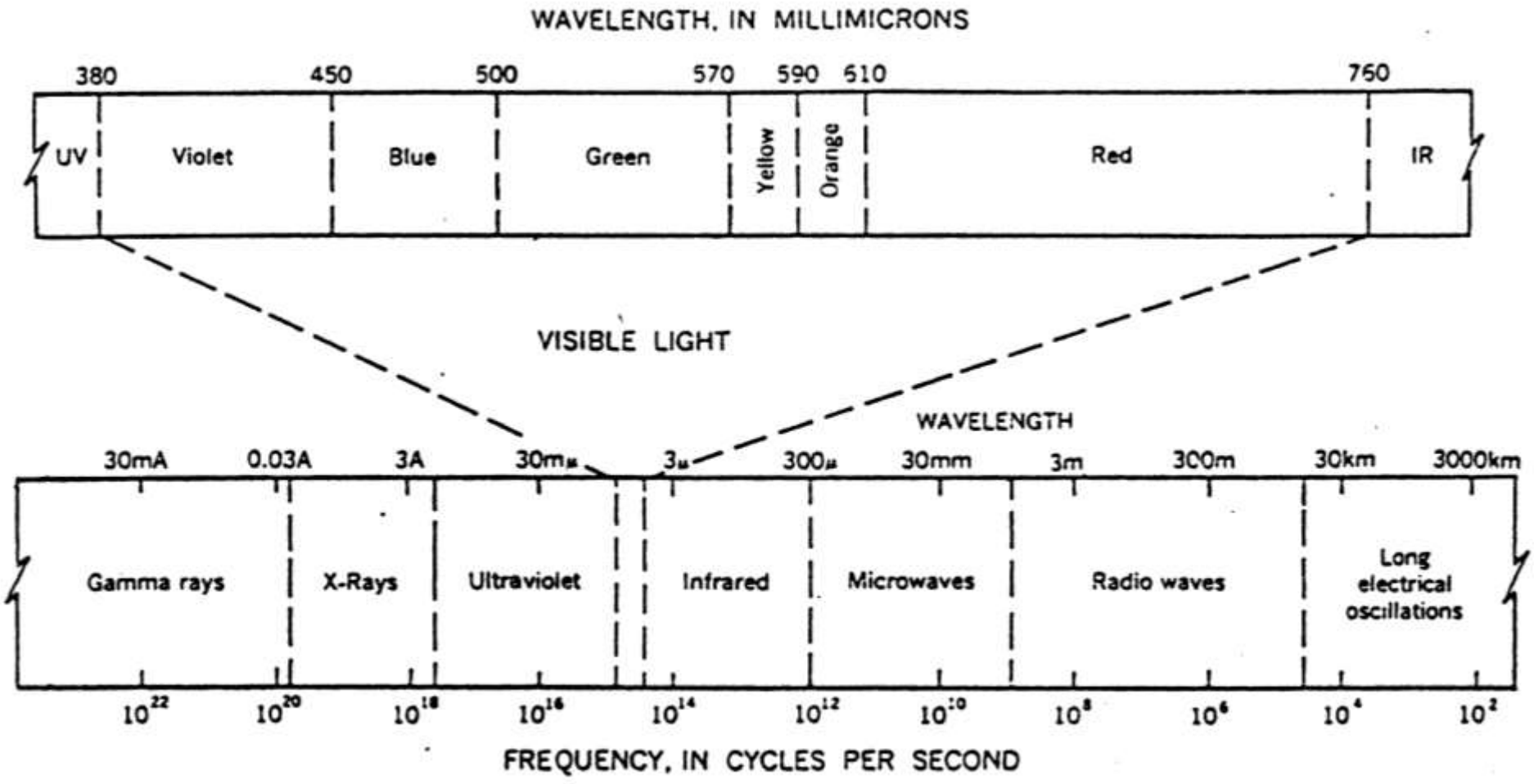
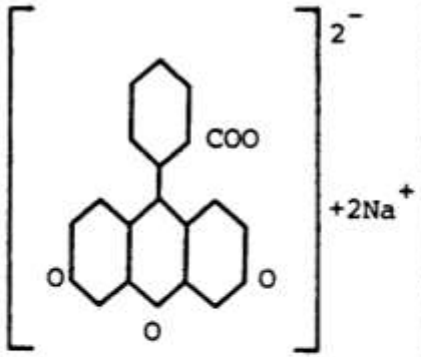
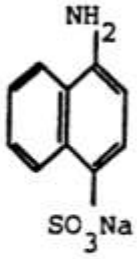
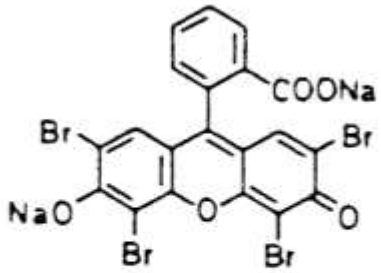
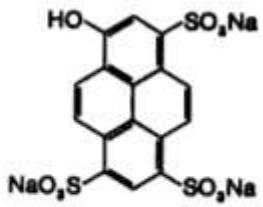
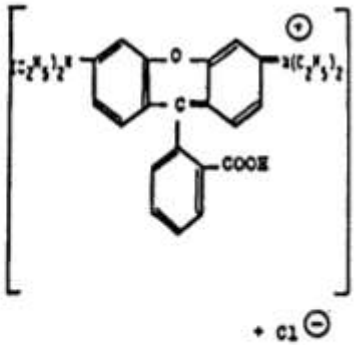
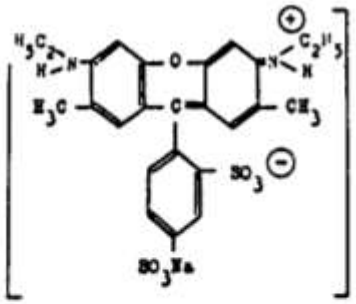
Spectrum



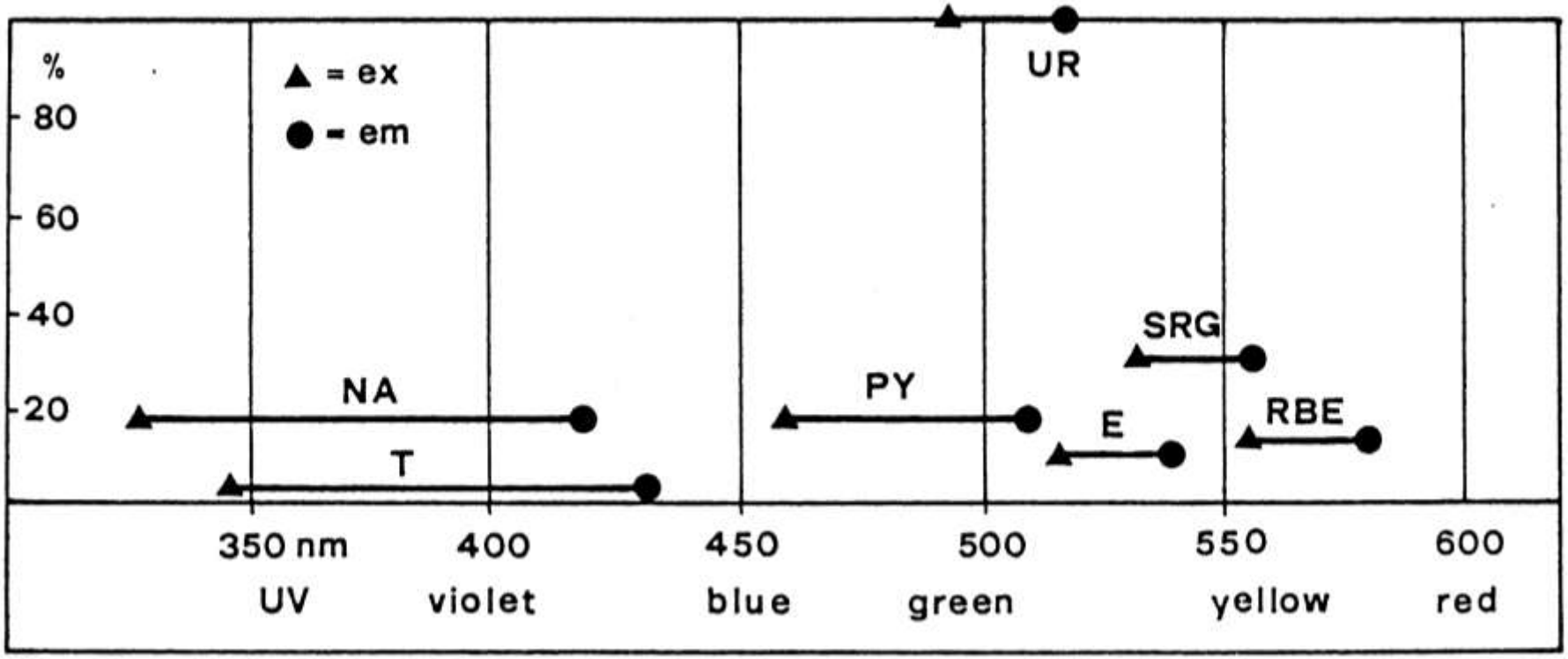
aus: WILSON (1968)

Chemical Substances

Handelsname	Uranin	Naphthionat	Eosin
Referenz-Farbindex	Acid Yellow 73 CI 45350		Acid Red 87 CI 45380
Chemische-Bezeichnung	Natrium-Fluoreszein	1-Naphthylamin-4-sulfon-säure Na - Salz;Naphthion-säure Natriumsalz	2',4',5',7',-Tetrabrom-fluorescein Dinatrium-salz
Summenformel	$C_{20}H_{10}O_5Na_2$	$C_{10}H_8NNaO_3S$	$C_{20}H_6Br_4Na_2O_5$
Strukturformel			
Molekulargewicht	376.15	245.23	691,88

Handelsname	Pyranin	Rhodamin B	Sulphorhodamin G extra
Referenz-Farbindex	Solvent green CI 59040	Basic Violet 10 CI 45170	Acid Red 50 CI 45220
Chemische Bezeichnung	8-Hydroxy-1,3,6-pyrentrisulphonsaures Natrium	NNNN Tetraethylrhodamin-Chlorhydrat	Diethyldiamino-3,6-dimethyl-3,7-phenyl-9 xanthylium disulfonat-2,7-Natriumsalz
Summenformel	$C_{16}\ H_7\ Na_3\ O_{10}\ S_3$	$C_{28}\ H_{31}\ O_3\ N_2\ Cl$	$C_{25}\ H_{25}\ O_7\ N_2\ S_2\ Na$
Strukturformel			
Molekulargewicht	524,38	479,02	631

SPEKTRALBEREICHE UND RELATIVE FLUORESZENZINTENSITAETEN EINIGER FLUORESZIERENDER TRACER



NA = Naphthionat, T = Tinopal, PY = Pyranin, UR = Uranin, E = Eosin, SRG = Sulphorhodamin, RBE = Rhodamin B extra.

Uranin

Uranin: red at high concentration

Naphtionat

white

Sulphorhodamin B

Principle of fluorescence measurement

Characteristic Absorption wave length for substance a: λ_a
Simultaneous emission: λ_e
$\lambda_a < \lambda_e$

$$I_e = I_0 * E * C * \phi_f * d_1$$

I_e= Intensity of emission
I_0 = Intensity of exitation
E = extinction coefficient at wave length λ_a
C = tracer concentration
ϕ_f = quantum efficiency of tracer
d_1 = thickness

$$I_e = f_{linear} (C) \text{ at constant exication}$$

Measurement

Art	Verfahren	Resultat
visual	Colorimetric	qualitative/ half-quantitative
Fluoroscope	Colorimetric	Half-quantitative
optical fluorometer	electric	quantitative reproducible

Filterfluorometer

fixed lamps + filtre

1 Fluorescence tracer

Spectralfluorometer

monochromator

Fibre optics

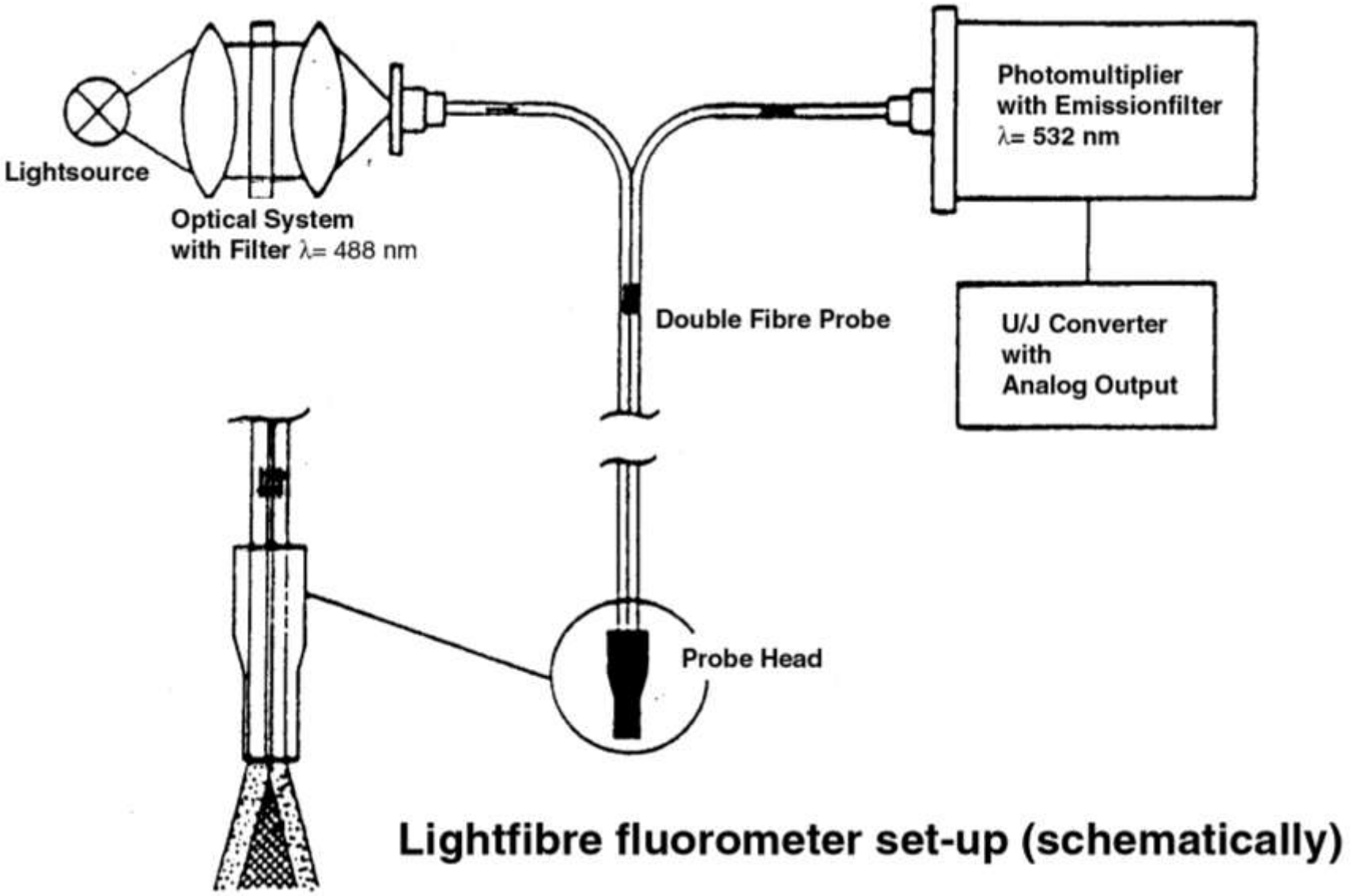
like Filter fluorometer
But more channels

several Fluorescence tracer

Measurement technique

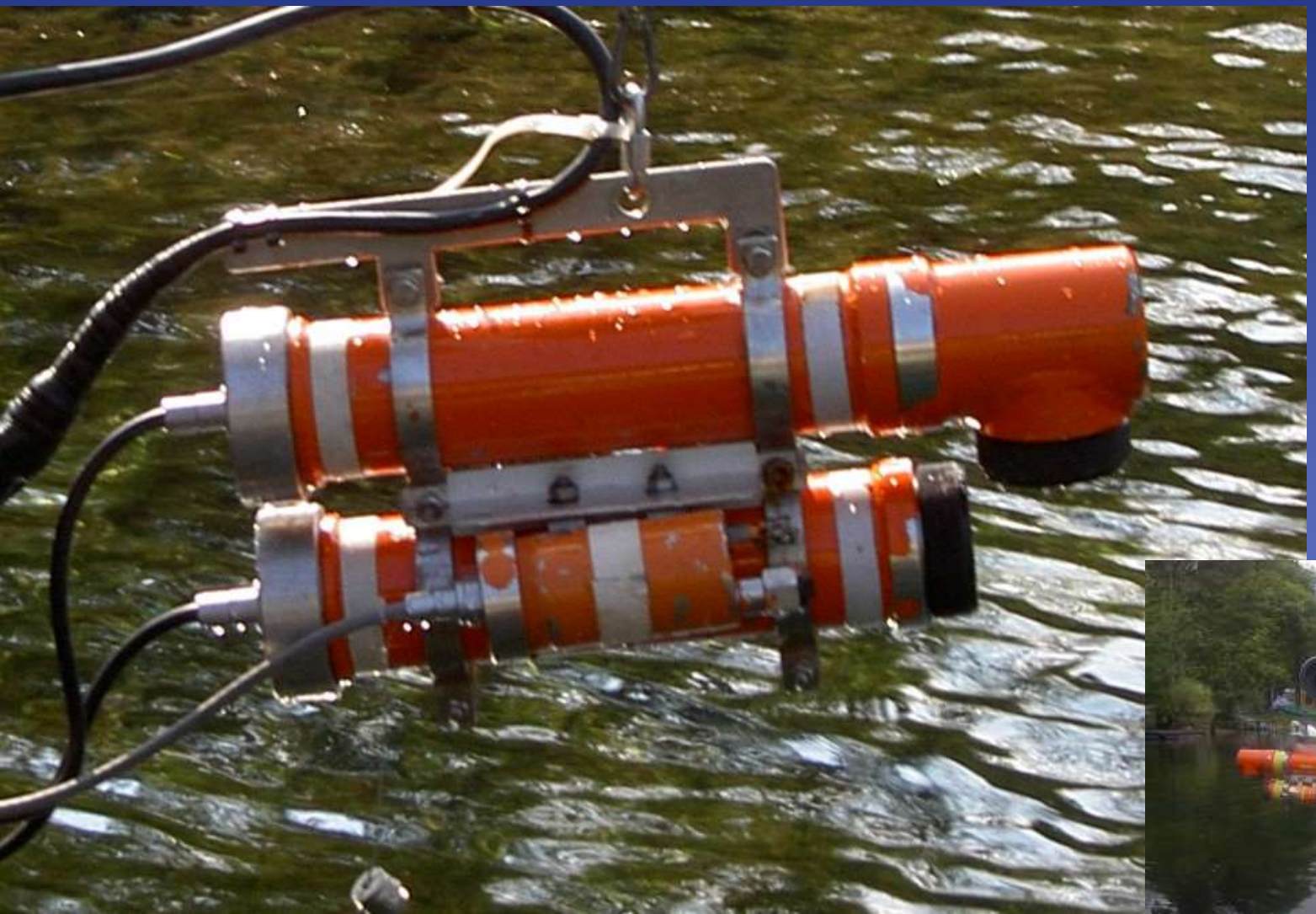
Technique	Principle	Result	Detection limit for Uranin (ppb)	Detection limit for Uranin (mg/m³)
Visual	-	qualitativ	ca. 50 ppb	ca. 20
Quarz lamp/ UV-Lamp	colorimetric	qualitative	ca. 5 ppb	ca. 2
Fluoroscope	colorimetric	half-Quantitative	ca. 1 ppb	ca. 0,5
Spectralphotometer	photometric	Quantitative	0,5 ppb	0,2
Optical Fluorometer	fluorometric	Quantitative	0,005 ppb	0,002
Fibre optics	optical	Quantitative	0,005 ppb	0,002

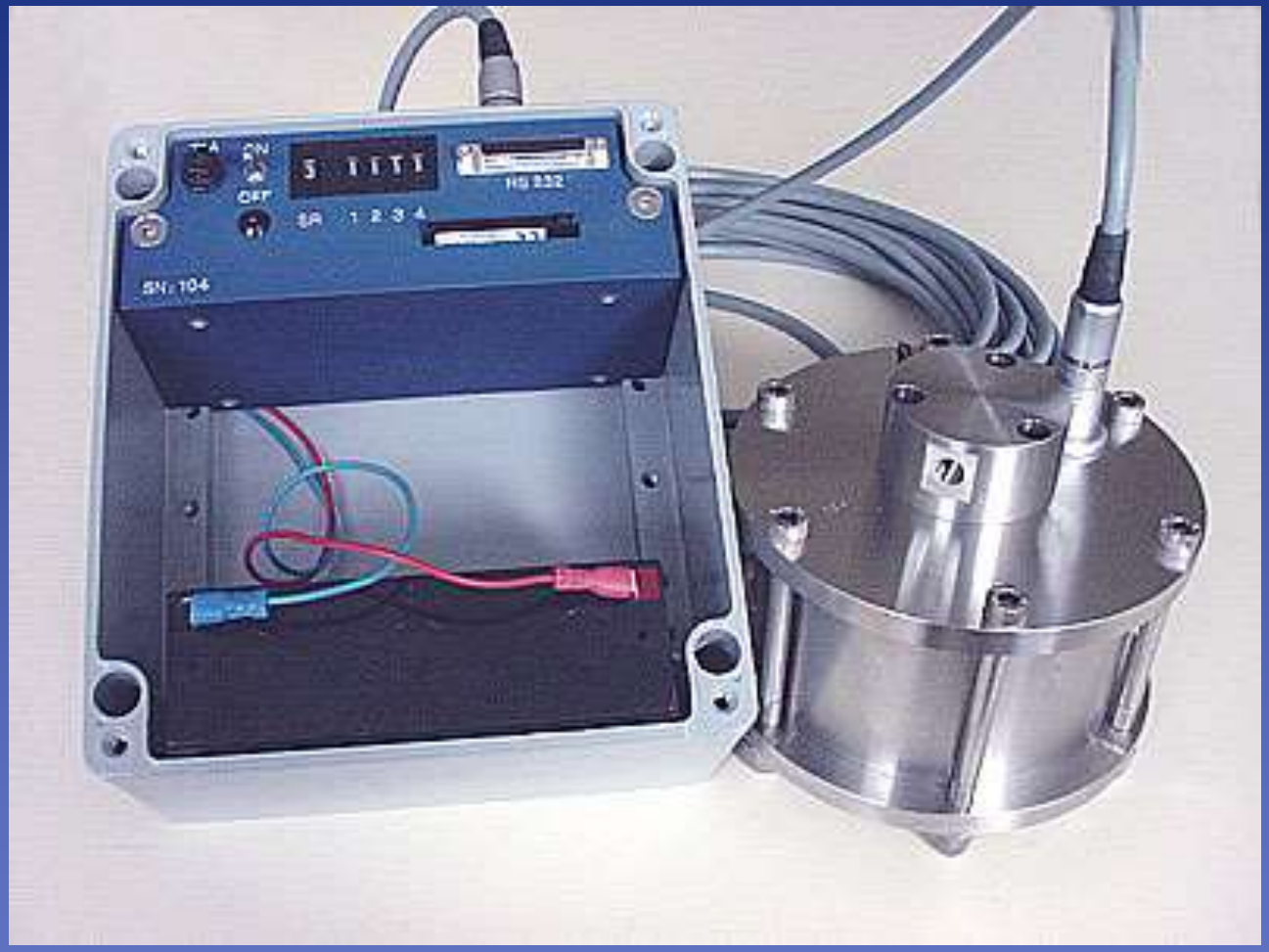
Fibre optics-fluorometer



Lightfibre fluorometer set-up (schematically)

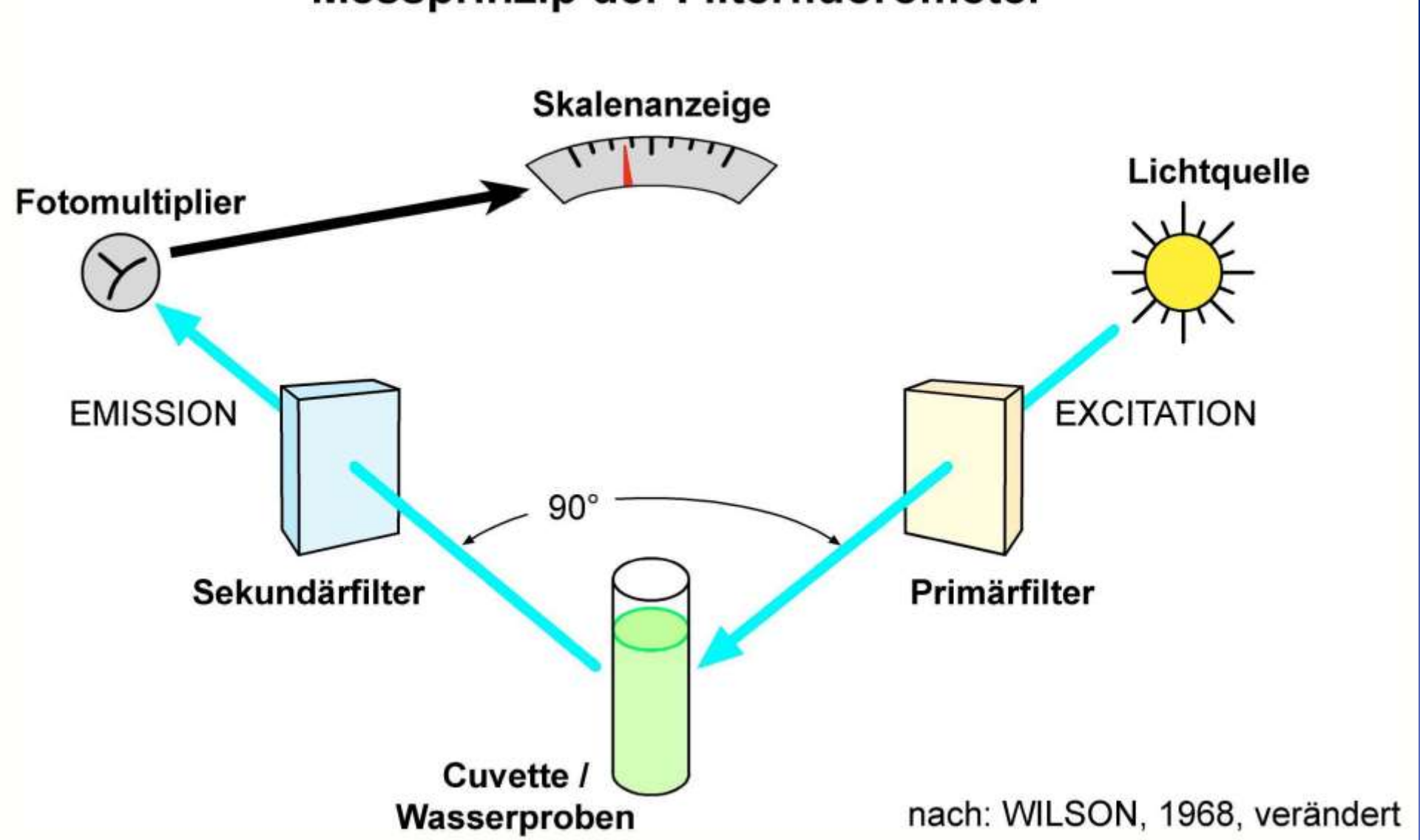
Variosens: Filterfluorometer

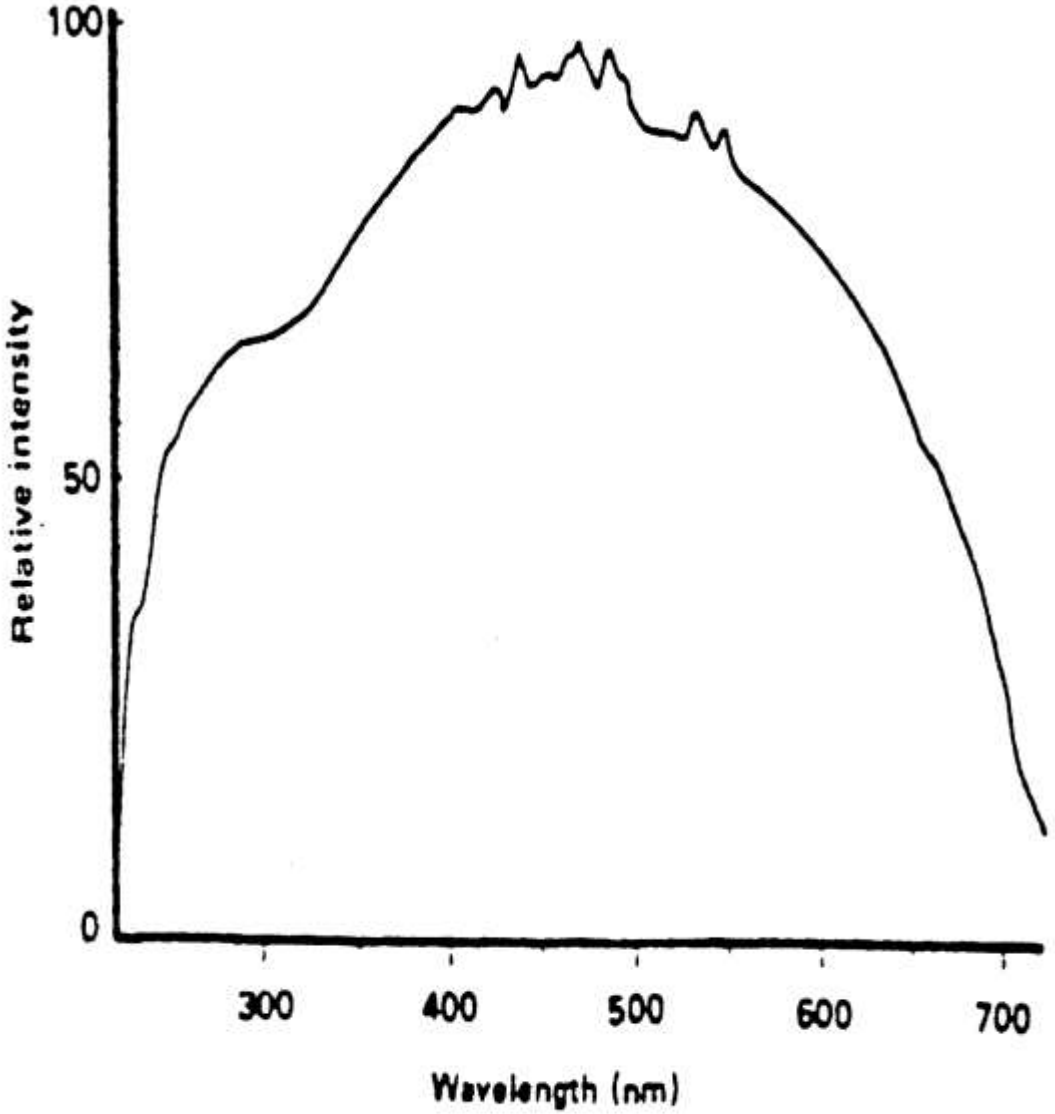




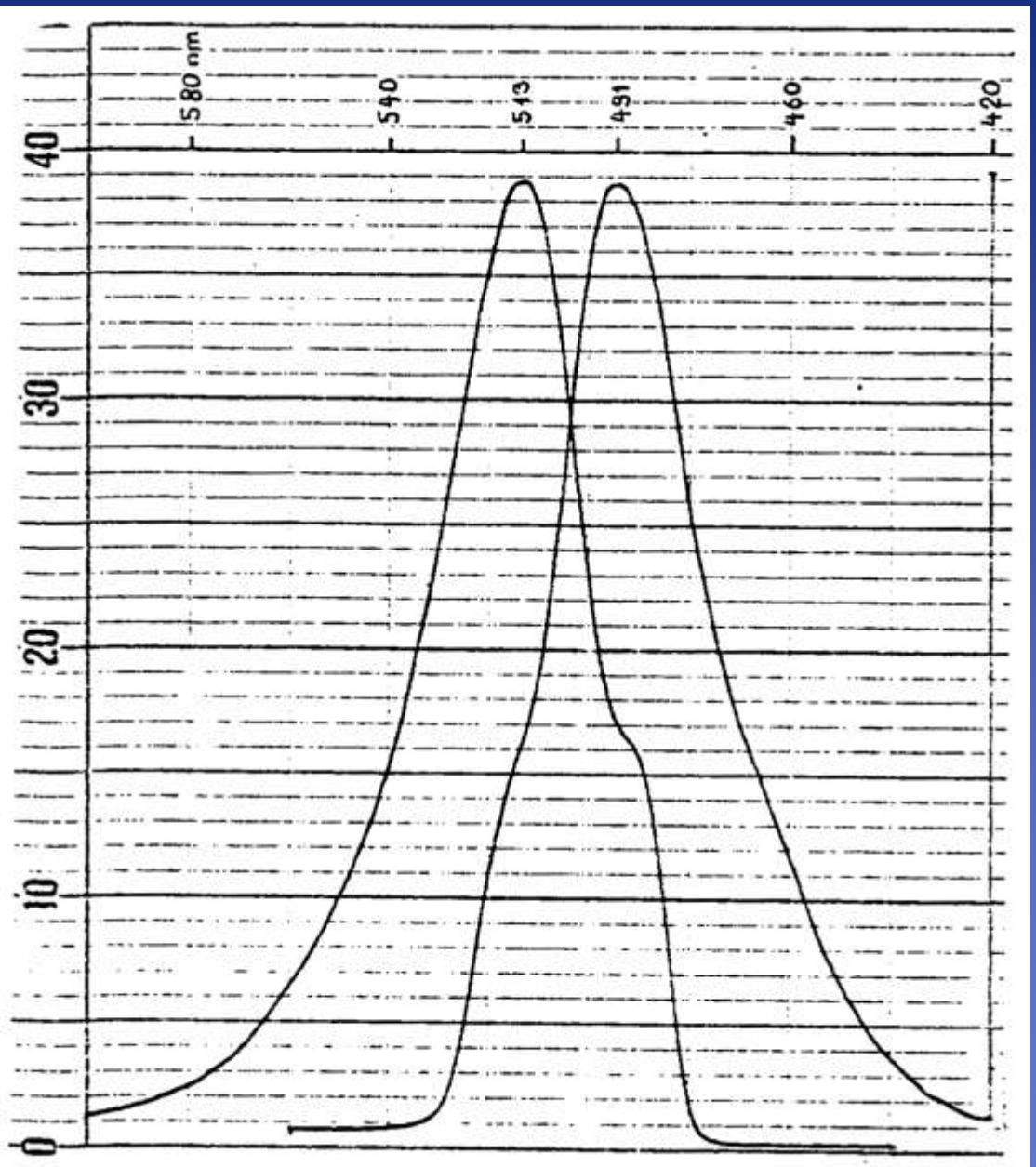
Filterfluorometer GGUN30

Perkin Elmer LS50B


Messprinzip der Filterfluorimeter
Skalenanzeige
Fotomultiplier
Lichtquelle
EMISSION
EXCITATION
90°
Sekundärfilter
Primärfilter
Cuvette /
Wasserproben
nach: WILSON, 1968, verändert



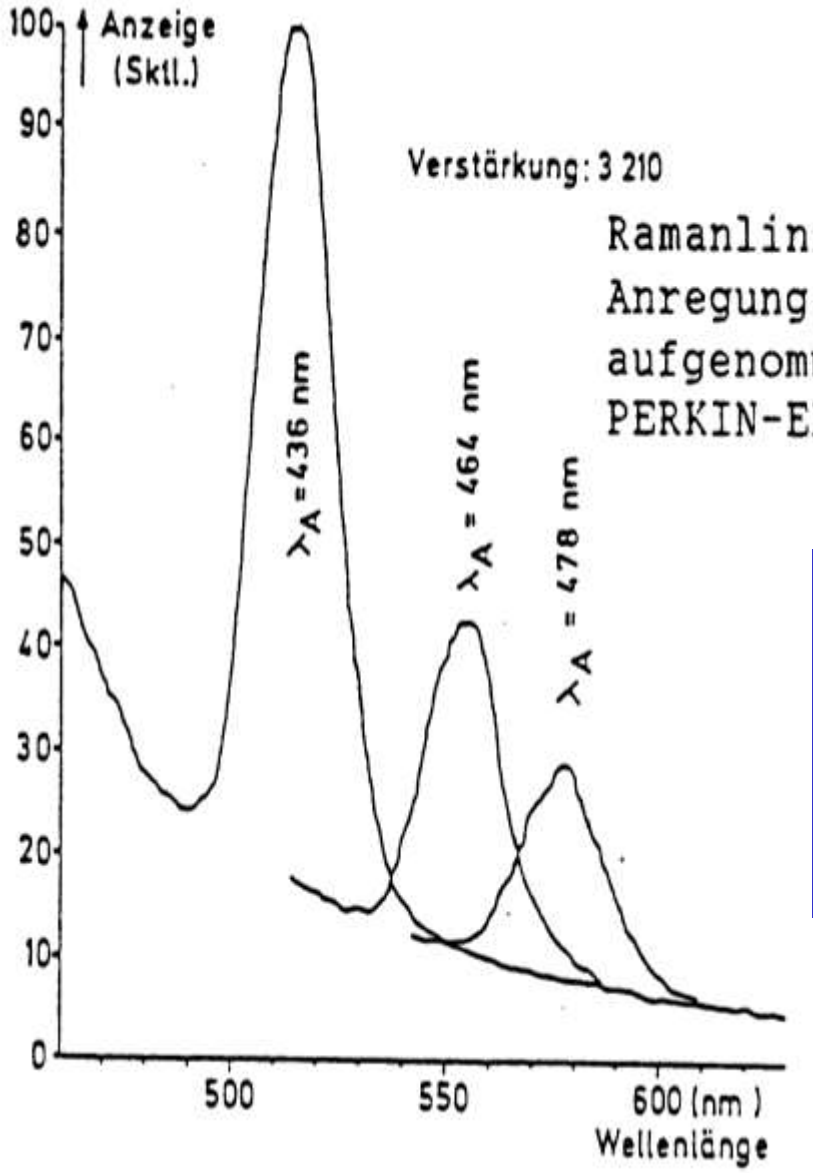
Emission spectrum of a high pressure Xe-lamp



Scans of Uranin

right curve:
Exitations spectrum
Emission 513 nm

linke Kurve:
Emissions spectrum
Exitation 491 nm

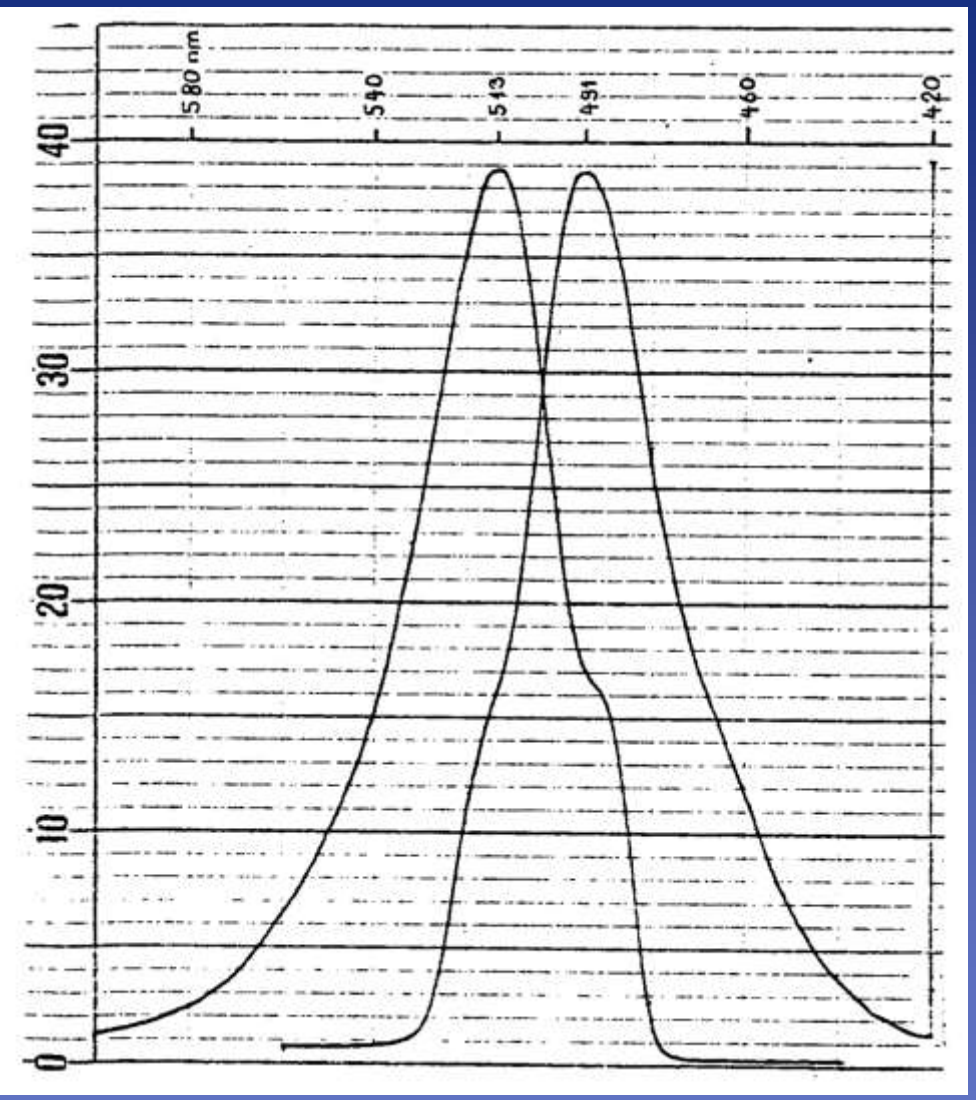


Ramanlinien des Wassers bei Anregung mit 436 464 und 478 nm, aufgenommen mit dem Spektralfluorimeter PERKIN-ELMER 203

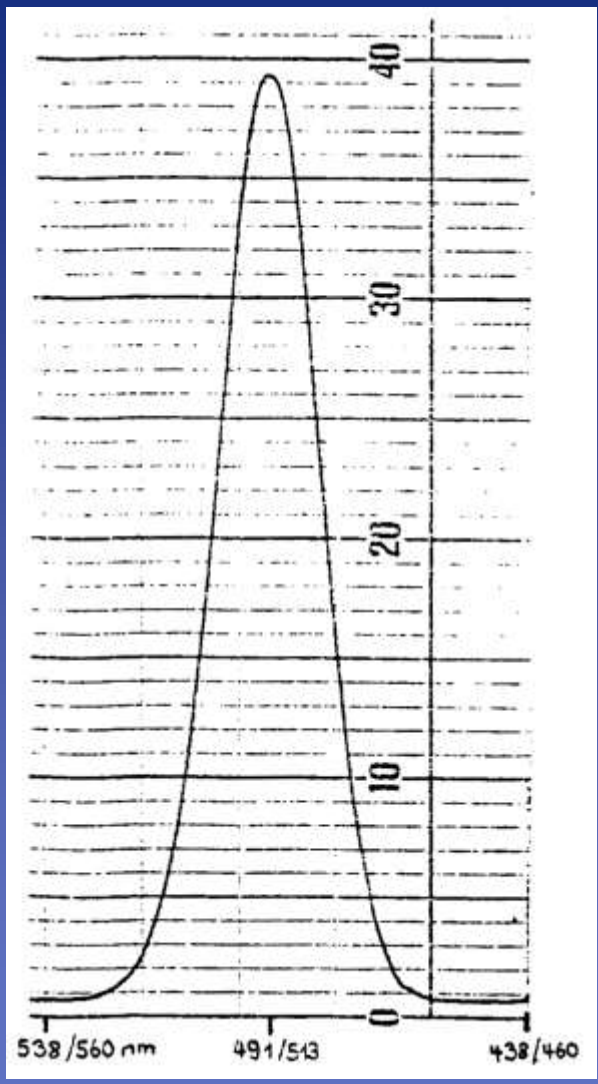
Raman effect can simulate fluoreszence

Solution

- Synchronscan (E-E-Spektrum, Double-scanning)
- distance tracer specific

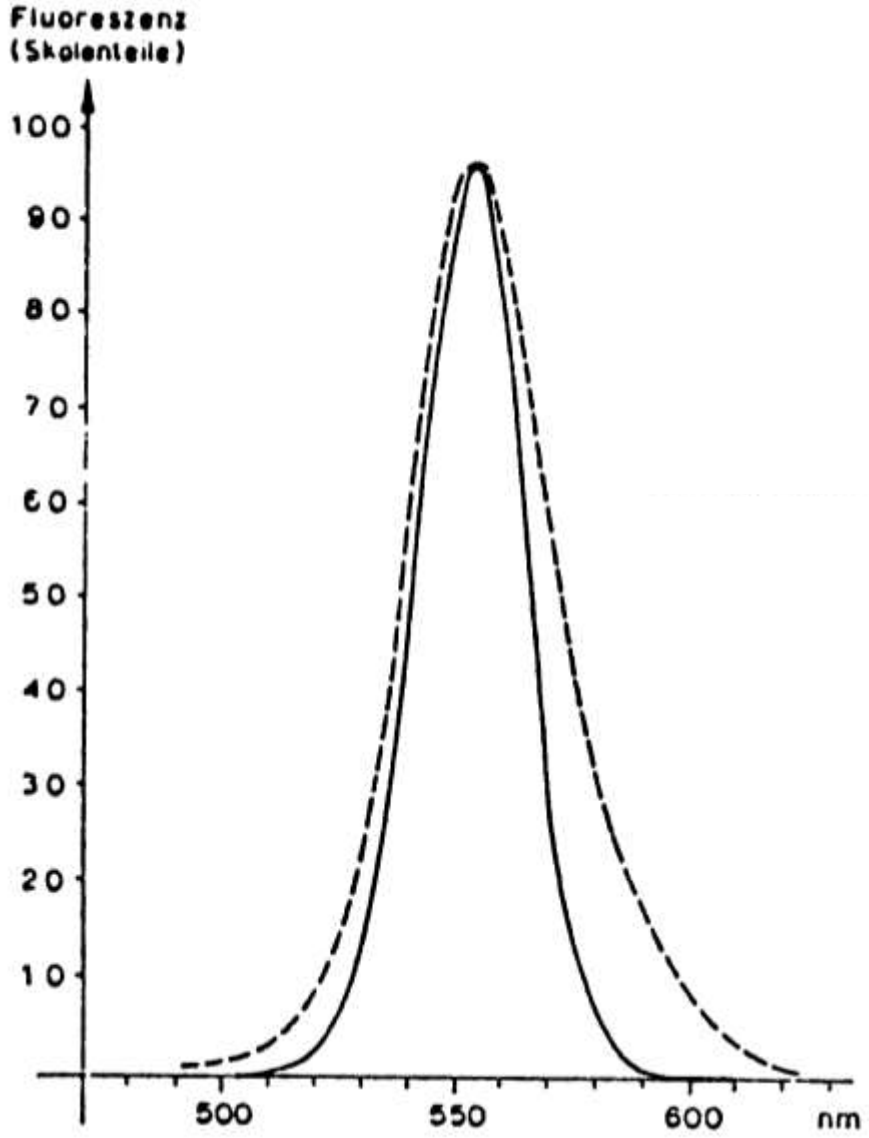


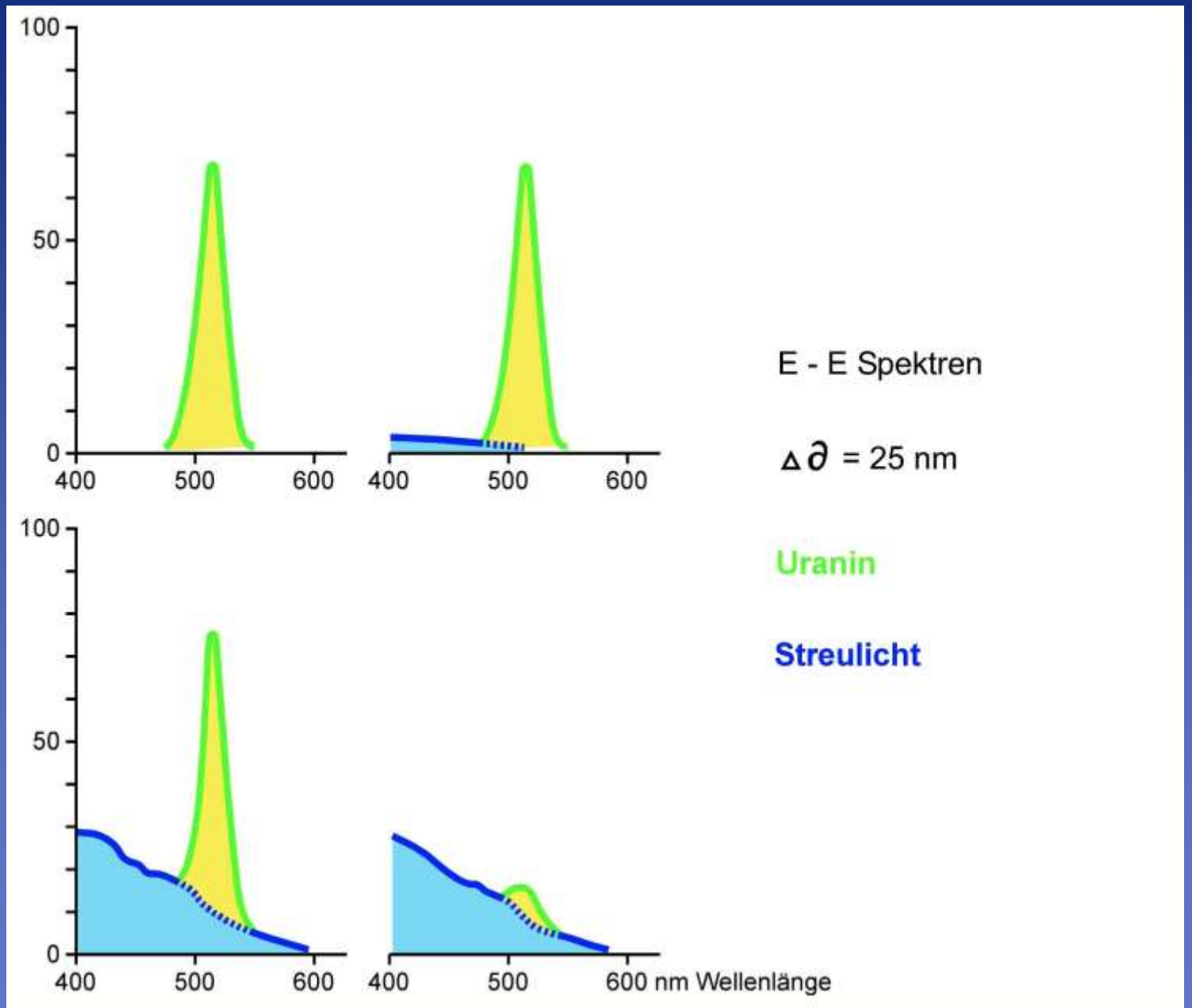
Scans
Von
Uranin

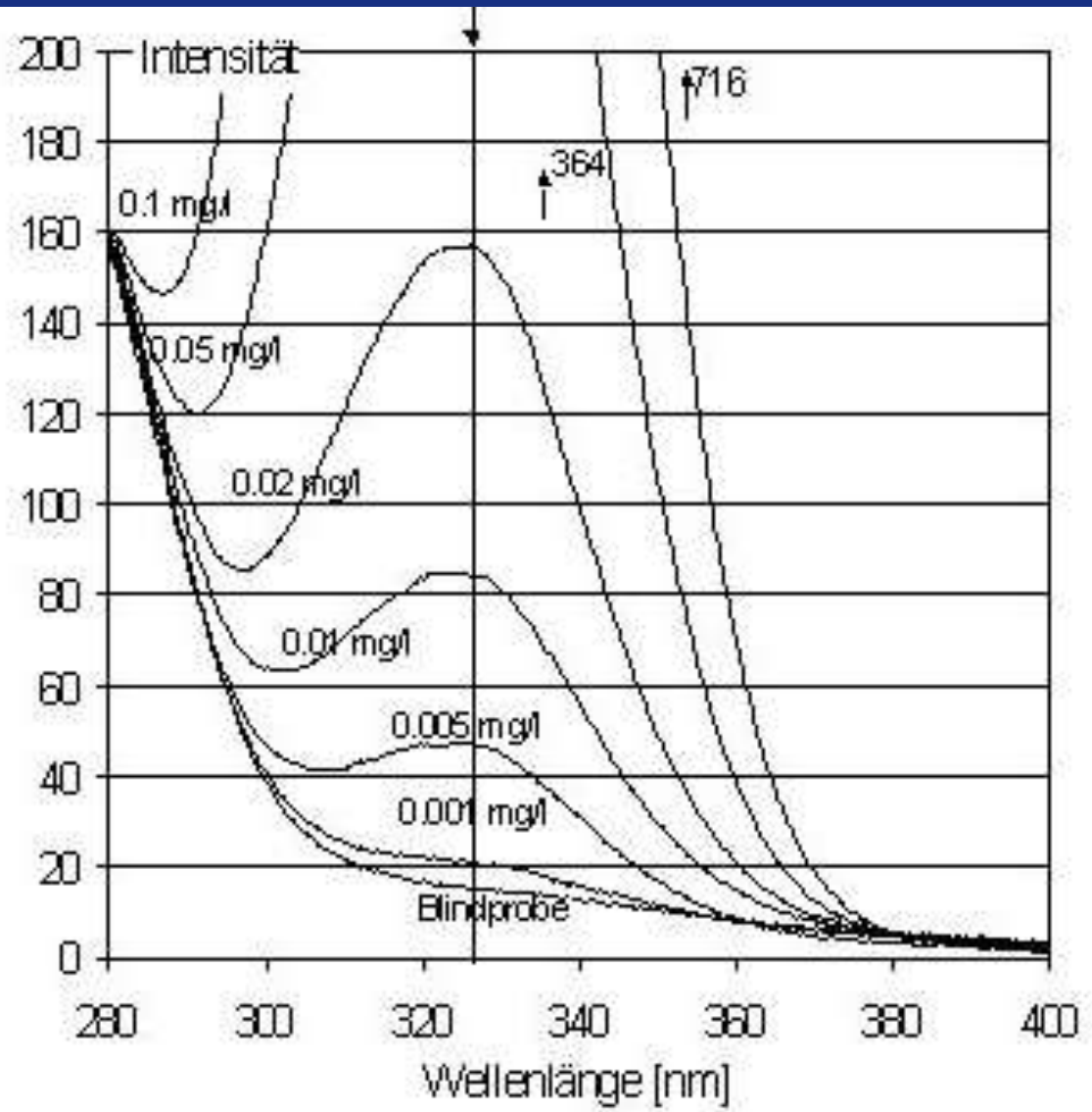


rechte Kurve: Exitationsspektrum, Emission 513 nm
linke Kurve: Emissionsspektrum, Exitation 491 nm

Synchronscan:
$\Delta\lambda$ = 25nm


Fluoreszenz
(Skalenteile)
100
90
80
70
60
50
40
30
20
10
500
550
600
nm

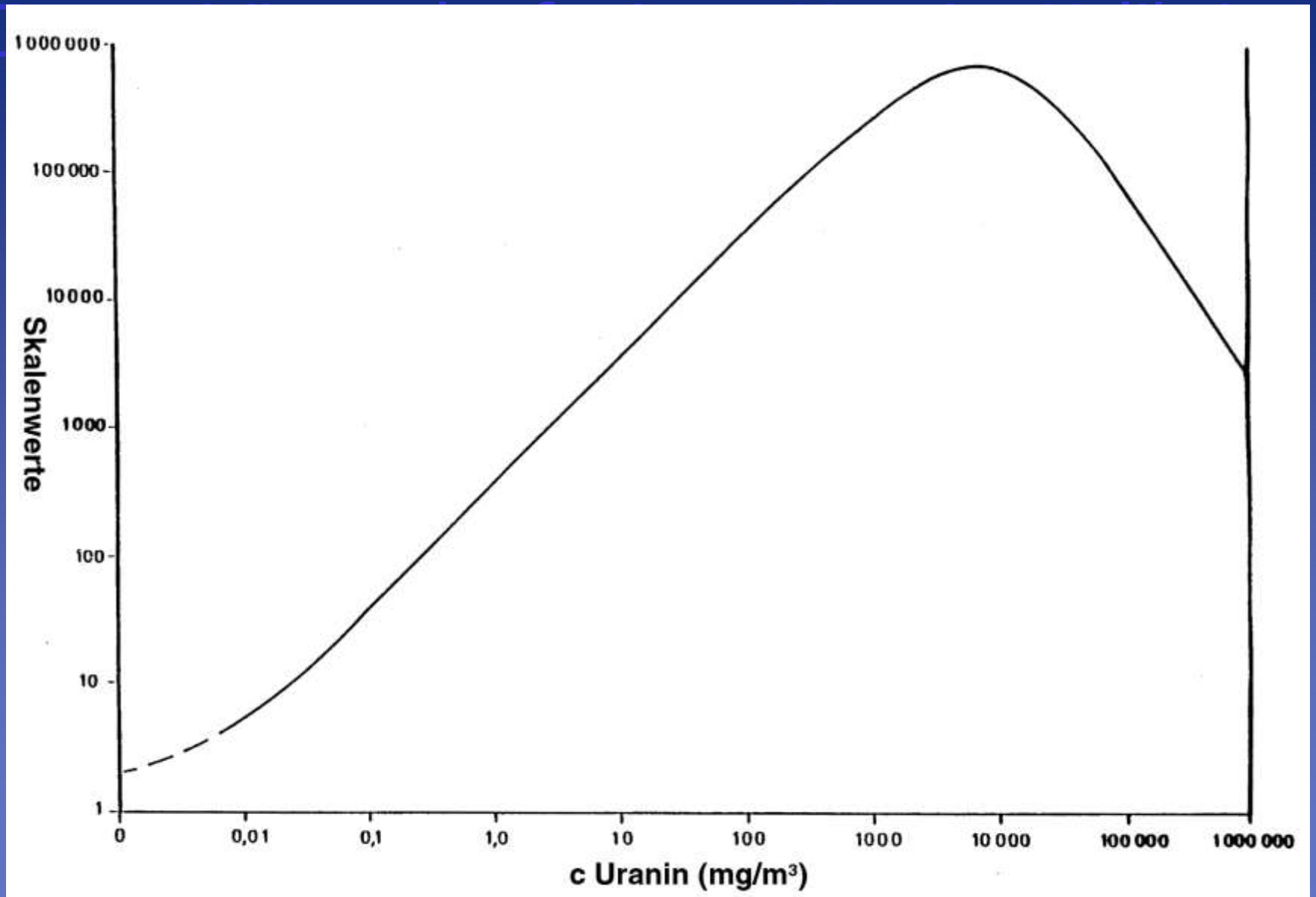

100
50
0
400
500
600
400
500
600
100
50
0
400
500
600
400
500
600 nm Wellenlänge
E - E Spektren
Δ∂ = 25 nm
Uranin
Streulicht



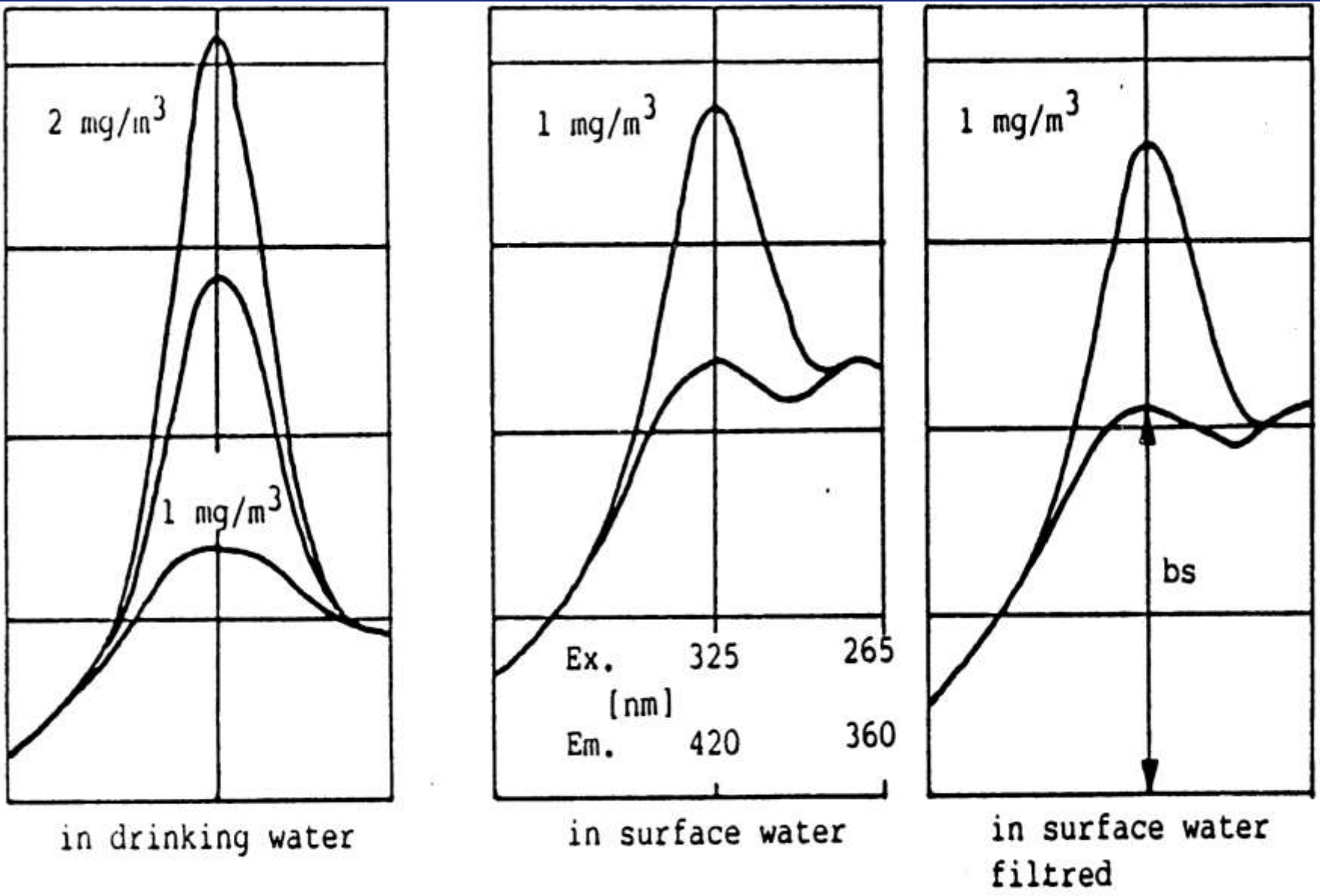
Eichkurven Naphthionat

calibration

Translate fluorescence measurement into concentration



Eichkurve Uranin (verändert aus: Käss 1992)



Spectral curves and background signals (bs) of Naphthionate in different waters.

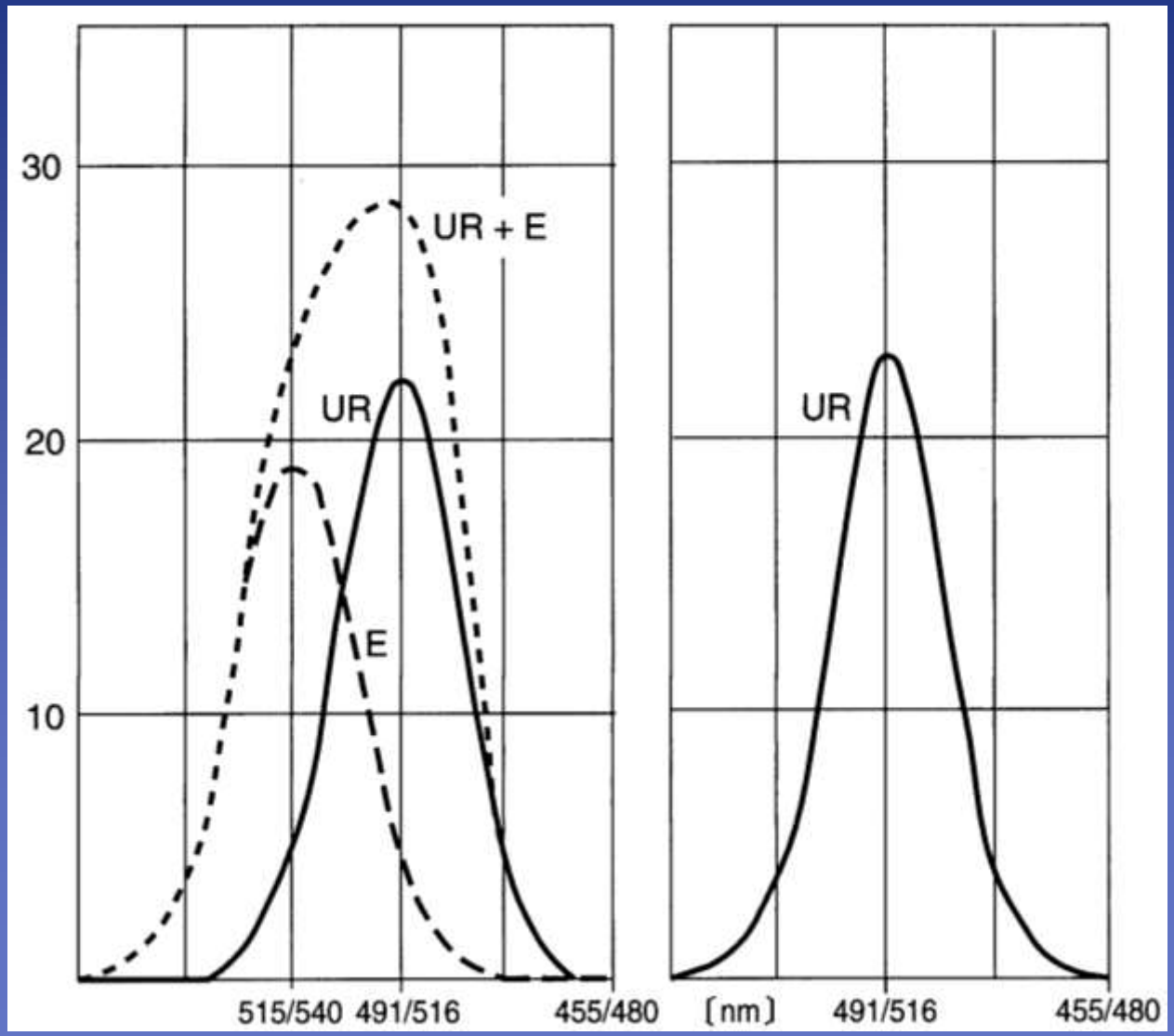
Dectection limit of different fluorescent dyes

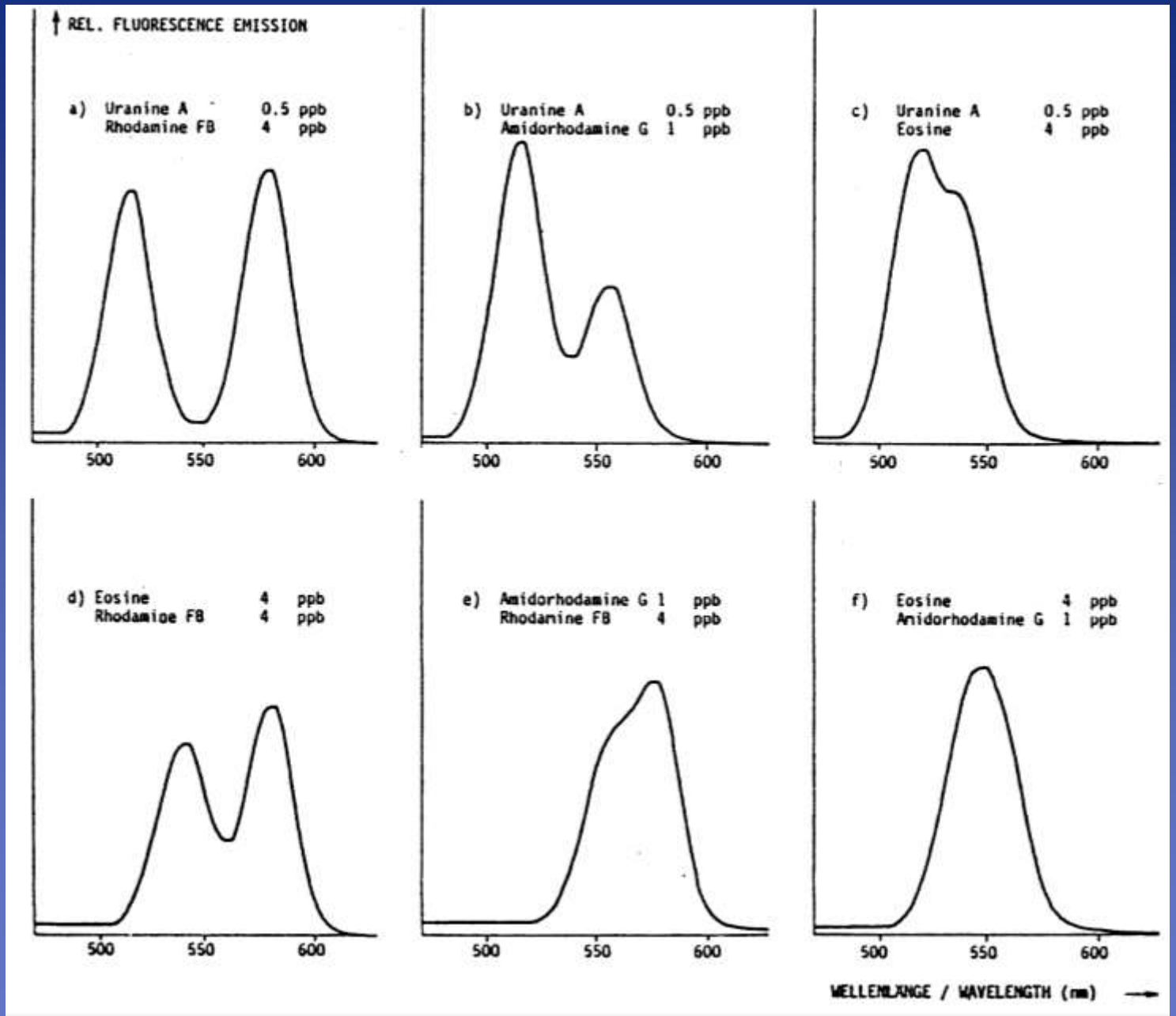
	Exitation/ Emission [nm]	Relative Fluorescene eff. [Uranin =100%]	Detection limit [mg/m³]
Uranin	491/516	100	**0,002**
Eosin	515/538	11,4	**0,02**
Amidorhodamin G extra	530/555	30	**0,005**
Rhodamin B extra	553/578	12,8	**0,01**
Rhodamin B	553/578	6,3	**0,02**
Tinopal	345/430	4	**0,4**
Pyranin	460/512	18	**0,02**
Naphtionat	325/420	18	**0,3-0,5**

Detection limit Uranin	0,001 mg/m³	
1mg	1000 m³	
1g	1 Mio m³	
1kg	1 Mrd m³	
10kg	10 Mrd m³	
100 kg	100 Mrd m³	
Aralsee	970 Mrd m³	970 kg
Bodensee	50 Mrd m³	50 kg
Grande Dixence	400 Mio m³	400 g

Detection limit Uranin	0,01 mg/m³	
1mg	100 m³	
1g	100000 m³	
1kg	100 Mio m³	
10kg	1 Mrd m³	
100 kg	10 Mrd m³	
Aralsee	970 Mrd m³	9700 kg
Bodensee	50 Mrd m³	500 kg
Grande Dixence	400 Mio m³	4 kg

Tracer mix: spectral separation by Synchronscan

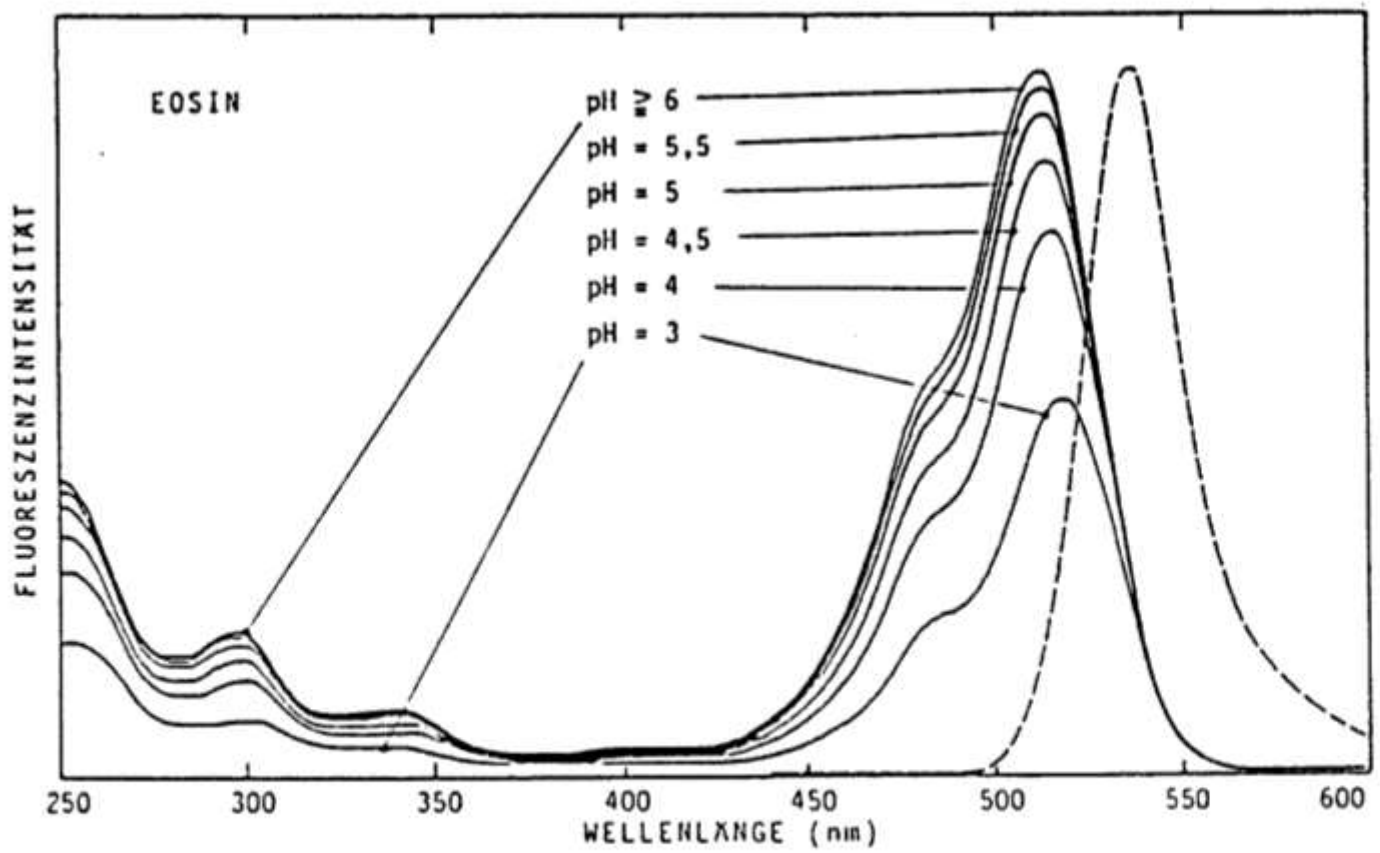



REL. FLUORESCENCE EMISSION
a) Uranine A 0.5 ppb
Rhodamine FB 4 ppb
b) Uranine A 0.5 ppb
Amidorhodamine G 1 ppb
c) Uranine A 0.5 ppb
Eosine 4 ppb
d) Eosine 4 ppb
Rhodamine FB 4 ppb
e) Amidorhodamine G 1 ppb
Rhodamine FB 4 ppb
f) Eosine 4 ppb
Amidorhodamine G 1 ppb
500
550
600
WELLENLÄNGE / WAVELENGTH (nm)

Solubility of fluorescent dyes

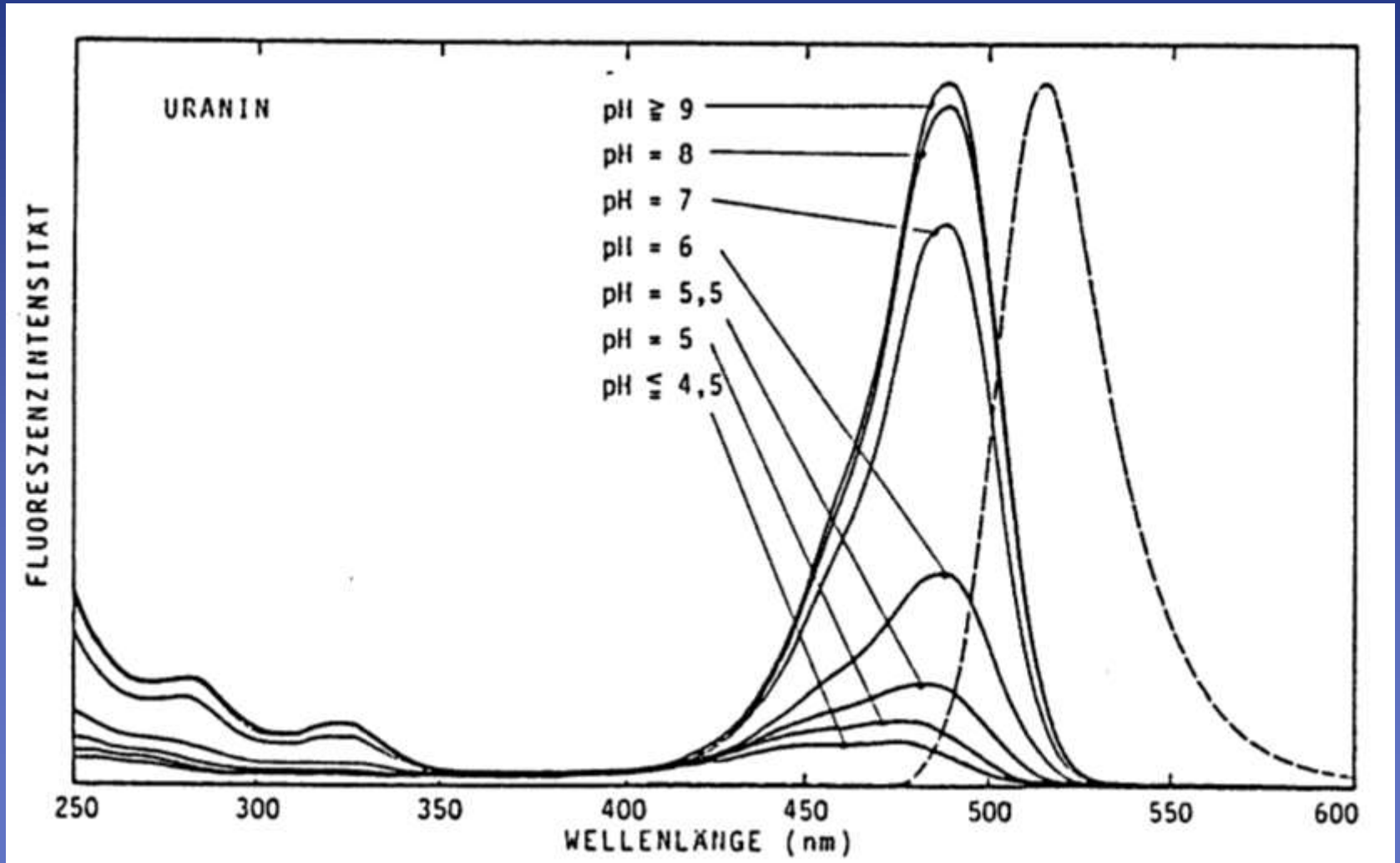
Naphthionat:	in Water (21°): 240 g/l
Tinopal:	difficult to dissolve only suspension
	Tinopal ABP als conz. Solution with 27%
Pyranin:	very good: up to 450 g/l
Uranin:	very high, up to 30%-solution (300g/l)
	at low temperature, cristalline
Eosin:	very high like Uranin (ca 300g/l)
Sulphorh. G extra:	at 20°C: 3 g/l, enhanced by Aethylenglycol
Rhodamin B:	at 20°C: 20 g/l, in weak acids 400 g/l
	enhanced by: Aethylenglycol
Sulphorhodamin B:	10 g/l suspension
Rhodamin WT:	200 g/l, only 20% solution

pH-dependency of eosine

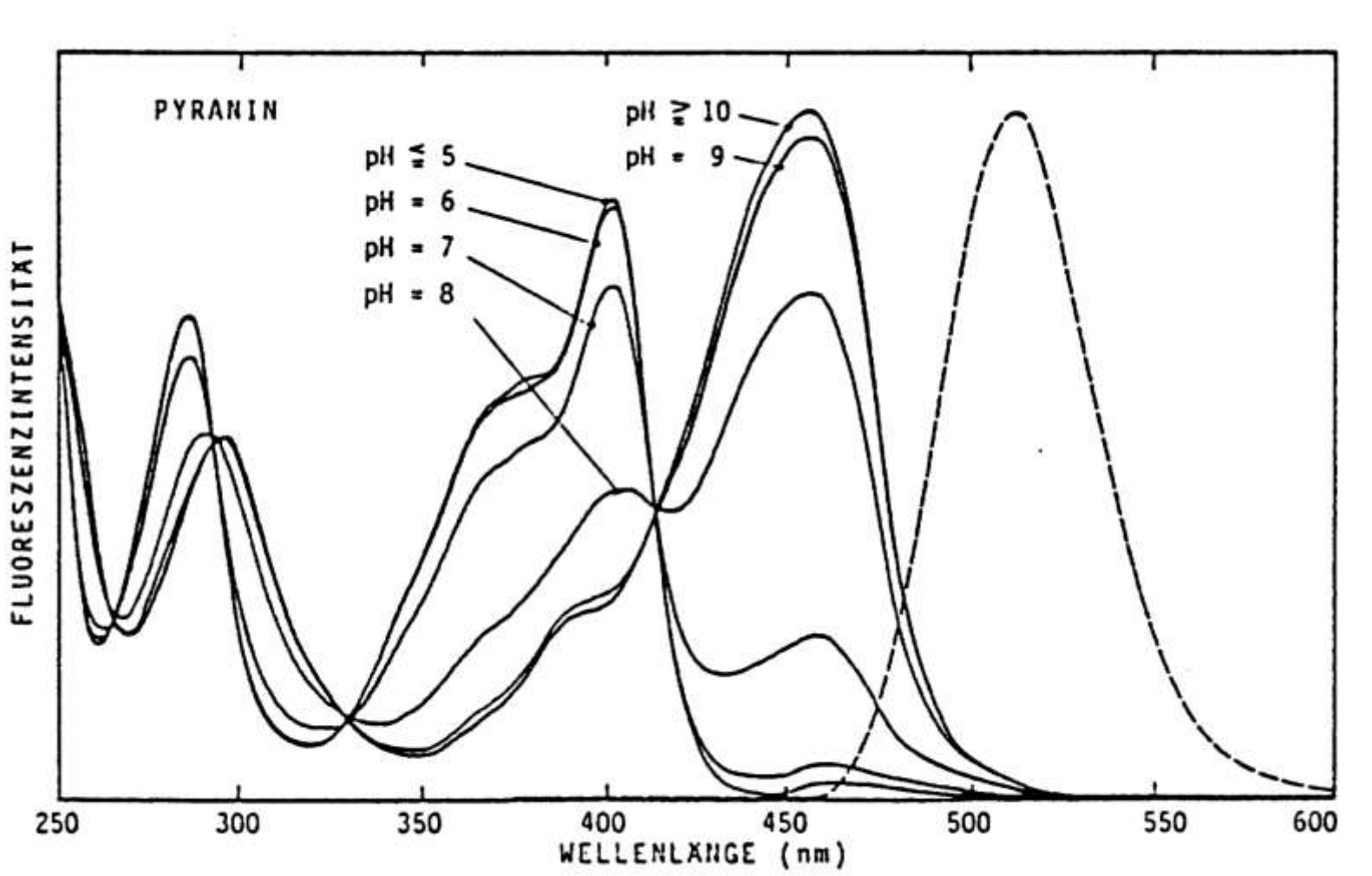


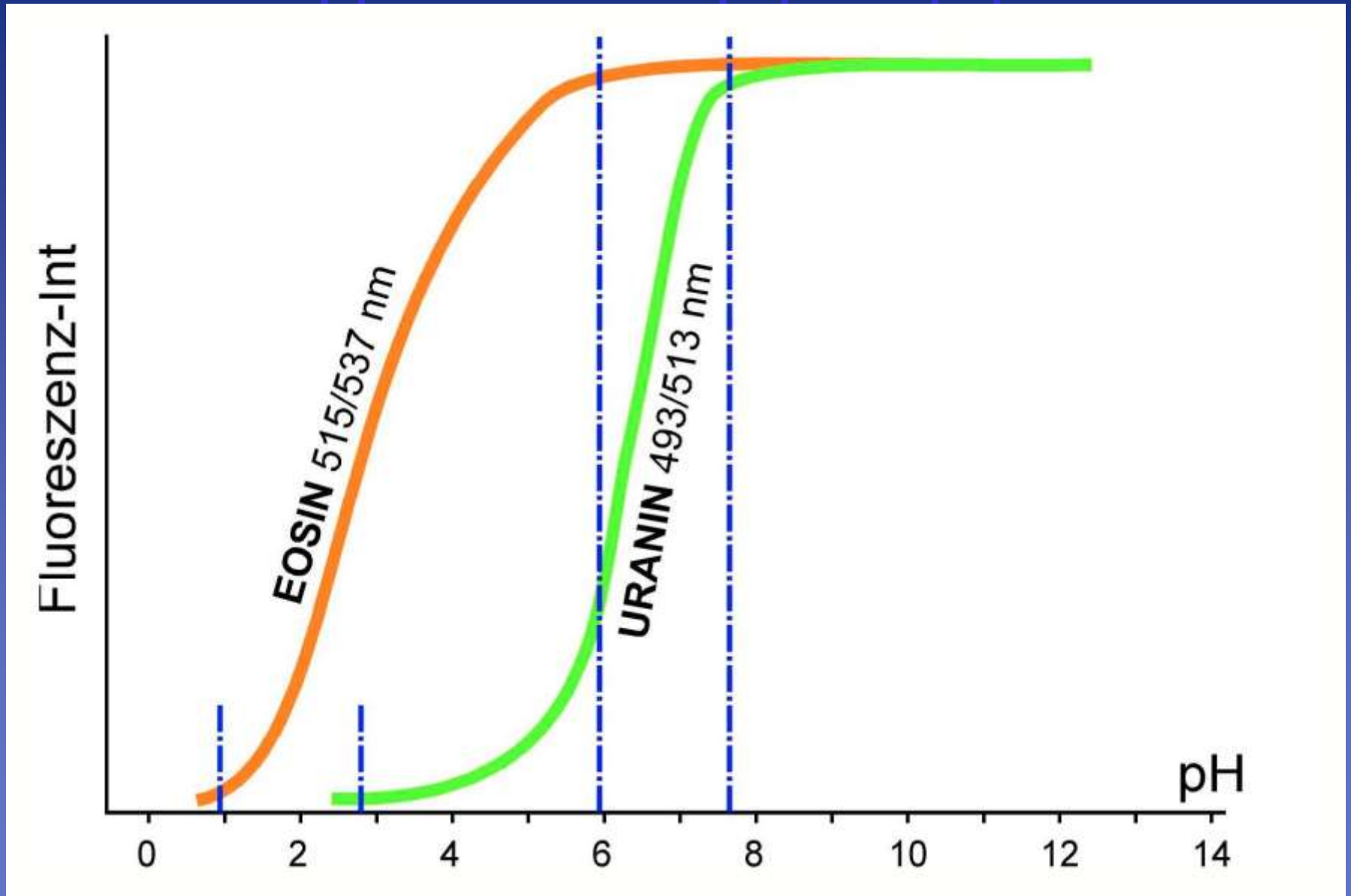
Abhängigkeit der Fluoreszenzanregung (-) von Uranin, Pyranin und Eosin vom pH-Wert.
(---): Emissionsspektren

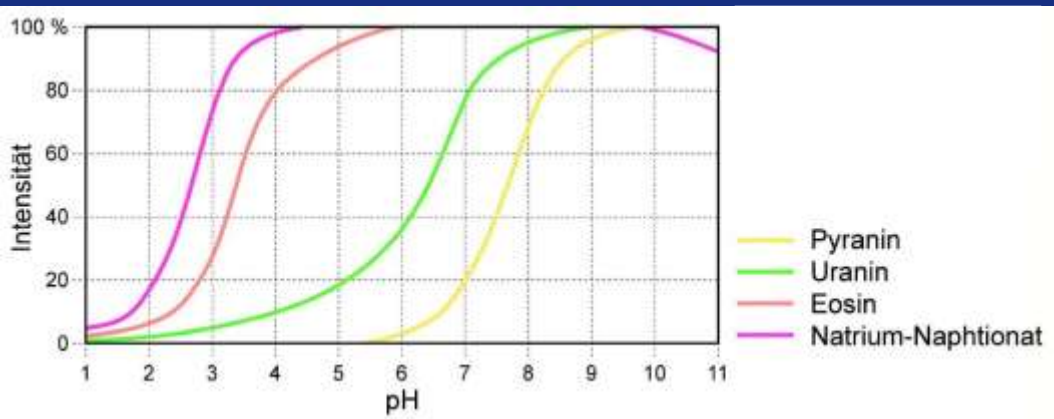
pH-dependency of fluoresceine

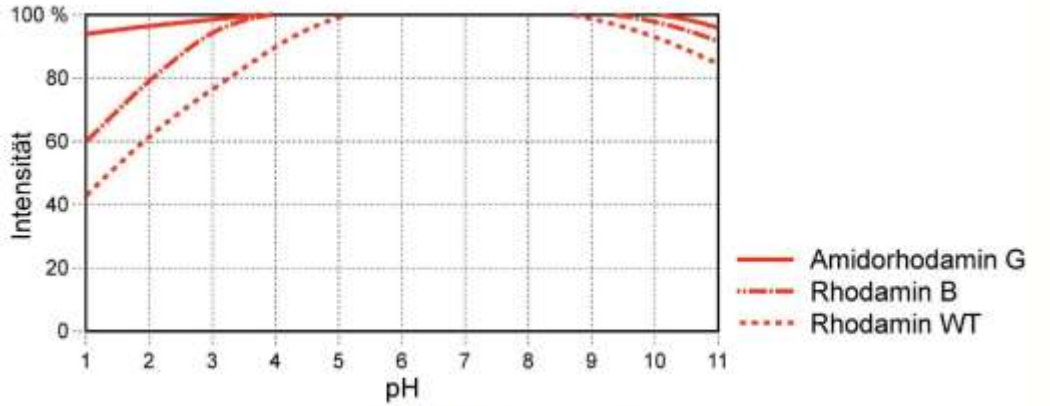


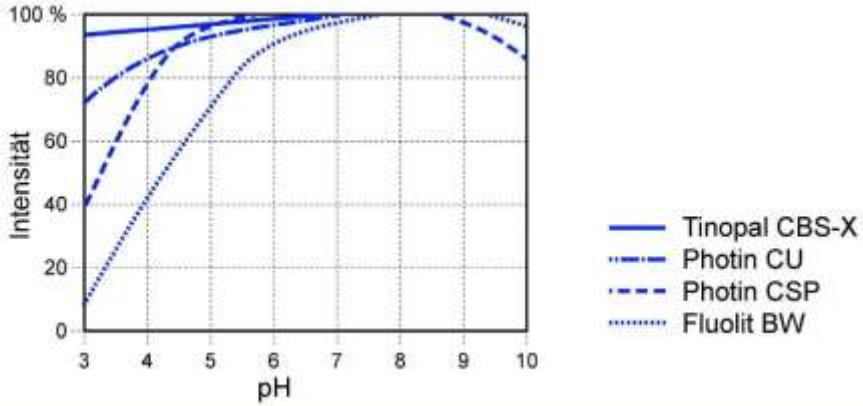
pH-dependency of pyranine



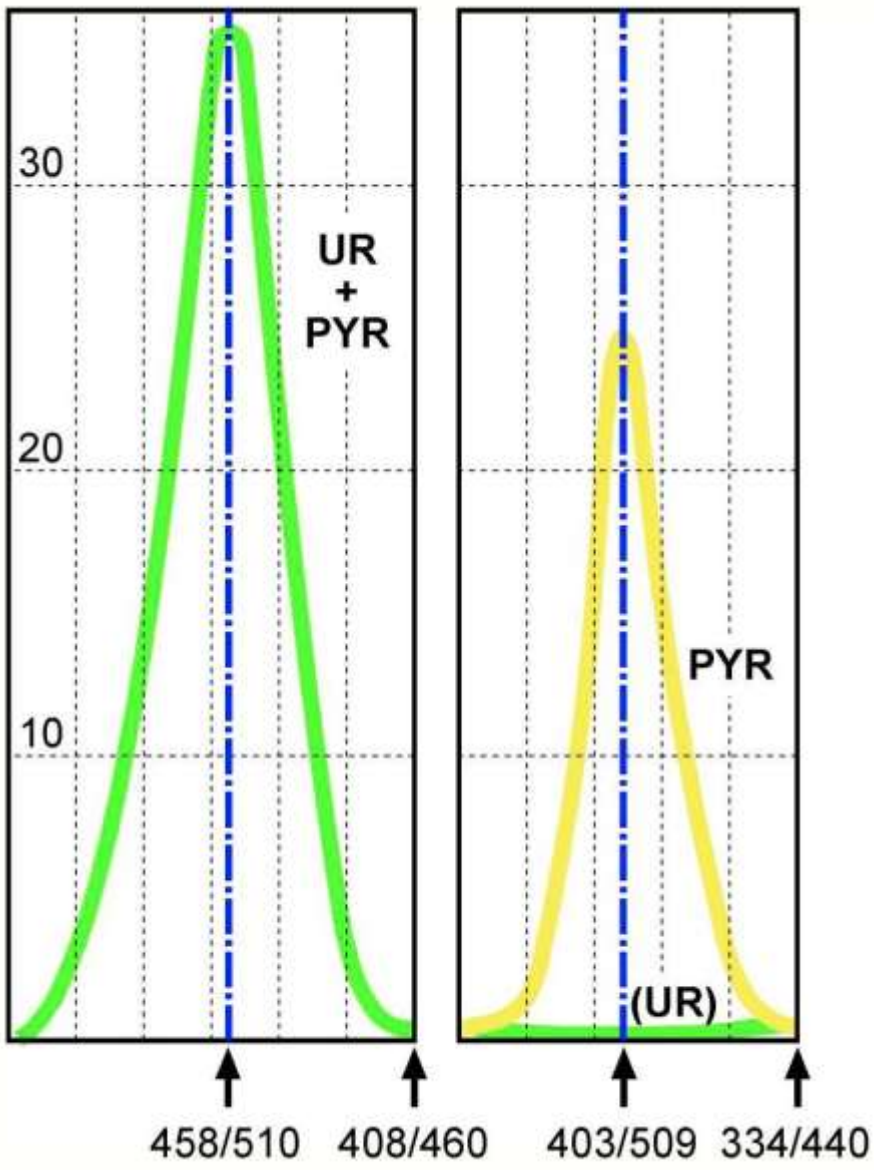

Fluoreszenz-Int
EOSIN 515/537 nm
URANIN 493/513 nm
pH
0
2
4
6
8
10
12
14







- Low pH affects:
  Uranin, Pyranin
- Strong dependency at pH < 5,5:
  Eosine and Naphtionate
- Low dependendy:
  Rhodamine and optical brightener

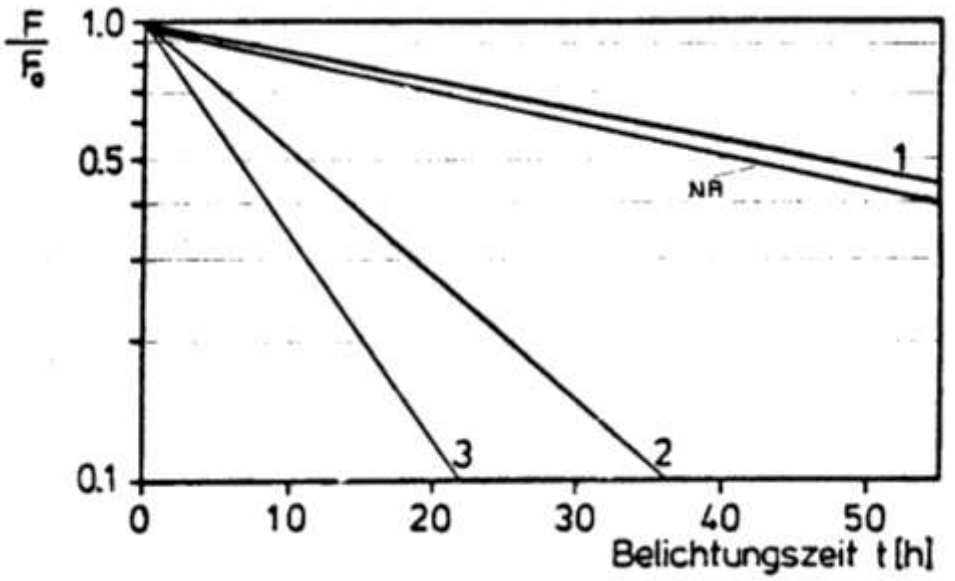


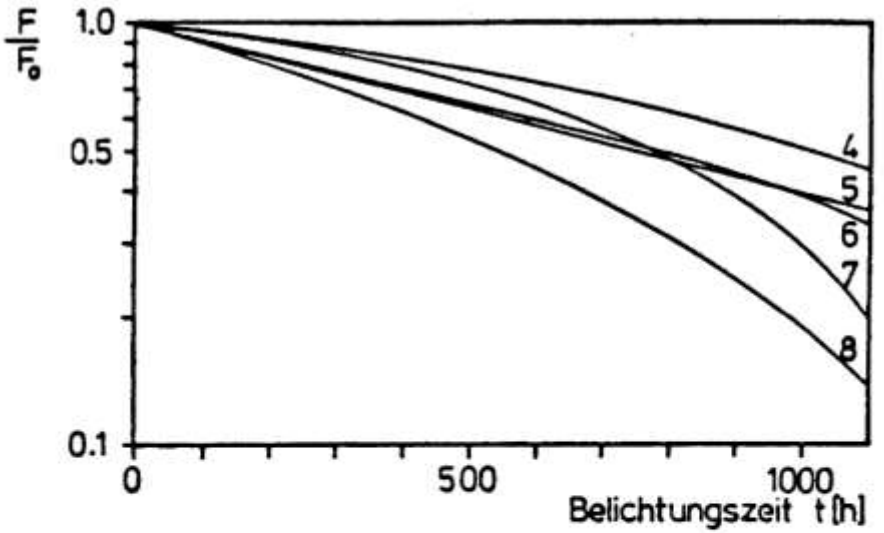
Pyranine measurement in the presence of Uranine

- Lower pH to 4.5
- Measure with 403/509 nm

Sensitivity to light

Photochemical effect





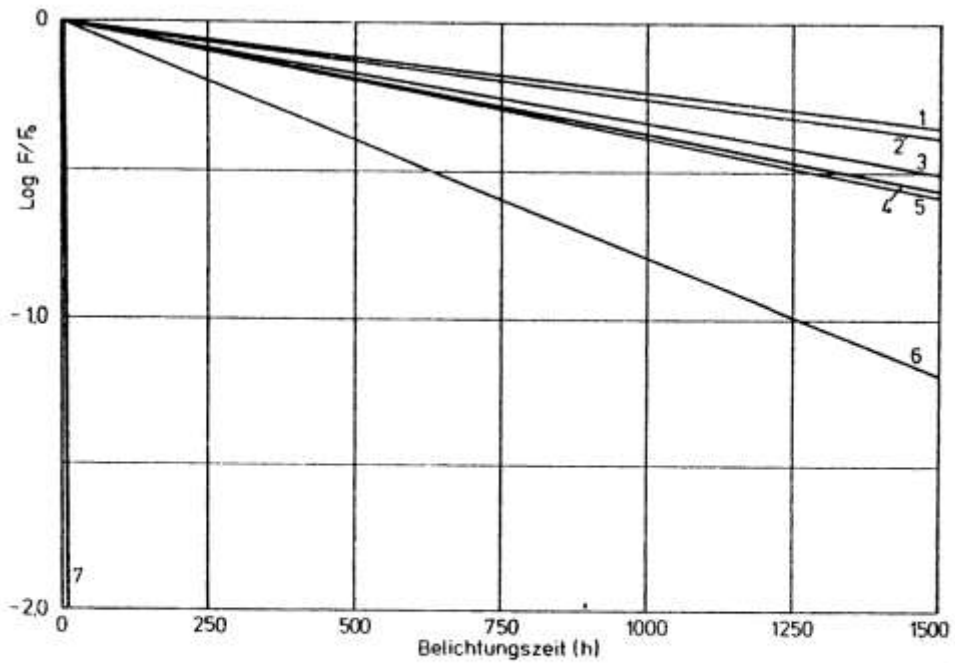
Darstellung der Abnahme der Fluoreszenzintensität von Fluoreszenzfarbstofflösungen (handelsübliche Qualität der Farbstoffe, bezogen von Fa. Brauns-Heitmann, Bad Aibling) in Abhängigkeit von der Belichtungszeit. Die Startkonzentrationen betrugen 10^{-7} bis 10^{-9} g/ml.

1 Pyranin, 2 Uranin, 3 Eosin, 4 Rhodamin WT, 5 Rhodamin FB, 6 Sulforhodamin B extra, 7 Brillantsulfoflavin FF, 8 Amidorhodamin G extra.

Photolysis = Reaction of 1. order

$$I = I_0 \bullet e^{-kt}$$

I = Fluorescence after time t
I_0 = Fluorescence at time t=0
k = Degradation coefficient
t = exposition time



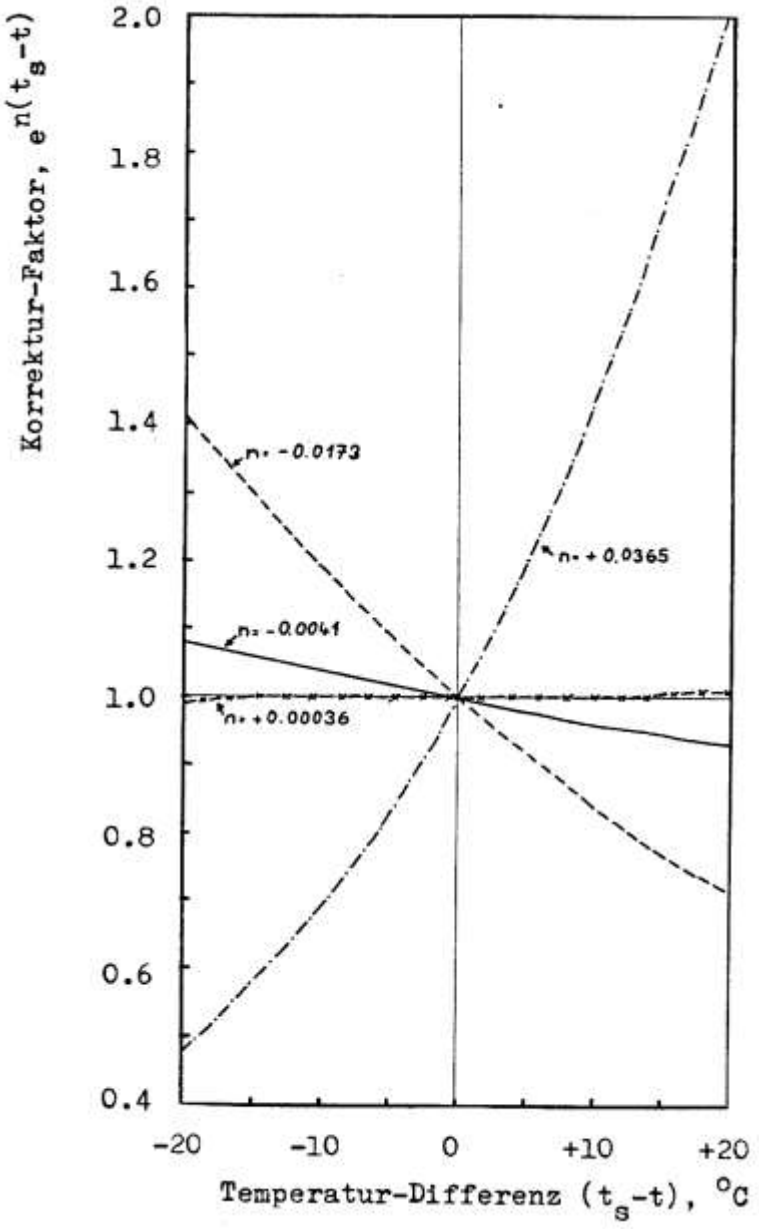
Abnahme des Fluoreszenzvermögens von wässrigen Lösungen ausgewählter Farbstoffe bei Belichtung mit künstlichem Tageslicht (Lösungsmittel: destilliertes Wasser).
Lösungen: 1 Rhodamin WT
2 Brillantsulfoflavin FF
3 Sulforhodamin B
4 Rhodamin B
5 Amidorhodamin G
6 Rhodamin 6G
7 Eosin

Fluorescence tracer	**Half life (h)***	**$\frac{T_{1/2}\ \text{tracer}}{T_{1/2}\ \text{Uranin}}$**
Eosin	6	0,5
Uranin	11	1
Tinopal CBS-X	17	1,5
Na-Naphthionate (NA)	-	3,7
Pyranin	47	4,3
Rhodamin 6G	375	34
Amidorhodamin G extra	-	~50
Amidorhodamin G	770	70
Rhodamin B	780	71
Rhodamin FB	-	~69
Sulforhodamin B extra	-	~71
Sulforhodamin B	820	75
Brillantsulfoflavin FF	1200	109
Rhodamin WT	1300	118

* Beleuchtungsstärke 2000±100 lx (Tageslichtspektum)
nach: gsf-Jahresbericht (1978/1979) und WERNLI (1985)

Temperature dependency of Fluorescence

- no problem in Laboratory
- ev. probleme in the field



Temperatur-Korrektionskurve für

——— Uranin (= SRG)

-·-·-· Tinopal

---- Rhodamin

**** Eosin

$F_s = F\ e^{n(t_s-t)}$

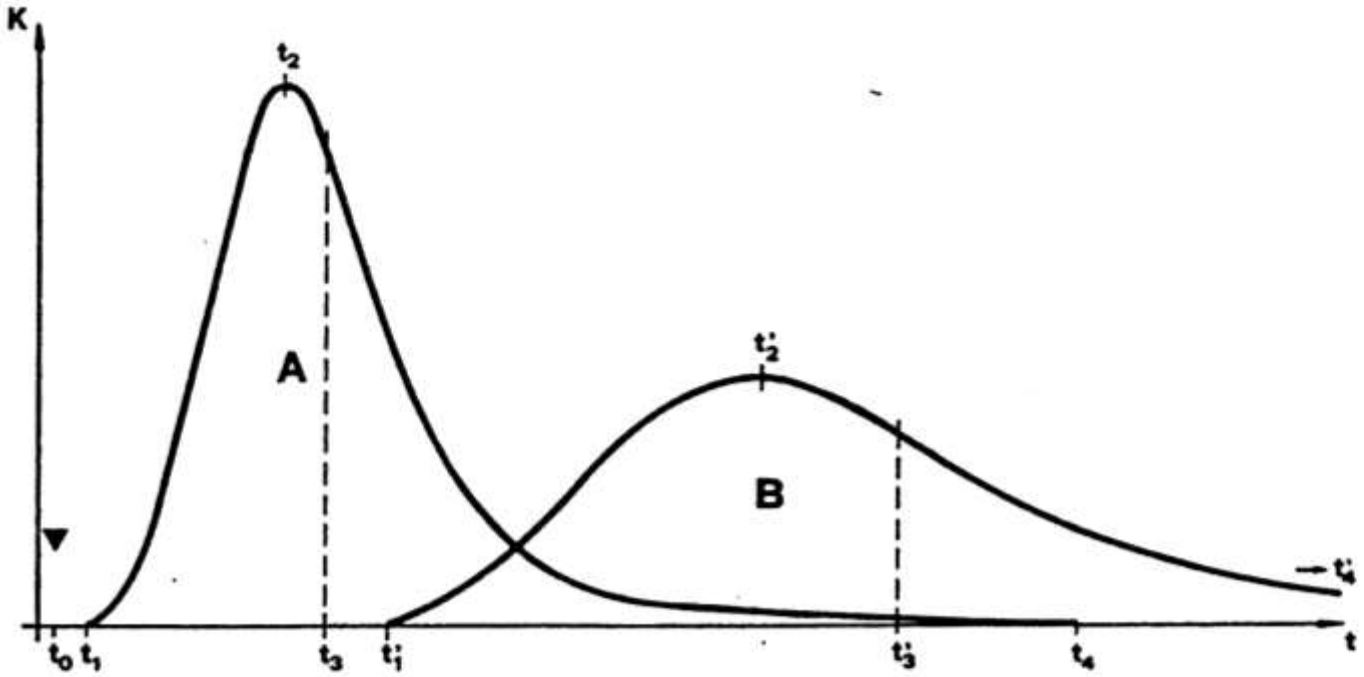
F_s = Fluoreszenz bei Standard-Temp. t_s

F = Fluoreszenz bei Proben-Temp. t

$t_s = 20^{\circ}$ C

(nach STAMPFLI aus LEIBUNDGUT 1978)

Adsorption



Aus: LEIBUNDGUT 1981

Schematisch dargestellt:

A: Durchgangskurve bei fehlender Adsorption, nur Dispersion wirksam.

B: Deformierte Durchgangskurve bei zeitlich beschränkter Adsorption des Tracers im Substrat (reversible Sorption) mit später vollständiger Ausbringung, Fläche B = A. Bei irreversibler Adsorption wird B < A.

Distribution coefficient (K_d) from batch-experiments

$$K_d = \frac{V \cdot C_0}{m \cdot C_1} [cm^3 / g]$$

with:
C_0: initial concentration (g/cm^3)
C_1: remnant concentration of substance in solution [g/cm^3)
V: substrate volume
m: dry weight

Execution:

100g Substrate + 250ml tracer solution in 1l glas bottle
Shake it for 24h (140 lifts/min)

Batch experiments in sand

	Initial concentration [mg/m³]	Adsorption [%]	coefficient K_d [cm³/g]
Naphthionat	10 100	0 0	0 0
Pyranin	10 100	0,5 9	0,025 0,25
Uranin	10 100	0 0	0 0
Eosin	10 100	0 1	0 0,025
Sulphorhodamin G extra	10 100	33 27	1,23 0,92
Rhodamin B extra	10 100	69 65,5	5,56 4,75

Fehler ca. ± 5 %

aus: LEIBUNDGUT und WERNLI, 1987

Retardation factor R_D from batches

$$R_D = \frac{v_a}{v_t}$$

R_D = Retardation factor
v_a = average velocity of ideal tracer
v_t = average velocity of tracer

$$R_D = 1 + \frac{\tau}{n} \cdot K_d$$

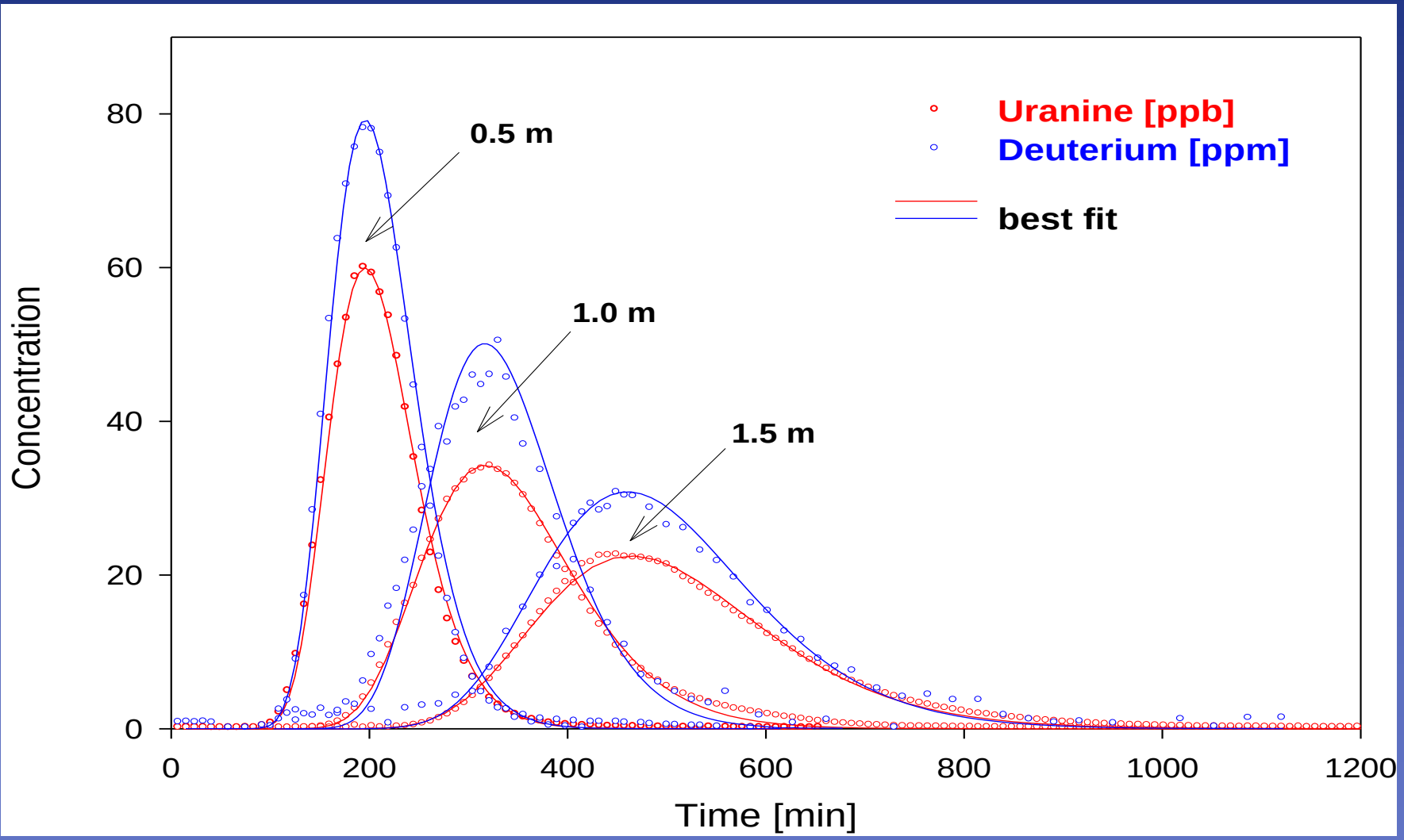
R_D = Retardation factor
K_d = coeffizient
τ = dry density of sediment
n = total Porosity of sediment

Deuterium and Uranin batch

preparation

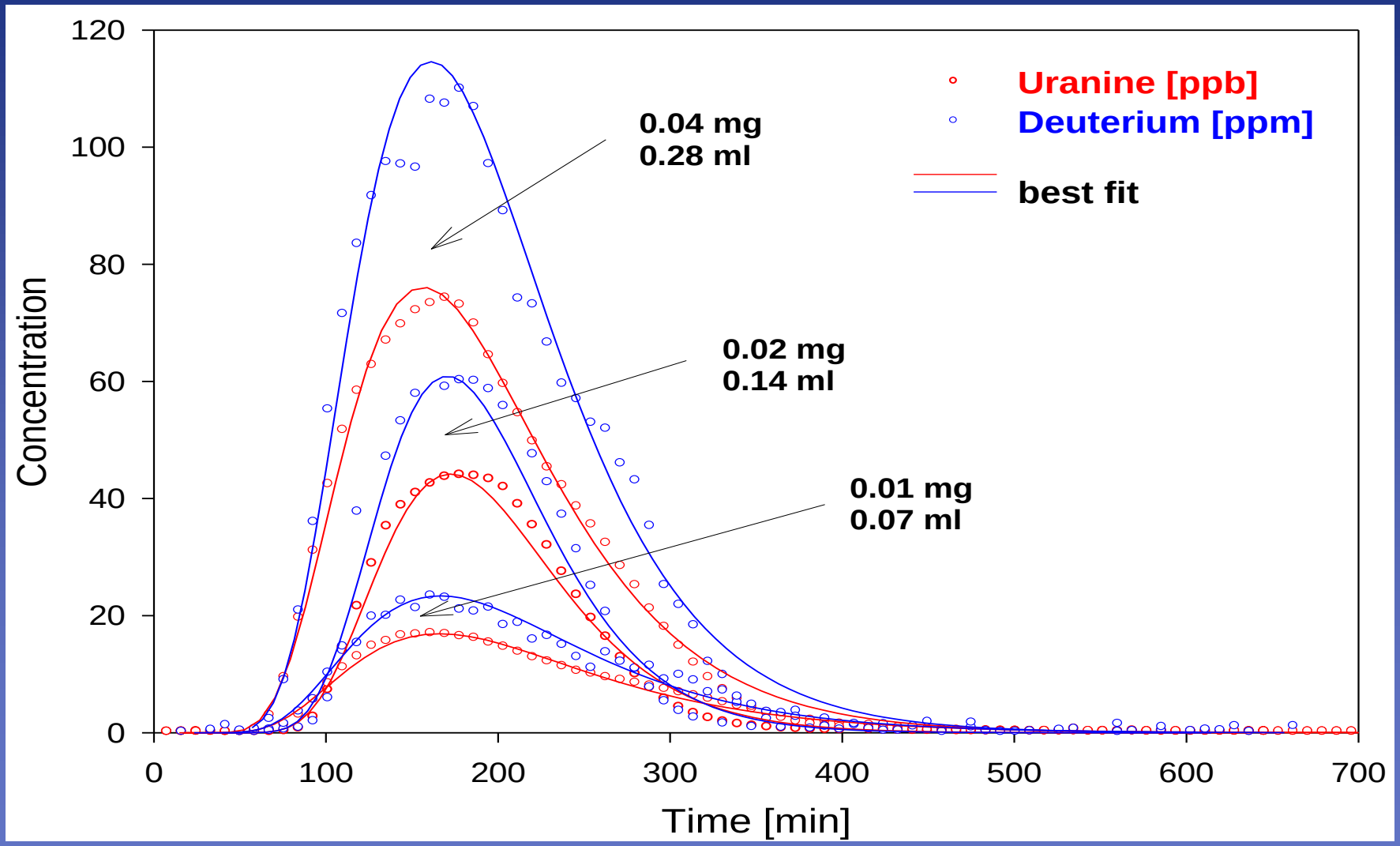

leere
PE-Flaschen

results



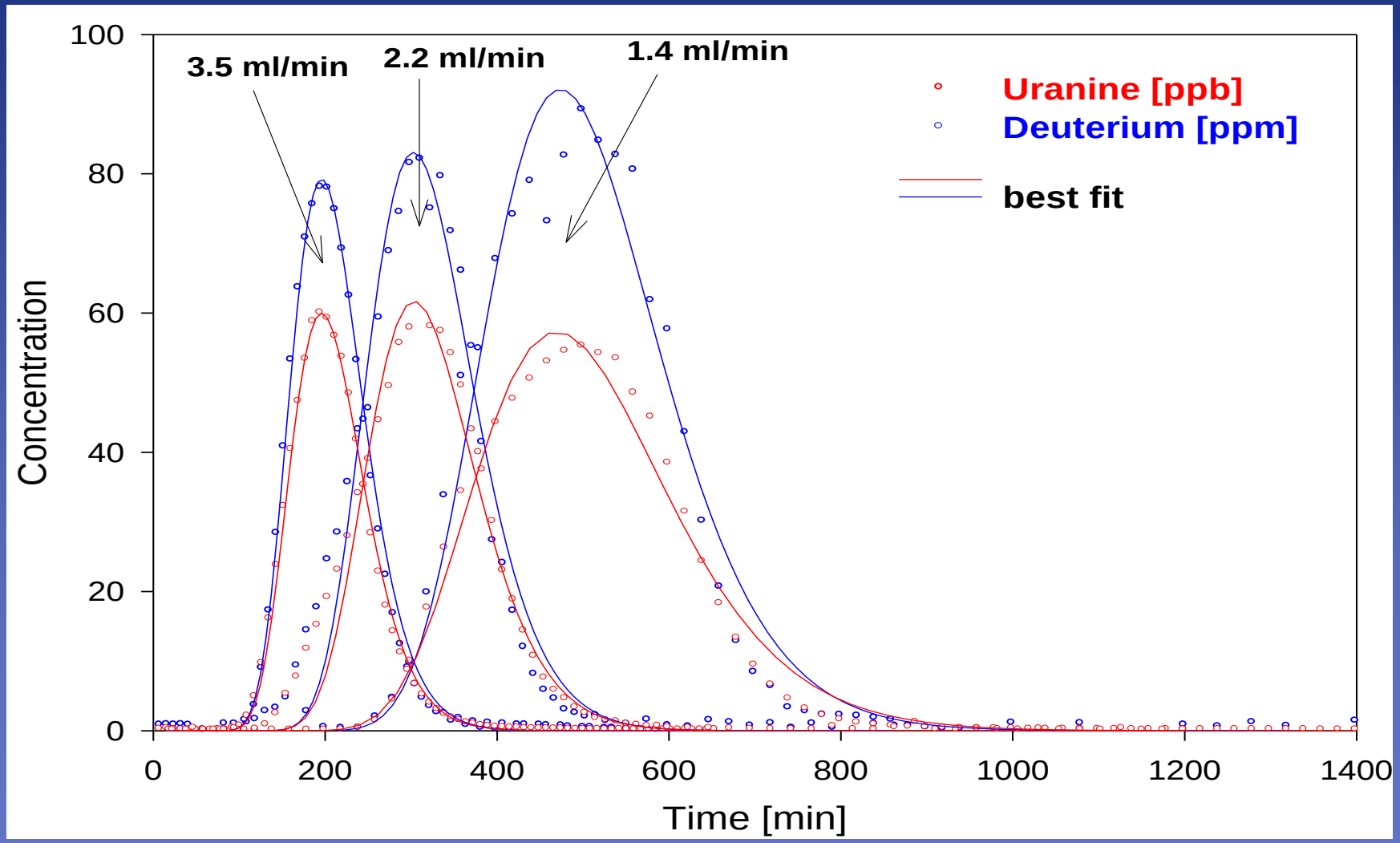
Königer et al, 2003

different tracer concentrations



Königer et al, 2003

Different flow rates



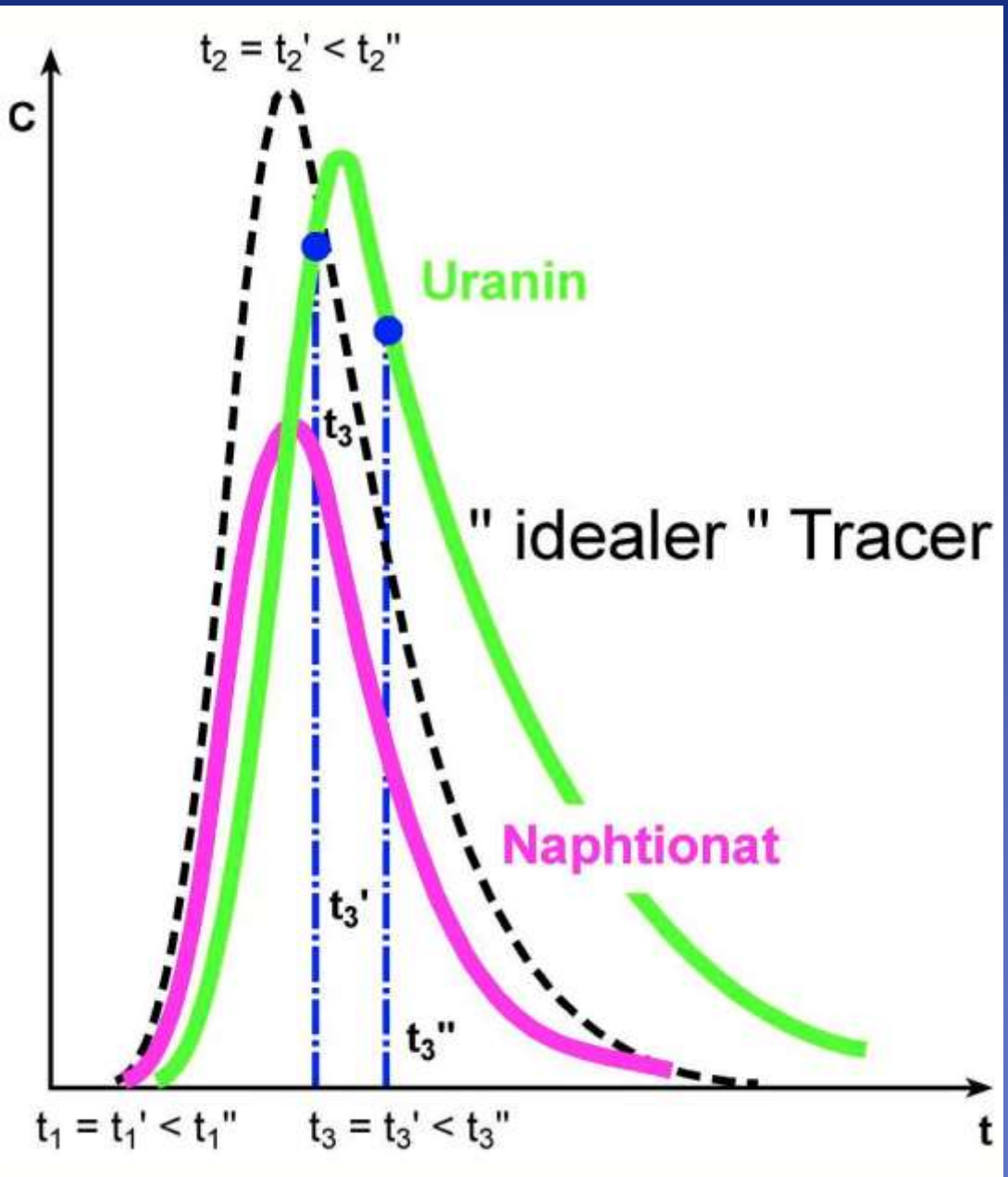
Königer et al, 2003

Distribution coefficients and retardation factors for different tracers

Tracer	Konz. Ausgangslösung (mg/m^3) c_0	c_0^*	Konz. Gleichgewichtslösung c_1	Substrat	K_d (cm^3/g)	R_D
RBE	1	0,68	0,17	Zweiglimmergneis Trockengewicht m = 16131 g	2,69	10,1
	10	6,81	1,92		2,31	8,8
	80	54,52	16,98		2,01	7,8
	100	68,10	26,23		1,45	5,9
	1000	681,00	462,90		0,43	2,5
ARG	30	20,44	13,17		0,50	2,7
	80	54,51	38,30		0,38	2,3
E	90	61,30	62,70		0	1,0
UR	10	6,81	7,08		0	1,0
RBE	80000	56700,00	4420,00	Molassesandstein m = 17160 g	9,71	40,3
ARG	30000	21300,00	2900,00		5,21	22,1
	80000	56700,00	15400,00		2,20	9,9
E	90	63,8	49,4		0,24	1,9
UR	10	7,09	7,20		0	1,0
RBE	80	75,50	50,10	Kalkstein m = 19437 g	0,28	2,4
ARG	30	28,30	25,40		0,06	1,3
E	90	84,90	82,20		0,02	1,1
UR	10	9,44	9,40		0	1,0

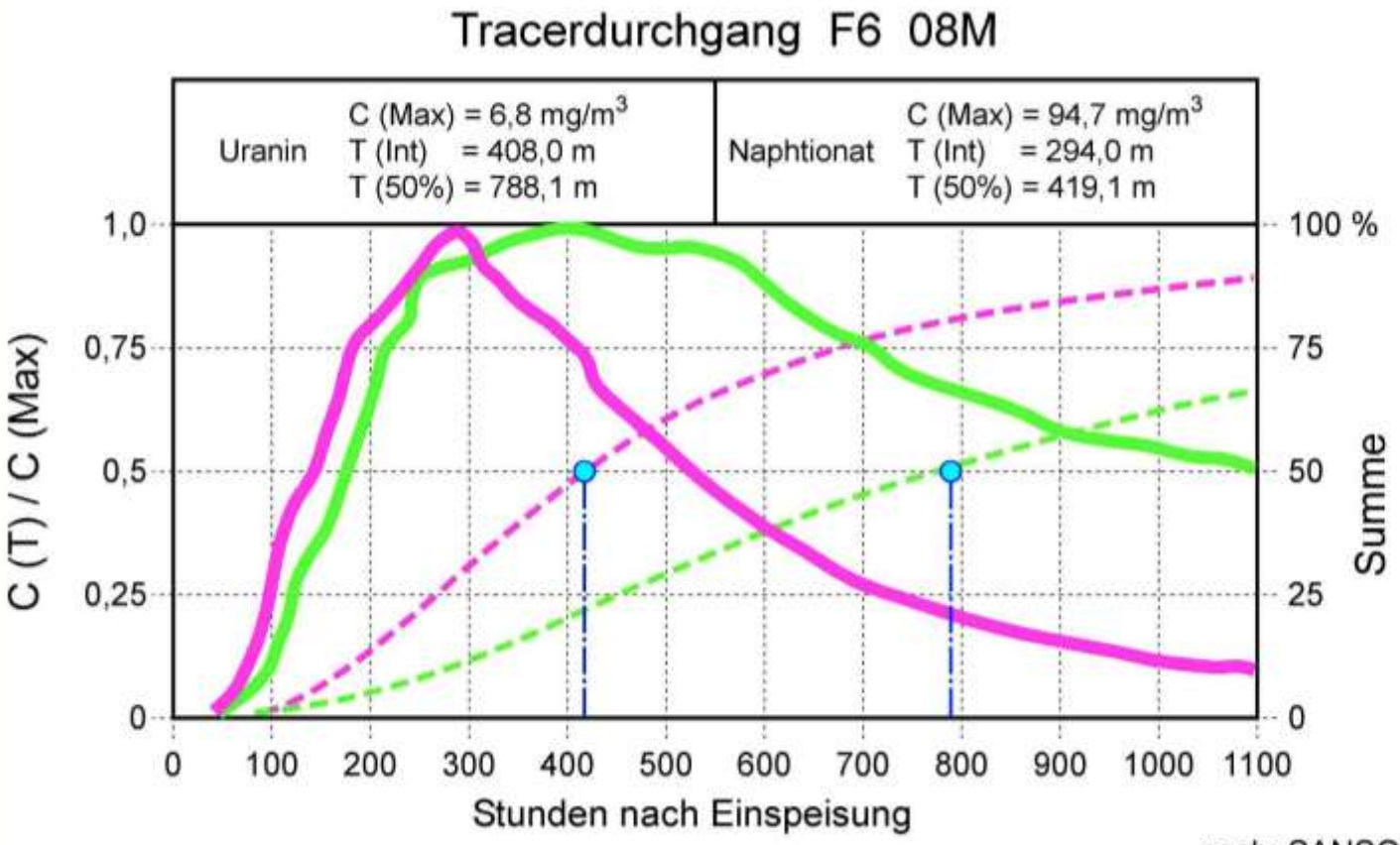
$$K_d = \frac{V \cdot C_0}{m \cdot C_1} [cm^3 / g]$$

$$R_D = \frac{v_a}{v_t}$$



nach Leibundgut & Wernli, 1986

Field tests in Wilerwald



Uranin:
Reversible
Adsorption

Naphtionat:
Irreversible
Adsorption

Uranin

Naphtionat

nach: SANSONI, SCHUDEL, WAGNER 1987

Final evaluation of fluorescent tracers by working group UBA

Tracer	Toxicological evaluation	Criteria
Uranine	harmless	T, L
Eosine	harmless	L, EK
Sulfo-rhodam.B	ecotoxicol. questionable	T
Amido-rhodam.G	harmless	T
Rhod.WT	not recommended	T
Rhod.B	not recommended	T, L
Rhod. 6G	not recommended	T, L
Nat.napht.	harmless	T
Pyranine	harmless	T
Tin.CBS-X	harmless	T
Tin.ABP liq.	harmless	T

T = Tox.Test **L** = Literature **EK** = Expert Knowledge

Conclusions - Recommendations

1. Hydrologists minimize the mass of tracers !

   - Calculation by known equation by modelling

2. Analysis only with high sensitive fluorometers

   - Use the low detection limit !

3. Restrict the use of Rhodamines as far as possible !

4. The right proportion:

   Potential danger by tracer : Gain of hydrological knowledge

Summary

Tracer	Ex/Em [nm]	rel. Fluor. efficiency	Detection limit [mg/m³]	Toxi-city	solubility [g/l]	Light sensi-tivity	Adsorption
Naphthionat	325/420	18	0,3-0,5	Low	240	high	Low
Pyranin	458/510	18	0,02	Low	350	High	Quite low
Uranin	491/516	100	0,002	Low	300	High	Low
Eosin	515/540	15	0,005	Low	300	Very high	Quite low
Sulpho-rhodamin G	530/555	32	0,003	Suffi-cient	3	Low	High
Rhodamin B extra	553/578	15	0,005	Suffici-ent	20	Low	Very high

pH-Optimum: Uranin 7 Pyranin 8 (alkalisieren) Naphthionat 6 – 8,5

costs

	Richtpreis (1988) SFR/kg	Kostenfaktor UR = 1	**Faktor der Fluoreszenz -ausbeute**	**Äquival. Einseise-menge zu 1 kg UR**	**Kostenfaktor unter Berücksichtigung der Fluoreszenzausbeute**
Naphthionat	35	0,4	18	**5,6**	2
Tinopale	25	0,3	4	**25**	6
Pyranin	110	1,2	18	**5,6**	7
Uranin	90	1	100	**1**	1
Eosin	140	1,6	15	**6,7**	10
Sulphorhodamin G extra	160	1,8	32	**3,1**	6
Rhodamin B	120	1,3	15	**6,7**	9