Aqueous Systems for Liquid Scintillation Counting: Use of Hydrotropes

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Dedicated to Professor *André M. Braun* on the occasion of his 60th birthday

Liquid hydrotropic systems, *i.e.*, mixtures of hydrotropes and water-forming hydrophilic and hydrophobic regions, allow the solubilization of organic scintillators in essentially aqueous media. Such systems were applied to liquid scintillation counting with 4-[4-(5-phenyloxazol-2-yl)benzyl]morpholine as scintillator, a 2,5-diphenyloxazole (PPO) derivative that proved well-soluble in acidic hydrotrope systems. Its fluorescence properties were studied. Phenylalanine labeled with ^{3}H or ^{14}C was used to test counting. While ^{14}C counting worked acceptably, ^{3}H counting was comparatively inefficient, probably due to the short lifetime of β-particles in aqueous environments.

Introduction. – The conversion of the energy of β-particles (emitted upon radioactive decay of unstable isotopes) to a corresponding number of fluorescence quanta is known as scintillation. Through collisions, the particles promote nearby atoms or molecules to electronically excited states capable of emitting fluorescence. The number of excited states generated depends on the energy of the β-particles, *i.e.*, on the decaying isotope. Since its first description, the phenomenon of scintillation was readily exploited for detection, counting, and identification of radioactive radiation [1]. Meanwhile common is the use of solid scintillator systems, such as inorganic materials like thallium-doped NaI [2–4], cerium-doped yttrium silicate [5–7], solid noble gases [8], and organic materials like 2,5-bis(2-benzoxazolyl)phenol in polystyrene [9], 2,5-diphenyloxazole (PPO) and 1,4-bis(2-methylstyryl)benzene (bis-MSB) in paraffin [10][11]. Liquid scintillation systems such as PPO and 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP) in aromatic organic solvents [12–14] are used as well. The latter systems commonly contain surfactants in order to form microemulsions, when water has to be taken up that is present in biological and medical samples containing radioactively marked substances in aqueous solution.

In this method (called liquid scintillation counting, LSC), β-particles initially produce excited solvent molecules (*e.g.*, benzene, toluene, liquid naphthalene derivatives), the excitation energy of which is transferred to (primary and secondary) scintillators, which then emit fluorescence pulses. LSC is superior in the detection of weak β-radiation from ^{3}H (tritium) or ^{14}C decays. Liquid scintillation counters and a great number of suitable scintillator solutions (cocktails) are commercially available. Although developed to almost perfect performance, there is one drawback of this technique, which results from the necessity of treating the waste, *i.e.*, weakly radioactive organic liquids are left, whose disposal is problematic.

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Therefore, there is growing interest in the use of low-vapor-pressure water-based systems especially for measuring aqueous samples stemming from biological, pharmaceutical, and medical research or diagnostics. Aqueous solutions, however, are generally considered inappropriate for LSC, since *i*) the lifetime of β-particles in H_2O is short, *ii*) H_2O is a bad solvent for scintillator molecules, which are usually hydrophobic organic molecules, and *iii*) upon collisions with β-particles, H_2O does not produce excited states capable of transferring excitation energy to scintillators.

In this work, we describe the first successful attempts employing hydrotropes to apply aqueous systems to LSC. Hydrotropy denotes the phenomenon of increasing solubility in H_2O of compounds normally insoluble or badly soluble, brought about by dissolved substances called hydrotropes. The term hydrotropy was first introduced by *Neuberg* in 1916 [15]. Later, there were very few further papers on the subject until *Balasubramanian et al.* and *Friberg* [16–23] started systematic investigations in recent years. According to these studies, the hydrotropy phenomenon is clearly distinct from salting-in effects [17], from cosolvency (continuous mixtures of H_2O and organic solvents such as dioxane or short chain alcohols), as well as from surfactant micelle formation [23]. There are, however, similarities to the behavior of the latter, *e.g.*, a minimum hydrotrope concentration must be present to observe the effect. Therefore, the solubility of hydrophobic substances follows a sigmoid curve as a function of the hydrotrope concentration. The minimum hydrotrope concentration, in some cases, depends on the kind of solubilizate. Its value is around 1 mol/dm^3, *i.e.*, it exceeds critical micelle-formation concentrations by orders of magnitude. The phenomenon of hydrotropy is ascribed to the formation of loosely structured solutions composed of extended fluctuating regions that are more hydrophilic and more hydrophobic. Defined aggregates, however, cannot be detected. The more hydrophobic regions – formed by the hydrotrope molecules – are capable of solubilizing organic hydrophobic molecules. Among the compounds exhibiting hydrotropy, there are (derivatives of) benzoic acid, salicylic acid, aminobenzoic acid, benzenesulfonic acid, toluenesulfonic acid, hydroxynaphthoic acid, hydroxybenzenes, caffeine, and the corresponding salts or hydrochlorides, respectively, depending on pH. Besides the aromatic anionics, aromatic cationics, and aromatic nonionics mentioned above, a few aliphatic and olefinic carboxylic acids as well as amino acids [20] are also known as hydrotropes. A review is given in [19].

Results. – *Choice of Scintillator Chromophore.* The basic principles of traditional liquid scintillation counting are expected to hold for cocktails containing hydrotropes: the fluorescence quantum yield of the scintillator chromophore should be as high as possible; reabsorption of fluorescence should be small in the system; the fluorescence maximum of the scintillator should be not too far from 420 nm to meet the maximum sensitivity of common detectors (photomultipliers) in commercial liquid scintillation counters. 2,5-Diphenyloxazole (PPO) and its derivatives have proved suitable in this respect and are widely used. In conventional LSC, however, PPO normally acts as a primary scintillator whose excitation energy has to be transferred to a secondary scintillator (such as 1,4-bis(2-methylstyryl)benzene) to match a center of gravity of the emission around 420 nm. We found that 4-[4-(5-phenyloxazol-2-yl)benzyl]morpholine (**1**; *Fig. 1*) in acidic aqueous solutions of aromatic hydrotropes shows the desired

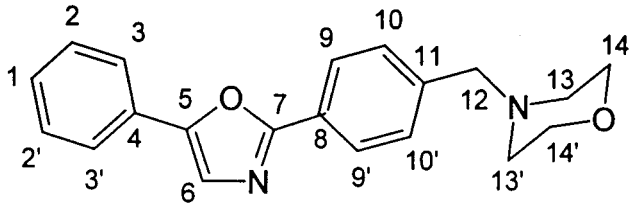


Fig. 1. *Scintillator molecule 4-[4-(5-phenyloxazol-2-yl)benzyl]morpholine* (**1**) *with arbitrary numbering of C-atoms*

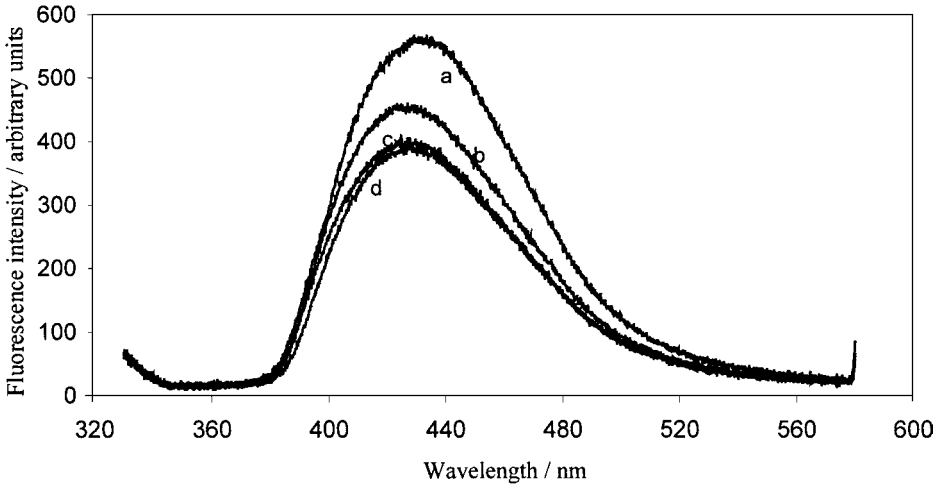


Fig. 2. *Fluorescence spectra of 4-[4-(5-phenyloxazol-2-yl)benzyl]morpholine* (**1**) *in hydrotrope solutions containing various amounts of TsOH* (**3**) *and* **1**. Excitation wavelength 310 nm (total absorption), pH <1. *a*) 3.5M **3**, 0.04M **1**; *b*) 4.0M **3**, 0.03M **1;** *c*) 3.0M **3**, 0.03M **1**; *d*) 3.5M **3**, 0.02M **1**.

spectral properties mentioned above (as shown in *Fig. 2*) without a wavelength-shifting secondary scintillator. Along with a fair fluorescence quantum yield of 0.94 (in cyclohexane), this feature qualified the compound for this study. Compound **1** was prepared by the reaction of 4-(5-phenyloxazol-2-yl)benzyl bromide [24] with morpholine. The low solubility in pure H_2O ($4.5 \cdot 10^{-5}$ mol/dm^3) promotes solubilization among the hydrotrope molecules in the systems studied here.

A couple of other scintillators proved much less soluble in hydrotrope systems, *i.e.*, 9,10-dimethylanthracene, bis-MSB, PPO, and POPOP. Counting efficiencies were less favorable than those of **1**, therefore, and these compounds were not investigated further.

Choice of Hydrotropes. According to the aim of the study, the hydrotropes used here have to take the role of the solvent molecules in ordinary organic LSC cocktails, *i.e.*, upon collisions with β-particles, transitions to electronically excited states must take place. Thus, aliphatics, amino acids, *etc.*, fall short of aromatic hydrotropes. We, therefore, concentrated on salicylic acid (2-hydroxybenzoic acid; **2**), TsOH (**3**), 3-hydroxy-2-naphthoic acid (**4**), benzene-1,2,3-triol (**5**) and benzene-1,4-diol **6** (or the Na salts, resp., depending on pH).

Incipient experiments ruled out **5** and **6** for inefficient counting (< 0.5% efficiency in ^{14}C) and for photochemical lability. Compound **4** proved less soluble in H_2O than **2** or **3** by an order of magnitude. We, therefore, continued the study with the latter two.

Fluorescence Properties of **1**. In *Table 1*, emission properties of compound **1** are listed for Ar-saturated solutions in cyclohexane, MeOH, and H_2O. Fluorescence quantum yields are given relative to PPO in cyclohexane. For this system, an absolute value $\Phi_F = 1$ is published [25]. Natural lifetimes τ_0 were calculated from measured lifetimes and fluorescence quantum yields according to *Eqn. 1*

$$\tau_0 = \tau_m / \Phi_F \quad (1)$$

which is valid when fluorescence (rate constant k_F), internal conversion (k_{IC}), and intersystem crossing (k_{ISC}) are the only processes deactivating the electronically excited fluorescent state, *i.e.*,

$$\Phi_F = k_F/(k_F + k_{IC} + k_{ISC}) \quad (2)$$

Stokes shifts $\Delta\nu$, *i.e.*, the energy loss between absorption and emission spectra, are calculated as the difference of the wave number of the 0-0 transition (ν_{00}) and that of the center of gravity of the emission-spectrum curve (ν_{CG}), respectively [25]. The intersection of normalized absorption and emission spectra was taken as ν_{00}. Large *Stokes* shifts are desirable in scintillators to suppress possible reabsorption of emitted fluorescence quanta.

While in the organic solvents and at neutral or high pH in H_2O, only small spectral shifts were found in the absorption ($\leq$ 4 nm) and emission ($\leq$ 21 nm) maxima of PPO and **1**, the fluorescence bands were considerably shifted to higher wavelengths (36–59 nm) in aqueous acidic solutions (*Table 1* and *Fig. 2*). This indicates the presence of a

Table 1. *Fluorescence Properties of PPO and* **1** *in Cyclohexane, MeOH, and H_2O at various pH.* $\lambda_{max.}$: Wavelength of the low-energy absorption maximum, $\lambda_{e,max}$: wavelength of the emission maximum. $\Delta\nu$: *Stokes* shift, Φ_F: fluorescence quantum yield relative to PPO in cyclohexane, τ and τ_0: measured fluorescence lifetimes calculated with *Eqn. 1*. pH Values were adjusted using HCl and NaOH.

Substance	Solvent	pH	$\lambda_{a.max}$/nm	$\lambda_{e,max}$/nm	$\Delta\nu$/cm^{-1}	Φ_F	τ/ns	τ_0/ns
PPO[a])	Cyclohexane		303	354	2860	1	1.7	1.7
PPO[a])	MeOH		302	362	2980	0.79	2.0	2.5
PPO	H_2O	7.1	302	374	3270	0.90		
PPO	H_3O^+	1.2	307	411	3740	0.79		
PPO	H_3O^+	0.1	310	414	3800	0.63		
PPO	OH^-	12.8	302	375	3100	0.81		
1	Cyclohexane		303	380	2300	0.94	1.8	1.9
1	MeOH		303	366	1240	0.20	< 1.5	< 4.8
1	H_2O	6.8	307	380	3160	0.50		
1	H_3O^+	1.3	310	416	4070	0.90	2.0	2.2
1	H_3O^+	0.1	313	420	3970	0.51		
1	OH^-	12.8	306	376	2670	0.43		

[a]) From [24].

protonated emitting chromophore species. In fact, in moderately acidic solutions (pH 3–4) two widely overlapping emission bands arise, the one at lower wavelength (nonprotonated species) diminishing with decreasing pH. Consequently, large stokes shifts are found in strongly acidic solutions.

Scintillation. Scintillation tests are limited with respect to concentrations: below the minimum hydrotrope concentration, LSC pulses could not be detected. Thus, the hydrotrope concentration can be varied between the minimum hydrotrope concentration and the solubility limit only, and, as a consequence, the scintillator solubility is also limited. For hydrotrope **2**, the maximum solubility at room temperature is nearly 3 mol/dm^3 and the minimum hydrotrope concentration is 0.9 mol/dm^3 [17], while the corresponding values for **3** are 5 mol/dm^3 and 0.7 mol/dm^3, respectively. ^{3}H- and ^{14}C-labeled phenylalanines were used to test scintillation.

The use of hydrotrope **2** in combination with scintillator **1** generally led to poor counting results not exceeding $S = 3\%$ for ^{14}C decays and 0.05% for ^{3}H decays, as shown in *Table 2*. At high pH values, the counting efficiencies were even worse. Attempts to measure counting efficiencies as a function of pH were discontinued, after it had turned out that the solubility of **1** in basic solutions of hydrotrope **2** is strongly reduced, and that, consequently, the measured counting efficiencies are extremely diminished (see *Table 2*).

Table 2. *Counting Efficiencies* S *for ^{3}H and ^{14}C Decay of Labeled* L*-Phenylalanine in Aqueous Hydrotrope Solutions of Sodium Salicylate* (**2**) *Containing 4-[4-(5-phenyloxazol-2-yl)benzyl]morpholine* (**1**) *at Different pH Values and Concentrations* c

pH	c(**1**)/mol/dm^3	c(**2**)/mol/dm^3	S(^{3}H)/%	S(^{14}C)/%
12.9	< 0.001[a])	1.5	0.011	1.2
12.0	0.003	2.5	0.017	1.55
12.0	0.005	2.5	0.02	2.01
7.5	0.003	2.5	0.049	3.05
7.5	0.005	2.5	0.044	2.87
7.4	0.005	2.2	0.039	2.74
7.2	0.005	1.8	0.026	2.69
7.1	0.005	1.5	0.023	2.38
7.1	< 0.075[a])	1.5	0.024	2.00

[a]) Solubility limit.

With hydrotrope **3**, better results were obtained. In *Figs. 3* and *4*, counting efficiencies S are given as a function of the concentration of hydrotrope **3** and scintillator **1**. At 0.04M **3** and 0.04M **1**, the S values appear to level off. Higher concentrations in both hydrotrope and scintillator could not be tested, since the solubility limits are almost reached. pH values around 1 were measured in these cocktails.

3. Discussion. – *Fluorescence.* The observation of a dual fluorescence in aqueous solutions of **1** (and of PPO) very probably reflects a protonation of **1** in acidic solutions. A protonation at the morpholine N-atom (*cf. Fig. 1*) explains the quite good solubility of **1** in the acidic hydrotrope systems (*Figs. 3* and *4*), but will not significantly influence absorption and emission spectra, since the morpholine group is separated from the chromophore by a CH_2 bridge. The protonation of the chromophore is required to

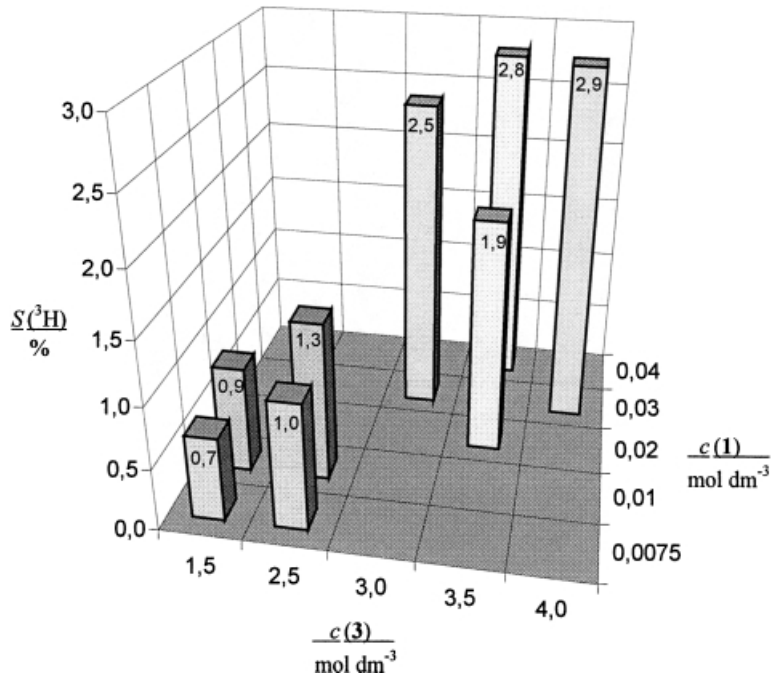


Fig. 3. *Counting efficiencies* S *for* ^3H *decay of labeled* L*-phenylalanine as a function of the concentration of hydrotrope* **3** (TsOH) *and scintillator* **1** (4-[4-(5-phenyloxazol-2-yl)benzyl]morpholine). All solutions exhibit pH values around or < 1.

affect the emission. The strongly increased *Stokes* shifts in the acidic systems might indicate that the protonation takes place in the excited state rather than in the ground state, since, in the latter case, a corresponding red shift of the absorption should be expected, as found, *e.g.*, in acidic solutions of acridine [26][27]. Moreover, protonated ground-state PPO chromophores might tend to rapid hydrolysis, which was not observed.

The drop in fluorescence quantum yield upon changing the pH from 1.1 to 0.1 (*Table 1*) may be due to chloride quenching [28][29] as the pH was adjusted by the addition of HCl.

Scintillation. We may discuss the results on the basis of the *Scheme*. Due to their kinetic energy, β-particles produced upon decay of ^3H or ^{14}C in phenylalanine excite a certain number of hydrotrope molecules *via* collision (*Step 1*). In good LSC systems (*i.e.*, toluene/PPO/bis-MSB + surfactant), the number of excited solvent molecules can amount to 15 in the case of ^3H decay and to 120 in the case of ^{14}C decay. The excitation energy is transferred to the scintillator **1** *via* (*Förster*-) energy transfer [30] (*Step 2*). The scintillators excited this way then emit a fluorescence pulse that is counted by the LSC instrument (*Step 3*).

The fact that LSC does not function below the minimum hydrotrope concentration proves *i*) the mechanism depicted in the *Scheme* and *ii*) the validity of the prerequisites of this study: the solubility of **1** under these conditions is too low to allow any detectable excitation of **1,** neither *via* collisions with β-particles (because of their rapid energy loss

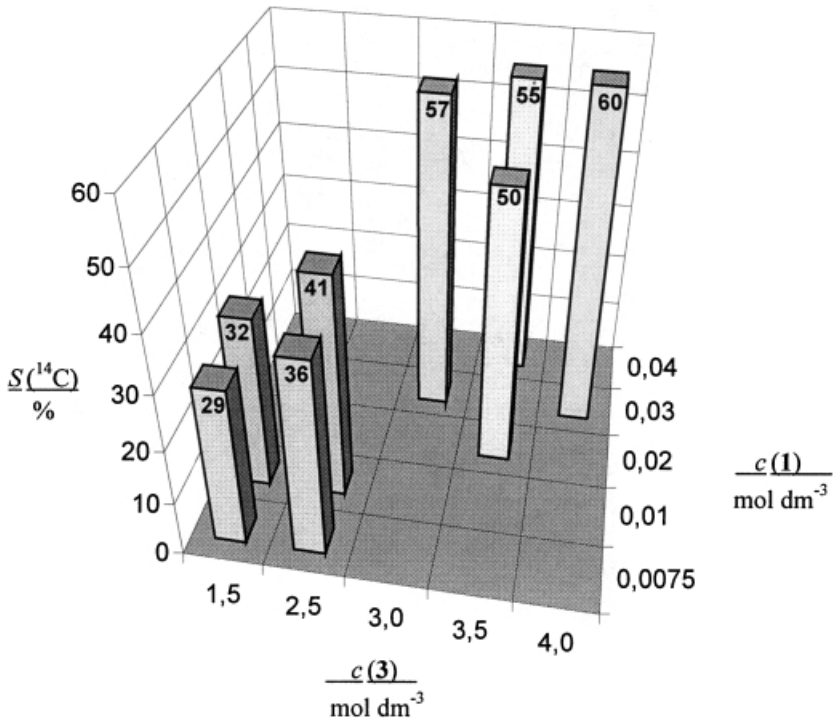


Fig. 4. *Counting efficiencies* S *for* ^{14}C *decay of marked* L*-phenylalanine as a function of the concentration of hydrotrope* **3** (TsOH) *and scintillator* **1** (4-[4-(5-phenyloxazol-2-yl)benzyl]morpholine). All solutions exhibit pH values around or < 1

in H_2O [31]) nor by energy transfer from a few presumably excited hydrotrope molecules. Above the minimum concentration, however, the scintillator concentration can be increased, and the scintillator molecules are largely sequestered within the more hydrophobic (hydrotrope-rich) regions of the systems, so that efficient energy transfer is possible.

In the absence of specific effects ratios of $S(^{14}C)$ over $S(^{3}H)$ should reflect the difference in β-particle energy, *i.e.*,

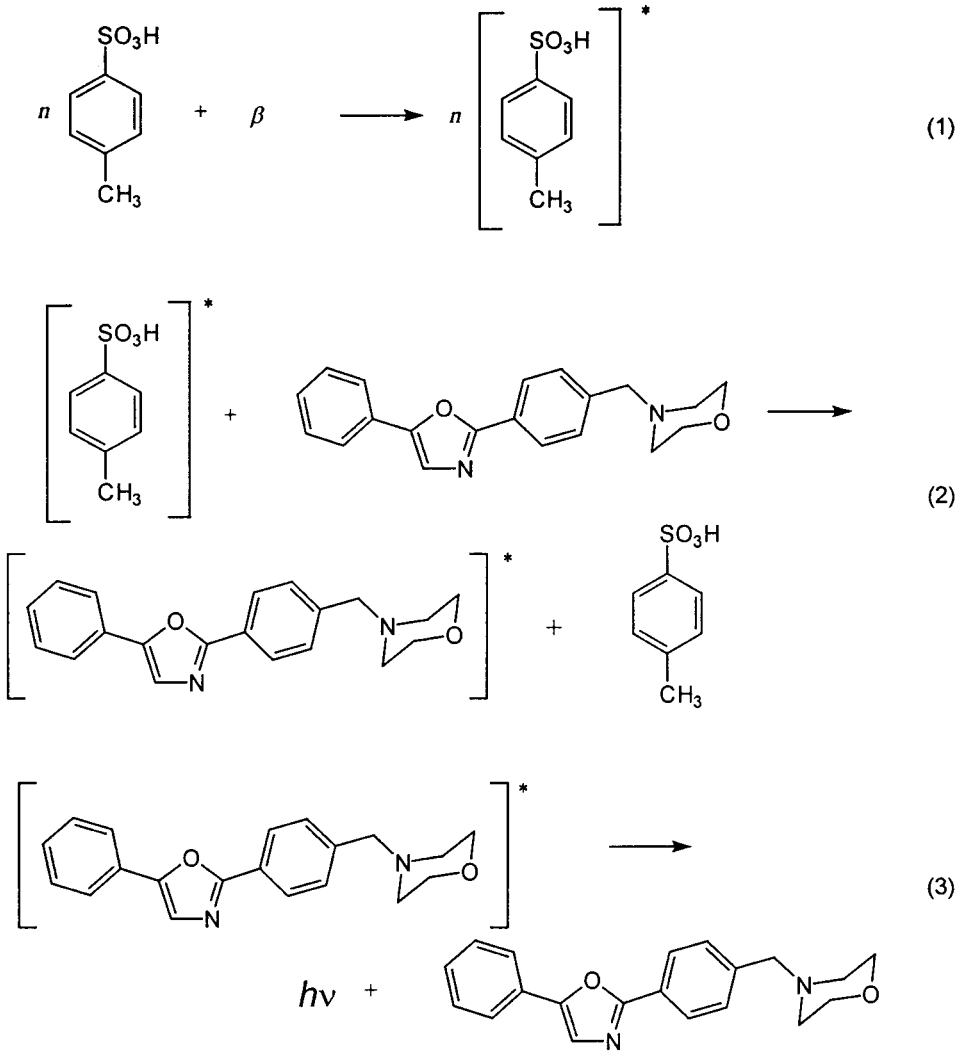
$$\frac{S(^{14}C)}{S(^{3}H)} = \frac{E(^{14}C)}{E(^{3}H)} \approx 8.6 \tag{3}$$

[1] as found in good LSC cocktails and also in several solid scintillators [24]. For the systems in *Figs. 3* and *4*, we calculate

$$\frac{S(^{14}C)}{S(^{3}H)} = \frac{E(^{14}C)}{E(^{3}H)} = \frac{60}{2.9} \approx 21 \tag{4}$$

The relatively much better ^{14}C counting was not expected: as the mean distance of hydrotrope molecules at 4 mol/dm^3 is 0.75 nm and can be expected less in the more organic regions of the hydrotrope, there are only a few H_2O molecules between them.

Scheme. *Scintillation Scheme in Hydrotrope Systems as Used in* Figs. 3 *and* 4



Thus, most of the β particles stemming from ^3H decay do reach a considerable number of hydrotrope molecules, since their mean range in H_2O is 0.8 μm [31]. Obviously, much of the initial β-particle energy is wasted in collisions with H_2O molecules, which do not lead to excited states.

^{14}C Counting yields efficiencies S up to 60%, which prove the potential of hydrotrope solutions in liquid scintillation counting but still fall short of good conventional LSC cocktails. The lacking 40% may be rationalized as discussed above for ^3H counting. In the system composed of **3** and **1**, further improvements are unlikely due to solubility limits.

Labeled phenylalanine was used for both ^3H and ^{14}C counting in order to exclude specific influences of the probe. More-hydrophobic probes, which tend to sequester in the more-hydrophobic regions of the hydrotrope systems, may yield better results.

4. Conclusions. – Aqueous systems containing hydrotropes are potentially of use in liquid scintillation counting with 4-[4-(5-phenyloxazol-2-yl)benzyl]morpholine as a scintillator. Common scintillators were not sufficiently soluble. Counting efficiencies vary with the concentration of hydrotrope and scintillator as well as with pH. ^{14}C Counting is much more efficient than ^3H counting.

We thank *Packard Instrument B.V.*, Groningen, NL, who mainly financed this work and also provided the scintillation counter. Further financial support from the *Fond der Chemischen Industrie* and by the *Deutsche Forschungsgemeinschaft* (Graduiertenkolleg '*Struktur-Eigenschafts-Beziehungen bei Heterocyclen*') are gratefully acknowledged. We are indebted to Dr. *D. Scheller*, TU Dresden, Institut für Organische Chemie, for NMR measurements and helpful discussions.

Experimental Part

Materials. Chemicals and solvents needed were taken from stocks of the Chemistry Department of the Technische Universität Dresden or purchased from *Sigma-Aldrich*, *Acros*, *Merck*, *Baker*, and *Lancaster*, or provided by *Packard Instrument BV*, Groningen. 4-[4-(5-Phenyloxazol-2-yl)benzyl]bromide was available from a previous investigation [24]. L-Phenylalanine (ring-2,6 ^3H and UL-^{14}C) was purchased from *Sigma*.

4-[4-(5-Phenyloxazol-2-yl)benzyl]morpholine (**1**). 4-(5-Phenyloxazol-2-yl)benzyl bromide (6.28 g, 20 mmol) was dissolved in 500 ml of CH_2Cl_2. To this soln., 100 g (*ca.* 100 ml, 1.15 mol) morpholine was added dropwise within 5 h at r.t. Then, the solvent (together with excess morpholine) was evaporated. The residue was treated with 40 ml of 2 N NaOH and 100 ml of Et_2O. The aq. and Et_2O phases were separated, and the aq. phase was extracted three times with 20 ml of Et_2O. The combined org. phases were extracted three times by 30 ml of H_2O. The org. phase was dried (Na_2SO_4), and the solvent was removed *in vacuo*. The oily residue (6.0 g = 18.7 mmol of **1**; 94%) converted to yellow crystals at r.t. after a couple of days. From a sat. soln. of **1** in boiling Et_2O, light yellow crystals were obtained at $-40°$: 3.2 g, 10 mmol, 50%. M.p. 85–86°. ^1H-NMR ($CDCl_3$)[2]): 8.05 (*d*, $^3J = 8.2$, H–C(9), H–C(9′)); 7.70 (*d*, $^3J = 7.6$, H–C(3), H–C(3′)); 7.41–7.45 (*m*, H–C(2), H–C(6), H–C(10), H–C(2′), H–C(10′)); 7.32 (*t*, $^3J = 7.5$, H–C(1)); 3.71 (*m*, H–C(14), H–C(14′)); 3.53 (*s*, 2 H–C(12)); 2.45 (br., 2 H–C(13), 2 H–C(13′)). ^{13}C-NMR ($CDCl_3$)[2]): 161.03 (C(7)); 151.07 (C(5)); 140.41 (C(11)); 129.44 (C(10), C(10′)); 128.87 (C(2), C(2′)); 128.35 (C(1)); 127.95 (C(4)); 126.31 (C(8)); 126.18 (C(9), C(9′)); 124.09 (C(3), C(3′)); 123.39 (C(6)); 66.94 (C(14), C(14′)); 63.05 (C(13), C(13′)); 53.60 (C(12)). Solubility in H_2O: 14.4 mg dm^{-3} ($4.5 \cdot 10^{-5}$ mol dm^{-3}) at 25°.

Fluorescence. Spectra for the data in *Table 1* were recorded at 25° on a *Shimadzu* (model *RF-5000*) spectral fluorimeter. Integrated fluorescence spectra obtained under identical absorption conditions (*i.e.*, at an absorption of 0.1 in 1-cm cuvettes at the excitation wavelength $\lambda = 337$ nm; slit widths were kept constant) were taken as measures for relative quantum-yields with PPO (scintillation grade, supplied by *Packard Instrument BV*) in cyclohexane as the reference [25]. Φ_F Values were corrected for refractive indices and for the photomultiplier sensitivity differing in certain wavelength regions. For the latter correction, solutions of fluorescers emitting in the respective region were used, whose corrected spectra have been published [25]. Measured values were reproducible within ±5%. Spectra from concentrated samples (such as used for LSC samples, see *Fig. 2*) were taken from the surface at an angle of 30° relative to the exciting light on a home-made apparatus described in [29].

Lifetimes were measured at r.t. employing an apparatus described in [32][33] modified in that *i*) a nitrogen laser from ILEE (model *NN-100*, pulse width 0.5 ns, emission wavelength $\lambda = 337$ nm) was used as excitation source, *ii*) a photodiode from *Soliton* (model *UPD-500UP*, rise time < 500 ps) was employed as the detector, and *iii*) signals were recorded on a *Hewlett-Packard* digital oscilloscope (model *54615 B*). Measured lifetimes were reproducible with deviations of ±0.2. Solns. for fluorescence quantum-yield determinations were deaerated by bubbling with Ar.

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[2]) For assignments, refer to the arbitrary C-atom numbering in *Fig. 1*.

Analyses. NMR Spectra were recorded on a *Bruker DRX-500* spectrometer at 500.13 MHz for ^{1}H and at 124.77 MHz for ^{13}C resonance. 2D-NMR was employed to exclude equivocal assignments. pH Values were measured using a *Präcitronic* pH-meter (model *MV 88*).

Scintillation Counting. A scintillation counter (model *Tricarb 2550 TR/LL*) as well as various scintillation cocktails were provided by *Packard Instrument B.V.*, Groningen, NL. In the determination of counting efficiencies *S* (counts per min/decays per min), reference was made to ^{3}H and to sodium ^{14}C marked L-phenylalanine in *Ultima Gold* cocktails (*Packard Instrument*). Counting efficiencies are averages of 3 to 6 measurements, which showed a reproducibility within $\pm 5\%$.

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