTRODUCTION

We have previously shown that room temperature phosphorescence in liquid solutions (RTPL) can be successfully applied as a detection method in continuous flow and chromatographic systems[1]. Attention was particularly focused on the sensitized phosphorescence of biacetyl, induced by the analyte. In principle, such a sensitized signal can be observed if the triplet state of the analyte is higher in energy than the triplet state of biacetyl. An exothermic energy transfer is thus possible, which is generally diffusion controlled[2].

In sensitized phosphorescence experiments the background signal originates mainly from the (inefficient) absorption of the excitation light by biacetyl itself, leading to direct room temperature phosphorescence in liquid solutions (RTPL). This background emission, whose intensity depends on the excitation wavelength, can also be applied for detection purposes as will be demonstrated in this paper.

If the triplet energy of the analyte is equal to or lower than that of biacetyl, a reversed energy transfer reaction must be taken into account. This leads to both a decrease in the sensitized and the direct RTPL signal of biacetyl[3]. In the present paper it is shown that measurement of such a partial quenching of the direct RTPL induced by the analyte can serve as a detection method in a continuous flow system. So, in

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addition to sensitized RTPL detection, another phosphorescence detection method is available, denoted as quenched RTPL detection. Consequently, in general, analytes with triplet state energies too low to produce sensitized RTPL can be detected by quenched RTPL. It is emphasized that the application of quenched RTPL instead of sensitized RTPL requires only an adjustment of the excitation wavelength.

Of course, in addition to triplet–triplet energy transfer, the analyte may also provide other deactivation pathways for the biacetyl triplet state, *e.g.*, electron transfer. For the polychloronaphthalenes (PCNs) and polychlorobiphenyls (PCBs) investigated here, most probably these processes are not effective, since it is found that the compounds with high triplet state energies, which are unable to quench the direct RTPL signal via energy transfer, do not produce any quenching at all[4].

The combination of UV detection, sensitized RTPL and quenched RTPL detection can give interesting results in the analyses of complex mixtures. This is demonstrated for some industrial mixtures of PCNs, which have frequently been used for impregnating purposes[5] and for the PCB mixture Aroclor 1221.

THEORETICAL

The processes leading to sensitized RTPL and quenched RTPL of biacetyl based on energy transfer are presented in Table I. It is noted that reaction (3) of the scheme leading to quenched RTPL is the reverse of reaction (3) leading to sensitized RTPL. Hence, the equilibrium position of the reversible reaction

$$^3A^* + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A + {}^3B^*$$

determines whether the analyte can be detected more sensitively by sensitized or by quenched RTPL.

As shown in Table I, for sensitized RTPL a suitable wavelength, λ_{ex}^{A}, is chosen for excitation of the analyte and the phosphorescence of biacetyl is detected. So the analyte produces a positive signal, superimposed on the background signal corresponding to the direct excitation of biacetyl at λ_{ex}^{A}.

TABLE I

REACTION SCHEMES OF SENSITIZED AND QUENCHED RTPL OF BIACETYL BY TRIPLET–TRIPLET ENERGY TRANSFER

Sensitized RTPL	*Quenched RTPL*
(1) Excitation of the analyte (A): $A + h\nu_{ex}^{A} \rightarrow {}^1A^*$	(1) Excitation of biacetyl (B): $B + h\nu_{ex}^{B} \rightarrow {}^1B^*$
(2) Intersystem crossing to the triplet state: $^1A^* \rightarrow {}^3A^*$	(2) Intersystem crossing to the triplet state: $^1B^* \rightarrow {}^3B^*$
(3) Energy transfer to biacetyl (B): $^3A^* + B \xrightarrow{k_1} A + {}^3B^*$	(3) Quenching of biacetyl phosphorescence by the analyte $^3B^* + A \xrightarrow{k_{-1}} B + {}^3A^*$
(4) Phosphorescence of biacetyl $^3B^* \rightarrow B + h\nu_{p}^{B}$	$^3B^* \searrow B + h\nu_{p}^{B}$

The intensity of the sensitized RTPL signal has been shown to be proportional to the analyte concentration, the efficiency of the intersystem crossing process of the analyte, the efficiency of the energy transfer to biacetyl and the phosphorescence efficiency of biacetyl[1], provided that the back transfer reaction is negligible. If the back transfer plays a rôle the linear dynamic range of the sensitized method is shortened[3].

In quenched RTPL a wavelength, λ_{ex}^{B}, is chosen where only biacetyl is excited, *i.e.*, about 420 nm, where the molar absorptivity of biacetyl is relatively high, about 20 M^{-1} cm^{-1} (ref. 6).

The presence of the analyte leads to a reduction of the direct RTPL intensity which is shown by a negative peak in the chromatogram. As indicated in Table I, such a negative peak can be expected if the deactivation of $^3B^*$ via energy transfer to the analyte is able to compete with the other deactivation processes. This is realized if k_{-t}[A] (where k_{-t} is the rate constant and [A] the analyte concentration) cannot be neglected with respect to $1/\tau_0^B$.

τ_0^B is the triplet lifetime of biacetyl in the absence of analyte and is about $5 \cdot 10^{-4}$ sec under the experimental conditions applied[3].

EXPERIMENTAL

The source and purification of acetonitrile, water, biacetyl and the pure PCNs and PCBs have been published[1]. The Halowax mixtures (Koppers, Pittsburgh, PA, U.S.A.), the dichloronaphthalene technical mixture (Eastman, Rochester, NY, U.S.A.) and the Aroclor 1221 (Monsanto, St. Louis, MO, U.S.A.) were supplied as indicated.

A detailed description of the continuous flow system has been given[1]. For the present experiments the apparatus used to deoxygenate the eluent was modified as depicted in Fig. 1. This was done to prevent pollution of the eluent due to contact between the eluent and the O-rings.

For the UV detection experiments, the fluorimeter Type SFM 22 (Kontron, Zürich, Switzerland) was replaced by a UV detector Type LC 3 UV (Pye Unicam, Cambridge, Great Britain) provided with a 8-μl flow cell.

The separations were performed on LiChrosorb RP-18 columns, $d_p = 5.5$ μm, length 11 cm, with a $1.0 \cdot 10^{-4}$ M biacetyl solution in azeotropic acetonitrile–water (83.7:16.3 v/v) as a mobile phase. The injection volume was 20 μl and the flow-rate 1 ml/min.

RESULTS AND DISCUSSION

The PCN mixtures

We have previously shown[3] that the triplet state energies of the lower chlorinated PCNs are distinctly higher than that of biacetyl. Hence, these PCNs produce only sensitized RTPL and no quenched RTPL of biacetyl. On the contrary, for the trichloro- and tetrachloro-substituted PCNs the reversed energy transfer is not negligible so that both sensitized and quenched RTPL detection is applicable. The highly substituted PCNs probably have triplet state energies too low to induce any sensitized RTPL of biacetyl. They are only detectable by quenched RTPL.

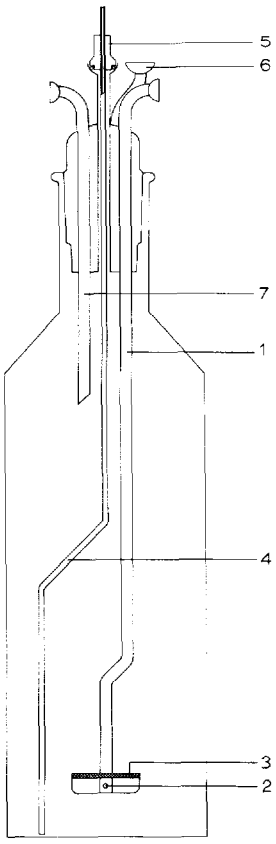

Fig. 1. Eluent vessel consisting of a 3-l glass bottle and a glass stopper B55 which fits well in a ground glass joint. Nitrogen gas enters the eluent via a glass tube (1), with spherical glass joint (cup, size 13/5, Rotulex®), via an opening (2) and a P3 glass filter (3); the outlet is via a glass tube (7). The deoxygenated eluent is pumped into the flow system via a stainless steel capillary (4), diameter 1/8 in. This capillary forms one unit with the stainless steel ball part (5) of a spherical joint. The glass cup of this joint (size 19/9, Rolulex®) is connected with a glass tube which ends in the bottom of the stopper. The entrance of oxygen via this joint is prevented by a Buna O-ring. 6 represents a construction identical to 4 and 5 for the inlet of eluent.

For sensitized RTPL, leading to positive peaks in the chromatograms, λ_{ex} is chosen in the range 275–310 nm. For quenched RTPL, giving negative peaks, λ_{ex} is about 415 nm. It is noted that also small negative peaks are possible in the chromatograms detected by sensitized RTPL since the background signal comes from direct RTPL of biacetyl. An efficient quenching leads to a reduction of the background signal; if this reduction is larger than the sensitized RTPL signal, a negative peak is observed.

The retention times and the limits of detection (LODs) based on sensitized RTPL under the present chromatographic conditions were measured for a number of pure PCNs, see Table II. The LODs, although corresponding to different λ_{ex} values, give an indication of the sensitivity of the sensitized RTPL detection for the particular compounds in the chromatogram. They were calculated from the chromatographic

peak heights, based on a signal-to-noise ratio of 3:1, obtained for 20-μl $1.0 \cdot 10^{-6}$ *M* sample solutions. The values are in the subnanogram or low nanogram range, once again demonstrating the sensitivity of the sensitized RTPL method.

TABLE II

CHROMATOGRAPHIC DATA OF THE SENSITIZED RTPL METHOD FOR PCNs λ_{em} = 520 nm.

PCN	λ_{ex} *(nm)*	t_R *(min)*	*LOD (pg)*
Naphthalene (N)	275	2.5	470
1-ClN	285	3.4	280
2-ClN	280	3.2	540
1,2-Cl_2N	285	4.5	1400
1,3-Cl_2N	290	5.1	1200
1,4-Cl_2N	295	5.1	410
1,5-Cl_2N	290	5.2	370
1,8-Cl_2N	300	4.0	310
2,6-Cl_2N	280	4.15	730
2,7-Cl_2N	285	4.25	930
1,3,8-Cl_3N	300	6.4	600
1,4,6-Cl_3N	300	8.1	1200
1,3,5,7-Cl_4N	300	14.5	2900
1,3,5,8-Cl_4N	310	11.35	1800
1,4,6,7-Cl_4N	305	12.25	1800

The dichloronaphthalene technical mixture and the Halowaxes 1031 and 1000

In Fig. 2 the chromatograms of the dichloronaphthalene mixture (Eastman) obtained by UV and sensitized RTPL detection are given. It should be noted that the concentrations of the sample solutions are different, *i.e.*, 50 ppm for the UV detected and 2.5 ppm for the RTPL detected chromatogram.

The small negative peak with a retention time of 1.5 min results from the lack of biacetyl in the injected sample solutions, and is detected in all the subsequent chromatograms.

The assignment of peaks 1–4 in Fig. 2 is straightforward using the data of Table II: peak 1 represents naphthalene (N); peak 2, relatively poorly detected by sensitized RTPL, belongs to 2-ClN and peak 3, being very intense, to 1-ClN. Peak 4 is ascribed to 1,2-Cl_2N. In view of its high LOD value (see Table II) its low intensity in Fig. 2B is not unexpected. Peak 5 may originate from three PCNs, *i.e.*, 1,3-Cl_2N, 1,4-Cl_2N and 1,5-Cl_2N. It is noted that, in addition to the sensitizing properties of the PCNs under study, also the excitation wavelength applied in the sensitized RTPL detection determines the peak heights in the chromatograms. In fact the low intensity of peak 2 is partly accounted for by the relatively low absorptivity of 2-ClN at 290 nm.

The chromatograms of this mixture are very similar to those obtained for Halowax 1031 (Koppers). The only difference is that the Halowax mixture gives a small additional peak at 8.1 min which, according to Table II and in agreement with Brinkman *et al.*[7], indicates the presence of 1,4,6-Cl_3N.

The chromatograms of Halowax 1000 show that this mixture contains the same PCNs but here the peak to be attributed to 1,3-, 1,4- and 1,5-Cl_2N is the most

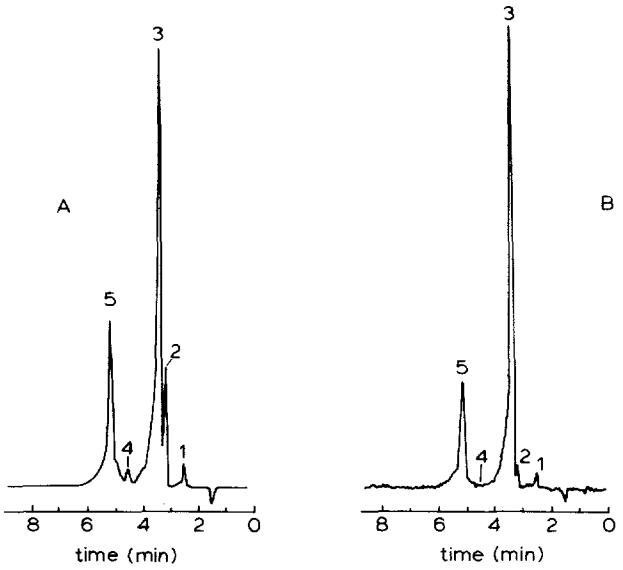

Fig. 2. Chromatograms of the dichloronaphthalene mixture (Eastman). A, UV detection; λ_{ex} = 226 nm; 1.28 a.u.f.s.; concentration of sample solution 50 ppm. B, Sensitized RTPL detection; λ_{ex} = 290 nm; λ_{em} = 520 nm; concentration of sample solution 2.5 ppm. The identification of the peaks is given in the text.

intense. Furthermore, additional peaks of 1,8-Cl_2N and 1,3,8-Cl_3N are found, although the latter is not confirmed in the literature[7]. In this context it is worth noting that according to ref. 7 the amount of 1,3-Cl_2N in Halowaxes 1031 and 1000 is almost negligible.

Halowaxes 1099 and 1001

These Halowaxes consist mainly of PCNs containing three or four chlorine atoms per molecule[7]. As noted above, for the analysis of these mixtures, in addition to sensitized RTPL, also quenched RTPL detection is important. It is our purpose to show that the combination of the two phosphorescence detection methods is very useful to characterize the samples.

The UV, sensitized RTPL and quenched RTPL detected chromatograms of Halowax 1099 are depicted in Fig. 3. Peak 1 shows the presence of at least one of the three compounds 1,3-, 1,4- and 1,5-Cl_2N, sensitively detectable by sensitized, but not by quenched RTPL. Peak 2 cannot be identified by using Table II. It may be attributed to a dichloronaphthalene, *i.e.*, 1,6-, 1,7- or 2,3-Cl_2N. Its triplet state energy must be higher than that of biacetyl. Furthermore, comparison of the relative intensities of peaks 1 and 2 in Fig. 3A and 3B reveal that the unknown compound is a relatively poor sensitizer. Peak 3 must be ascribed to 1,3,8-Cl_3N, which has both sensitizing and quenching properties[3]. Peaks 5 and 11, only very weakly detected in the UV chromatogram (Fig. 3A), are well detectable in the sensitized one (Fig. 3B) since no influence of quenchers with the same retention time is encountered (Fig. 3C). The quenching effect is pronounced for peaks 9 and 10, which are intense in Fig. 3A but hardly or not visible in Fig. 3B.

Peaks 6 and 7 deserve special attention. In the UV detected chromatogram

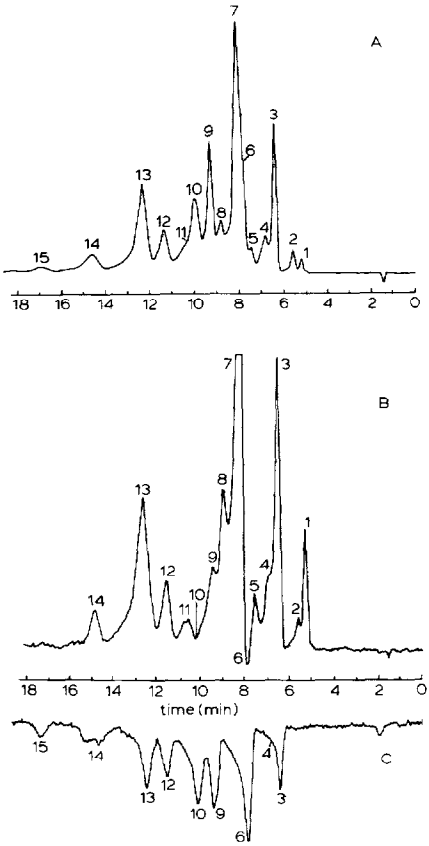

Fig. 3. Chromatograms of Halowax 1099. A, UV detection; λ_{ex} = 233 nm; 0.32 a.u.f.s. B, Sensitized RTPL detection; λ_{ex} = 300 nm; λ_{em} = 520 nm. C, Quenched RTPL detection; λ_{ex} = 415 nm; λ_{em} = 520 nm. Injected amount of sample in all chromatograms: 1 μg. The identification of the peaks is given in the text.

they are not separated. However, in the sensitized RTPL detected chromatograms they are clearly separated into a negative and a positive peak. This can be simply explained on the basis of Fig. 3C which shows that peak 6 belongs to an efficient quencher, whereas peak 7 is not detectable by quenched RTPL. We presume that peak 6 corresponds to 1,4,5,8-Cl_4N[7], which is a strong quencher because of its low-lying triplet state[8] and that peak 7 corresponds to 1,4,6-Cl_3N.

Peaks 12 and 13 consist of higher chlorinated PCNs which can have sensitizing as well as quenching properties. According to Table II, 1,3,5,8-Cl_4N may contribute to peak 12 and 1,4,6,7-Cl_4N to peak 13; 1,3,5,8-Cl_4N is also mentioned in ref. 7. Comparison of peak 14 in the three chromatograms reveals unambiguously that it is composed of at least two components, one being only detectable by quenched RTPL and the other by both sensitized and quenched RTPL.

Halowax 1099 appears to be almost identical to Halowax 1001. In the latter mixture also traces of 1-ClN are found by sensitized RTPL. Their amounts seem to be too low for UV detection.

In Fig. 4 the sensitized and quenched RTPL chromatograms of Halowax 1013

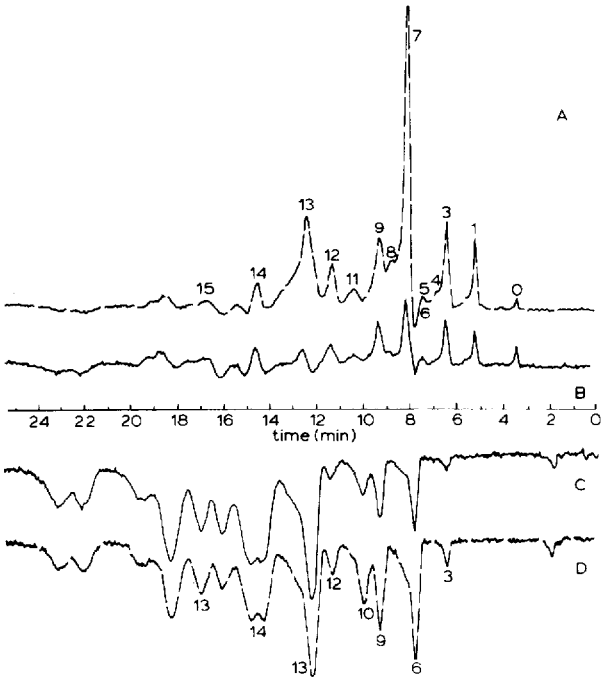

Fig. 4. Chromatograms of Halowaxes 1013 (dashed lines) and 1014 (solid lines). A and B, Sensitized RTPL detection; λ_{ex} = 300 nm; λ_{em} = 520 nm. C and D, Quenched RTPL detection; λ_{ex} = 415 nm, λ_{em} = 520 nm. Injected amount of sample in all chromatograms: 1 μg. The identification of the peaks is given in the text.

and 1014 are collected. The numbered peaks are also found in the chromatograms of Halowax 1099, given in Fig. 3.

Comparing the chromatograms in Fig. 4, it is obvious that in Halowax 1013 and 1014 the same components are present but in different amounts. (The chromatograms were recorded under exactly the same conditions; peak 0 is attributed to 1-ClN.) For instance, the 1013 mixture contains much more 1,4,6-Cl_3N (peak 7) than the 1014 mixture. In general, Halowax 1013 is richer in sensitizers than Halowax 1014.

TABLE III

CHROMATOGRAPHIC DATA OF THE SENSITIZED RTPL METHOD FOR PCBs λ_{em} = 520 nm.

PCB	λ_{ex} *(nm)*	t_R *(min)*	*LOD (pg)*
Biphenyl (B)	260	2.8	400
2-ClB	255	3.0	9400
3-ClB	260	3.5	420
4-ClB	265	3.6	310
3,4-Cl_2B	265	4.55	5600
3,5-Cl_2B	265	5.1	620
3,3′-Cl_2B	260	4.5	800
4,4′-Cl_2B	270	4.4	210
3,5,3′,5′-Cl_4B	260	9.15	3400
3,4,5,3′,4′,5′-Cl_6B	275	14.1	1600

As noted above, many of the components of the Halowaxes 1013 and 1014 are also present in Halowax 1099. In the latter mixture, however, quenchers with long retention times, as observed in Fig. 4, are lacking (see Fig. 3). Most probably these peaks indicate the presence of penta-, hexa- and heptachloronapthalenes, which is in line with the results obtained by Brinkman *et al.*[7] for Halowax 1014.

The PCB mixture

The PCBs have triplet state energies distinctly higher than biacetyl[4]. Therefore, in principle, they are all sensitizers, so that application of quenched RTPL detection is not appropriate.

Nevertheless it is interesting to use phosphorescence detection to investigate PCB mixtures, since combination with UV detection strongly enhances the selectivity. The reason is that the sensitivity of the sensitized RTPL method varies strongly within a series of PCBs, as can be seen in Table III. PCBs substituted at the 2-position are especially poor sensitizers, because of their short triplet lifetimes[1]. Therefore, they are almost undetectable by sensitized RTPL, the only exception being 2-ClB.

The selectivity aspect is clearly shown in Fig. 5, where the UV and sensitized

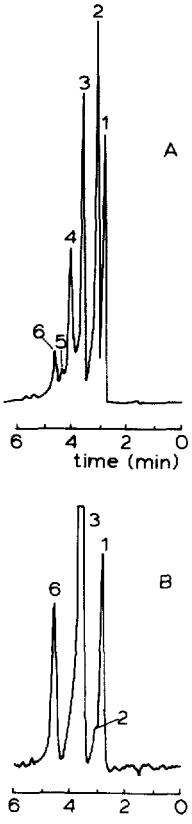

Fig. 5. Chromatograms of Aroclor 1221. A, UV detection; λ_{ex} = 244 nm; 0.32 a.u.f.s.; concentration of the sample solution 50 ppm. B, Sensitized RTPL detection; λ_{ex} = 265 nm; λ_{em} = 520 nm; concentration of the sample solution 10 ppm. The identification of the peaks is given in the text.

RTPL detected chromatograms of Aroclor 1221 are depicted (again, the sample concentrations used are different). The former chromatograms exhibits six peaks but the latter only four. The composition of Aroclor 1221 is well known[9,10]; the main components are biphenyl (B), 2-ClB, 4-ClB, 2,2′-Cl_2B and 4,4′-Cl_2B, corresponding to peaks 1, 2, 3, 4 and 6, respectively. All these compounds are detected in the UV chromatogram. As expected, the sensitized RTPL chromatogram is simpler: 2,2′-Cl_2B is not detected at all, while 2-ClB produces only a very weak signal.

CONCLUSIONS

This paper shows that the combination of UV detection and phosphorescence detection in liquid chromatography provides new perspectives for identification of complex mixtures such as polychloronaphthalenes and polychlorobiphenyls. For the PCNs, in addition to sensitized RTPL, quenched RTPL can be applied successfully as a detection method. This method is based on the partial quenching of the direct RTPL of biacetyl due to energy transfer to the analyte.

We expect that the quenched RTPL detection method will be quite generally applicable. The reason is that energy transfer, which requires a relatively low triplet state energy for the analyte, is not the only possible quenching reaction. Many analytes will be able to quench the biacetyl phosphorescence rapidly by other mechanism, for instance electron transfer. Some promising results for compounds such as phenothiazines and anilines have already been obtained. A detailed study of the quenched RTPL detection method is currently being undertaken.

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