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Measurement of liquid flow in open channels — Tracer dilution methods for the measurement of steady flow —

Part 4: Fluorescent tracers

[ISO 9555-4:1992](https://standards.iso.org/iso/9555-4:1992)

<https://standards.iso.org/iso/9555-4:1992> *Mesure de débit des liquides dans les canaux découverts — Méthodes de dilution en régime permanent utilisant des traceurs --*

Partie 4: Traceurs fluorescents



Reference number
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Annex

A General characteristics of commonly used fluorescent tracers 9

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 9555-4 was prepared by Technical Committee ISO/TC 113, *Measurement of liquid flow in open channels*, Subcommittee SC 4, *Dilution methods*.

[ISO 9555-4:1992](#)

ISO 9555 consists of the following parts, under the general title *Measurement of liquid flow in open channels*:
Tracer dilution methods for the measurement of steady flow:

- Part 1: *General*
- Part 2: *Radioactive tracers*
- Part 3: *Chemical tracers*
- Part 4: *Fluorescent tracers*

Annex A forms an integral part of this part of ISO 9555.

Introduction

The former standard series ISO 555 was subdivided into parts on the basis of the method of field measurement, i.e. constant-rate injection method and integration (sudden injection) method. Since the choice of the type of tracer to be used in a field measurement will often depend on the expertise and the laboratory facilities available, this new series of standards ISO 9555 is divided into parts based on the type of tracer used. This revision has enabled the unnecessary repetition of text of the various parts to be avoided and will, it is hoped, prove to be a more convenient form of presentation for the user.

ISO 9555 deals with the measurement of steady flow in open channels by dilution methods using tracers. The methods described may also be applied to the measurement of slowly varying flow, but they may only be used when flow conditions ensure adequate mixing of the injected solution throughout the flow.

For the measurement of very large flows, tracer methods can be onerous in terms of tracer costs and measurement times. However, the use of tracers often reduces danger to personnel during flood periods.

ISO 9555-1 presents the general principles of the methods of constant-rate injection and integration (sudden injection). ISO 9555-2, ISO 9555-3 and ISO 9555-4 deal with the specific aspects of the use of radioactive, chemical and fluorescent tracers, respectively, as well as specific analytical procedures.

This approach has been adopted for the following reasons:

- to facilitate subsequent updating, additions or revisions which concern only ISO 9555-2, ISO 9555-3 or ISO 9555-4;
- to provide a more practical document for the user, who is often obliged to choose the tracer best suited to the available analytical equipment.

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Measurement of liquid flow in open channels — Tracer dilution methods for the measurement of steady flow —

Part 4: Fluorescent tracers

1 Scope

This part of ISO 9555 deals with the use of fluorescent tracers in discharge measurements by the dilution method. Apparatus and methods of general application are set out in ISO 9555-1 and are not repeated here, with the exception of those relating specifically to fluorescent tracers.

The use of fluorescent tracers is attractive because of the small amounts of tracer needed to make a discharge measurement. Certain fluorescent tracers can be measured at concentrations of less than 1 µg/l. Fluorescent tracers are easy to handle and their concentrations can be readily determined.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 9555. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this part of ISO 9555 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 772:1988, *Liquid flow measurement in open channels — Vocabulary and symbols*.

3 Definitions

For the purposes of this part of ISO 9555, the definitions given in ISO 772:1988 and the following definitions apply.

3.1 fluorescence: The emission of electromagnetic waves of characteristic energy when atoms or molecules decay from an excited state to a lower energy state. The excitation may be induced by subjecting the substance to radiation of slightly higher energy (shorter wavelength) than that of the characteristic emission, and it ceases as soon as the external source is removed.

3.2 fluorimeter; filter fluorimeter: Instrument equipped with a lamp or other means of exciting fluorescent radiation in a sample, with filters and with a detector to measure relative fluorescent intensities caused by variations in concentration of the substance under examination. If the wavelengths are determined using a monochromator, the instrument is called a spectrofluorimeter.

3.3 fluorescence quenching: The reduction of fluorescence intensity, due to an interaction of the dye molecules with other chemicals present in the water. Concentration quenching is a phenomenon which appears to be similar to fluorescence quenching but is not a true quenching. Instead, it is a reduction in the rate of increase of the flowmeter readout with increasing dye concentration due to the increasing optical density of the dye itself. Concentration quenching occurs only at very high concentrations.

4 Tracers used

4.1 General

A number of fluorescent dyes have been used as tracers for measuring open-channel flow. Stream waters generally have lower background fluorescence in the orange than in the green and blue wavelengths. Consequently, the orange wavebands

allow for greater sensitivities than do the green and blue wavebands.

Six fluorescent dyes are discussed for use in discharge measurements: fluorescein and rhodamine B (see 4.2 and 4.3) have been used extensively in the past but are no longer recommended for use in making discharge measurements. The other four tracers: acid yellow 7, sulfo rhodamine B, pyranine and rhodamine WT (see 4.4 to 4.7) have good properties for making discharge measurements and will be discussed further in this part of ISO 9555. Other tracers such as eosine (acid red 87) have not yet been widely studied but might be used in the future.

The general characteristics of commonly used fluorescent tracers are given in annex A.

4.2 Fluorescein

Fluorescein, also known as sodium fluorescein, was one of the earliest dyes used as a tracer in water. However, it is highly susceptible to photochemical decay and its fluorescence response is subject to changes at pH values less than 6.5. Also, many streams exhibit high background fluorescence similar to that of fluorescein. Therefore, this dye is not recommended for the measurement of flow in open channels.

4.3 Rhodamine B

Rhodamine B has been extensively used as a tracer in water. However, it is readily adsorbed onto sediments, sample bottles and test equipment. Also, it has been shown to be somewhat toxic to aquatic organisms under certain conditions. Generally, however, the high concentrations of dye required to produce toxicity problems exist only for insignificant periods of time at the point of dye injection in an open channel during the measurement of flow. Rhodamine B is not recommended for discharge measurement, primarily because of its high losses as a result of adsorption.

4.4 Acid yellow 7

Acid yellow 7 is also known as lissamine FF, lissamine yellow FP, brilliant sulfo flavine FF, and brilliant acid yellow 8G.

4.5 Sulfo rhodamine B

Sulfo rhodamine B is also known as pontacyl brilliant pink B, lissamine red 4B, kiton rhodamine B', acid rhodamine B'', kenacid-G, and aminorhodamine-g extra.

4.6 Pyranine

Pyranine is also known as solvent green 7.

4.7 Rhodamine WT

Rhodamine WT has been widely used as a water tracer while sulfo rhodamine B, acid yellow 7 and pyranine have less use as a tracer in water.

5 Tracer measurement

5.1 Principles

Fluorimetric analysis, or fluorimetry, is based on the physical phenomenon called fluorescence.

Fluorescence is a result of an almost instantaneous sequence of events as follows:

- a) absorption of energy from an outside source such as the sun or an ultraviolet lamp;
- b) excitation of some of the electrons of the fluorescent substance, resulting in enlarged electron orbits, called the "excited state";
- c) emission of energy in the form of photons (light), as the excited electrons return to their normal positions or the "ground state".

The emitted (fluoresced) energy nearly always has longer wavelengths and lower frequencies than the absorbed energy, because some energy is lost in the process (Stokes' law). It is this property of dual spectra, i.e. the different specific combination of excitation and emission spectra for each fluorescent substance, which is utilized in fluorimetry to make it an accurate and sensitive analytical tool.

Most substances are at least mildly fluorescent, and most fluorescence occurs in the 200 nm to 800 nm range of wavelengths, i.e. ultraviolet and visible light. Strongly fluorescent substances convert a high percentage of absorbed energy into emitted energy. Fortunately, most strongly fluorescent substances fluoresce in the ultraviolet-to-green part of the spectrum, while fewer substances, including the tracer dyes recommended for use in flow measurements, fluoresce in the yellow-orange band. These dyes are strongly fluorescent in dilute solutions. Fluorescent materials likely to be found in some streams include algae and other naturally occurring organics, certain minerals, and man-made pollutants such as paper and textile treated with optical cleaning agents, certain petroleum products and laundry detergent brighteners.

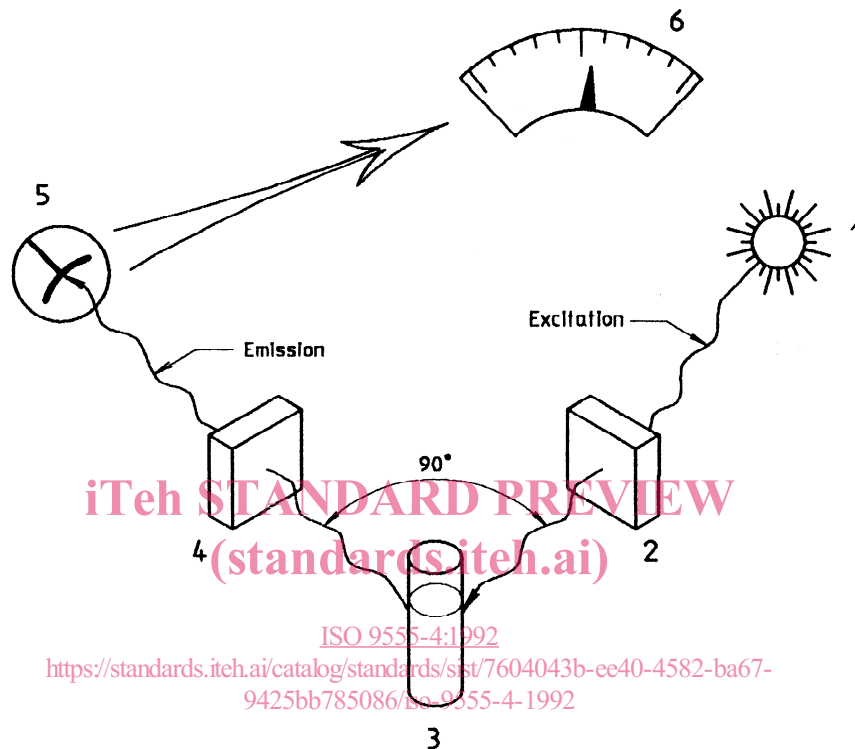
Fluorescence intensity is affected in varying degrees by certain physical and chemical factors, such as the type of solvent, the concentration, the tempera-

ture, the pH, photochemical decay and fluorescence quenching.

The fluorimeter, or filter fluorimeter, is the basic piece of equipment used to measure fluorescence. The fluorimeter is an instrument which gives a relative measure of intensity of light emitted by a sam-

ple containing a fluorescent substance; the intensity of fluorescent light is proportional to the amount of fluorescent substance present.

A fluorimeter consists of six basic components as shown in figure 1. All commercial fluorimeters have the same basic structure.



Key

- 1 Energy source
- 2 Primary filter
Passes only a selected band of the source's output spectrum matching a selected band of the dye's excitation spectrum.
- 3 Sample holder
Right angle to the light path minimizes the amount of scattered light reaching the sensing device.
- 4 Secondary filter
Passes only a selected band of the dye's emission spectrum and preferably none of the light passed by the primary filter.
- 5 Sensing device
Responds to the spectral band passed by the secondary filter.
- 6 Readout device
Gives values proportional to the light reaching the sensing device.

Figure 1 — Basic structure of most filter fluorimeters