4
Artificial Tracers

Artificial tracers are substances that offer additional information of value in the investigation of hydrological systems and subsystems. Artificial tracers must be introduced actively into the hydrological system under investigation. The scales of application are limited in both time and space. In general, artificial tracers are used in systems which have a residence time smaller than one year. However, it is possible to label a specific, well defined hydrological feature. Sometimes even the volume of water is defined. The general characteristics and the function of tracer hydrology were described in Chapter 2.

Typical fields of applications are: the detection of hydrological connections, flow paths and flow directions in catchments and aquifers, delineation of catchments and aquifers (qualitative), determination of flow velocities and further aquifer flow parameters based on the tracer breakthrough curves, hydrodynamic dispersion, runoff separation, residence time, infiltration and runoff generation processes, convection-diffusion processes in surface water, simulation of contaminant transport and discharge measurement applying dilution methods.

In principle, the guideline followed is that an ideal tracer represents the water flow, but nonideal tracers can also be useful for special applications. Four main groups of artificial tracers can be distinguished based on their chemical appearance (see Table 4.1). Up until the 1980s, during the pioneer phase of tracer hydrology, a wide spectrum of artificial tracers was being tested for their suitability. However, most did not exhibit the right combination of properties and, therefore were deemed to be ill suited to serve as water tracers. The tracers which are considered to be suitable water tracers are presented in Table 4.1, and only these substances will be dealt with in the remainder of this book. Of the six groups of artificial tracer listed in Table 4.1, fluorescence tracers are the most important and most often applied, followed by the salt and the advanced tracers. Drifting particles are tracers of a different physiochemical origin, and are used to assess special problems, such as the filtration capacity of unsaturated zones.

In order to perform experiments successfully in both the field and in the laboratory, a sound knowledge of the characteristics of the tracer substances and the respective measurement techniques is required. The publications prepared by the following international organizations dealing with tracer methods are an excellent resource for all

Tracers in Hydrology Christian Leibundgut, Piotr Maloszewski, and Christoph Külls

Table 4.1 Artificial tracers

Artificial tracers					
Soluted or in aqueous solution					Solid
Fluorescence tracers	Salt tracers	Radioactive tracers	Activatable tracers (radioactive)	Advanced tracers	Drifting particles
Naphthionate	Chlorid (Na^+Cl^-, K^+Cl^-)	Tritium ^3H	Bromide (^{80}Br)	Gases (e.g. SF_6)	Lycopodium spores
Pyranine	Li^+Cl^-	Chrome (^{51}Cr)	Indium (^{116m}In)	'Heavy' water (^2H)	Fluorescent particles
Uranine	Bromid (K^+Br, Na^+Br^-)	Indium (^{114m}In, ^{114}In)	Manganese (^{56}Mn)	Fluorobenzoic acids	Bacteria/viruses
Eosine	Iodide (K^+I^-)	Cobalt (^{58}Co, ^{60}Co)	Lanthanum (^{140}La)	Nonfluorescence dyes	Phages
Amidorhodamine G		Bromide (^{82}Br)	Dysprosium (^{165}Dy)	Temperature	DNA
Rhodamines					

matters concerning the methodological aspects and the application of artificial tracers:[1] the International Association of Tracer Hydrology (ATH, 1967–2001), the International Commission on Tracers (ICT-IAHS, 1993–2000) and the International Atomic Energy Agency (IAEA, 1983–1991).

The objective of the application of tracers in hydrology is the investigation of water in all its various guises, behaviours and characteristics within the different media and substrates represented in the water cycle. Consequently, conservative tracers representing the flow of water are required. Conservativity exists if the tracer is physico-biochemically stable (nonreactive in natural water) and not sorptive. Good water solubility is also essential (see section 4.1.2.1).

4.1 Fluorescent tracers

Fluorescent tracers are the most important of the artificial tracers. They are popular among tracer hydrologists because of their relatively easy handling, the seemingly simple analysis, the high sensitivity of the analysis, the low detection limit and, consequently, the small quantity of tracer needed in field experiments. Fluorescent tracers are also attractive because of the linearity of the calibration curve in the measuring scale, and their toxicity levels are very low compared to other tracer substances; some are entirely nontoxic.

Since the first known experiment using the fluorescent tracer Uranine more than one hundred years ago, much effort has been expended in the search for additional dye tracers. However, of the great number of potential fluorescent tracers only a few substances are truly suitable (Tables 4.1 and 4.2). The fundamental methodological basics have been discussed in publications by many authors.[2] As most of the research work in this area occurred prior to and during the 1980s, only a few papers dealing with the fundamentals of fluorescent tracers have been published in recent years.

Due to the need for toxicity tests, it is unlikely that the tracers currently available will be supplemented by new tracers. Research to find or to develop suitable substances has been largely unsuccessful. Viriot and André (1989) and Netter and Behrens (1992) suggested a few new tracers, none of which have received further application in the field. Hadi (1997) synthesized two new conservative dyes, succinylfluorescein disodium

[1] ATH: Maurin and Zötl (1967); Käss (1972); Gospodaric and Habic (1977); Leibundgut and Weingartner (1982); Morfis and Paraskevopoulou (1986), Hötzl and Werner (1992); Kranjc (1997b); Seiler and Wohnlich (2001); ICT-IAHS: Peters *et al.* (1993), Leibundgut (1995), Adar and Leibundgut (1995), Pointet (1997), Peters and Coudrain-Ribstein (1997), Leibundgut, Gunn and Dassargues (1998), Leibundgut, Mc Donnell and Schultz (1999), Kovar *et al.* (1998), Dassargues (2000); IAEA: Drost (1983), Plata (1983), Rao (1983), Florkowski (1991), Mandel (1991), Margrita and Gaillard (1991), Navada (1991), Roldao (1991).

[2] Feuerstein and Selleck (1963), Käss (1964, 1967a, 1998), Moser and Sagl (1967), White (1967), Drew (1968), Wilson (1986), Gygax and Schwab (1969), Behrens (1971, 1973, 1982), Smart (1976), Smart and Smith (1976) and Smith and Laidlaw (1977), Leibundgut (1974, 1981a), André and Molinari (1976), Leibundgut and Hirsig (1977), Grisak and Pickens (1980), Behrens, Hötzl and Maurin (1981), Davis *et al.* (1985), Behrens (1986), Quinlan (1986); Benischke and Schmerlaib (1986), Leibundgut and Wernli (1986), Mull *et al.* (1988), Hadi *et al.* (l997), Wernli (2003), Flury and Wai (2003).

Table 4.2 The fluorescent tracers suitable for hydrological purposes, ordered according to the fluorescence emission maxima

Commercial name	Ex/Em max nm	Compound class	CI: generic name	CI number	Chemical name	Chemical formula	Molecular weight
Naphthionate	325/420	Aminonaphtalensulfonic acid		—	4-Amino-1-naphtalensulfonic acid sodium salt	$C_{10}H_8NNaO_3S$	245.23
Naphtionate Sodium-salt							
Pyranine	Circa 460/510	Anthraquinone	Solvent Green	59040	1-Hydroxy-pyren-3,6,8-trisulfon-trisodium	$C_{16}H_7Na_3O_{10}S_3$	524.39
D&C Green 8							
Uranine	491/516	Xanthene	Acid Yellow 73	45350	Hydroxy-6-oxo-9-(2-carboxyphenyl)-xanthene	$C_{20}H_{10}O_5Na_2$	332.31
Sodium-fluorescein, D&C Yellow 7							
Eosine	515/540	Xanthene	Acid Red 87	45380	3-Hydroxy-6-oxo-2,4,5,7-tetrabromine-9(−2′-caboxyphenyl)-xanthene-disodium	$C_{20}H_6Br_4Na_2O_5$	691.88
Eosine Yellow D&C Red 22							
Amidorhodamine G	Circa 530/555	Xanthene	Acid Red 50	45220	3,6-Bis-ethylamino-2,7-dimethyl-9-2′,4′-disulfophenyl-sodium	$C_{25}H_{25}N_2NaO_7S_2$	552.59
Sulforhodamine G							
Sulforhodamine B	Circa 560/585	Xanthene	Acid Red 52	45100	3,6-Bis-diethylamino-9-(2′,4′-disulfophenyl)-sodium	$C_{27}H_{29}N_2NaO_7S_2$	580.65
Rhodamine B	Circa 555/570	Xanthene	BasicViolet 10	45170	3,6-Bis-diethylamino-9-(2′carbophenyl)-xanthylium-chloride	$C_{28}H_{31}CIN_2O_3$	479.02
Rhodamine WT	Circa 560/585	Xanthene	Acid Red 388	—	3,6-Bis-diethylamino-9-(2′,4′-dicarboxylphenyl)-xanthylium-sodium	$C_{29}H_{29}N_2NaO_5$	480.55

salt (SF) and 5(6-)-carbocylfluorescein trisodium salt (CF), on the basis of xanthene structures, both of which may be used to extend the spectrum of fluorescent tracers used for special purposes. However, both are not a substitute for Uranine (Hadi *et al.*, 1997). Similarly, Einsiedl and Maloszewski (2005) developed a new fluorescent dye, pyrene-1, 3, 6, 8-tetra sulfonic acid (PTS), but its application is limited. Furthermore, as these new substances cannot yet be synthesized industrially they are very expensive. Rhodamine WTS (CI 555/580 nm) is a new fluorescent tracer that has recently been mentioned as a potential tracer for use in the investigation of drains. However, a detailed description outlining its suitability is not yet available.

The fluorescent tracers deemed suitable for hydrological purposes are presented in Table 4.2. The substance's common name is in bold font. Unfortunately, certain tracers have several commercial names. There are also examples of different tracers with the same name. The only way to overcome this problem is to identify the substances by the colour index (CI) name and number (Quinlan and Smart, 1977; Flury and Wai, 2003), also provided in Table 4.2. It is recommended that the colour index reference be cited in all scientific and applied communications. Other important specific characteristics such as the dye class and atomic weight provide an initial impression of each tracer substance.

Although Pyranine is listed in Table 4.2, and is indeed suitable for special applications, it is not a commonly used fluorescent tracer due to the fact that it is problematic from an analytical perspective and because it is extraordinarily expensive. Consequently, Pyranine has hardly been used in decades. It was applied successfully in a multi tracer test with Deuterium and Uranine in a fissured aquifer (Himmelsbach, Hötzl and Maloszewski, 1998). Smart and Smith (1976) reported on experiments using Pyranine in Jamaica, in which the tracer could be evaluated over distances of up to 3 km. Pyranine was used successfully to investigate macropore flow (Smart and Wilson, 1984).

The potential water tracer Rhodamine WTS is not listed. The reason for this is that not all of the relevant information (e.g. CI) is available for the substance, and references regarding hydrological experiments are also missing. However, Wernli (1996) has investigated carefully the substance's suitability as a hydrological tracer. Rhodamine WTS is characterized by an excitation/emission wavelength of 558/578 nm. Its solubility in water is high. Except for its low temperature dependence, the other relevant characteristics are similar to those of the Rhodamines generally. The substance is not available in highly concentrated form, which may be another limitation in terms of its broader application as a hydrological tracer.

4.1.1 Basics of fluorescence

Fluorescence is a luminescence that occurs where energy is supplied by electromagnetic radiation. The substances used for tracing purposes are situated within the small range of visible light between the higher ultraviolet and the infrared wavelengths (c. 350–750 nm).

Fluorescence is one of the two luminescence phenomena, together with phosphorescence. Whereas phosphorescence takes energy from chemical processes and has an emission pulse of longer than 10^{-4} s, fluorescence has a very short pulse of 10^{-18} s. Fluorescence describes the ability of chemical compounds to emit an activating light impulse as longer-wave radiation. The excitation energy source kicks an electron of an atom from a lower energy state into an 'excited' higher energy state; the electron then releases the energy in the form of light (fluorescence) at which point it reverts to a lower energy state. The emission takes place just as long as the activation occurs, causing only transient fluorescence effects compared to longer-lived phosphorescence (Bandow, 1950; Eisenbrand, 1966).

The intensity of fluorescent emission follows a linear dependence involving the intensity of incident light and the tracer concentration:

$$I_e = A^* I_0{}^* \varepsilon(\lambda_{ex})^* \Phi(\lambda_{em})^* c^* d \tag{4.1}$$

I_e = fluorescence intensity
A = instrumental constant
I_0 = incident light intensity
$\varepsilon((\lambda_{ex})$ = molecular extinction coefficient at wavelength λ_{ex}
c = tracer concentration
$\Phi(\lambda_{em})$ = quantum yield
d = sample layer thickness

The extinction coefficient depends on excitation, while the quantum yield depends on emission wavelengths. Hence, an excitation scan reflects the properties of the extinction coefficient and an emission scan those of the quantum yield of the respective tracer.

For tracer concentrations in a certain range the intensity of emission is proportional to the tracer concentrations.

$$c \propto I_e \tag{4.2}$$

The linear correlation simplifies the finding of the relationship between intensity measurement and sample concentration, which is done by calibration (Figure 4.2 for Uranine). The upper boundary condition for this is that:

$$c < 1 \big/ (2^* \varepsilon(\lambda)^* d) \tag{4.3}$$

and if not fulfilled, it leads to self-shadowing effects. If the concentration of the sample exceeds the upper boundary, the sample must be diluted to meet the linear range. The usual concentrations in tracer test samples are within the range 0.01–100 mg/m^3. The chemical explanation for the fluorescent behaviour of a compound is the organic ring-structure with double bonds (Figure 4.1).

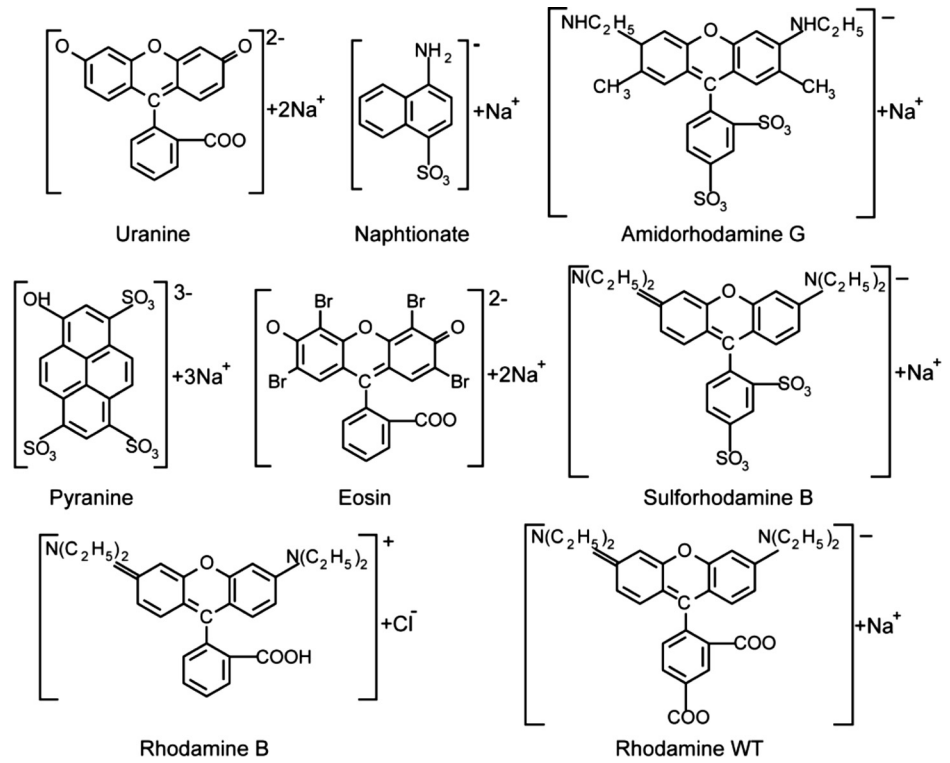

Figure 4.1 Structural formulae of the dyes listed in Table 4.2.

4.1.1.1 Spectra

The maxima of the spectra of fluorescent tracers are characteristic and constant. Having relatively small fluorescence ranges, tracers are well suited for application as hydrological tracers. When analysing fluorescent compounds two spectra, characteristic for each substance, are commonly processed: the excitation-spectrum and the emission-spectrum. They are inverted and their peaks stand apart at a specific wavelength-difference, referred to as 'Stokes shift' ($\Delta\lambda$). In the example below (Figure 4.2: Uranine), the two spectra of Uranine are indicated, with peaks at 491 and 516 nm. The characteristic $\Delta\lambda$ for Uranine is between 20 and 25 nm and is conventionally set to 25 nm to run synchronous scans. It is recommended, however, that the positions of the peaks be checked for each new charge of tracers, particularly in the event of a change of manufacturer.

The units of the spectral analysis are arbitrary. Therefore, in order to gain information about the concentration of the tracer, calibration curves must be produced by running spectral analyses of known tracer concentration samples, as presented in Section 4.1.2.2. The maximum intensity is realized with excitation and emission in the respective peaks of wavelength (Figure 4.2).

In order to avoid interferences between excitation and emission spectra using the classical spectral technique, the *synchronous scan* technique is widely used today

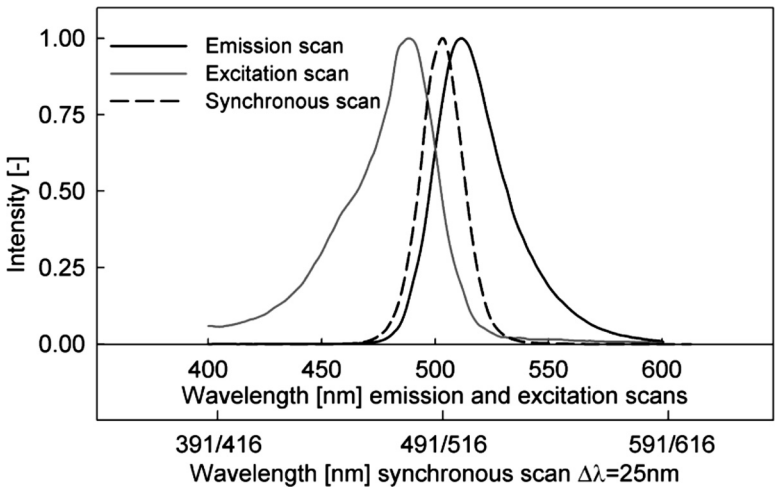

Figure 4.2 Excitation-spectrum (peak $\Delta\lambda = 491$ nm), emission-spectrum (peak $\Delta\lambda = 516$ nm) and synchronous scan spectrum of Uranine (normalized).

(Figure 4.2). Employing this technique, the two spectra (excitation and emission) are run synchronous with the characteristic $\Delta\lambda$ of the measured substance. The specific $\Delta\lambda$ for Uranine is depicted in Figure 4.2. The most important fluorescent tracers, namely Uranine, Eosine and the Rhodamines, all have the same $\Delta\lambda$ of approximately 25 nm. Although this method alternates the spectra it still possesses the advantages of narrowband spectra, with little light scattering and, therefore, no Raman scattering (see section 4.1.3.3.1). It also allows for the measurement of multiple tracers in one scan.

A comparison between the Stokes shifts and relative fluorescence intensities of some commonly applied fluorescent tracers are shown in Figure 4.3. The dominant role of Uranine is clearly apparent, due to its much higher fluorescent intensity as compared to any of the other substances. This, and other beneficial properties associated with fluorescent tracers, makes Uranine the most suitable fluorescent tracer generally, and for groundwater in particular, as has been proven in a great number of tracer experiments.

Due to different ionic forms, the excitation and emission spectra of the fluorescent tracers might change. However, the problem can be solved using the synchronous scan technique and, if needed, the adjustment of the sample in the required pH range of the maximal intensity range. Only in event of extraordinary analytical problems is there a need to consider this aspect carefully (Wolfbeis *et al.*, 1983; Behrens, 1986; Benischke and Schmerlaib, 1986).

4.1.2 Chemical and physical characteristics of dye tracers

The family of molecules that appears most suitable for use as water tracers features the basic structure of xanthene dye. The chemical explanation for the fluorescent behaviour of a compound is the organic ring-structure with double bonds (Bandow,

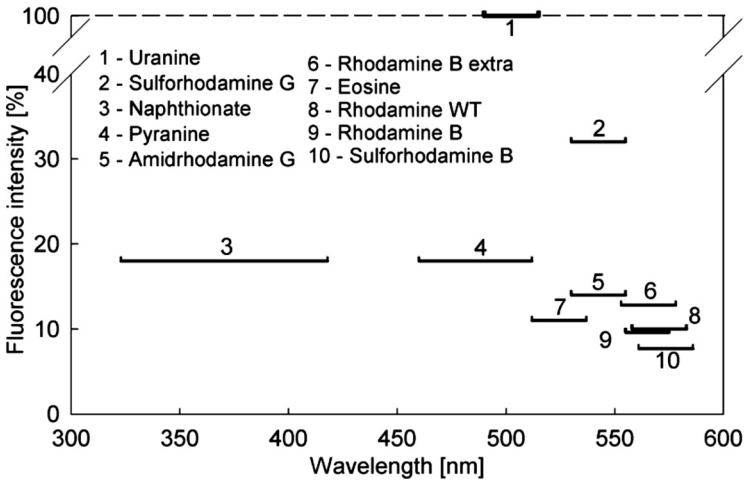

Figure 4.3 Fluorescent tracers: Stokes shifts (nm) and intensities (%) of different tracers. Note the break on the ordinate axis.

1950; Hadi *et al.*, 1997). Hence, the prime molecules suggested as hydrological tracers are the sulfonated xanthene derivates, where the ring structure (R) is CH_3, COOH or $CH_2CH_2CO_2H$ (see Figure 4.4).

The sulfonic acid functional groups, and their sodium salt derivatives, increase the solubility of the molecules. If the chemical structure is destroyed, the fluorescence is also lost. Deterioration may be caused by sunlight, chemical reaction or biological degradation.

A changing pH has the potential to change the electrical charge of the molecule from negative, through neutral to a positive value, and vice versa. If the medium reaches a certain degree of acidity, the protons will break up some of the double bonds, as a result of which the compounds lose their fluorescence until the proton concentration drops again (Figure 4.5). The process is discussed in detail in Hadi *et al.* (1997, p. 32) and Flury and Wai (2003, pp. 2–12).

The xanthenes include many commonly used dyes such as Uranine (sodium fluorescein), succinylfluorescein disodium salt and 5(6-)-carbocylfluorescein trisodium salt, Eosine and all the Rhodamines. Other chemical substances featuring fluorescence are,

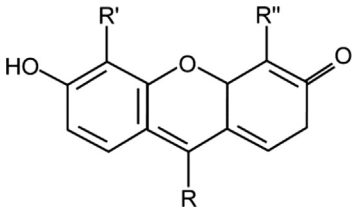

Figure 4.4 Crude sulfonated xanthene derivates, with R = CH_3, COOH or $CH_2CH_2CO_2H$; R′ = R″ = SO_3Na or H (modified from Hadi *et al.*, 1997).

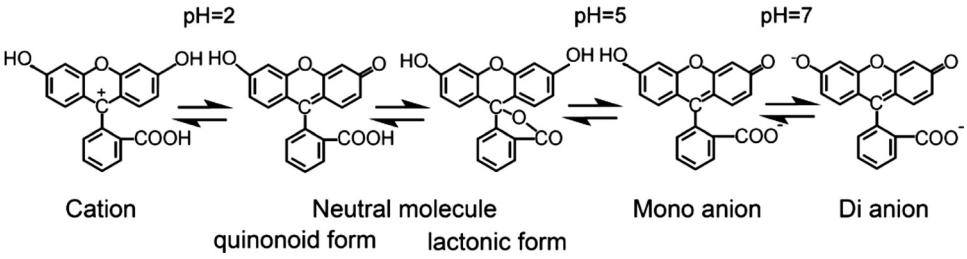

Figure 4.5 Change to the chemical structure and the net charge of Uranine as affected by pH (modified from Lindqvist, 1960; Behrens, 1986).

for example, Pyranine (anthraquinones) and Naphthionate (naphthylamine sulfonic acid). A more detailed summary of the chemical classes of dyes can be found in Käss (1998) and Flury and Wai (2003).

Each tracer application is individual and many things have to be taken into consideration. In addition to the most important property discussed above, conservativity, there are certain basic requirements that a tracer should meet. These requirements of tracers in general, and of artificial tracers in particular, are listed in Table 4.3.

4.1.2.1 Solubility

The solubility of tracers in water is a crucial requirement of tracers used to investigate water flows in the hydrological cycle as the tracer should be as close to the characteristics of water as possible. As fluorescent tracers are organic dyes, the solubility of nearly all is low, and some are even hydrophobic. The more soluble tracers include those listed in Table 4.2. Their solubility depends on both the temperature and the pH of the water.

Table 4.3 Required properties of artificial tracers in general and of fluorescent tracers in particular. The properties in bold are the main characteristics of conservative tracers

Properties to be considered	Requirements of an ideal (conservative) tracer
1. Solubility in water	High
2. Fluorescence intensity	High
3. Detection limit	Low
4. ***pH dependence***	Low
5. ***Temperature dependence***	Low
6. ***Photolytic stability***	High
7. ***Sorption processes***	Negligible
8. ***Chemical and biological stability***	High
9. Toxicity and related environmental effects	None or minimal
10. Costs and other practical aspects	Low or moderate

The higher the pH and temperature, the higher the compound's solubility. Thus, the more hydrophilic functional groups the molecule has, the higher its solubility in water. A substance's solubility is correlated inversely with adsorption (Bailey and White, 1970; Leibundgut and Wernli, 1986; Shiau, Sabatini and Harwell, 1992). The extent of the solubility of an organic compound in water depends upon its ability to form hydrogen bonds and van der Waals interactions.

The soluble tracer amounts (g/l) for pure water at a temperature of 20 °C are as follows. Pyranine (178), both Uranine and Eosine (300) and Naphthionate (240) are characterized by good to very good solubility. By contrast, the solubility of the Rhodamines (3–20 g/l) is considerably lower (see Table 4.10). It is worth mentioning that, as a neutral molecule, fluorescein is poorly soluble in water, but as a charged molecule (from sodium fluorescein – Uranine) it has very good solubility (Hadi *et al.*, 1997; Flury and Wai, 2003).

The solubility of a substance can be increased by adding appropriate chemicals. However, each chemical added to water in nature represents a contamination. Therefore, chemicals should only be used in special situations, such as in the case of experiments in glaciers at low temperatures. In field experiments the dissolved amount of tracer can vary from the solubility measured in the laboratory, due to the presence of salts or other materials, and factors such as pH and temperature.

4.1.2.2 Fluorescence intensity – detection limit

The fluorescence intensity of a trace substance depends on its physical properties, namely quantum yield, extinction coefficient and tracer concentration, as described in section 4.1.1. This feature plays a crucial role insofar as the detection limit depends, on the one hand, on the fluorescence intensity to which it is positively correlated and, on the other, on the sample background. Due to higher scattering at wavelengths in the UV range (300–500 nm), the sample background concentration of those tracers is higher and, consequently, the detection limit lower. This mainly concerns Naphthionate and Pyranine. This becomes apparent in Table 4.4, where fluorescence intensity and

Table 4.4 Detection limits in pure natural water (groundwater) under optimal technical measuring conditions

Dye	Relative fluorescence intensity [Uranine = 100%]	Detection limit [mg/m^3]	Excitation/ emission [nm]
Naphthionate	18	0.2	325/420
Pyranine	18	0.06	455/510
Uranine	100	0.001	491/516
Eosine	11.4	0.01	515/540
Amidorhodamine G	32	0.005	530/555
Rhodamine B	9.5	0.02	555/575
Rhodamine WT	10	0.02	560/585
Sulforhodamine B	7	0.03	561/586

detection limits are considered. The detection limit is clearly not the same for the different measurement devices. Using modern optical fluorometers the detection limits of fluorescent tracers in pure natural water are very low. The limits quoted in Table 4.4 are applicable for tracers measured in pure water by means of both optically and electronically optimal measurement sets. Using an optical fluorometer some of the lower detection limits stated are neither realistic nor reliable.

By the application of advanced techniques like HPTLC/AMD (Weiss *et al.*, 2008) lower detection limits are attainable. However, the costs and the time consuming analysis may restrict the operational use remarkably. Further information concerning advanced measurement techniques is discussed in Section 4.1.3.8.

When analysing surface water samples, or other nonoptically pure water, the background is relevant. Consequently, the detection limit may differ due to the scattering of light caused by the presence of suspended particles or green-blue fluorescents in natural water. The higher the background signal the lower the detection limit (Figure 4.6).

In order to improve the situation it is often sufficient to leave the sample to settle overnight so as to allow for the sedimentation of the suspended material. Should filtering be required, the use of membrane filters (1 μm) or 0.45 μm filters will suffice.

The intensity of fluorescence follows a function that is linear to the tracer concentration. This simplifies the measurement of fluorescence considerably. The shape of the curves is that of a straight line passing through the origin (Figure 4.7).

However, the linearity is only valid within a constricted concentration range of each tracer. According to Wilson (1968), linearity is given for up to several hundred mg/m^3 of the common tracers. Käss (1998) stated that Uranine, for example, exhibits a linear relationship, even up to approximately 1000 mg/m^3. Experience with modern devices indicates the range of linearity to be smaller. When measuring higher concentrations a self-shadowing effect occurs (concentration quenching), which prevents a direct measurement. The upper molecules may reduce the excitation beams of deeper molecules and, in the event of an overlap of excitation and emission spectra, the emitted light

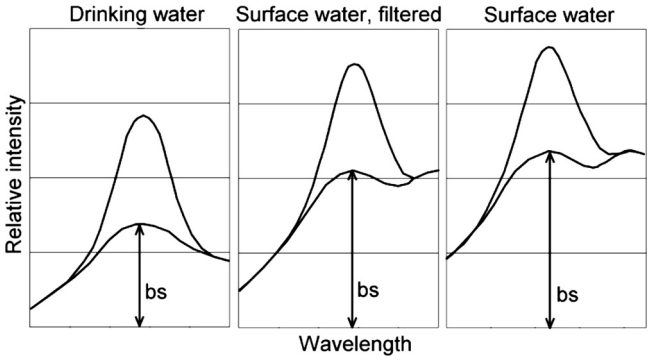

Figure 4.6 Spectral curves and background signals (bs) of Naphthionate (1 mg/m^3) in different waters. The excitation and emission peaks lie at 325 and 420 nm, respectively. The relatively high background signals are caused by high scattering and the occurrence of blue fluorescents (Leibundgut and Wernli, 1986).

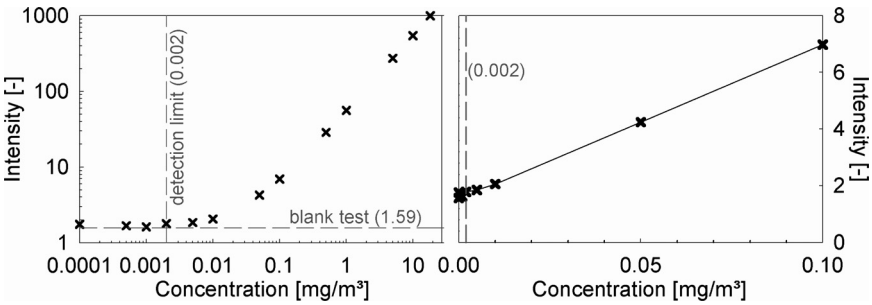

Figure 4.7 Calibration curve of Uranine in the range of linearity up to a fluorescence intensity of approximately 20 mg/m^3 ($y = 54.553x + 2.26213$; $R^2 = 0.99$) (for concentration >0.002 mg/m^3). Note the nonlinearity in the range of very low intensities around the detection limit.

may be reabsorbed (Rost, 1991). In this case the sample must either be diluted to an adequate concentration range, or grey filters or smaller slits must be attached to lower the excitation light.

The favourable linearity of the fluorescence over a wide spectrum is much lower due to the measurement range of the fluorometers. Usually, samples with a concentration >20 mg/m^3 have to be diluted. For practical purposes, it is recommended that the samples always be diluted to the measurement range quoted for the device used. The effect of this is to ensure that the nonlinearity of higher concentrations remains negligible and a modifying effect of the device and the layer thickness of the sample is avoided.

Near the origin the concentration/intensity curve is not strictly linear, which introduces an uncertainty for very low tracer concentrations around the detection limit. This is a fundamental problem and careful consideration is necessary. As the user becomes familiar with the calibration of the fluorometer it is normal that, once it has been established, the calibration remains valid for a long time, and the number of standards needed is reduced. However, occasional spot checks should be carried out. A recalibration is necessary whenever changes are made to a device; for example after removing the lamp.

Naphthionate, Eosine and the Rhodamines exhibit linearity similar to that of Uranine. The detection limit for Pyranine is more complicated, due to the high dependence upon the pH value, and the corresponding effect on the wavelength. A detailed description of Pyranine analysis was provided by Launay *et al.* (1980) and Benischke and Schmerlaib (1986). The analyses of tracer concentrations near the detection limit require careful consideration of all of the relevant dependencies – both the physical and the chemical – in order to avoid misinterpretation (Figure 4.7). Several repeat measurements of the samples are recommended, sometimes in combination with additional techniques, as described in Section 4.1.2.4.

4.1.2.3 Effects of dependencies

The properties presented in italics in Table 4.3 are the main characteristics defining conservative tracers, which are crucial particularly for quantitative investigations of

the water flow and hydrodynamic properties of groundwater and surface water. The dependence of fluorescence not only upon pH, temperature and light, but also upon chemical and microbiological effects (metabolism), can result in the degradation of the fluorescence in water samples and in natural water bodies. Consequently, when performing experiments using fluorescent tracers careful consideration of these aspects is required in both the planning of these experiments and in the analysis. Generally, the issue of pH dependence is of particular importance in experiments involving groundwater and soil water. Photolytic dependence, except in the case of surface water and temperature dependence, is usually not especially problematic.

However, the pH value and the photolytic effect can also be helpful in solving certain analytical problems such as, for example, the measurement of two or more tracers in the same sample (see Sections 4.1.2.4 and 4.1.2.6).

4.1.2.4 pH dependence

As was mentioned previously, a changing pH has the potential to change the net charge of the molecule from negative, through neutral to positive, and vice versa (see Figure 4.5). If the medium reaches a certain degree of acidity, the protons will break some of the double bonds, causing the compounds to lose their fluorescence until the proton concentration falls again. Variations in the pH value of the traced water have a twofold impact on fluorescent tracers: (i) on the analysis and (ii) on the degree of sorption affinity of the tracer.

4.1.2.4.1 Analytical problems Due to the different structures discussed above, the sensitivities of fluorescent tracers to pH vary, as illustrated in Figure 4.8.

Uranine and Pyranine react very sensitively to pH values below the range of most natural waters. Solutions in low mineralized water or in pure water must be alkalized before measuring. The fluorescence intensity of Pyranine in low buffered water may fluctuate strongly. Pyranine detection is more complicated due to the high dependence upon the pH value and the subsequent wavelength shift. A synchronous scan using a wavelength interval $\Delta\lambda = 25$ nm is optimal in the maximum of 445 nm. For alkaline solutions a $\Delta\lambda = 50$ nm is recommended, and for intermediate pH values (5–7) a $\Delta\lambda = 107$ nm (Benischke and Schmerlaib, 1986). A slightly different instruction is provided by Launay *et al.* (1980). Eosine and Naphthionate only react in a more acid environment, at pH values <5.5. Therefore, they are usually easy to handle in the lab. The Rhodamines are less sensitive to pH and usually pose no analytical problems in natural waters.

As the pH dependence of dye tracers is reversible under natural conditions the problem of pH dependence can be managed quite simply. A sample showing a pH value below the critical boundary for a certain dye tracer (Figure 4.8) can be brought into the range of its maximum fluorescence intensity by means of adequate buffering. In principle, all solutions of the pH sensitive tracers should be buffered. In the case of highly alkaline solutions complexation is required to avoid the precipitation of alkaline earths.

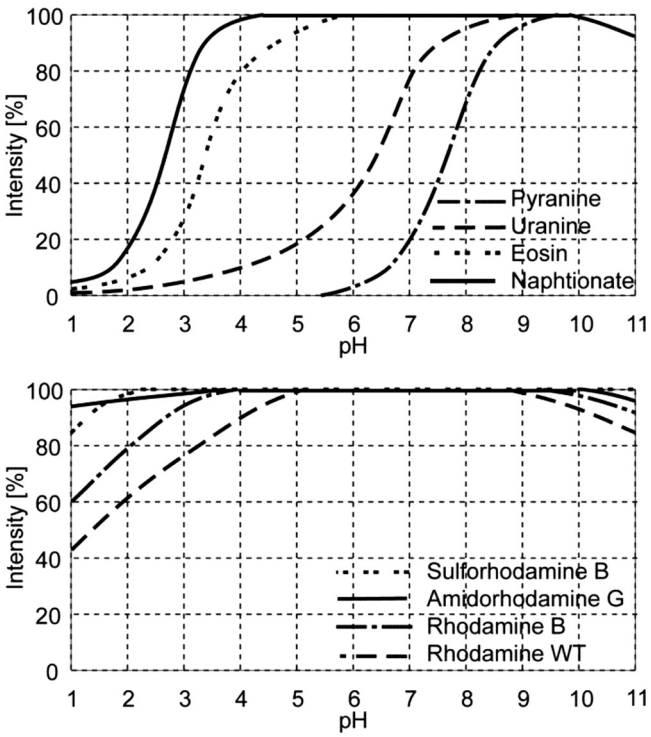

Figure 4.8 The pH dependencies of the fluorescence tracers measured in a synchronous scan at a wavelength interval of $\Delta\lambda = 25$ nm (modified and supplemented after Smart and Smith, 1976; Käss, 1998).

The reversibility of the pH dependence can also be advantageous in tracer analysis. Having conducted a multitracer test with two or more fluorescence tracers, the sample may contain a mixture of tracers. Due to the different pH dependencies, it is possible to separate them. The procedure will be described in Section 4.1.3.

The pH problem is more difficult to manage when performing in situ experiments, as here it is not possible to take advantage of the reversibility of pH dependence. pH should be measured during the online detection of fluorescence. However, using the curves presented in Figure 4.8, the necessary correction can be calculated when conditions within the medium are stable. The pH of natural waters is usually stable during measurement, except in low mineralized waters, which are poorly buffered (e.g. in crystalline catchments). Long term monitoring in the headwaters of catchments with differentiated geology, where there are changing pH values, may prove to be a serious problem.

4.1.2.4.2 Sorption problems The sorption affinity of the tracers also depends on the pH value. As was mentioned above, changing pH values have the potential to change the electrical charge of the molecule. This leads to the sorption effect of pH dependence. The higher the pH the lower the sorption. Fluorescent tracers are mostly organic molecules with various functional groups attached to the molecular kernel. In

addition, the functional groups protonate and deprotonate depending on pH, thereby changing the net charge of the molecule (Flury and Wai, 2003).

Consequently, certain tracers tend to be absorbed in different proportions within acidic media and substrates. In the case of experiments to be carried out in such media, it is necessary to consider carefully whether it is possible to use fluorescent tracers or not. Negative analyses caused by pH-induced absorption may lead to a complete misinterpretation of an experiment if the result is interpreted as an inexistent hydrological connection. The problem is discussed in Leibundgut (1974), Behrens, Oerter and Reinwarth (1982) and in the Chapter 7.5.2. In natural waters problems occur principally in acid soils, peat-bog and swamp regions, and generally in crystalline geological settings. Since these features are characteristic for the tropics and subtropics as well as the arctic, the use of fluorescent tracers is to be handled carefully in these regions. Typically only a few experiments are known to have been performed in these regions (Smart and Smith, 1976).

4.1.2.5 Temperature dependence

The temperature dependence of the fluorescent tracers is usually unproblematic. Fluorescence intensity and temperature are inversely proportional. For analytical purposes this dependence may be neglected during analysis and calibration, provided the laboratory temperature is 20 °C and measurement lasts no longer than 30 s (Feuerstein and Selleck, 1963; Leibundgut, 1974, 1978; Behrens and Demuth, 1992). The standardization of deviant temperatures can be made applying the equation resulting in the constants in Table 4.5 (Leibundgut, 1978):

$$F_s = F^* \exp(h(T_s - T)) \tag{4.4}$$

F_s – fluorescence at temperature T_s
F – fluorescence at temperature T
h – tracer dependent coefficient (1/°C)
T_s – standard temperature (°C)
T – measurement temperature (°C)

Again, the products of different producers may exhibit a slightly different constant value (h). In order to be certain a controlling calibration is recommended.

Measurements made during in situ tests always require a standardized water temperature of 20 °C using the constants in Table 4.5. This may be difficult during tests in which there are rapid variations in temperature; for example in surface water tests (Leibundgut and Zupan, 1992). Further information on this subject is provided in Chapters 6 and 7.

4.1.2.6 Photolytic dependence

Unlike pH dependence, exposure to light has an irreversible effect on fluorescence. Therefore, the use of fluorescence in the study of surface waters is feasible only to a

Table 4.5 Fluorescent's tracer constants (h) of temperature dependence

Tracer	Coefficient (h)
Naphthionate[a]	−0.0056
Pyranine[b]	−0.0019
Eosine[c]	0.00036
Uranine[c]	−0.0041
Rhodamine B[c]	−0.0172
Amidorhodamine G[c]	−0.0041

[a]Analyzed at the tracer laboratory of the institute of hydrology, Freiburg, in 2008.
[b]Smart and Smith (1976).
[c]Leibundgut (1978).

limited extent. In soil and groundwater experiments photolytic decay is usually not a problem.

As neon light has a short wavelength the samples in the lab also undergo degradation during exposure. Therefore, only the sample currently being analysed should be handled in full light, whether neon or daylight. The other samples need to be covered. This applies in both the lab and out in the field.

The photolytic degradation of a substance is described by a first-order equation and depends on the energy of the excited state formed. The higher this energy and the longer the irradiation time (t), the greater the decomposition of the molecule of the excited substance. The smaller that (t) is, the less time the molecule has to react (Viriot and André, 1989):

$$I(t) = I_0{}^* \exp(-kt) \tag{4.5}$$

I(t) = fluorescence after irradiation time t
I_0 = fluorescence at irradiation time t = 0
k = degradation coefficient
t = irradiation time

The decay rate is correlated inversely to the tracer concentration. The lower the concentration, the higher the decay rate. This is probably partly due to the filter role of the molecules at high concentrations. At high concentrations the molecules in the bulk of the solution are shielded from the incident light by the high absorption near the surface (quenching effect) and, therefore, fewer molecules degrade (Leibundgut, 1978). However, Feuerstein and Selleck (1963) claimed that the decay rates are independent of concentration in the range 1–100 mg/m^3. Temperature is another factor involved in photochemical decay. However, as the temperature correction (Equation (4.4)) indicated, a difference of less than 2%, which is within the error deviation range (Behrens and Demuth, 1992), can be neglected.

Many of the researchers who have investigated the rapid photochemical decay of fluorescent tracers, mostly that of Uranine, did so under controlled conditions in the

Table 4.6 Benchmark values of the measured and the relative half-life values of dye tracers due to photolysis. The data are not strictly comparable due to different test conditions

Fluorescence tracer	Measured half-life $T_{1/2}$ (h)	$T_{1/2}$ tracer/$T_{1/2}$ Uranine
Naphthionate	41	3.7
Pyranine	47	4.3
Uranine	11	1
Eosine	6	0.5
Amidorhodamine G	550	50
Rhodamine B	790	71
Rhodamine WT	1300	118
Sulforhodamine B	820	75

lab (Leibundgut, 1974, 1978; Werthemann, 1980a, b; Behrens and Teichmann, 1982; Behrens and Demuth, 1992; Hadi, 1997). Even so, the results differ considerably. The boundary conditions of the lab experiment, such as radiation amount, wavelengths, temperature, type of bottle used and their radiation transmission, differ to the extent that only benchmark values can be provided (Table 4.6 and Figure 4.9).

Behrens and Teichmann (1982) discovered that for some dyes the process of photolytic decay does not necessarily follow a first-order equation as described above. As can be seen in Figure 4.9 (left), the rates of decay of Pyranine, Uranine, Eosine and Naphthionate follow straight lines, which contrasts with those of the Rhodamines (Figure 4.9, right). To the surprise of the investigators, however, when dissolved in pure water the Rhodamines also plotted as straight lines following a first-order equation.

Table 4.6 and Figure 4.9 clearly indicate two groups of tracers. The first are fluorescent tracers with excitation maxima below 520 nm (Eosine, Uranine, Pyranine, Naphthionate) and the second are those with maxima above 520 nm (Rhodamines). The latter are less sensitive to photochemical decay. The values given in Table 4.6 indicate the different sensitivities of tracers to light and should be consulted when planning the use

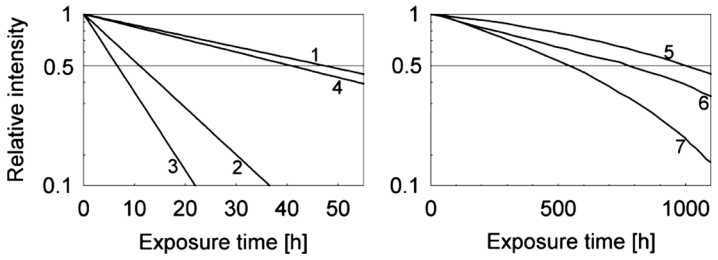

Figure 4.9 Photolytic decay of various fluorescence tracers caused by exposure to light. Left: tracer group with an excitation maximum below circa 520 nm (1: Pyranine, 2: Uranine, 3: Eosine, 4: naphthionate). Right: tracer group with an excitation maximum above 520 nm (5: Rhodamine WT, 6: Sulforhodamine B, 7: Amidorhodamine G) [source: Behrens and Teichmann, 1982; Wernli, 1986].

of fluorescent tracers in experiments. In principle, the two standard tracers, Uranine and Eosine, are clearly not suitable for surface water experiments.

Under ideal laboratory conditions photolysis follows a strict law (Equation (4.5)), which is generally not applicable under field conditions. Here photolytic decay depends on the intensity of the radiation to which the tracer molecule is exposed. This intensity is not constant in space and time. It changes with different atmospheric conditions, the shading effects of trees and turbidity and/or turbulence effects (Hadi, 1997). The half-life lengths and decay rate values quoted in Table 4.6 and Figure 4.9 are, therefore, benchmark or guideline values to be referred to when planning field experiments. They allow for a rough estimation of photochemical decay under the conditions of a specific experiment. Consequently, they make an assessment of the suitability of a certain tracer for a particular experiment, and the calculation of the required tracer mass possible, but they do not allow for precise corrections. To be safe, the values should be considered to be minimum values as the decay rates under natural conditions may be higher rather than lower. Further information is given in Section 7.3.4.

Experience shows that even the parallel measurement of radiation conditions during an experiment is problematic, due to the highly variable surrounding conditions described above, and also the water itself where turbulence and the varying layer depths result in further uncertainties (Petermann *et al.*, 1989). However, provided a successful determination of the photolytic decay during tracer experiments in rivers is achieved, a decay correction term can be introduced in the calculation of the flow parameters (Petermann *et al.*, 1989; Naturaqua, 1994). For further detail refer to Section 7.4.2.2.

Whereas no problems are to be expected in groundwater experiments, except where the sampling is affected in springs, it is readily apparent that tracer tests carried out in surface waters need to be rather of a relatively short duration. Great caution must be exercised when making quantitative measurements employing fluorescent tracers in rivers and lakes (see Section 7.4). In the case of surface waters the optimal time for tests using fluorescent tracers is at night. All in all, intelligent test arrangements may provide good solutions to this problem; for example, the use of the highly sensitive Uranine in a lake experiment carried out to determine the residence time of water in Lake Bled, Slovenia. The half-life time for Uranine of 11 h was used to calculate the decrease in the concentration and quantity of Uranine (Leibundgut and Zupan, 1992). Photolytic decay is crucial when using the tracer dilution method to determine runoff quantitatively. In this case, the problem can be eliminated or minimized only in experiments of very short duration carried out under adequate weather conditions (clouded), along shaded stretches of river or at night (see Chapters 6 and 7).

This dependence can also be useful, however. It is an effective way to eliminate specific tracers from a sample mixture (see Section 4.1.3.3). Photolytic decay can, in principle, also be turned into an advantage by using the relationship between irradiation and decay to measure the irradiation by assessing the decay of a known tracer substance over time. Leibundgut (1978) investigated this technique, developed the so-called fluorescence-actionometer (a bulb of special glass) and applied a number of them as free floating measuring points beneath the surface of a small lake. Field monitoring experiments lasting several weeks and requiring low maintenance are possible. The same approach was investigated systematically by Behrens and Demuth (1992).

4.1.2.7 Sorption processes

Sorption is the most important property relevant to the use of artificial tracers generally, and fluorescent tracers in particular. Sorption is a crucial process in the performance of experiments in the saturated and unsaturated zones. A first indication of the sorption behaviour of a tracer is provided by the solubility. The higher the solubility of a tracer substance, the lower its sorption (see Table 4.10). An exception is Amidorhodamine G, which has a low degree of solubility of approximately 3 g/l but possesses comparatively good sorption characteristics. Depending on their molecular make-up, fluorescent tracers exhibit widely contrasting reactions upon contact with different substrates. Generally anionic and neutral substances are less susceptible to sorption than cationic. Cationic tracers usually interact more strongly with the substrates, but both groups usually react in a way that is referred to as reversible sorption, the effect of which is chromatography (Leibundgut, 1981b).

The amphiphilic nature of fluorescent tracers and the fact that the electrical charge of the molecule is subject to change mean that their interaction with solid surfaces is complex. Sorption of dyes to solid surfaces involves one or a combination of the following interactions: hydrophobic, van der Waals, ion exchange, covalent bonding and hydrogen bonding (Zollinger, 1991; Schwarzenbach, Gschwend and Imboden, 1993; Flury and Wai, 2003). Rhodamine WT consists of two isomers with different sorption characteristics, which can lead to retardation (Shiau, Sabatini and Harwell, 1992; Sutton *et al.*, 2001; Hadi, 1997).

However, the averaged transport velocitiy of dissolved anionic tracers through sediments may be greater compared to that of the water molecules due to anion exclusion (Gvirtzman and Gorelick, 1991). The electrostatic repulsion by negatively charged solid surfaces forces the tracer anions into the centre of the pores where the velocity is faster. The effect has been shown for Naphthionate in laboratory and field tests (Leibundgut and Wernli, 1986; Sansoni *et al.*, 1988).

The identification of sorption in a tracer experiment is quite difficult. The reason for this is that several processes, such as adsorption, absorption and so on, result in the 'sorption' of tracers. For tracer hydrological purposes, the phenomenon of the ratio of injected tracer mass captured by one or more of these processes is usually important. Leibundgut (1981b) proposed, therefore, that the definition of sorption in tracer hydrology be 'the total of the sorpted tracer caused by all of the processes involved'. The fundamentals of the sorption processes themselves will not be discussed further here, but their impact on a tracer breakthrough will be presented in Figure 4.10.

The surfaces of soil and aquifer particles are usually negatively charged. The degree of sorption in these substrates rises from 0 to 100%. The latter number reflects an irreversible sorption, and a hydrological tracer which is consequently unusable.

The problem of reversible sorption is shown clearly in Figure 4.10. Curve A (ideal tracer) represents the breakthrough of water movement ($v_{water} = v_{tracer}$), the behaviour of the substance we wish to know more about. Curve B alternatively represents the breakthrough of a nonideal sorptive tracer ($t'_o = t_t$ – mean transit time of tracer),

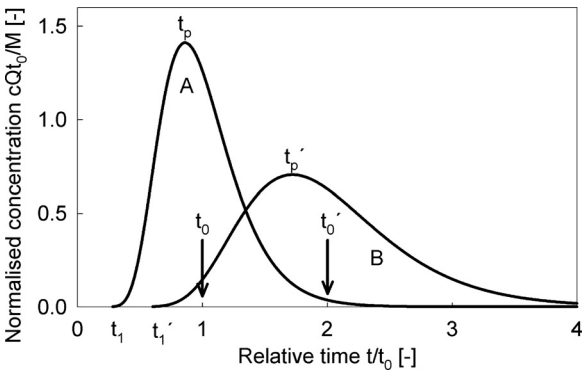

Figure 4.10 Effect of chromatography caused by reversible adsorption depicted in a tracer breakthrough curve. Curve A: ideal tracer breakthrough (t_o – mean transit time of water); that is without any sorption effects. Curve B: reactive tracer breakthrough following a reversible reaction with an instantaneous equilibrium ($t'_o = t_t$ – mean transit time of tracer). In both cases the recovery of the tracer is complete (100%).

resulting in lower flow velocities. The second tracer (curve B) follows reversible sorption with instantaneous equilibrium, which leads to a slower transport of the tracer relative to the flow of the water (see Equation (4.6)).

A precondition of the investigation of the flow dynamics of water is an ideal tracer representing the water flow, or at least a nearly ideal tracer. The mathematical description of the ideal tracer transport phenomenon is given in Chapter 5. However, not all of the available tracers are strictly conservative. Different mathematical models can be used to describe the reaction of a nonideal tracer with the matrix (e.g. Carnahan and Remer, 1984). Most tracer hydrologists, however, generally assume that a nonideal tracer reaction is followed by instantaneous equilibrium and a linear reaction isotherm, which is described by Equation (4.6). In this case, the transport of the nonideal tracer is slower than the flow of the water (curve B in Figure 4.10) by the retardation factor (R_d), described by Equations (4.7) and (4.8). This type of reaction is seldom observed in practice.

It is known from the literature that, in the case of nonideal tracers, two or even three reactions may often occur simultaneously. The conceptual outline of some of the types of reaction that occur between water (solute) and a porous matrix is provided in Figure 4.11. In the first-order reversible kinetic reaction model, the parameters k_1 and k_2 are the forward and backward reaction rate constant, respectively ($k_1 > 0$; $k_2 > 0$). The irreversible first-order reaction in which k_{irr} is the irreversible reaction rate constant (e.g. radioactive decay, biodegradation) is also incorporated in Figure 4.11. In the event that the first-order kinetic reaction becomes irreversible ($k_2 = 0$), the forward reaction rate is equal to k_{irr} and the irreversible process is represented by the sum of k_1 and k_{irr} ($k_1 + k_{irr}$). The parameter R_3 describes the retardation of pollutant movement relative to the flow of water in the case of an instantaneous equilibrium reaction. When both first-order kinetic (reversible or irreversible) and linear equilibrium reactions occur simultaneously the so-called two-site (or combined) reaction model introduced

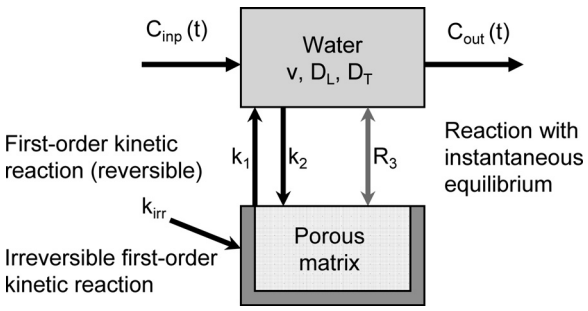

Figure 4.11 Dispersive transport in water (convection and dispersion). Presentation of some theoretical reactions between water (solute) and a porous matrix.

by Cameron and Klute (1977) applies. This particular model combines two reactions: instantaneous equilibrium with a linear sorption isotherm and a first-order (reversible or nonreversible) kinetic reaction. Some typical tracer breakthrough curves yielded by the two-site reaction model are shown in Figure 4.12, where they are compared to the curve of an ideal tracer, which follows only convective-dispersive transport within flowing water.

In practice it is very important that tracer mass recovery be calculated according to Equation (5.40) in Chapter 5. The curve can help reveal the type of reaction that occurs between the matrix and the pollutant follows (Figure 4.13).

The analytical solution for convective-dispersive transport coupled with a combined (two-site) reaction model was developed by Klotz, Maloszewski and Moser (1988), and applied to evaluate the migration of two radioactive pollutants (strontium and europium). Hendry, Lawrence and Maloszewski (1997, 1999) used the same model

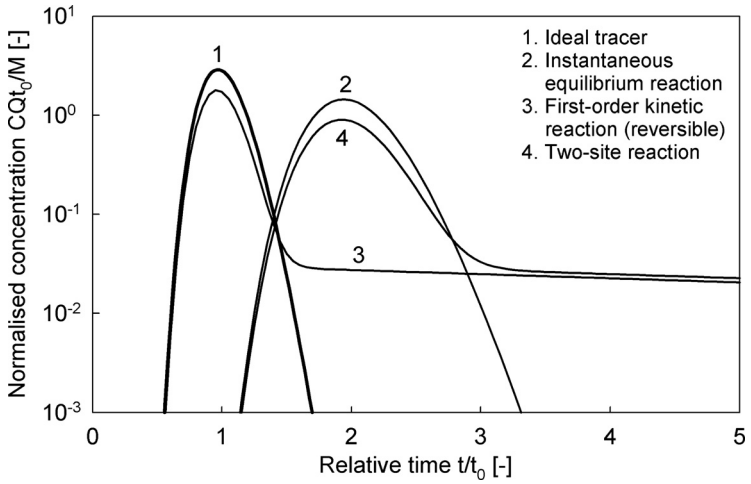

Figure 4.12 Tracer breakthrough curves observed after instantaneous injection of two tracers: ideal (1) and nonideal (curves 2–4).

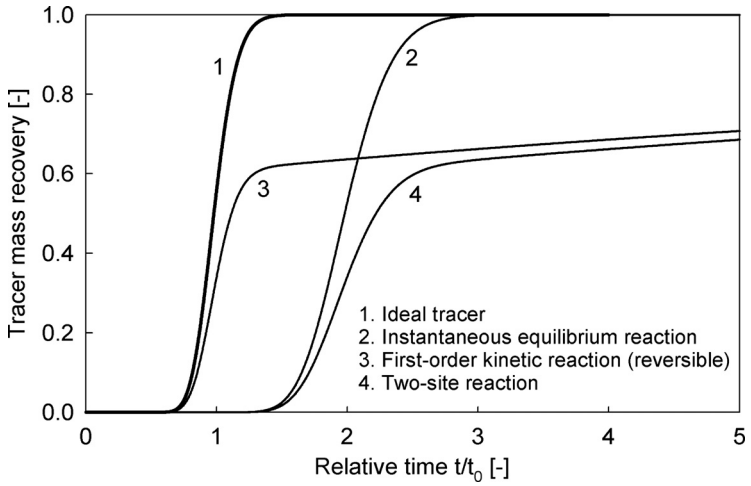

Figure 4.13 Tracer mass recovery curves calculated for the examples illustrated in Figure 4.12.

successfully to describe bacteria migration in a sand column, while Maloszewski *et al.* (2003) used it to describe herbicide transport in a porous aquifer. Maloszewski and Zuber (1990) also employed it in the interpretation of a multi-tracer test performed in fractured marls. The transport of nonideal tracers (contaminant transport) is not, however, the focus of this book. Some key publications are listed below for the benefit of the interested reader.[3]

The purpose of the following general considerations in relation to sorption is intended to illustrate the process. In general, tracer experiments in sandy substrates using suitable tracers (Table 4.2) allow for a complete, or nearly complete, recovery of the tracer, permitting a correct calculation of the aquifer parameters (Figure 4.14). Experiments in clayey material, however, usually result in incomplete recovery (Figure 4.15). The two Figures show two column experiments to investigate the tracer recovery of Uranine in a sandy (Figure 4.14) and in a clayey (Figure 4.15) substrate. Phase A_i represents the column transits by the tracer solution, including both saturation of column water and sorption processes, and phase B_i flushing transits with clear water and desorption. The simulation in the lab replicates the natural conditions present during a tracer experiment with an instant injection (Dirac impulse). In natural experiments the two processes occur simultaneously (Leibundgut, 1981b).

As well as aiding in the selection of tracers for field experiments and in the calculation of the amount of tracer to be injected, the two parameters K_d (distribution coefficient) and R_d (retardation coefficient) characterizing sorption processes are commonly used when the instantaneous equilibrium reaction with a linear sorption isotherm is applicable. The parameters are discussed in the following paragraphs.

[3]Cameron and Klute (1977); Carnahan and Remer (1984); Hendry, Lawrence and Maloszewski (1997, 1999); Klotz, Maloszewski and Moser (1988); Maloszewski and Zuber (1990); Maloszewski *et al.* (2003).

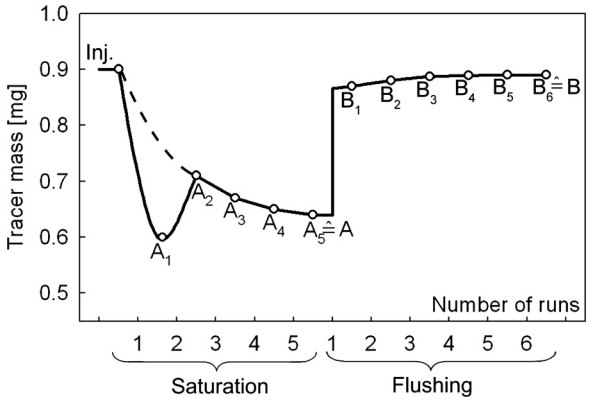

Figure 4.14 Tracer breakthrough curve of Uranine in a sandy substrate showing complete tracer recovery. Sorption during phase A is caused by saturation of the column water, whereas phase B represents the flushing effect.

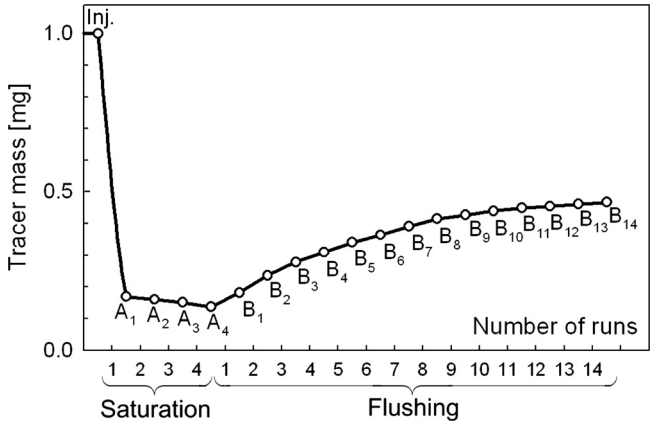

Figure 4.15 Tracer breakthrough curve of Uranine in a brown soil substrate showing incomplete tracer recovery with sorption of approximately 50% of the injected tracer mass.

4.1.2.7.1 The distribution coefficient K_d Distribution coefficients are commonly determined in batch experiments using the following equation:

$$K_d = {}^{V}\!/_{m}{}^{*} \frac{c_i - c_s}{c_s} [\mathrm{cm}^3/\mathrm{g}] \tag{4.6}$$

where

V = volume of the solution,
m = mass of the dry substrate,
c_i = initial tracer concentration and
c_s = dissolved tracer concentration (equilibrium solution).
K_d = depends on the ionic composition of the exchanger and the solution.

K_d represents the mobility of the tracers in groundwater, describing the thermodynamic equilibrium of the tracer between substrate and solution. Obviously, the tracer concentration (isotherms) has a strong impact on the sorption level of the tracer. The higher the K_d value, the higher the sorption. This process assumes the linear sorption isotherm with instantaneous equilibrium. One should take into account that most sorption/desorption processes follow a kinetic linear or nonlinear reaction. Hence, Equation (4.6) is not always applicable. For the batch experiment a short instruction is given in the box below.

The distribution coefficient K_d is determined by means of a batch test as follows:

Add 250 ml tracer solution (volume V with a concentration c_i) to a glass bottle containing 100 g of substrate (m) dried at a temperature of 105 °C. Shake for 24 h at a rate of 140 shakes per minute. After 24 h the tracer concentration in the equilibrium solution (c_s) is measured. K_d is calculated according to Equation (4.5).

Numerous sorption tests have been performed and further distribution coefficients can be found in the literature.[4] In general, distribution coefficient values depend not only on the tracer properties, but also on the substrate (e.g. texture, material, grain size) and on the concentration, and so exhibit a high degree of variability (e.g. Ptak and Schmid, 1996; German-Heins and Flury, 2000). Values resulting from laboratory sorption experiments are maximum values considered to represent the upper boundary (Table 4.7). They facilitate the assessment of the suitability of a certain tracer for a given investigation, and the calculation of the required tracer mass. They cannot be used for linear corrections of field experiments.

4.1.2.7.2 The retardation coefficient The retardation coefficient can be calculated with:

$$R_d = \frac{v}{v_t} \tag{4.7}$$

where

v = mean flow velocity of water and ideal tracer respectively,
v_t = mean transport velocity of the tracer

or

$$R_d = 1 + \rho^* \frac{(1 - n_e)}{n_e} * K_d \tag{4.8}$$

[4]For example, Feuerstein and Selleck (1963); Leibundgut (1974); Smart and Laidlaw (1977); Sabatini and Austin (1991); Leibundgut *et al.* (1992); Hadi *et al.* (1997); Allaire-Leung, Gupta and Moncrief (1999); Kasnavia, Vu and Sabatini (1999); Kranjc (1997); and others.

Table 4.7 Distribution coefficients (K_d) from batch experiments. The higher the K_d value, the higher the sorption

Tracer	c_i [mg/m^3]	K_d [cm^3/g][a]	K_d [cm^3/g][b]	K_d [cm^3/g][c1]	K_d [cm^3/g][c2]	K_d [cm^3/g][c3]
Naphthionate	10	0	0.38	—	—	—
	100	0	0.28			
Pyranine	10	0.03		—	—	—
	100	0.24				
Uranine	10	0	0.4	0	—	—
	100	0	0.28	0	0	0
Eosine	10	0	5.51	0	—	—
	100	0.03	4.37	0.025	0.09	0.24
Amidorhodamine G	10	1.23	—	1.23	—	—
	100	0.92		0.92	0.19	0.75
Rhodamine B	10	5.56	—	5.61	—	—
	100	4.75		4.75	1.25	9.18
Rhodamine WT		—	—	—	—	—
Sulforhodamine B	10	—	36.6	—	—	—
	100		29.4			

[a]Wernli (1986), substrate: tertiary molasse sand; 250 ml tracer solution.
[b]Mägdefessel (1990), substrate: 79.6% sand, 18.1% silt, 2.3% clay; 250 ml tracer solution.
[c]Dervey (1985), substrate: (1) mica gneiss; (2) coarse lime sand; (3) fine lime sand.
Further distribution coefficients from batch experiments can be found in Klotz (1982a, b) and Sansoni *et al.* (1988).

where

ρ = dry density of substrate,
n_e = porosity of substrate and
K_d = distribution coefficient.

In order to provide the reader with an impression of the suitability of the fluorescent tracers in different soil and aquifer substrates, benchmark values obtained from batch and column experiments are listed in Tables 4.7 and 4.8. Two concentrations (c_i 10 and 100 mg/m^3) are selected in Table 4.7 to highlight the link between concentration and distribution coefficient. R_d describes the retardation of tracer transport caused by an instantaneous equilibrium reaction (Table 4.8). R_d values are obtained by means of column tests (e.g. Klotz, 1982a, b; Dervey, 1985).

Although the reference values obtained from lab experiments may not be transferred directly to field conditions, they provide a useful guideline for the estimation of the sorption losses of a given tracer and, consequently, the estimation of the tracer injection mass required. The formulae to estimate the tracer injection mass will be provided in Chapter 6. The following procedure is recommended for the preparation of an experiment:

1. Complete a batch test employing the tracers that may potentially be used in the substrates to be investigated.

Table 4.8 Retardation coefficients from column experiments

Tracer	c_i [μg/l]	$R_d{}^{a1}$	$R_d{}^{a2}$	$R_d{}^{a3}$	c_i [μg/l]	$R_d{}^{b1}$	c_i [μg/l]	$R_d{}^{b2}$	c_i [μg/l]	$R_d{}^{b3}$
Pyranine	20	1.18	1.22	1.04						
Uranine	10	1.18	1.22	0.99	10	1	10	1	10	1
Eosine	20	1.69	1.55	1.12	90	1	90	1.9	90	1.1
Amidorhodamine G					30	2.7	30000	22.1	30	1.3
					80	2.3	80000	9.9		
Rhodamin B	50	>6	>5	>8	10	8.8	80000	40.3	80	2.4
					100	5.9				

[a]Klotz (1982a, b), substrate: (1) fluvio-glacial gravel; (2) drift and valley sand; (3) tertiary gravel sand.
[b]Dervey (1985), substrate: (1) mica gneiss; (2) molasse sandstone; (3) limestone.

2. Select the optimal tracer.

3. Estimate the tracer injection mass using a loss parameter for sorption.

As a result of the experience gained in many tests a general assessment of the tracer's suitability is listed in Table 4.9.

The use of artificial tracers in the vadose zone may involve additional criteria compared to the application in the saturated zone. In order to investigate macropore flow even sorptive tracers like Rhodamines and nonfluorescent dye tracers are needed to visualize the water flow. To simulate the leaching of compounds such as pesticides via macropores shortly after a heavy precipitation events (strong) sorptive tracers, for example the Rhodamines, are suitable (Brandi-Dohrn *et al.*, 1995).

Tracer experiments, particularly in crystalline and in swampy areas, may be confronted with acidic water. In aqueous acidic solutions, some of the xanthene tracers may switch to a nonfluorescent form (see Chapter 4.1). Consequently, the tracer in the sample cannot be measured by means of a fluorescence analysis without readjusting the sample to an adequate pH-range, as discussed in Section 4.1.2.3.

In acidic environments some fluorescent tracers (e.g. Uranine) may change to a cationic state. This modifies the transport behaviour significantly. The positively charged molecule can be absorbed on the soil and aquifer matrix of which the tracer is lost entirely. Many tracer hydrologists know by experience that Uranine tracer tests have a higher failure rate in humic soils or acidic aquatic environments with low pH. In such

Table 4.9 Suitability of tracers based on the sorption effect for different fields of application

Hydrological compartment	Suitable tracer based on the sorption criteria
Porous groundwater	Uranine, Pyranine, Eosine, Naphthionate
Karst groundwater	Uranine, Pyranine, Naphthionate, Eosine, Rhodamines
Fissured rock groundwater	Uranine, Pyranine, Naphthionate, Eosine
Unsaturated (vadose) zone	Uranine, Eosine
Surface water	All fluorescent tracers
Glaciated areas	All fluorescent tracers, except Pyranine

cases, the application of Rhodamines represents an alternative due to their resistance to low pH-values. However, the high sorption affinity of Rhodamines prohibits the use in aquifers, especially in soils and porous aquifers. For shorter distances, Bromide or Deuterium as an artificial tracer may represent alternatives.

4.1.2.8 Chemical and biological stability

Fluorescence quenching is used as an umbrella term for several processes causing a reduction or suppression of fluorescence intensity. Quencher molecules are molecules that are involved in these processes, and which either hinder molecules becoming excited or transfer the excited molecule radiation back to ground state. The latter can occur when excited fluorescent molecules and quenchers collide, and the energy is transformed into heat energy (dynamic Stern–Volmer relationship), or when the excitation energy is passed to the quencher due to resonance energy transfer (Förster resonance energy transfer). Complex building by fluorescent molecules and quenchers can limit or terminate the ability to fluoresce (static Stern–Volmer equation), or result in a colour change (Laitinen, 1960). A high energy state is more readily reactive than the base state.

Dye tracers may be readily quenched and/or decomposed as a consequence of oxidation and other chemical changes. However, oxidative processes affect the dye tracers to different degrees. Whereas the Rhodamines are more resistant, the other dyes will be irreversibly quenched. Consequently, chlorinated water should not be used to prepare calibration solutions, nor should it be used in tests of water supply installations with chlorination or ozonization facilities if it is not possible to take samples prior to treatment (Wilson, 1968; Leibundgut, 1974; Käss, 1998).

Salinity may also affect fluorescence but generally to a much lesser degree than either pH or light. High concentrations of salt decrease the fluorescence signal, but do not change the spectrum itself (André and Molinari, 1976; Smart and Laidlaw, 1977; Flury and Wai, 2003). Magal *et al.* (2008) reported the same effect for sea water. In more saline sea water, the fluorescence signal is inversely proportional to the salinity, and the sorptivity increases. For example, in water from the Dead Sea the fluorescence intensity is 10–15% of that of pure water.

Chemical quenching may occur due to conversion of the dye to a nonfluorescent iodate derivate (Gaspar, 1987; Hadi *et al.*, 1997). Potentially more problematic is fluorescence quenching or metal complexation leading to reduced fluorescence, or even to a change in colour. A more detailed description of these processes can be found in the original literature published by Stern and Volmer (1919) and Förster (1952, 1982) and so on.[5]

Another form of fluorescence quenching is concentration-quenching, which occurs at very high concentrations (see Section 4.1.2.2). The quenching is caused by a self-shadowing effect of the molecules, which reduce the fluorescent intensity. Photolytic decay, the chemical alteration of the fluorescent molecules brought about by exposure

[5]Williams and Bridges (1964); Gaspar (1987); Van Der Meer, Coker and Chen (1994); Flury and Wai (2003).

to light, is sometimes considered to be a form of fluorescence quenching, but unlike quenching processes it effects a change to the net charge of the molecule (pH). Photo bleaching is irreversible.

Microbial degradation of fluorescent tracers is also known to occur, in natural waters and in samples. However, no clear rules can be formulated to correct the measured concentration values of dyes (Zupan, 1982, 1989). Obviously, this kind of degradation can lead to misinterpretation of tracer experiments as shown by Goldscheider, Hötzl and Kottke (2001) for Naphtionate. It is noticeable the decay did not occur in the aquifer but in the samples stored at room temperature. Microbial decay of Uranine is widely described in the literature (Behrens, 1986; Behrens and Demuth, 1990; Behrens and Leibundgut, 1992; Hadi, 1997; Käss, 1998).

There are hints of metabolism of fluorescent tracers in contact with other chemicals and during long term experiments due to a shift of fluorescent maxima (see Section 4.1.2.4). Under special conditions, Sulforhodamine B appears to undergo the process of dealkylation quite quickly, which leads to a shift of the spectrum towards the emission peak of Eosine. Mägdefessel (1990) observed this phenomenon in a sample taken only eight months after injection in a porous aquifer. The analysis was performed by means of D,C-chromatography (Figure 4.16). Empirical data were provided by Weiss *et al.* (2008) based on reddish groundwater from an aquifer traced 30 years ago. In addition to the original tracer Sulforhodamine, its metabolites (amine derivates) were also identified. However, studies of this nature require advanced analytical techniques (HPTL/AMD, Nano-Chip-LC/QTOF-MS) (see Section 4.1.3.8).

As demonstrated by the study above, fluorescent tracers appear to remain stable over very long periods of time in unpolluted groundwater aquifers. Other concurring examples have been reported by Bauer (1969) and Käss (1998). According to a personal communication by Wernli relating to an ongoing long term tracer experiment in the Swiss Tertiary Molasse (Wernli and Leibundgut, 1993), Eosine remained stable over a period of 24 years.

Samples of fluorescent tracer may remain stable for a long time provided the water is pure. Samples should be taken in brown glass bottles or, if using plastic,

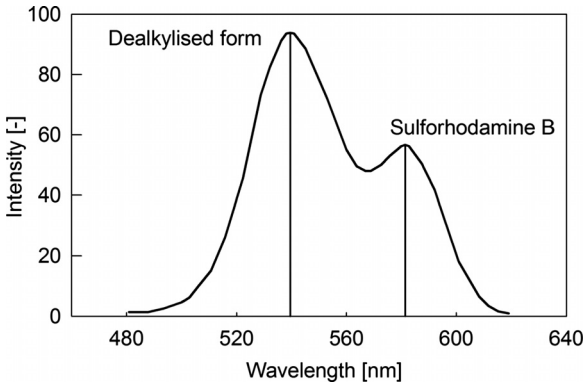

Figure 4.16 Shift of the emission peak of Sulforhodamine B brought about by a dealkylation process (Mägdefessel, 1990).

in polyethylene or polysulfone bottles. As the chemical compositions of plastic bottles differ, it is recommended to test the effects on sorption on all kind of bottles to be used.

In the case of field experiments dealing in particular with karst water, polluted water and sewage water, microbial decay must be considered carefully. It is recommended that the samples be analysed as quickly as possible in order to avoid the problem to the greatest degree possible.

4.1.2.9 Toxicity and related environmental effects

Each injection of artificial tracer in a hydrological system is in a sense a contamination of the water body in question. However, carefully planned and correctly prepared tracer experiments generally involve only minimal quantities of fluorescent tracer substances in the range of grams, or kilograms at most. Therefore, the 'contamination' is usually tolerable. When preparing a tracer experiment it is vitally important that national regulations pertaining to tracer experiments are consulted. It will be necessary to make a formal application in some cases.

The commonly used hydrological tracers have been investigated intensively in several studies.[6] Summarizing the results in relation to human and eco-toxicological aspects the following can be stated: Uranine is harmless; Eosine, Pyranine and Naphthionate appear to be harmless. It is suspected that the Rhodamine group as a whole is toxic, except Amidorhodamine G and Sulforhodamine B which are less problematic (Table 4.10). When planning tracer experiments it must also be considered that problems may occur in future by metabolism of fluorescent tracers, as described in the previous chapter (Figure 4.16). A new study using the TDI approach (tolerable daily intake) indicated a slightly different assessment for Uranine (Brüschweiler, 2007). All authors agree, however, that a correct evaluation of the injection mass of a tracer taking into consideration the expected tracer concentrations in the water used is required (cf. Section 6.2).

4.1.2.10 General assessment

Of practical interest is the price of tracer substances, which is not negligible. The cost of the tracer required for an experiment can be calculated by multiplying the relative fluorescence yield by the quantity (g) and the price. A comparative evaluation of the costs is not provided in Table 4.10 since the prices fluctuate considerably. An internet search is recommended in order to find the most reasonable current prices.

In order to aid in the evaluation of tracer substances a summary assessment of the commonly used tracers is provided in Table 4.10.

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[6]UBA (1996); Käss (1967a); Little and Lamb (1973); Nestmann, Kowbel and Ellenton (1980); Smart (1982); Hofstraat *et al.* (1991); Leibundgut and Hadi (1997); Käss (1998); Behrens *et al.* (2001).

Table 4.10 Summary of the characteristics of the fluorescent tracers

Tracer	Ex/Em [nm]	Relative fluorescence yield	Detection limit [mg/m^3]	Toxicity	Solubility [g/l]	Light sensitivity	Absorption behaviour
Naphthionate	325/420	18	0.2	Harmless	240	High	very good
Pyranine	455/510	18	0.06	Harmless	350	High	Good
Uranine	**491/516**	**100**	**0.001**	**Harmless**	**300**	**High**	**Very good**
Eosine	515/540	11,4	0.01	Harmless	300	Very high	Good
Amidorhodamine G	530/555	32	0.005	Sufficient	3	Low	Sufficient
Rhodamine B	555/575	9.5	0.02	Toxic	3–20	Low	Insufficient
Rhodamine WT	561/586	10	0.02	Toxic	3–20	Very low	Insufficient
Sulforhodamine B	564/583	7	0.03	Sufficient	10 (10 °C)	Low	Insufficient

4.1.3 Measurement techniques

Fluorometry is by far the best of the techniques available for the measurement of fluorescent tracers. Depending on the wavelength ranges of the dye tracers, the analyses cover the visible and the near UV range of the electromagnetic spectrum. A high pressure xenon lamp used as a light emitting source covers a spectrum which includes the peak of most tracers, at wavelengths of approximately 400–550 nm. The emission intensity is used in the analysis of fluorescent tracers. Putting the physical laws to use, the measurement of fluorescence tracers dissolved in water is conducted nowadays by fluorometers. Several types of devices (filter and spectral fluorometer) are available.

Both techniques follow the general principle of fluorescence analysis using the fluorescence capacity of the tracer, and feature a light source that excites the sample and a detector that measures the emitted light. In order to analyse the characteristic wavelengths of the dyes (excitation, emission) filters or monochromators are used to extract from the wider wavelength range of the light source the excitation wavelengths, which is generally a narrow band. The excited solution scatters the light and fluorescence. A second set up of filters or monochromators separates the emitted scattered light from the fluorescence light, which is measured with a detector (photomultiplier). Usually the excitation light and the emission detector are arranged at right angles to one another to avoid distortion by the transmitted light (Figure 4.17). This way the strong transmission beam does not disturb the weak fluorescent light.

In order to avoid scattering light it is best to use narrow band filters or monochromators with a slit standard width of 10 nm. Overly narrow filters (<5 nm) are not useful as the drop in intensity is too great.

4.1.3.1 Filter fluorometer

Only one tracer can be measured at a time, which depends on the set of filters attached. Filter fluorometers are not entirely suitable for lab analyses as the fixed lamp/filter combination only allows for the detection of fluorescence intensity within a certain wavelength range as a sum of the background concentration plus the fluorescence

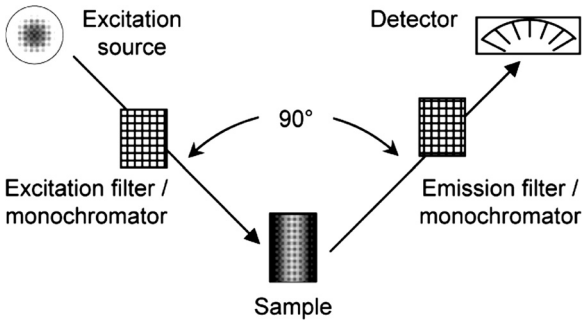

Figure 4.17 Basic fluorescence measurement set-up of a fluorometer.

signal. Therefore, it is impossible to state with certainty whether it is the fluorescence of the tracer applied or 'wild' fluorescence stemming from another substance. Therefore, a spectro-fluorometer is necessary.

However, in case of in situ measurements in field experiments, filter-fluorometers are the most appropriate device, in particular for monitoring purposes and in the case of discharge measurements using a known tracer. When performing multi-tracer experiments, each tracer applied necessitates one filter-fluorometer with the adequate light source/filter equipment. The filter required is a combination edge filter and double band interference filter.

4.1.3.2 Spectral fluorometer

Today the most common laboratory device is the spectral fluorometer. Its advantage derives from the two continuous adjustable monochromators, enabling it to run scans. To eliminate the fluctuation of the excitation energy a part of the excitation light is sidelined to a Rhodamine cell, which provides the needed reference signal. This method returns more precise qualitative and quantitative spectra. The fluorescence of the tracer and of the background at both ends of the spectrum can be identified, and separation of the background signal is possible. The measured fluorescence intensities are analysed directly by a software program. The repeatability of an analysis where a good device is set up optimally is $\pm 1\%$. The technical features of the spectral fluorometer are contained in the corresponding user manual.

4.1.3.3 Synchronous scan technique

The technique has been a feature of chemical analysis since it was presented by Lloyd (1971), and was introduced in the analysis of fluorescent tracers by Behrens (1971), Leibundgut (1973) and André *et al.* (1977). The application of qualitative fluorescent tracer analyses was only made possible by the development of the first spectrofluorometers for the analysis of fluorescence in water samples in the 1960s.

The synchronous scan technique is the most effective way to analyse fluorescence spectra (cf. Figure 4.2). All further information concerning measurement techniques relates to this technique. Instead of running separate scans of excitation and emission spectra, the synchronous scan runs through the spectrum with a constant wavelength distance $\Delta\lambda$, corresponding to the difference in the wavelength between the excitation and the emission spectrum. The optimal $\Delta\lambda$ is tracer specific and is between 20–25 nm for most of the xanthenes (Figure 4.18). However, the difference in intensity is negligible using $\Delta\lambda$ 25 nm instead of the absolutely specific $\Delta\lambda$ depicted in Figure 4.2. The spectrum of the synchronous scan (I_S) is the product of intensities of excitation (I_{Ex}) and emission (I_{Em}) scans: $(I_S) = (I_{Ex}).(I_{Em})$. For operational use a $\Delta\lambda$ of 25 nm is set.

As the scattering signals arise with a narrower $\Delta\lambda$, due to the overlapping of the transmission ranges of the monochromators, a $\Delta\lambda$ of 25 nm is the minimal distance. Pyranine and Naphthionate have a wider $\Delta\lambda$. The synchronous scan spectrum is

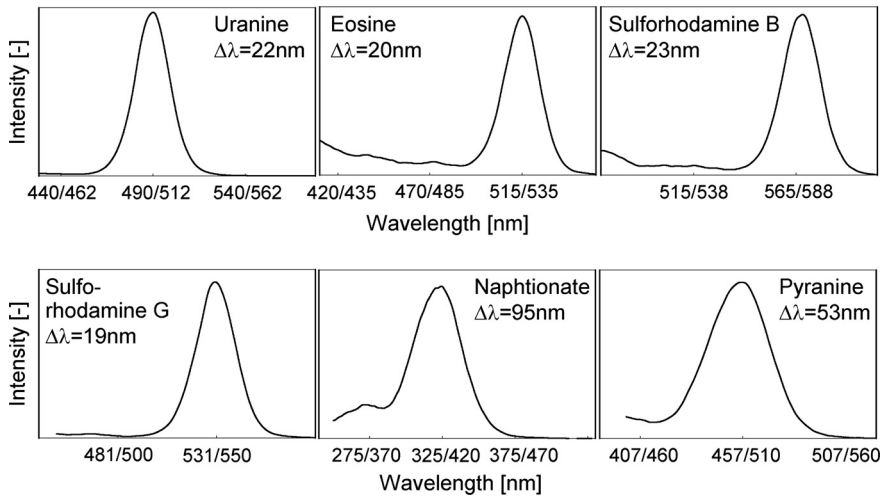

Figure 4.18 Synchronous scans of commonly applied fluorescent tracers. Rhodamine B and Rhodamine WT were omitted based on their similarity to Sulforhodamine B and Amidorhodamine G. 440/462 and so on: excitation/emission wavelength (nm).

considerably smaller than the emission spectrum of a fluorescent dye. It allows for a more precise qualitative identification of a substance.

The synchronous scan technique is essential when conducting multitracer tests. The technique allows for the separation of tracer mixtures in samples. Spectral analyses using synchronous scan provide a means to detect more than one tracer in a sample in only one run, provided the emission maxima of the respective tracers have approximately the same $\Delta\lambda$ and their fluorescence maxima lie in a minimum distance of about 50 nm from each other. Consequently, mixtures of Uranine/Rhodamines and Eosine/Rhodamines (except Amidorhodamine G) can be analysed elegantly in this way running the scan at $\Delta\lambda$ 25 nm (Figure 4.19).

Mixtures of Naphthionate and all other fluorescent tracers can also be detected by means of a synchronous scan. Although no further treatment is required, two runs are necessary; one at $\Delta\lambda = 95$ for the detection of Naphthionate and one at $\Delta\lambda = 25$ nm for the tracers with wavelengths higher than that of Eosine (Figure 4.20).

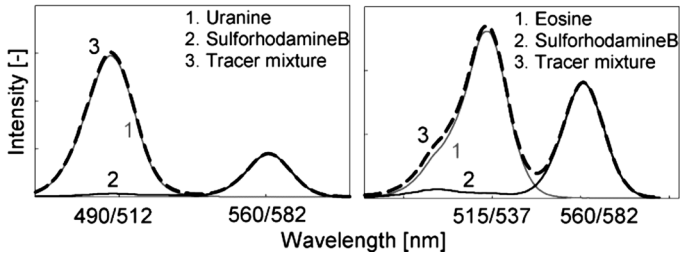

Figure 4.19 Fluorescence spectra of tracer mixtures measured with $\Delta\lambda$ 25 nm. Left: Uranine and Sulforhodamine B; right: Eosine and Sulforhodamine B.

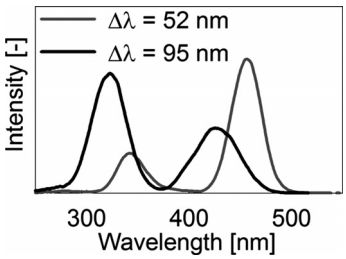

Figure 4.20 Synchronous scans of a Naphthionate-Pyranine tracer mixture require two scans ($\Delta\lambda = 95$ nm: Naphthionate; $\Delta\lambda = 52$ nm: Pyranine).

If the fluorescence maxima of the tracers in a mixed sample differ by $\Delta\lambda < 50$ nm, the (elegant) synchronous scan alone is not sufficient to separate the tracers. In Figure 4.21 the intensities of the two single tracers Uranine (1) and Eosine (2) are added on the mixture (3). Self-evident the spectra of the single tracers are covert within that of the mixture. In such situations further analytical techniques are needed to detect the individual tracers accurately.

Problems of overlapping occur between Pyranine/Uranine, Uranine/Eosine and Eosine/Amidorhodamine G. Conveniently, the pH dependence proves helpful in solving at least part of the problem by bringing the pH to a value sufficient to quench one of the tracers (see Figure 4.8).

Uranine/Eosine mixture – detection of Eosine:

1. The combined signals of both tracers are measured in a synchronous scan.

2. The sample must be acidified to a pH value of five, which suppresses the Uranine to a large extent.

3. The excitation-emission scan provides the Eosine intensity at the fluorescence maximum for higher Uranine concentrations directly (the remaining Uranine does not affect the result). Where concentrations of both tracers are almost equal, a lower pH is required. As a consequence the Eosine level will also drop. Further calculation is necessary according to the instructions given in Figure 4.23.

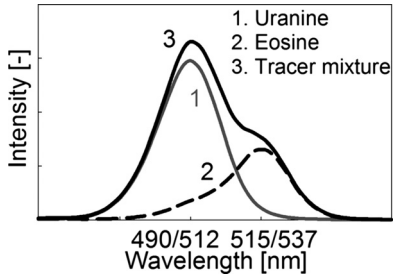

Figure 4.21 Fluorescence spectra of tracer mixtures: Uranine and Eosine.

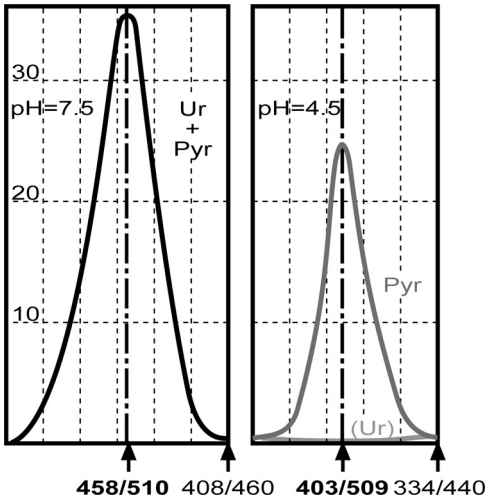

Figure 4.22 Pyranine detection by acidification of the Pyranine/Uranine tracer mixture. Measurement is at pH 4.5 ($\Delta\lambda = 106$ nm).

Uranine/Eosine mixture – detection of Uranine.
A combination of pH dependence and photolysis is required:

1. The combined signals of both tracers are measured in a synchronous scan.

2. The sample must be acidified to a pH value of 4.3.

3. Expose the acidified sample to daylight for a number of hours. The result will be to destroy the fluorescence of Eosine but that of Uranine will not be affected.

4. An excitation-emission scan after (re)alkalinising reveals the Uranine fluorescence.

Pyranine can be determined in a Pyranine/Uranine mixture in an acid solution with a pH of 4,5 (Figure 4.22). When the concentrations of both tracers are equally low, the pH must be lowered to 3.0. Here Uranine has only a minimal fluorescence in the emission maximum of Pyranine at 405 nm. A synchronous scan at $\Delta\lambda = 25$ nm in the maximum of 445 nm is the best means to separate Pyranine from Uranine (Benischke and Schmerlaib, 1986).

Analysing Pyranine by suppressing Uranine fluorescence:

1. The combined signals of both tracers are measured in a synchronous scan ($\Delta\lambda = 25$ nm).

2. The sample must be acidified to a pH value of 4.5.

3. The excitation-emission scan provides the Pyranine intensity at the fluorescence maximum of Pyranine directly (Figure 4.22).

4. Bear in mind the shift in the wavelength due to acidification.

Pyranine/Eosine mixtures can be treated applying the same procedure at pH 1. Again, bear in mind the shift in the wavelength due to acidification.

A more rigorous option for the separation of a tracer mixture is to combine the pH and light dependence, acidifying the mixture and destroying the active tracer after measuring irradiation with a short-wave light, before then alkalizing it to measure the second tracer.

4.1.3.3.1 Separation of fluorescence dyes using ratio calculation This method utilizes the fact that the ratio of two fluorescence intensities at two different wavelengths is independent of the tracer concentration. The technique is demonstrated for Uranine/Eosine below (Figure 4.23). In the example the broken line represents a mixture of Uranine and Eosine measured with $\Delta\lambda = 25$ nm. More information can be found in Käss (1998) and Wernli (2003).

The synchronous technique with spectral fluorometers avoids another serious problem associated with fluorescence analysis. When changing the wavelength of only one monochromator, while keeping the other stable, as is done under the classical technique, three problems occur: (i) scattered light blurs the resulting spectrum, (ii) the signal gets too 'high' and, even more importantly, (iii) the *Raman effect*, as a form of scattering light, can fake fluorescence (Figure 4.24). The Raman effect is observed in all spectral ranges following excitation and is the result of the molecular oscillation of the water molecules. Its wavelength is as much higher as the oscillation frequency of water to that of the excitation light (Behrens, 1971; Harris and Bertolucci, 1989).

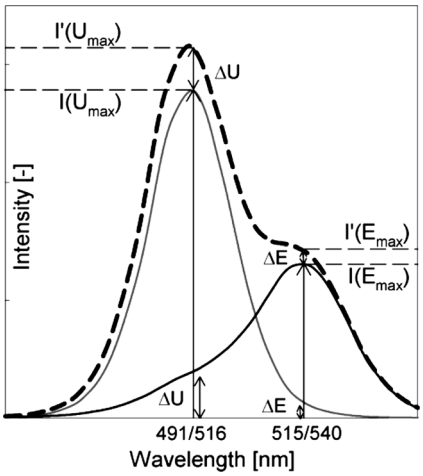

Figure 4.23 Separation of fluorescence dyes using ratio calculation. Broken line: mixture of Uranine and Eosine measured with $\Delta\lambda = 25$ nm.

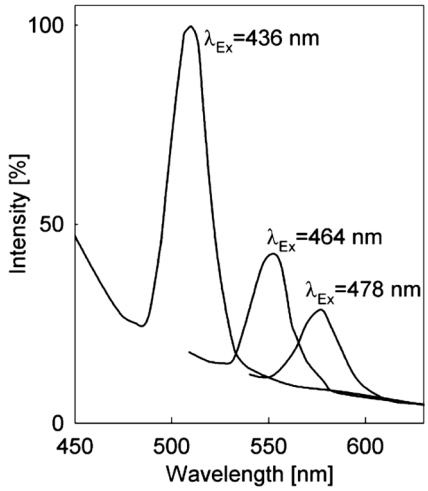

Figure 4.24 Raman scattering of water in the wavelength ranges of most fluorescence tracers (after Behrens, 1971).

4.1.3.4 Background signals and light scattering

Another advantage of synchronous scans is the masking of the blank value. Background signals (blank values) can be caused by high scattering and in the presence of the blue wavelength, especially at low tracer concentrations (Figure 4.25).

These background signals are also caused by electronic noise, and may additionally be brought about or amplified by suspended material. When measuring fluorescence in pure water one expects the background concentrations to be low. Where the correspondence between the amplification (gain) and the concentration is optimal, the background is equal to zero. Very low concentrations require a higher amplification in

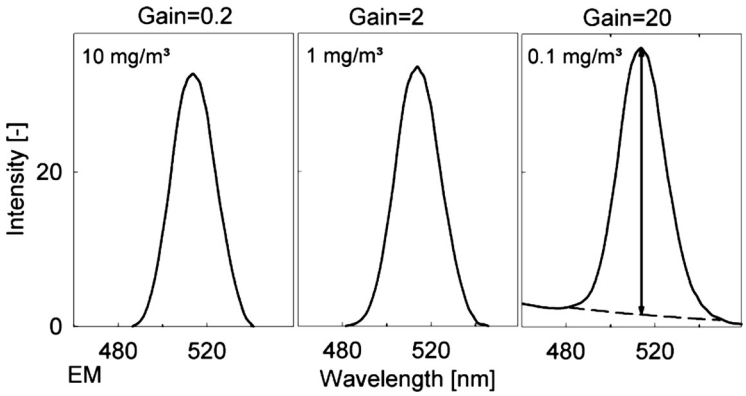

Figure 4.25 Spectral curves of Uranine in pure water revealed by a synchronous scan at $\Delta\lambda = 25$ nm. In the event of high amplification of the signal, the background signal has to be interpolated (see spectral curve to the right).

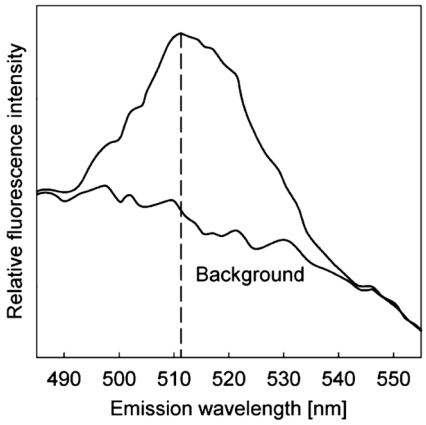

Figure 4.26 Uranine scan in turbid water. The turbidity distorts the spectral curve (modified from Wernli, 2003).

order to get a distinct signal (Figure 4.25). The example of 0.1 mg/m^3 in Figure 4.25 shows how to separate the background from the effective fluorescence signal. Additional problems may occur towards the end of the life of the energy source (xenon lamp). The stability of the fluorometer drops and consequently the background fluctuation rises.

Light is scattered in both pure and in unclear water. In pure water the background is nearly zero. Suspended material, colloids, humins and air or gas bubbles are common in samples of surface and karst water, and in samples from springs after heavy rainfall. The background signal then ranges from high to very high (Figure 4.26). The problem is more pronounced in the lower wavelength ranges (blue), particularly in the UV ranges. This is due to higher degrees of scattering. A very high background signal near the respective fluorescence peak may even result sometimes in a shift of the fluorescence peak.

The following procedure is recommended as a means to overcome the scattering problem:

1. Let the sample settle over night, allowing the suspended material to sink.

2. Filter the water (1 μm) if still unclear.

3. Alternatively, the sample can be centrifuged.

However, these measures are not effective where colloids are present in the sample.

In the future, very advanced multi-coupled analytical techniques such as HPTL/AMD(*h*igh *p*erformance *t*hin *l*ayer *c*hromatography with *a*utomated *m*ultiple *d*evelopment) and Nano-Chip-LC/QTOF-MS (nanochip *l*iquid *c*hromatography/*q*uadruple *t*ime-*o*f-*f*light *m*ass *s*pectrometry) will become important instruments in fluorescent tracer analyses. The methods have been applied successfully to special problems measuring fluorescent tracers or their derivates (Weiss *et al.*, 2008).

4.1.3.5 Fibre optic fluorometer (FOF)

Fibre optic fluorometers are used in the lab and in the field for in situ measurements and monitoring. The characteristics of the instruments available differ; that is the number of channels (multitracer measurements), detection limits, size, cable characteristics and applicability (surface waters, groundwater). There is a variety of suppliers and instruments on the market, which are easy to find on the internet. FOFs work according to the principals of the filter fluorometer. The light source used to stimulate fluorescence is either a halogen bulb or diodes (LED). Modern LEDs with a high spectral density are advantageous in terms of their robustness, reliability, maintenance and energy consumption. After passing through an excitation filter, the excitation signal is sent through a fibre optic cable (duplex fibre optic) to the probe head in the measuring volume (c. 1 cm^3) introduced into the medium of interest. A part of the fluorescent light emitted isotropically by the tracer is collected by a second fibre optic cable and guided back to the photomultiplier with an emission filter and an I/U converter. The converted electrical signal is connected to the analogue output (Figure 4.27).

The excitation light can be selected according to the fluorescence range and irradiation density required for the tracer used. The detection limit for Uranine is in the range of 0.02 mg/m^3. Provided the water is very pure, some devices with an optimal measuring set allow, from experience, a detection limit up to 0.002 mg/m^3. Linearity between the FOF signal and tracer concentration is given up to the range of 10 mg/m^3 (Hodel and Stoller, 2000).

Fibre optics allow for continuous measurement with a high spatial and temporal resolution. The number of measurement channels varies from type to type, but usually up to six channels are available and allow the individual to choose the measurement interval (1 s to several hours). The most important fluorescent tracers (all xanthenes) can be measured. Several channels allow for the measurement of more than one tracer at

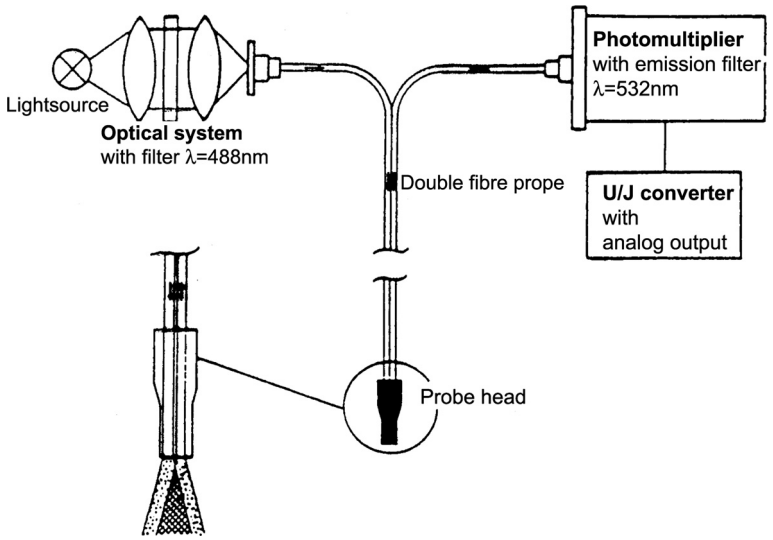

Figure 4.27 Fibre optic fluorometer set-up, schematic (Schmid and Barczewski, 1995).

a time at multiple sites. Due to this configuration, the technique is especially suitable for the measurement of fluorescent tracers within fractures, lakes or for (river) discharge measurements using dilution methods.

A weakness of the fibre optics approach, particularly in relation to surface water experiments, is the effect of ambient light on the probe head. In order to avoid disrupting the measurement signal as much as possible a phase sensitive detection (lock in technique) is used (Hodel and Stoller, 2000). However, the effect of ambient light cannot be entirely avoided when making measurements in surface waters. A protection tube around the probe head further reduces the influence of ambient light. A protection is recommended in surface water measurements generally to avoid damage to the probe. However, this precaution slightly slows the free water flow around the probe head, which is important for simultaneous in situ measurements. So, when a protection tube is used, its potential influence on the flow field needs to be taken into consideration. Further detailed technical information about fibre optics is provided in (Barczewski and Marschall, 1992; Benischke and Leitner, 1992; Schmid and Barczewski, 1995; Hodel and Stoller, 2000; Hodel, Stoller and Diem, 2004).

4.1.3.6 Field fluorometer for in situ measurements

Field experiments involve contrasting and more varied technical conditions than are found in the lab, but there are also advantages to the measurement of samples directly in the field. In situ measurements reduce the number of the required water samples drastically, with only control samples needed. Further advantages are less transport and the lack of a time lag between sampling and analysis. Thus, the risk of sample contamination and/or ageing with the associated potential negative consequences such as photolytic, biological and chemical degradation is eliminated. Furthermore, an immediate analysis and interpretation of the measurements means an onsite plausibility check is possible, and allows the researcher to extend or to shorten the measurement duration, or even to change the test arrangement and to repeat the experiment if necessary. With the development of low energy consuming diodes, the carrying of many or heavy batteries to the experimental site is no longer a problem.

However, unlike laboratory measurements, field experiments with in situ measurement require consideration of basic parameters such as water temperature and pH value. Variations in these parameters often result in fluorescence characteristics that are not consistent with the calibrations known from laboratory tests, as referred to previously. This may as a result lead to inaccurate results. Another factor that can affect and distort the measurement is turbidity (especially in surface water). Turbidity reduces excitation and emission radiation due to absorption and reflection of particles. Operators using the field fluorometer must accept that there is no possibility to prepare or to treat the sample so as to obtain better signals and results (e.g. adjusting pH value).

A much more successful approach than trying to correct the values measured in situ is, to calibrate the measurement directly in the field with the water to be measured in order to equalize the measurement conditions (e.g. calibration with 'shaken' river water) using the dilution method. However, this approach cannot be applied for all experiments. An example of a situation where this method does not work is in the case of

measurements made within lakes at the interface of epi- and hypolimnion, where there are quickly changing temperature conditions. The potential for calibration or instant calibration techniques varies considerably according to the type of field fluorometers.

Four types of device, each based on different principles, are applied in practice: the fibre-optic fluorometer, flow-through fluorometer, pocket fluorometer and in situ fluorometer (Variosens, others). So far applications of the spectral fluorometer have been limited to laboratories.

4.1.3.6.1 Fibre optic fluorometer (FOF) FOF measures the fluorescence applying a concept described in Section 4.1.3.6. FOFs are generally best suited for in situ measurements. They are especially suitable in field experiments investigating the unsaturated zone, fissured rock aquifers, the hyporheic interstitial and when measuring river runoff using the dilution method (Barczewski and Marschall, 1992; Schmid and Barczewski, 1995; Hodel, Stoller and Diem, 2004; Selker *et al.*, 2006). The use of FOFs is restricted by the limited cable length and problems with scattering light; for example in lake research.

4.1.3.6.2 Flow-through fluorometer The flow-through fluorometers take samples by pumping water into the device and sending the data to a data logger. The underwater or downhole fluorometers are a new type of flow–through-fluorometers. The data are logged into the device and are later transferred to a computer for evaluation. The detection limit is low, allowing the operator to take advantage of the advantages of fluorometry. However, suspended material causes problems when measuring directly in the field. The devices are simultaneously in situ fluorometers as they are watertight and can be placed in the water under investigation, such as rivers, lakes, groundwater boreholes and springs. An additional function of this device is the measurement of turbidity, enabling a correction for the fluorescence response. However, the correction is problematic and does not necessarily lead to absolutely correct values. When using the dilution method to measure runoff in rivers it is, therefore, also recommended that the 'instant calibration' be used. Further information pertaining to the devices, the measurement techniques and the application is provided in Schnegg and Kennedy (1998); Schnegg and Bossy (2001); Schnegg (2002); Flynn *et al.* 2005; Schegg and le Doucen (2006).

4.1.3.6.3 Pocket fluorometer Another means of measuring fluorescent tracers in field experiments is by using the pocket fluorometer. It is designed for Uranine and Rhodamines. As a handheld device it is very easy to transport to any site. However, the detection limit for Uranine (0.1 mg/m^3) is approximately 50 times higher than that of a spectro- and fibre optic fluorometer. Consequently, the pocket fluorometer is not suited to tracer hydrological investigations involving low tracer concentrations. On the other hand, it is very useful for discharge measurements using the dilution method, dealing with tracer concentrations in the range of $\geq$2 mg/m^3. A large effective range between approximately 0.1–200 mg/m^3 is sufficient for most experiments (Wernli, 2007).

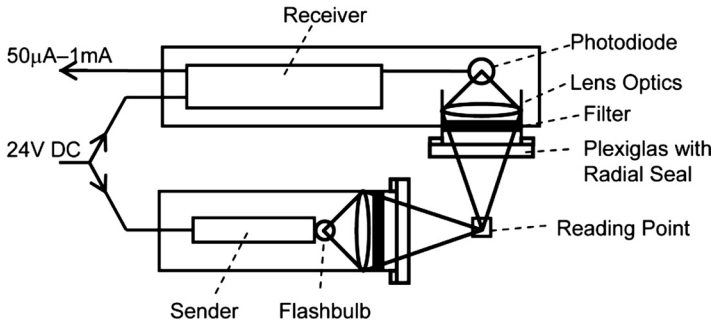

Figure 4.28 Set-up of the Variosens measurement device.

4.1.3.6.4 Variosens The in situ fluorometer 'Variosens' is based on a light pulse technique. Producing three decades' worth of concentration in just one second, it allows for the rapid measurement of fluorescence in the water body itself. The set-up is illustrated in Figure 4.28. The instrument covers a concentration range of four decades (0.01–100 ppb) by a linear current output of 0–1 mA. It operates with a power consumption of between 6 and 10 Watt. With a stainless steel housing (weight: 12 kg) it can be used for water depths of 0–1000 m, or 0–200 m with an aluminium housing (weight: 8 kg). Power is supplied to the device through an electrical cable extending from the boat from which the measurements are being made. The tracer is excited by a xenon flash bulb (frequency: 10 Hz) and the signal picked up by the receiver is transmitted to the boat where it is recorded continuously (Früngel, 1972, 1989; Früngel and Koch, 1974; Hirsig, Leibundgut and Nydegger, 1982). A similar device, 'Back Seat', is available for the applications in surface water (Goudsmit *et al.*, 1997).

Its application is known mainly from surface water experiments, particularly in oceanography where it was developed and used primarily for measuring chlorophyll (Herrmann, 1977). There have also been many studies carried out in lakes and rivers investigating diffusion and dispersion processes and other surface water specific processes, such as the horizontal and vertical distribution of river water in the epi- and hypolimnion of lakes and chlorophyll measurements respectively (e.g. Hirsig, Leibundgut and Nydegger, 1982; Stevens, Lawrence and Hamblin, 2004). Details of the practical application of the Variosens in field experiments will be presented in Chapter 6 and in the Lake Bled case study in Chapter 7.

4.1.3.7 Laser measurement

A laser (*l*ight *a*mplification by *s*timulated *e*mission of *r*adiation) is a short wave radiation light source featuring various properties such as a narrow, low divergence beam with a well defined wavelength. These characteristics are used for 'laser spectroscopy'. The application of the laser technique to measure fluorescence is a promising area of measurement techniques in tracer hydrology. Essentially, three different laser-spectroscopy methods can be used for hydrological investigations, namely (i) absorption spectroscopy, (ii) laser Doppler velocimeter and (iii) laser-induced fluorescence. The

latter is directly related to the fluorescence characteristics of fluorescent dyes. Laser-spectroscopy is the experimental analysis of interactions between radiation (emitted by a laser) and matter (gas, fluid or solid). Due to the high radiation efficiency and the narrow spectral range, laser-spectrometry is very suitable for fluorescence measurements. However, there have only been a few applications in hydrology as of yet. Further information on laser-induced fluorescence techniques is provided by Brumley and Farley (2003) and Englert (2003).

4.1.3.8 Further measurement techniques

Where analytical problems are unsolvable using fluorometry, such as the separation of tracers in problematic mixtures and that of the separation of eluates of activated charcoal bags, recently advanced techniques are being offered increasingly.

High Performance Liquid Chromatography (HPCL) is a promising analytic for fluorescent tracers. The detection limits for Uranine (1, 7 mg/m^3) and Sulphorhodamine B (4, 7 mg/m^3) are in the same order of magnitude as with optical fluorometers (Franke, Westerholm and Niessner, 1997). Recently advanced analytical techniques such as HPTL/AMD (*h*igh *p*erformance *t*hin *l*ayer *c*hromatography with *a*utomated *m*ultiple *d*evelopment) and Nano-Chip-LC/QTOF-MS (nano-chip *l*iquid *c*hromatography/*q*uadruple *t*ime-*o*f-*f*light *m*ass *s*pectrometry) have been applied successfully to special problems with measuring fluorescent tracers or their derivates (Weiss *et al.*, 2008). The detection limit is approximately ten times lower when using these techniques. Additionally, the tracers can be measured simultaneously. By enrichment of the tracers using solid-phase extraction (SPE), the detection limit can be reduced by a factor of 10–100 compared to optical fluorometry. The opportunity to automatize the analyses using HPCL is an advantage over the conventional techniques. In future, the advantages of the advanced analytic may even compensate for the much higher costs for material and for time consuming preprocessing (oral information A. Leis, Graz).

4.1.3.9 Long term sampling using active charcoal bags

A completely different approach in the sampling of fluorescent tracers is the adsorption of the tracer substances by placing active charcoal bags (probe, fluocapteur) in the water under investigation, with the subsequent extraction of the tracer from the charcoal in the lab. As a solute, the elution is treated much like common samples and is measured using a fluorometer. As there is no strong correlation between the volume of water and adsorption in active charcoal, the technique provides qualitative, or perhaps semiquantitative results for the time period of exposure. Simultaneous monitoring of the concentration by both sampling and active charcoal gives reliable results for porous aquifers (Figure 4.29) and glaciers (Lang, Leibundgut and Festel, 1979; Leibundgut, 1981a; Behrens *et al.*, 1986). The active charcoal technique is successful in pure water, such as in springs and groundwater. The presence of organic matter may reduce the effectiveness of this technique when sampling in surface water, karst springs and in

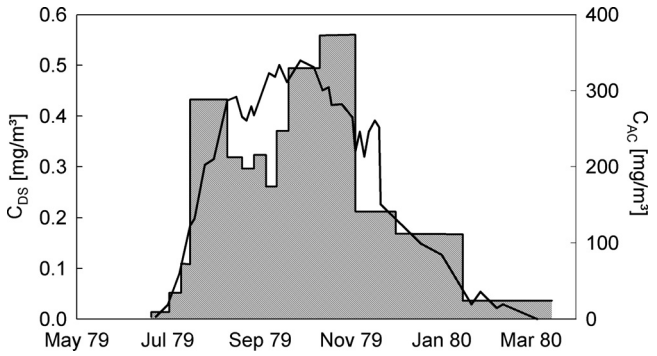

Figure 4.29 Comparative representation of a tracer breakthrough sampled by direct sampling (C_{DS}) and by active charcoal (C_{AC}) in a porous aquifer.

glaciated areas (Drew and Smith, 1969; Bauer, 1972; Brown and Ford, 1973; Smart and Smith, 1976; Perlega, 1977; Wernli, 2003).

The technique is used to sample at remote sampling points and where site access is difficult, such as in karst caves or glaciers (White, 1967; Hötzl, 1973; Lang, Leibundgut and Festel, 1979). Lange *et al.* (1998) described the usefulness of this technique in determining transmission losses during episodic flooding in small arid streams. Smart and Wilson (1984) utilized the activated charcoal technique to investigate the ephemeral nature of pipe flow (macropore). The 'passive detector method,' as so-called by the authors, proved to be an efficient technique for the definition of general network characteristics and function. Furthermore, tracer concentrations below the detection limit can be captured by collecting a high volume of water in the charcoal bag. Under ideal conditions, the concentration increases by up to 1000 times. However, the progression in sensitivity will usually be limited due to the rise in background concentration. The detection limit may increase due to the presence of organic matter and the associated high background concentration. For tracer experiments related to drinking water supplies, charcoal bags offer the opportunity to monitor over long periods of time. In springs and boreholes also, where tracer breakthrough is not expected, charcoal bags can be used to make sure that no tracer passes unobserved. Suitable charcoal bags, 'fluocapteurs,' are described in Bauer (1972), Leibundgut (1981a) and Wernli (2003). Fluocapteurs must allow for sufficient water flow through the sample in order to ensure that the tracer can be adsorbed onto the active charcoal.

Analysis in the laboratory involves more steps than the 'normal' analysis. The general procedure is to extract the tracer from the active charcoal and to measure the fluorescence of the elution after a sedimentation phase. A solvent is needed to elute the tracer. For Uranine, methylamine 40% can be used. As methylamine degrades Rhodamine to a dye with a different fluorescence spectrum, it is not suitable to extract Rhodamine or mixtures of Uranine and Rhodamines. Other solvents are pure water, ethanol-caustic potash extraction mixture and ethanol-ammonia mixture. The latter is preferable as it does not turn yellowish during the sedimentation phase. Details of the different methods can be found in Perlega (1977) and Wernli (2003).

Independent of the extraction method, it is important to take into consideration a shift in the fluorescence maxima. For example, ethanol-ammonia mixture: an Uranine maximum lies at 526 nm; in dilutions of more than 1:4 the fluorescence wavelength drops to 516–518 nm (Wernli, 2003). In an ethanol-caustic potash mixture the Uranine maximum lies at 530 nm (Perlega, 1977).

4.2 Salt tracers

In hydrology, salts (i.e. their ions) represent environmental tracers as well as artificial tracers (Table 4.1). Salt tracers are used to investigate aspects of hydrological systems in the same way as all other artificial tracers. They are widely used to measure smaller discharges in brooks and springs (cf. Section 6.3). Other specific fields of application are tracer tests in the saturated and the unsaturated zone (Singha and Gorelick, 2005), the combined use of salt tracers and geophysical methods to determine groundwater flow, or to investigate problems of leakage from rivers and sewage pipes (e.g. Zellweger, 1994; Armbruster *et al.*, 1992; Reeves, Henderson and Beven, 1998; Kollmann, Meyer and Supper, 1992; Einsiedel, 2005). Furthermore, salt tracers are an alternative to fluorescent tracers in multi-tracer experiments.[7]

Prior to the 1960s, artificial salt tracers were used widely in experiments in karst hydrology (Gospodaric and Habic, 1977). In later years, fluorescent tracers gradually became the main tracers employed. Salt tracers are generally less sensitive than the fluorescent tracers, which limits their suitability and thus their application in recent times. For this reason, salt tracers will only be discussed in this introduction to the tracers, in order to provide readers with a basis upon which to make their own judgement about the potential use of salt tracers to meet their requirements. Out of the potentially numerous salts only a few are considered to be suitable water tracers (Table 4.11). A successful application of salt tracers is only feasible in small scale experiments, because relatively high tracer masses are needed. Salt tracers can be used for studies in soil profiles in the vicinity of springs or boreholes, and in small surface water bodies (creeks).

4.2.1 Chemical and physical characteristics of salts

Salts are inorganic compounds, which break up into cations and anions when dissolved in water. Ionic compounds in the solid salt form have a high melting point, a brittle consistency and are highly soluble in polar solvents. In solution, and when melted, their electrical conductivity is high due to the mobile ions. Their volatility is low due to strong binding energy (ionic bridges) in the ionic grid.

Again, sorption is a crucial property in applications of salt tracers in groundwater, in the unsaturated zone and in soils. Mineral and organic matrix particles with a large surface area (clay, iron oxides, humins) adsorb molecules and ions onto their surfaces.

[7] For example, Batsche *et al.* (1970); Gospodaric and Habic (1977); Müller and Zötl (1980); Leibundgut and Harum (1981); Behrens *et al.* (1992); De Carvalho-Dill *et al.* (1992): ATH (1992).

Table 4.11 The available salt tracers and their properties

Salt	Molecular formula	Water solubility at 10 °C [g/l]	Ionic radius [Å]	Ionic potential (charge/radius)	Molecular weight [g/mol]	ion	Analysis method
Sodium chloride (mine salt)	NaCl	357	Na^+: 1.02	Na^+ 1.0	58.44	Cl^-	EC, ISE, flame photometry
			Cl^-: 1.81	Cl^- −0.6		Na^+	
Potassium chloride	KCl	313	K^+: 1.38	K^+ 0.7	74.55	Cl^-	EC, ISE
			Cl^-: 1.81	Cl^- −0.6		K^+	ISE, flame photometry
Bromide	NaBr	850	Br^-: 1.96	Br^- −0.5	102.89	Br^-	IC, ISE
Lithium	LiCl	820 (20 °C) 672 (0 °C)	Li^+ 0.76	Li^+ 1.3	42.39	Li^+	Spectroscopy, flame photometry
	$LiCl\ 3H_2O$				96.38		
Borax (sodium borat)	$Na_2B_4O_7{*}10H_2O$	16.2	—	—			
Iodide	NaI	184 (25 °C)	I^- 2.2	I^- −0.5	149.89	I^-	IC, ISE

EC: Electrical conductivity, ISE: Ion sensitive electrode.

Humins, for example, have a specific surface area of 800–1000 m^2/g. Ionic adsorption is reversible and occurs only in an aqueous solution between the dissolved and solid phase. When tracers are applied to investigate processes in the unsaturated zone, that is, in soils, one must bear in mind that the matrix surface of soils also constitutes an ion exchange capacity (Leditzky, 1978). The ions absorbed in the matrix tend to exchange with dissolved ions in the soil water, and therefore also with the tracing ions. Cations and anions exhibit different sorption behaviour. For many soils the cation exchange capacity is higher, and anions are generally characterized by low to very low sorption. In clays, soils containing (Fe, Al)-hydroxides and in soils with a high content of organic matter, however, anions can also be affected by significant sorption. The humic acids contained in such materials often lead to surface complexation, which appears as a sorption effect in a tracer breakthrough. As a result cations offer suffer a significant loss in passing the matrix due to the negative electrical potential of clays and humins. The nature of the ion exchange is dependent primarily upon the ionic potential (Table 4.11). The higher the potential, the smaller the exchange (Appelo and Postma, 2007). Ions with a higher charge especially exhibit a stronger tendency to sorption. Secondary effects depending on ion radius, concentration or specific affinity of ions, as in the example of potassium and illite, have also to be considered. The dynamics of ion exchange in soils are described, for example, in Scheffer and Schachtschabel (2008), Appelo and Postma (2007).

In contrast to the sorption of ions is the so called anion exclusion, which may enhance the transport velocity of anions in porous media due to electrostatic repulsion by negatively charged solid surfaces (Gvirtzman and Gorelick, 1991).

Consequently, *salts possess only limited suitability as tracers.* The factors favouring the use of salt tracers are easy handling, availability and the potential for continuous recording. There are also, however, several attributes complicating the application of salt tracers. When using salts as tracers one is confronted, to differing degrees, with the following: high natural background and pollution, high detection limits, large injection masses, transport problems, relatively laborious analyses and the problem of sorption and ion exchange. Potassium cations and iodide anions are strongly affected by sorptive processes. In addition, the latter (I^-) are unstable and tend to interact with other tracers or substances (e.g. S, Cd, Br, Cl). It also has an effect on water flavour and therefore should not be applied in potable water systems. Borax is limited to short distances in groundwater and the analysis of low concentrations is difficult. In summary, borax, iodide and potassium cations are poorly suited to applications in hydrological tracing and will not be described further. A summary is provided in Käss (1998).

Although salt tracers are of only limited suitability as tracers, there have been many salt experiments performed in the past.[8] In the initial phase of tracer experiments in karst areas especially, salts served as important tracers alongside fluorescent tracers. The success of these experiments varied considerably. As the suitability of each of the few salt tracers available is quite different, the tracers will be characterized individually briefly in the following.

[8]For example, Verga and Zuppi (1986); Ramspacher *et al.* (1986); Dounas *et al.* (1982); Behrens (1981); Bögli, Leibundgut and Zojer (1981); Feyen *et al.* (1999); Maloszewski *et al.* (2006a, b); Deeks *et al.* (2008); Batsche *et al.* (1970); Gospodaric and Habic (1977); Müller and Zötl (1980); Leibundgut and Harum (1981); Behrens *et al.* (1992); De Carvalho-Dill *et al.* (1992).

4.2.1.1 Sodium chloride (NaCl)

Sodium chloride was the most widely applied artificial salt tracer in the past, due to its easy handling, good sensitivity, nontoxicity, low price and ubiquitous availability. When using this tracer detection is usually based on measurements of the chloride ion (Cl^-). It is assumed to be a chemically stable and conservative tracer with high geochemical mobility. Sorption is usually negligible. A widespread application of sodium chloride as a tracer is in the measurement of discharges in brooks up to several m^3/s using the dilution method. This approach is outlined in greater detail in Section 6.3. A disadvantage is the high natural background concentration of chloride generally, accentuated by pollution arising from activities such as road salting and so on. As a consequence, a large quantity of salt is needed. An elegant solution is the injection of brine by a tanker truck or some similar vehicle (Käss, 1972; Müller and Zötl, 1980; Leibundgut and Harum, 1981).

To estimate the tracer mass required for a particular field experiment the approximate ratio of sodium chloride to Uranine used is 10 000:1. This high tracer mass and the considerable transport costs negate the low price of the product itself. The large mass also means that instantaneous injection in a porous aquifer is not possible. An adequate input function has to be found (see Chapter 5). Furthermore, the large amounts needed may lead to a change in the hydraulic flow by changing the specific weight of the traced water mass and will probably cause stratification in the aquifer (e.g. Eissele, 1963). A typical example of this is a karst siphon, where stratification may occur due to the higher density of the traced water, often resulting in misinterpretation of the data. In porous aquifers also, the salt tracer plume may sink down towards the aquiclude. Furthermore, the mixing of the salt with the water requires a long time, which implies an adulteration of the water flow determined by the tracer breakthrough curve. Both phenomena were discussed by Leibundgut (1981a) in the context of a multi-tracer experiment in a fluvioglacial gravel aquifer.

The sorption potential of chloride is relatively low. In many cases it is even virtually an ideal tracer. Its suitability has been demonstrated particularly in karst experiments, but also in porous aquifers (summarized in Käss, 1998) and in the unsaturated zone (Scanlon, 1991; Flury and Wai, 2003; Ptak, Piepenbrink and Martac, 2004). The additional/simultaneous determination of the *Na-cations* in a tracer experiment may provide an interesting insight into the processes of ion exchange (Käss, 1967b; Batsche *et al.*, 1970). However, for technical reasons it is more or less impossible to monitor the Na-cation using ion selective electrodes (Müller and Zötl, 1980).

4.2.1.2 Bromide (Br^-)

In natural hydrological systems Br^- usually occurs at a very low background concentration, usually below the detection limit. For this reason, and also because of its high solubility (circa 850 g/l at 10 °C), it is easier to handle in the field than other salts. However, bromide also requires a high injection mass, due to its lower sensitivity, approximately 3000 times higher than that of Uranine.

Bromide is assumed to be chemically, biologically and photolytically stable, and due to its negative charge sorption is very low in mineral soils, where it acts mostly as an ideal, or nearly ideal, conservative tracer. Adsorption may occur in humic soils. Bromide is often used for tracer experiments in the vadose zone, and as a reference tracer for comparison purposes (e.g. Onodera and Kobayashi, 1995; Lennartz and Kamra, 1998; Sambale *et al.*, 2000; Ginn *et al.*, 2002; Stamm *et al.*, 2002; Parsons, Hayashi and Van Der Kamp, 2004; Gish *et al.*, 2004). Bromide may be an alternative in certain situations, particularly in problematic test areas with a pH range unsuited to Uranine (Didszun, 2004; Einsiedel, 2005; Hangen *et al.*, 2005; Leibundgut and Uhlenbrook, 2007).

In the case of multi-tracer tests potential interference with Eosine must be taken into consideration. Bromide metabolizes during the processes of chlorination and ozonation in water supply installations. Unlike sodium chloride, bromide can be monitored successfully using ion selective electrodes. However, the technical detection limit is quite high (50 μ/l).

Due to its relatively high charge density, Br^- transport generally occurs through the middle of pores, so that it passes through soil and groundwater systems faster than water molecules (Flury and Wai, 2003). As a consequence, the flow velocities measured using bromide do not correspond to those measured using other tracers like Uranine.

4.2.1.3 Lithium

The soft alkali metal lithium is employed as either lithium hydroxide (LiOH) or lithium chloride (LiCl) in hydrological investigations. Lithium cations rarely occur in natural soils and so the background concentrations are very low, or nonexistent. Of the salt tracers, lithium has the lowest affinity for ion exchange.

It has been shown to be a suitable tracer in karst aquifers (e.g. Behrens *et al.*, 1992) as well as in porous (e.g. Käss, 1994; Vereecken *et al.*, 2000; Ptak, Piepenbrink and Martac, 2004). However, some authors reported problems in relation to the detection limit and ion exchange over longer distances; as stated by Käss *et al.* (1986), who detailed a tracer experiment carried out in the karst of Peloponnesus. In porous aquifers lithium is only a suitable tracer over short distances (<200 m).

Given its positive charge and that its retaining properties in the soil matrix are similar to those of metals, lithium is often used to investigate contaminant flow (e.g. heavy metals) in soils (e.g. Bencala, McKnight and Zellweger, 1990). Haase *et al.* (1996) injected lithium chloride into survey wells of different depths to investigate the rooting depth of semiarid vegetation (shrubs). Care needs to be taken when dissolving lithium since the dissolution reaction of, for example, LiBr or LiCl releases heat. To avoid this add sufficient water first and then add the tracer.

4.2.1.4 Iodine

Dissolved iodine occurs as iodide- (I^-) or as iodate- (I_O^{3-}) anions, depending on the reducing or oxidizing characteristics of the aquatic environment. The iodate anion is

more reactive and so is less suited to use as a hydrological tracer than iodide. This fact should be kept in mind when iodide is applied as a tracer in an environment with the potential to convert iodide to iodate or iodine. The use of iodine in medicinal products means that it can act as a pollution tracer. Iodide is chemically instable, as a result of which its use is limited to short distance experiments. In combination with starch, however, it forms a starch-iodide complex that can be used to illustrate flow paths in soils (van Ommen *et al.*, 1988; Lu and Wu, 2003). Tracers used for the purposes of visualization in the vadose zone are discussed in Section 4.5.4.

As a redox-sensitive element, iodine can exist in various forms, including iodide and iodate. In reducing environments, aqueous iodine usually occurs as the mobile iodide anion (I^-). Under more oxidizing conditions, iodine may be present as the more reactive iodate anion (IO_3^-), which is characterized by retarded transport due to the fact that iodate interacts with clays and organic matter (Couture and Seitz, 1983; Yoshida, Muramatsu and Uchida, 1992; Hu and Moran, 2005).

Of the halides, iodide is recommended for geothermal tracer studies; bromide and chloride are useful only when their natural background concentrations are low. However, iodide may not be an ideal tracer as it has a tendency to adsorb onto laboratory cores (Chrysikopoulos, 1993).

4.2.2 Measurement techniques

In the past, the analysis of salt tracers was quite complicated and involved several chemical methods. A thorough description of these is provided by Käss (1998). The current state of the art in the analysis of the salt tracers is ion exchange chromatography (IC) and inductively coupled plasma mass spectrometry (ICPMS). In the case of sodium chloride, electric conductivity can be used as a proxy value in small scale experiments. There are ion sensitive electrodes available for the monitoring of chloride and bromide.

4.3 Drifting particles as tracers

Drifting particles, such as spores, phytoplankton, bacteria, viruses, phages and microspheres, constitute another group of artificial tracers, their most characteristic feature being that they are not in solution. Although applied mostly only in very specific situations, drifting particles have been shown to be good qualitative tracers for the investigation of flow paths and hydraulic connections. Traditionally they were widely applied in studies of karst systems. They also have considerable potential as tracers for special applications, particularly in the investigation of hygiene-related issues in the water supply. Nowadays they are being used to study and investigate the flow behaviour of micro-organisms and particles in saturated, unsaturated and surface water systems (Zötl, 1974; Leibundgut and Lüthi, 1977; Benischke *et al.*, 1980: Hötzl, Käss and Reichert, 1991; Auckenthaler, Raso and Huggenberger, 2002; Flury and Wai, 2003; Zvikelsky and Weisbrod, 2006; Göppert and Goldscheider, 2008).

4.3.1 General characteristics of drifting particles

Disregarding certain 'exotic' particles used in the past (such as chaff, etc.) that have proved to be unsuitable in modern hydrology, the Lycopodium spores (club moss spores) were the first particle tracers used successfully in karst systems to verify flowpaths and hydraulic connections. However, like all other particle tracers, they do not allow for an evaluation of the tracer breakthrough curve leading to correct flow parameters as sometimes presented without comment in the publications. Other particle tracers include bacteria, phages, viruses and microspheres. The particle sizes limit the through flow of the tracers through larger pores, and so tracer breakthrough probably occurs earlier than is the case for ideal tracers. An accurate quantification of the traced water body is not possible using nonsolute tracers. Nevertheless, particle tracers have potential uses as tracers for special purposes, particularly in the investigation of the filtration capacity of the unsaturated zone and aquifers. They are also useful with respect to the infiltration of contaminants in sewage or irrigation water, and for all applications with hygiene implications, and impacts on water supply installations and water protection zones, subject to the principles outlined by Leibundgut and Lüthi (1977). The sizes of these particles range from 30 μm diameter in the case of *Lycopodium clavatum* to 0.01 μm for the Poliomyelitis virus (Figure 4.30).

The suitability of the particle tracers is limited for several reasons:

- only qualitative, perhaps semi-quantitative evaluation is possible,
- all particles tend strongly to 'adsorption' onto the surfaces of solid particles,

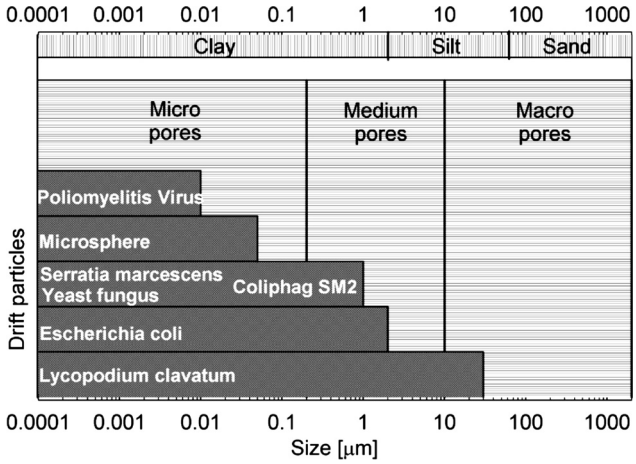

Figure 4.30 Comparison of drift particles (size plotted logarithmically) related to the pore size of substrate.

- the preparation and conducting of both the experiment and the analysis are labour intensive,
- analyses of viruses and bacteria can only be carried out in specialized laboratories,
- sampling bottles need to be uncontaminated and sterile.

Leibundgut and Lüthi (1977) provided a comparison of the application of soluble fluorescent tracers, the bacteria Streptococcus and Lycopodium spores, which is also presented as a case study in Section 7.2.2 of this book. The study deals with the determination of the filtration capacity of an aquifer and its unsaturated zone near a well.

4.3.1.1 Lycopodium spores

Clubmoss (*Lycopodium clavatum*) spores are so far the most commonly used particle tracers for hydrological studies. They have a tetrahedral form with convex surfaces and are almost spherical. They measure c. 30 μm in diameter and possess a density similar to water (a little higher). The spores' surfaces are coated with a fine network (reticula), which serves to increase adhesion. Injection occurs as a suspension in water. It is necessary to add a detergent or ethyl alcohol as the spores are hydrophobic. One kilogram of spores amounts to between 100–300 billion individual spores. In order to analyse the samples, they must be processed using a caustic potash solution so as to eliminate detritus. After sedimentation the solid fraction is centrifuged and analysed with a microscope (100–200$\times$ magnification). The methods and analyses were outlined by Zötl (1967); Bauer (1967), Dechant (1967) Hötzl, Maurin and Zötl (1976), and discussed in detail by Zötl (1974).

In addition to the 'traditional' detection method, namely counting spores, it is also possible to add a fluorescent coloured dye (Käss, 1982; Dechant and Hacker, 1986; Käss and Reichert, 1986; Benischke, Goldscheider and Smart, 2007) and to subsequently measure applying fluorescence measurement techniques. The availability of multi-coloured spores allows for their application in combined or multi-tracer experiments.

4.3.1.2 Bacteria and bacteriophages (or phages)

Bacteria and bacteriophage tracers are used mainly to investigate the dissemination of germs in various systems, and especially to monitor pollution in potable water. Examples of the bacteria species employed include *Escherichia coli*, *Staphylococcus crus*, *Streptococcus faecalis* ATTC, *Serratia marcescens* and *Pseudomonas fluorescens* (e.g. Auckenthaler, Raso and Huggenberger, 2002; Silliman *et al.*, 2001; Gunn *et al.*, 1998; Sinton and Ching, 1987; Leibundgut and Lüthi, 1977). However, when applied in the unsaturated zone post-experimental treatment (e.g. with sodium hydroxide solution, as described

by Käss, 1998) is required due to the potential for proliferation. Bacteria and phages tend strongly to adsorption onto the surfaces of solid particles.

Bacteriophages (or phages) have been used as hydrological tracers in many studies in the past, and are still widely employed today, but apart from their low detection limit (1 phage per 100 l) and the large range of possibilities for application, the obstacles to their widespread use still outweigh the advantages. The preparation and conducting of experiments is extensive, the sampling bottles must be uncontaminated and the analyses can only be carried out in specialized laboratories with electron microscopes. Bacteria and phages also tend to be adsorptive, even in karstic aquifers (Mallen *et al.*, 2005). Furthermore, their applicability as viable tracers depends very much on the environment. These particles have been used as hydrological tracers in many studies; in groundwater, in the vadose zone and in surface water. The methods of application have been described in great detail (Kinnunen, 1978; Aragno and Müller, 1982; Rossi, 1992, 1994; Rossi *et al.*, 1994; Bricelj and Sisko, 1992; Käss, 1982, 1998; Flynn *et al.*, 2006).

Bacteriophages can supplement the set of applicable tracers only where the flow paths in groundwater and surface water are of interest, and where no further quantitative and mathematical evaluation of the field experiments is required. Thus, tracer experiments with phages do not allow for an evaluation of the tracer breakthrough curve, needed to provide correct flow parameters according to the theoretical needs (cf. Chapter 5) as is sometimes claimed. For this reason, and due to the high adsorption affinity, phages are not suitable for state of the art experiments in aquifers and unsaturated zones. There is also no evidence to suggest that the phages continue to be the preferred tracers in lakes and seas (Käss, 1998). The potential benefits of stable isotope and fluorescent tracers are much greater, as will be discussed in Chapter 7.

In addition to the general restrictions mentioned above, the particular limitations with respect to the suitability of bacteria and bacteriophages are:

- deactivation as a result of rapid natural decay (loss of viable phages) following a first-order kinetic decrease (Bricelj and Sisko, 1992);
- phages have a strong tendency to adsorption onto the surfaces of solid particles;
- their applicability as viable tracers depends greatly on the environment (i.e. pH, chemistry, oxygen, organic matter and especially temperature);
- each type of phage reacts individually; not all react in the same way to physiochemical influencing factors such as pH, polarity and so on.

However, as mentioned above, the sensitivity of phages is high and they are potentially useful as tracers for special purposes, particularly in the investigation of the filtration effects of contaminants in sewage and irrigation water (Kinnunen, 1978), and for all studies concerning hygiene implications and impacts on water supply installations and

water protection zones (Leibundgut and Lüthi, 1977; Marti *et al.*, 1979; Auckenthaler, Raso and Huggenberger, 2002; Flury and Wai, 2003).

4.3.1.3 Fluorescent microspheres

Although fluorescent microspheres are applied widely in medical studies, they have only a limited recognized use as hydrological tracers. Microspheres are synthetic colloidal polystyrene latex particles. They are available in various sizes ranging from 0.05 to 0.90 μm, and may possess different surface characteristics. Only sizes <2 μm have been used for hydrological purposes as yet. Incorporated into the polystyrene latex beads are hydrophobic fluorescent dyes. Two types of beads are available: plain and carboxylated microspheres (Ward *et al.*, 1997). They can be supplied as an aqueous suspension in pure deionized water.

The analysis process is similar to that of fluorescent Lycopodium spores, involving epifluorescence microscopy of a membrane-filtered sample (see Section 4.3.1) and requiring no further preparation. Ward *et al.* (1997) developed the technique further by dissolving the microsphere matrix, which releases the fluorescent dye into a solution. The result is a much easier analysis. The costs are moderate and no interference with other tracers are known. The technical details and examples of several microsphere tracer tests were reported on by Harvey *et al.* (1989), Ward *et al.* (1997) and Käss (1998).

As with all of the other particle tracers, microspheres are only really suitable for special applications, such as the investigation of colloid or particulate transport processes and the simulation of the propagation of pathogenic bacteria. For the latter, a microsphere diameter of approximately 1 μm is recommended. Microspheres serve especially well in karst aquifers.

4.3.2 Measurement techniques and sampling of particle tracers

4.3.2.1 Measurement

The detection method is in the best case semi-quantitative but generally solely qualitative. In order to detect spores, bacteria and microspheres, the water from the investigated source is filtered, and the accumulated particles are counted under the microscope. The Lycopodium spores require further treatment before measurement (see Section 4.3.1.1). Bacteria and bacteriophages can be detected by cultivation. These methods require time and so are unsuitable for field measurements. Though a monitoring technique, similar to fluorescent tracers, is technically not realized.

In addition to the 'counting' method, it is also possible to measure fluorescent microspheres, and bacteria and spores dyed with fluorescent colours, employing fluorescence measurement techniques (Käss, 1982). Niehren and Kienzelbach (1998) presented an online microsphere counter with a detection limit of one microsphere (diameter: 1 μm)

per ml water. The availability of multi-coloured spores allows for their application in combined or multi-tracer experiments.

4.3.2.2 Sampling

The sampling of drift particles often proves difficult. Spores and microspheres must be sieved out. Naturally the pore size of the filter (plankton net/nylon net) must be smaller than that of the particles. A pore size of 25 μm is recommended for spores (e.g. Käss, 1998; Hötzl, Maurin and Zötl, 1977). Microspheres with a smaller diameter require a smaller mesh size. To avoid clogging of the filter by other suspended materials, Wolkersdorfer (2001) recommended an upstream net with a larger mesh size.

4.4 Radioactive tracers

Radioactive tracers were used frequently to solve hydrological problems in the past, under the guidance of the International Atomic Energy Agency (IAEA).[9] Nowadays radioactive substances may be applied to hydrological subsystems as artificial tracers upon receipt of special permission from a local Atomic Energy Commission.

4.4.1 Basics of radioactivity

Every chemical element stands for various isotopes of nuclides, all of which have the same atomic number (i.e. number of protons), but different mass numbers (i.e. number of protons + neutrons). Some nuclides are stable, some are unstable; the latter being radioactive isotopes. The unit of radioactivity is the Becquerel (Bq), defined as decay per second. The old unit, Curie (Ci), is seldom used today ($1\text{Ci} = 3.7 * 10^{10}\text{Bq}$). Essentially, radioactive decay can occur in three ways:

- α-Radiation: He-nuclides are emitted, resulting in a high ionization density. Thus, α-radiators are not used as tracers.
- β-Radiation: electrons are emitted, a medium ionization density allows for the use of a few substances as tracers.
- γ-Radiation: electromagnetic radiation takes place with a high diffusion capacity. γ-radiators are good tracers.

The decay law is the basic formula for the analysis of radioactive tracers. Decay is a first-order reaction, which means that the number of decaying atoms per unit

[9]For example, Moser and Rauert (1980): Drost (1983); Plata (1983, 1991); Rao (1983); Florkowski (1991); Mandel (1991); Margrita and Gaillard (1991); Navada (1991); Roldao (1991).

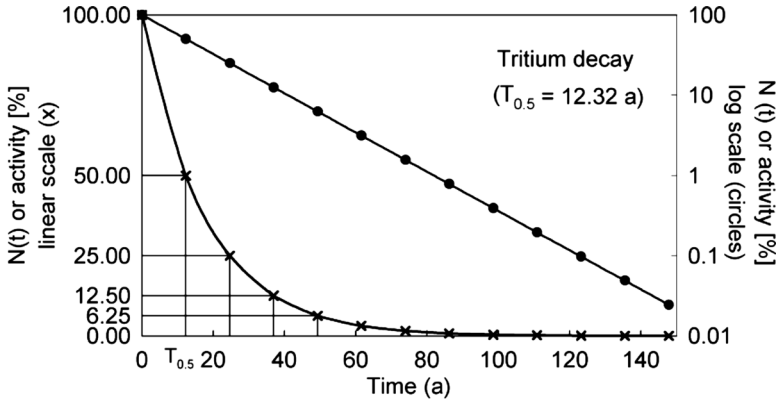

Figure 4.31 Decline of radionuclide activity with decay time; linear (left axis, x) and semilogarithmic (right axis, dots) graphs.

of time is proportional to the total number of atoms of the nuclide (where λ is the decay constant). After a certain time (t), from the initial N_0 atoms only N(t) are left (Figure 4.31):

$$N(t) = N_0{}^* \exp(-\lambda^* t) \tag{4.9}$$

A characteristic quantity is the time at which half of the original number of atoms has decayed, called a half-life time ($T_{1/2}$). It shows the following relationship:

$$N(t = T_{1/2}) = 0.5N_0 = N_0 \exp(-\lambda^* T_{1/2}) \tag{4.10}$$

So, the decay constant is related to the half-life by:

$$\lambda = \ln(2)/T_{1/2} = 0.693/T_{1/2} \tag{4.11}$$

Generally, the radiation and the decay per unit time are measured using counting devices such as the Geiger counter, semiconductor counter and scintillation counter. Depending on the device, these can be used to measure α, β or γ radiation.

The Geiger counter and similar devices record activity and decay. The output (count) is a summation of potentially more than one radioactive element, as the Geiger counter cannot distinguish between different nuclides. Due to the varying gamma quantum energies of different radionuclides, the semi-conductor and the scintillation counters can measure gamma spectra and so distinguish between different nuclides. Further information on the measurement of radioactivity can be found in Moser and Rauert (1980).

4.4.2 Radioactive tracers

The potential health and environmental risks posed by radioactive substances mean that the use of artificially applied radioactive tracers is very limited nowadays. Those that still prevail are those with either low half-life times or low radiation energies, such as ^{3}H, ^{51}Cr, ^{114}In and ^{82}Br. Radioactive substances which have been used for water tracing are given in Table 4.12.

The advantages of radioactive tracers are very sensitive and selective detection, the disappearance of the tracer from the system due to decay, and the ability to follow the flowpath of water and tracer using a Geiger counter. The disadvantages are the potential health risks, the very high costs of measurement and materials, and the fact that it is unlikely that a permit will be granted for the tracer test. The disadvantages generally hinder the application of radioactive tracers in countries with modern environmental legal standards. Even so, they are very suitable for studies addressing specific problems and they disappear rapidly after their half-life period has expired.

Table 4.12 Radioactive substances used for water tracing (source: Moser and Rauert, 1980)

Radioactive nuclide	$T_{1/2}$	Chemical compound	Radiation	Characteristics
^{3}H	12.35 a	^{3}HHO (Water)	β	Chemically identical to the labelled water
^{51}Cr	27.7 d	EDTE - chelat	γ	Low sorption
^{114m}In	50 d	EDTE - chelat	γ	Low sorption
^{114}In	72 s	EDTE - chelat	β	Low sorption
^{58}Co	70.8 d	$[Co(CN)_6]^3$-chelat	γ	Low sorption
^{60}Co	5.3 a	$[Co(CN)_6]^3$ – chelat	γ	Low sorption
^{82}Br	36 h	Br^- – Anion	β	Very low sorption, chemically very stable
^{131}I	8.05 d	I^- – Anion	β	Chemically unstable, sorption by oxidation
^{24}Na	15.0 h	Na^+ - Kation	β	Sorption, can be used in channels
Activation product				
^{80}Br	17.6 min	Br^- - Anion	γ	Low sorption
^{116m}In	54 min	EDTE - chelat	γ	Low sorption
Rare earth elements		EDTE - chelat	γ	Low sorption

Tritium (^3H) – tritiated water, as a water molecule, is an ideal tracer. It can be used as an artificial tracer but only in applications that do not disturb measurements of environmental tritium. At the present time, tritium is mainly used in lysimeter and laboratory-column experiments, and in strongly contaminated sites, where the dye tracers are not suitable due to strong sorption processes. Tritium has declined in importance as an artificial tracer due to concerns over its toxicity and its relatively long half-life.

^{51}*Chrom* is still used in many tracer experiments; for example by Jonsson, Johansson and Wörman (2004) as a reactive radioactive tracer in the hyporheic interstitial. ^{114}*Indium* was used a lot more in the past than is the case today. It is a gamma-emitting radionuclide with a half-life of 49.5 days. It emits gamma-energies of 190, 558 and 725 MeV. To be suitable as a water tracer it must be used in the proper chemical form. The EDTA-complex has been proven to be perfect. Although the EDTA-complex provides a kind of negative shield around the indium-cation, sorption onto sediments can play a role when analysing the tracer quantitatively.

4.4.2.1 Single-well technique

One of the most important methods when using radioactive tracers is the single-well technique. The single-well technique makes use of radioactive isotopes artificially injected into the system under investigation in order to measure filter velocity and flow direction (Drost and Neumaier, 1974). Further information concerning the method and its application is contained in Chapter 7.1.3.

4.5 Other tracers

4.5.1 Fluorobenzoic acids (FBA)

A variety of fluorinated derivates of benzoic acids (fluorobenzoates) have been proposed as tracers (Malcolm *et al.*, 1980; Stetzenbach, Jensen and Thompson, 1982; Bowman, 1984a; Bowman and Gibbens, 1992; McCarthy, Howard and McKay, 2000). Their use in hydrological applications has received considerable attention over the past 20 years. There are 16 FBA isomers or derivatives that exhibit similar physicochemical properties and environmental behaviour, due to the number and the position of the fluorine atom in the benzene ring (Hu and Moran, 2005). According to Juhler and Mortensen (2002), all of these can serve as tracers. FBAs typically chosen for hydrological studies are Difluoro-Benzoic Acids (DFBAs).

Fluorobenzoic acids do not occur naturally and are, therefore, suitable for use as hydrological tracers. Their pK_a values (acid dissociation constant) are relatively low. This indicates that FBA tracers are predominantly negatively charged under most environmental conditions. As anions they are readily soluble in water and are nonvolatile. In terms of their transport behaviour, Fluorobenzoic acids have often been compared

with the inorganic anion bromide (Bowman and Gibbens, 1992; Benson and Bowman, 1994; Jaynes, 1994; McCarthy *et al.*, 2000; Dahan and Ronen, 2001). FBAs are generally described as being conservative tracers with low levels of sorption and degradation under laboratory and field conditions. Recent investigations, however, reveal a more differentiated view. In substrates rich in clay or organic matter, both significant degradation and retardation are possible (Bowman and Gibbens, 1992; Jaynes, 1994). Seaman (1998) also observed the adsorption of FBAs to hydrous Fe-oxides. The suitability of FBAs as tracers for a specific material should, therefore, be evaluated prior to field experiments, especially in the presence of organic carbon, clay and Fe-oxides (Hu and Moran, 2005). Given the dependence upon pH conditions, low pH levels are generally problematic. Results from sorption and transport experiments presented by McCarthy *et al.* (2000) indicate that FBAs can be useful as nonreactive tracers as long as the pH is approximately 2 pH units above the FBA's specific pK_a. The stability of FBA isomers under strictly anaerobic conditions has yet to be investigated (Benson and Bowman, 1994). In summary, according to Flury and Wai (2003), benzoate and fluorobenzoates are useful tracers that migrate under most pH conditions found in soils and aquifers, similar to bromide. Under low pH conditions, mobility usually decreases. Sorption and transport of fluorobenzoates can be affected by the presence of organic carbon, clay and Fe-oxide. The behaviour of the tracer in the presence of these three compounds should be evaluated by means of sorption or column tests prior to conducting the experiment.

McCarthy *et al.* (2000) assessed the aquatic toxicity of four Fluorobenzoic acids tracers using a Ceriodaphnia 96-h acute toxicity test. The LD50 (lethal dose resulting in the mortality of $\geq$50% of the test organisms) of the tested isomers was above 100 mg/l. The toxicity of FBAs for humans has not been established (Wright and Hull, 2004). FBAs should not be used as tracers where there is a risk of causing contamination to drinking water until more definite human toxicity information becomes available.

4.5.1.1 Suitability/potential applications

Fluorobenzoic acid tracers are applied in the investigation of water flow and solute transport in the unsaturated zone and in both porous and fractured aquifers (Dahan and Ronen, 2001). Recent studies have demonstrated that FBAs may not exhibit ideal conservative behaviour in certain experimental constellations. Nevertheless, FBAs were in the past often proposed as tracers for vadose zone hydrology because of the lack of background concentrations and the relatively good mobility properties. FBAs might be useful as alternative tracers where other common anionic tracers are not suitable. Unlike salts, most FBAs are quite expensive (Caldiga and Greibrokk, 1998; Turin *et al.*, 2002). As the aqueous diffusion coefficients of FBAs are about a third of those of the halides, diffusivity-tracer approaches using FBA and halide tracers simultaneously have also been employed to investigate solute dispersion and diffusive mass transfer between fast- and slow-moving flow regions (Hu and Moran, 2005 and references therein).

The greatest potential use of FBAs would appear to be in multi-tracer tests due to the wide variety of available isomers in this tracer family displaying similar characteristics (Dahan and Ronen, 2001; Juhler and Mortensen, 2002; Turin *et al.*, 2002; Wright

and Hull, 2004; Hu and Moran, 2005). Multiple FBA tracers can be applied either simultaneously or sequentially. This makes it possible to conduct several simultaneous leaching studies at the same location without tracer interference (e.g. Kung *et al.*, 2000; Gish *et al.*, 2004; Wright and Hull, 2004; Hu and Moran, 2005) and to tag individual injection boreholes (e.g. Turin *et al.*, 2002).

A systematic evaluation of the characteristics of FBAs as suitable tracers similar to that of fluorescent tracers taking into account the required properties for hydrological tracers is still needed (cf. Table 4.3).

4.5.1.1.1 Analytical methods and detection limits The detection limits of FBA tracers depend on the analysis method. In general, FBAs can be analysed using ion chromatography (Pearson, Comfort and Inskeep, 1992), high performance liquid chromatography (HPLC) with ultraviolet detection (Bowman, 1984b), HPLC combined with a reverse phased separation method (Dahan and Ronen, 2001), gas chromatography-mass spectrometry (GC-MS) analysis of derivatives (Caldiga and Greibrokk, 1998) and liquid chromatography-tandem mass spectrometry(LC–MS–MS) (Juhler and Mortensen, 2002). According to Hu and Moran (2005), the most widely used method for FBA analysis uses high-performance liquid chromatography (HPLC) with UV detection after separation by means of a strong anion exchange (SAX) column. For multi-tracer tests, the possibility of simultaneous analyses of multiple FBA isomers employing chromatography techniques is advantageous, and single run capacities might become crucial for cost efficiency (Hu and Moran, 2005).

4.5.2 Deuterium ^{2}H as a tracer

The isotopes ^{18}O, ^{2}H and ^{3}H are essentially attractive hydrological tracers. The stable isotopes of water are the most commonly used environmental tracers. Being constituents of the water molecule themselves, they are considered to be those most capable of representing the true flow of water. Usually these isotopes are used as natural tracers, but the isotopes may potentially also be employed as artificial tracers. In both natural and artificial applications the physics and measurement techniques are identical (cf. Chapter 3).

In recent times the application of tritiated water (^{3}H) as an artificial tracer has become rather infrequent due to radiation risks and eco-toxicological concerns. Therefore, the most promising isotopes are the stable oxygen and hydrogen isotopes. Both deuterium (^{2}H) and oxygen 18 (^{18}O) are nontoxic, completely soluble, chemically and biologically stable and are not subject to photolytic decay.

As deuterated water, ^{2}H is available in concentrations of almost 100%. Double labelled water (^{2}H and ^{18}O enriched) can also be purchased and applied as a tracer. Experiments using environmental isotopes as a label (enriched or depleted) of the water in the system under investigation have also been reported. Such studies do not influence the environment and may be more affordable.

Successful applications of deuterated water have been reported in laboratory tests, lysimeter and groundwater studies, and for investigations of solute transport in the

unsaturated zone and of water flow in the soil-vegetation system.[10] The advanced application of deuterated water for the estimation of tree transpiration and the investigation of plant water uptake in the xylem flow has been discussed in several papers (Calder *et al.*, 1986; Calder, 1992; Kalma, Thorburn and Dunn, 1998).

Comparing deuterium with other groundwater tracers, Leis and Benischke (2004) found that deuterium and bromide showed the highest degree of conservativity. Consequently, deuterium is often regarded as a reference in multi-tracer tests. However, the diffusion rate of H^2HO in water is high when compared with other conservative solute tracers. It is particularly important that this characteristic be taken into consideration in the presence of immobile water zones (Becker and Coplen, 2001).

Deuterium can be purchased in concentrated form (^2H_2O), but because of the high costs only applications involving relatively small volumes of water are feasible. The sensitivity of the analytical equipment necessitates that special attention be paid to the determination of the injection amounts, to avoid an inappropriately 'heavy' signature of the water samples (Königer, 2003). This includes the conversion of the concentrations of the injected deuterated water to the δ notation used for sample analyses (cf. Chapter 3). In practice, a level of precision of 2‰ of the hydrogen-isotope analysis results in a minimum detectable concentration of about 0.1 mg/l above the background concentration (Becker and Coplen, 2001).

The alteration of the natural isotope signature is another disadvantage of the use of deuterium as an artificial tracer in large scale experiments, as this may inhibit its usefulness as an environmental tracer. In spite of these restrictions, deuterium would appear to be an appropriate artificial tracer, particularly for investigations in the unsaturated zone. For this purpose the feasibility of analysing quite small sample volumes is also beneficial (Königer, 2003).

4.5.3 Dissolved gas tracers

The use of injected dissolved gas tracers was proposed by Carter *et al.* (1959). However, technical difficulties related to the injection, sampling and analysis of the gases have been obstacles to their widespread use as tracers. Many of these problems have been overcome in recent times and since the 1990s their application has increased (Solomon, Cook and Sanford, 1998). In addition to the naturally occurring noble gases, man made fluorinated compounds (SF_6, CFCs, PFCs) display properties meeting the requirements for use as artificial tracers. In oceanography, sulfur hexafluoride (SF_6) has been applied in tracer experiments for many years (Watson and Ledwell, 2000), and its use in hydrological investigations seems to be increasing.

[10] Garcia Gutiérrez *et al.* (1997); Himmelsbach, Hötzl and Maloszewski (1998); Schwinning *et al.* (2002); Stamm *et al.* (2002); Königer (2003); Benischke, Leis and Stadler (2004); Leis and Benischke (2004); Hangen *et al.* (2005); Cencur Curk, Bricelj and Stichler (2006); Mali, Urbanc and Leis (2007); Wenninger (2007); Stumpp (2008); Königer and Marshall (2008); Königer *et al.* (2009).

4.5.3.1 Common gas tracers and tracer characteristics

The most commonly used gas tracers include helium, neon and stable isotopes of krypton and sulfur hexafluoride. The favourable characteristics of noble gases are their inert and nontoxic nature in hydrological systems (Solomon, Cook and Sanford, 1998). Furthermore, concentrations of dissolved gas in many orders of magnitude greater than background concentrations can be obtained without any difficulty, even for large volumes of traced water. Argon is an exception due to the comparatively high natural background concentrations and for this reason is generally not used. Xenon gas is also rather expensive, which limits its practical application as a tracer substance (Solomon, Cook and Sanford, 1998).

Like the noble gases, fluorinated compounds are relatively inert in water, nontoxic and can be detected even at low levels (Solomon, Cook and Sanford, 1998). Significant degradation of CFCs is possible under highly reducing conditions (Busenberg and Plummer, 2000). Noble gases, CFCs and SF_6 have the potential to serve as environmental tracers for hydrological investigations (cf. Chapter 3). However, the choice of SF_6 as an artificial hydrological tracer should be considered carefully, given the high potential greenhouse gas effect of SF_6 gas (Busenberg and Plummer, 2000). In future its use may become subject to restrictions due to the potential for negative environmental impacts.

4.5.3.2 'Gas-specific' methods/technical equipment

Obviously dissolved gases differ from other tracers primarily on the basis of their volatile nature, with specific requirements in terms of tracer injection, sampling and analysis to prevent degassing.

Measures must also be taken at the time of injection to avoid the formation of air bubbles. Usually injection is by means of blowing the gaseous tracer directly into the water through diffusers, as described by Sanford, Shropshire and Solomon (1996). A near constant injection rate can be maintained during hours or days. A suitable injection porous pipe needs to be found through which gas diffuses slowly enough to be dissolved completely. In fact, the cost of the gas applied is the limiting factor for constant injection experiments. By contrast, gas tracers are less suited for instantaneous injection. Sugisaki and Aoki (1993), Gupta, Moravcik and Lau (1994), Uddin, Dowd and Wenner (1999) and Shapiro *et al.* (2008) provided examples of how a virtually instantaneous injection can be achieved in aquifers. This usually requires the prior preparation of a gas tracer solution.

The methods and the equipment required for injection, sampling and the preparation of aqueous solutions of tracer gases were described in detail by, for example, Sanford, Shropshire and Solomon (1996), Sanford and Solomon (1998) and Wilson and McKay (1993, 1996).

Generally the analysis of gas tracers is carried out by means of gas chromatography, which is common to many laboratories (Sanford, Shropshire and Solomon, 1996). However, advanced methods using a gas chromatograph equipped with an electron capture detector (GC-ECD) improve sensitivity and yield an enlarged concentration range for

tracer applications. For example, Law, Watson and Liddicoat (1994) reported a range up to seven orders of magnitude greater for SF_6. There are commercial devices available for the detection of SF_6 based on spectrography with diode lasers. Although the detection is less accurate than with GC-ECD, these devices can be useful for online measurements.

4.5.3.3 Applications

The volatility requires that in field applications the gas tracers are used without the presence of a gas phase, if a quantitative evaluation is intended. Exchange with air entrained in unsaturated media and gradual losses from the solution through the air-water interface of shallow aquifers or surface water bodies reduce the validity of tracer tests (Wilson and McKay, 1993; Gupta, Moravcik and Lau, 1994; Engblom, Sanford and Stednick, 2004). However, by injecting a second nonreactive gas along with the tracer gas and monitoring the distributions of both gases, the gas transfer velocity can be quantified and the mass balance closed (Watson, Upstill-Goddard and Liss, 1991; Clark *et al.*, 1996). Gas tracers can also be useful for largely qualitative tracing of hydrological connections between surface waters and local aquifers (Engblom, Sanford and Stednick, 2004; Gamlin *et al.*, 2001; Avisar and Clark, 2005; Harden *et al.*, 2008). Several applications taking advantage of the volatile nature of gas tracers have been proposed; for example to delineate unsaturated zones (Upstill-Goddard and Wilkins, 1995) or use as a partitioning tracer to detect pools and residual zones of NAPLs in the subsurface (Wilson and McKay, 1995; Cirpka and Kitanidis, 2001; Divine, Sanford and McCray, 2003). Returning to the classical applications of artificial tracers, dissolved gases are regarded as being especially suitable for experiments involving large volumes of water (Solomon, Cook and Sanford, 1998; Gamlin *et al.*, 2001). This is attributed to the wide concentration range possible, which renders them cost effective. However, their potential can only be fully utilized if advanced analysis methods are available. One must also always take into consideration the laboratory costs. One may very well opt to use a dissolved gas in situations where other tracers are not applicable for certain reasons; for example in very sensitive environments.

4.5.4 Nonfluorescent dyes

Inevitably all dyes used for staining experiments make far from ideal tracers. Nonfluorescent dyes may be valuable tracers for transport studies but, particularly in terms of travel times and distances, they do not adequately represent the water itself. However, staining techniques have attracted remarkable interest as a tool for demonstrating the occurrence of preferential flow in soils.[11]

In contrast to the classical idea of tracer applications, staining experiments rely on the usually problematic sorption effect of the tracer to a degree, ensuring its distinct

[11] Van Stiphout *et al.* (1987); Van Ommen *et al.* (1988); Andreini and Steenhuis (1990); Ghodrati and Jury (1990); Hatano and Booltink (1992); Flury *et al.* (1994); Petersen, Hansen and Jensen (1997); Perillo *et al.* (1999); Öhrström *et al.* (2002); Weiler (2001); Weiler and Näf (2003); Morris and Mooney, (2004).

visibility in the porous media. Thus, when choosing a tracer, two competing criteria must be considered, namely visibility and mobility (Flury and Flühler, 1995). The major outcome of such tracer experiments are not breakthrough curves but stained profiles visualizing the spatial flow patterns of infiltrating water and solutes. Tracer visualization experiments can also be conducted by using iodide or bromide, if an indicator solution is applied in order to trigger a colour reaction (Van Ommen, 1985; Lu and Wu, 2003). A similar method using ammonium carbonate and pH indication was proposed by Wang *et al.* (2002).

A variety of tracers is available, fluorescent dyes included. Nonfluorescent dyes were tested for visualization purposes in soils by Corey (1968), Smettem and Trudgill (1983), Flury and Flühler (1995), Mon, Flury and Harsh (2006) and others. A comprehensive review of the application of dye tracers in the vadose zone was provided recently by Flury and Wai (2003). Most fluorescent and nonfluorescent dyes are organic molecules with varying functional groups. Apart from fluorescence itself, the chemical and physical principles relevant for tracing, discussed in Section 4.1.2, are also largely applicable to nonfluorescent dyes. The interaction of a dye with solid materials depends on its type of functional groups and the pH conditions. For instance, methylene blue (CI Basic Blue 9), which exhibits excellent visibility in most soils, has been used extensively for flow path visualization (e.g. Bouma and Dekker, 1978; Smettem and Collis-George, 1985; Van Stiphout *et al.*, 1987; Hatano *et al.*, 1992). However, due to its cationic form in aqueous solutions it adsorbs strongly onto most subsurface media and is not a good indicator of water movement. With respect to sorption behaviour in soils generally, acid dye tracers are preferable (Corey, 1968; Flury and Wai, 2003; Mon, Flury and Harsh, 2006). They are regarded as being relatively mobile and the best suited of the dyes for visualization experiments aimed at characterizing flow.

After dye-tracing conducted as part of a sprinkling experiment in an excavated soil section provides only one picture of the cumulative flow pattern, allowing at best for integral statements about major flow processes (Weiler, 2001). As the dye pattern does not match the infiltration pattern of water exactly, it must be interpreted with caution. The complex sorption mechanisms must be taken into account in order to avoid misinterpretations (Bouma and Dekker, 1978; Flury and Wai, 2003). The dye front is always retarded when compared to the wetting front and to conservative tracers. Furthermore, the pattern formed depends on the sorption characteristics of the dye, the varying chemical and physical conditions throughout the soil profile and the application rate and the resultant differences in contact time (Corey, 1968; Flury and Flühler, 1995; Perillo *et al.*, 1998; Ketelsen and Meyer-Windel, 1999; German-Heins and Flury, 2000; Kasteel, Vogel and Roth, 2002; Mon, Flury and Harsh, 2006).

Depending on the purpose behind the visualization and analysis of the spatial patterns, specific evaluation techniques are required for dye-tracing experiments if more than a qualitative illustration is intended. Usually this involves applying image analysis procedures to photographs taken from excavated stained soil sections after the tracer experiment.[12] The use of binary images revealing simply either the presence or absence

[12]Hatano *et al.* (1992); McBratney *et al.* (1992); Droogers *et al.* (1998); Ogawa *et al.* (1999); Forrer *et al.* (1999); Forrer *et al.* (2000); Weiler (2001); Vanderborght *et al.* (2002); Kulli *et al.* (2003); Weiler and Flühler (2004); Persson (2005); Schlather and Huwe (2005a); Mooney and Morris (2008); Bogner *et al.* (2008).

of the dye was common in the past. Recently considerable progress has been made in the development of methods enabling actual estimates of concentration.

4.5.4.1 Brilliant blue

Introduced to soil-hydrology by Flury and Flühler (1995), the food dye Brilliant Blue FCF (CI Acid Blue 9 also known as FD&C Blue 1/CI Food Blue 2) has become the most prominent tracer used in visualization experiments in the vadose zone. The bright greenish-blue colour of the dye provides a good contrast to most soils, and tracer fronts are likely to be very sharp. Another advantage is that as a food dye it possesses a relatively low toxicity, explaining its preferred use in field tracer experiments. Sorption behaviour is strongly nonlinear and generally complex, but it has been studied extensively in comparison with other potential dye tracers (German-Heins and Flury, 2000; Ketelsen and Meyer-Windel, 1999; Kasteel, Vogel and Roth (2002); Stamm *et al.*, 2002). The costs are also reasonable. According to many authors, Brilliant Blue FCF is one of the best tracers currently available for visualization experiments in the vadose zone.[13]

[13] Flury and Flühler (1994); Flury *et al.* (1994); Flury and Flühler (1995); Perillo *et al.* (1998); Ketelsen and Meyer-Windel (1999); German-Heins and Flury (2000); Ketelsen and Meyer-Windel (1999); Kasteel, Vogel and Roth (2002); Flury and Wai (2003); Morris and Mooney (2004); Mon, Flury and Harsh (2006).