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Original Article

Fluorescence Quenching of Organic Dyes by Metal Ions: Mechanisms, Stern–Volmer Analysis and Applications in Selective Metal-Ion Determination — A Critical Review

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ABSTRACT

A metal ion can be estimated by noting how strongly it reduces the glow of an organic dye. Many laboratories like this idea because it is inexpensive and fast. However, published reports use no common rules for naming the quenching mechanism or for presenting analytical results. This review first explains what happens to the excited dye at the molecular level: collisional and complex-forming quenching, electron and energy transfer, paramagnetic and heavy-atom effects, and apparent quenching caused by light absorption in the solution. Next we examine how reliable the Stern–Volmer equation is, what its straight and bent plots mean, and why measurements at one temperature cannot fix the mechanism. Flavins, xanthenes, coumarins and carbon-based emitters are compared as probes, and ions that quench strongly (Cu^{2+}, Co^{2+}, Fe^{3+}, Pb^{2+}, Hg^{2+}) are contrasted with ions that barely react. We also discuss pH, time, temperature and dye concentration, the way detection limits and selectivity are reported, and uses in water, food, drug and biological samples. Studies up to 2026 are included. In our opinion, quenching assays are quick and sensitive but are not selective on their own. Good studies should prove the mechanism, correct for light absorption, test mixtures of ions and report figures in a uniform way. Ratiometric, lifetime-based and array-based designs seem the best way forward.

Keywords: fluorescence quenching, Stern–Volmer analysis, organic dyes, metal ions, selectivity, metal-ion determination

INTRODUCTION

Metals can help or harm, depending on the amount. Copper and iron are needed by many enzymes, but an excess disturbs redox balance inside cells. Lead has no useful role in the body; it accumulates and mainly injures the kidneys and nerves. Because of this, health authorities fix maximum limits for these metals in drinking water [1]. Such limits are normally verified with atomic absorption, plasma techniques or voltammetry. These methods are accurate, but the instruments are expensive and need trained operators and a permanent laboratory, so they are not convenient for quick testing at the sampling site.

Fluorescence is an attractive alternative. It is sensitive, needs only a small volume and can be done with compact instruments. Quenching assays are the easiest form: a dye is mixed with the sample and the emission becomes weaker if the metal ion is present. A custom-made receptor is not always needed, and common commercial dyes frequently work [2,3]. For instance, a naphthalimide dye sold as an air-conditioner leak detector was quenched by Cu^{2+} and gave a detection limit of roughly 1.7 μM [4]. This type of work began with studies of flavins and transition-metal ions [5]. Since then, many more dye–ion combinations have been described, including nanomaterials, paper strips and mobile-phone readers.

This review has two purposes. One is to gather the reported work. The other, and the more difficult one, is to judge whether the evidence supports the conclusions drawn. We start with basic photophysics, then discuss dyes and metal ions, and then the experimental conditions that influence the signal. The remaining sections cover analytical figures, applications, recurring problems and future directions. Work up to 2026 is covered, and older references are retained where they are still the standard source.

2. Mechanisms of Fluorescence Quenching

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After absorbing light, a fluorophore remains excited for only a few nanoseconds. Emission is only one of the routes by which it returns to the ground state. When another route is available, fewer molecules emit and the quantum yield drops. The substance that opens this route is the quencher. Quenching is classed as dynamic, static or a combination of both [2,6].

2.1 Dynamic (collisional) quenching

Here the quencher collides with a dye that is already excited and takes away its energy without light emission. The dye is not altered chemically. As the quencher must reach the dye by diffusion, the effect increases in warm or thin solutions. The lifetime of the excited state decreases in the same proportion as the intensity [7], while the absorption spectrum is unchanged.

2.2 Static quenching

In static quenching, the dye and quencher bind beforehand and give a ground-state complex that is non-fluorescent. Only the free dye can emit, so the intensity falls, yet the lifetime of that free dye does not change. Complexes often become less stable on heating, so quenching often decreases as the temperature rises. This is only a general tendency. If the binding absorbs heat, the association constant may increase with temperature [7]. A change in the absorption spectrum is another common sign and is easy to check.

Table 1: Practical Differences Between Dynamic and Static Quenching

Feature	Dynamic	Static
Time of dye–quencher contact	After excitation of the dye	Before excitation (ground-state complex)
Effect of higher temperature	Usually more quenching	Usually less quenching (not always)
Fluorescence lifetime	Decreases	Unchanged
Absorption spectrum	Unchanged	May change

2.3 Stern–Volmer analysis

The usual way to analyse quenching data is the Stern–Volmer equation [8]:

$$F_0/F = 1 + K_{sv}[Q]$$

F_0 and F are the intensities without and with the quencher, [Q] is the quencher concentration and K_{sv} is the Stern–Volmer constant. A linear plot of F_0/F against [Q] is generally taken to mean a single process, and its slope reflects the sensitivity of the dye to the ion. Two points need care. First, linearity alone does not show that quenching is dynamic, since binding in the ground state can give a very similar line when the quencher is far in excess [7]. Second, if binding is strong, or the dye is not much less concentrated than the quencher, static quenching alone gives an upward curve. The simple linear form works well only when the product of the binding constant and the dye concentration is under about 0.05 [7].

So an upward curve may indicate mixed quenching, strong one-to-one binding, or the sphere-of-action effect, where a quencher already sitting beside the dye at the moment of excitation quenches it immediately [2]. A downward curve usually suggests that some of the dye is inaccessible to the quencher. Most papers give an R^2 value with K_{sv} to show the quality of the fit, but R^2 does not reveal the mechanism. The equation is more than one hundred years old, and more complete treatments are summarised by Gehlen [9].

2.4 Other routes of signal loss

The emission can fall for other reasons too. In photoinduced electron transfer, an electron shifts between the excited dye and the ion; this principle is the basis of many fluorescent chemosensors [10,11]. Förster energy transfer [12] requires the donor emission to overlap the acceptor absorption, whereas Dexter transfer [13] needs the two partners to be extremely close so that their orbitals overlap. Paramagnetic ions can drive the dye to the triplet state by intersystem crossing, and heavy atoms do this through stronger spin–orbit coupling. Finally, the inner-filter effect occurs when the ion or its complex absorbs the excitation or emission light. It only imitates quenching and must be corrected or excluded [14]. As a rough rule, an absorbance above about 0.05 at either wavelength can lower the signal falsely [7].

3. Organic Dyes and Related Emitters as Sensing Partners

A good dye for quenching-based sensing should be bright, fairly stable to light, water soluble and able to

interact with the target ion. Riboflavin and related flavins satisfy many of these conditions. They are cheap, non-toxic and emit around 530 nm under blue light. Their photophysics is well known [15], and transition-metal ions are known to change their emission [5]. The drawbacks are decomposition by light during measurement and strong dependence of emission on pH.

Fluorescein and rhodamines are xanthene dyes. They are bright and widely available, and the opening and closing of their ring structure allows selective detection of some metal ions. Coumarin and naphthalimide dyes are easy to modify, so binding groups can be added to improve selectivity; as mentioned, even an unmodified commercial naphthalimide dye responded to Cu^{2+} [4].

Carbon dots and semiconductor quantum dots were introduced later as probes [16–18]. They are more resistant to bleaching than organic dyes and their surface groups can bind ions, but results may differ between batches. Nitrogen-doped carbon dots prepared from biomass by a fast microwave method responded to Fe^{3+}, Cu^{2+} and Hg^{2+} with detection limits of 6.0, 1.41 and 1.36 μM, and the quenching was attributed to electrostatic and π–π interactions, inner-filter effects and energy transfer [19]. The number of suggested causes shows that the mechanism is unsettled. Some reports favour static quenching through binding at the surface [20], some describe a combination of surface interaction, aggregation and, for iron, an inner filter [21], and some find a major dynamic part [22]. A 2025 simulation study proposed that the cations do not form stable complexes on the dot surface, so quenching is dynamic [23]. Lifetime measurements should therefore be made before a mechanism is claimed for carbon dots.

Luminogens with aggregation-induced emission [24] act in the opposite manner to ordinary dyes because they emit more when they aggregate. In sensors based on them, a metal ion either causes aggregation or breaks it, and the signal changes accordingly [20].

4. Metal Ions as Quenchers

The response of a dye depends mainly on the electronic structure of the ion. Ions with unpaired d electrons, chiefly Cu^{2+}, Co^{2+} and Fe^{3+}, are often good quenchers. They have low-energy levels that accept energy or electrons from the excited dye, or they favour intersystem crossing because of their paramagnetism [5,25]. Zn^{2+} and Cd^{2+} have filled shells and usually quench weakly; in some receptor-based probes they even increase emission by stiffening the receptor. Pb^{2+} and Hg^{2+} quench mainly by the heavy-atom effect and, in many sensors, by binding to donor atoms of the dye [26]. Alkali and alkaline-earth ions have almost no effect at moderate concentration. Ni^{2+} is paramagnetic but frequently gives only a small effect, commonly explained by slower transfer and weaker binding.

Two recent studies show that one ion can act in very different ways. Cu^{2+} quenched a commercial naphthalimide dye according to a modified Stern–Volmer model, and since the emission decreased at higher temperature the authors described the process as dynamic [4]. In contrast, a porphyrin probe with a dipicolylamine receptor was almost fully quenched by Cu^{2+} while its lifetime changed only from 8.65 to 8.31 ns, which the authors interpreted as static quenching; K_{sv} was 1.39×10^6 M^{-1} and the detection limit 26.3 nM [27]. Hg^{2+} probes are also varied: one 2023 review grouped them into seven mechanisms, with static quenching being just one of them [20]. Fe^{3+} needs a different warning. Iron (III) absorbs far into the near-UV [7], and for carbon dots the apparent quenching by iron has been linked partly to the inner-filter effect rather than true interaction [21].

Table 2: General Behaviour of Selected Metal Ions Toward Fluorescent Dyes

Ion	Main quenching routes	Remarks
Cu^{2+}	Electron or energy transfer, paramagnetism, complex formation	Often the strongest quencher; static and dynamic behaviour both reported [4,27]
Co^{2+}	Paramagnetism, energy transfer	Moderate to strong; plots often linear
Fe^{3+}	Electron transfer, inner-filter effect, hydrolysis	Strongly pH dependent; colour and UV absorption can interfere [21]
Pb^{2+}	Heavy-atom effect, complex formation	Usually weaker; selective if the dye has suitable binding groups
Hg^{2+}	Heavy-atom effect, strong binding to soft donor atoms	Very effective with sulfur- or nitrogen-containing dyes; many probe mechanisms [20]

Ion	Main quenching routes	Remarks
Zn^{2+}, Cd^{2+}	Very little quenching (filled shell)	May increase emission in receptor-based probes
Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}	Negligible at moderate levels	Rarely interfere

5. Factors Governing the Quenching Response

pH. Acidity influences both partners. Protonation alters the charge and emission of the dye, and at higher pH many metal ions form hydroxide precipitates or hydrolysed species with different behaviour. A pH study in suitable buffers is therefore the first step. Acidic to weakly acidic media have often given the strongest quenching, possibly because the free hydrated ion is available. The pH should also be rechecked after adding the metal solution, as acidic salts may change it.

Time. Binding and collisions take some time. The signal normally falls during the first few minutes and then becomes steady. The measurement time should be chosen from this steady region so that small timing errors do not matter. Some probes respond faster; a water-soluble porphyrin probe for Cu^{2+} gave a response within five minutes [28]. A later increase or drift may mean photodecomposition of the dye or slow side reactions.

Temperature. As Table 1 indicates, the trend with temperature gives a hint about the mechanism, but only a hint. Weaker quenching on heating suits static quenching, yet there are systems in which binding absorbs heat and quenching increases with temperature [7]. In optimisation work the response may also reach a maximum and then fall because complexes dissociate or the dye decomposes. A thermostatted cell holder is therefore useful.

Concentration. The dye must be concentrated enough to give a stable signal but not so much that self-absorption or aggregation appears, because both damage the calibration. The quencher range should be chosen so that the working region is linear or follows a clear model. This is more important for strong binders, since the plot curves when the dye concentration is not small relative to the quencher [7].

Matrix and interferents. Real samples contain other ions, organic ligands and coloured substances that may alter the signal. The dye should be tested against several ions, alone and in mixtures. For the porphyrin probe mentioned earlier, eight other metal ions at ten equivalents each hardly changed the emission, and Cu^{2+} still quenched it, whereas Ni^{2+} produced only partial quenching in the same mixture [27]. A masking agent can be added when an interferent cannot be avoided.

6. Analytical Performance

Quenching methods are assessed with the same parameters as other analytical methods. Linear range and sensitivity are taken from the Stern–Volmer plot. The detection limit is usually three times the standard deviation of the blank divided by the slope of the calibration line, and the quantitation limit is ten times this ratio [29]. Precision comes from repeated readings and accuracy from the recovery of a known added amount in real samples. Since definitions differ between papers, the way a detection limit was calculated should be checked before methods are compared.

Even with the three-sigma rule, the value depends on the number of blank readings. In a 2026 study of a Cu^{2+} probe the blank deviation was based on four readings [27], which is usual practice but a small basis for a value quoted to three significant figures. Reported limits range from micromolar for the commercial naphthalimide dye (1.7 µM) and biomass carbon dots (1.36 to 6.0 µM) [4,19] to nanomolar for porphyrin probes (26.3 nM and 6.43 nM) [27,28]. Reproducibility improves when authors correct for the inner-filter effect and bleaching and state the buffer, excitation wavelength and slit widths.

7. Applications

Water analysis is the most common use. Dye-based quenching methods have been described for copper, iron, lead and mercury in tap, river and ground water, with detection limits from micromolar to nanomolar depending on the dye and ion. A 2023 review on Hg^{2+} probes points out that most studies use water samples, while food, medicines, cosmetics and biological samples are examined less and usually need lengthy pretreatment [20]. In food and beverages the same principle is applied to screen toxic metals; a recent copper probe, for example, was tested in food samples and in vivo [30]. Even so, samples generally need digestion or dilution to remove proteins and pigments.

In drug analysis, some drugs and vitamins are naturally fluorescent, so their estimation can be linked to ion binding. In biology, dyes and nanoparticles that enter cells allow imaging of loosely bound metal pools [31], and newer probes such as a water-soluble porphyrin for Cu^{2+} have been used in living cells [28]. Many studies also fix the dye on paper or a polymer and read the colour or emission with a mobile-phone camera, which is suitable for work outside the laboratory.

8. Critical Discussion, Limitations and Outlook

Assigning the mechanism. The commonest mistake, in our view, is to name the mechanism from one Stern–Volmer plot. A straight line at one temperature cannot separate static from dynamic quenching because both follow the same equation, and as stated in Section 2.3 strong static binding can curve the plot upward [7]. A sound assignment needs constants at more than one temperature, measured lifetimes, and absorption spectra of the dye with and without the ion. Some authors use the bimolecular quenching constant: a value above the diffusion limit, about 10^{10} M^{-1} s^{-1} (closer to 7×10^9 M^{-1} s^{-1} in water at 25 °C), is presented as proof of static quenching. This needs an independently measured lifetime, which many papers lack, and long-range energy transfer can also give a constant above the diffusion limit [7]. A lifetime check is simple but often omitted; where it was done, as for the porphyrin probe above, the conclusion was clearer [27].

Comparing performance. Detection limits from different papers are rarely comparable. Some come from the blank deviation, some from the lowest concentration distinguishable by eye, and a few from extrapolation beyond the measured range. Selectivity is usually shown by adding one interferent at one level, while real samples contain mixtures in which the target may be much less abundant than the interferents. Good recovery in tap water is useful but says little about coloured or salty samples.

Artefacts. Several effects imitate dye–ion quenching. Examples are metal salts absorbing at the excitation or emission wavelength, pH changes from acidic metal solutions (Fe^{3+} and Cu^{2+} hydrolyse easily), hydroxide precipitation and bleaching during repeated scans. Iron (III) with carbon dots is a standard example, since much of the apparent quenching may be an inner-filter effect [21]. Control additions of buffer alone and of pH-matched solutions should be routine.

Recommended practice. Authors should report the buffer, the pH after addition, the excitation and emission wavelengths and the slit widths. Work should be done at two temperatures at least, with inner-filter correction, and the detection limit should be defined as three times the blank deviation over the slope using replicates. Selectivity should be tested in mixtures and in at least one real matrix. Recovery of the signal, for example by adding EDTA to a Cu^{2+}-quenched probe [27], supports the view that the interaction is reversible binding and not a side reaction.

The simplicity of turn-off sensing is also its main weakness. A drop in signal is not specific, as anything that lowers emission, such as scattering particles, absorbing molecules or a pH change, can be mistaken for the analyte. Several ions quench the same dye, so selectivity must be designed through the choice of dye, pH, masking agents or a ratiometric format. Turn-off signals are also harder to read at very low concentration than turn-on signals, because a small decrease has to be seen against a large background.

We expect progress in four directions. First, attaching dyes to selective binding sites or nanomaterials should improve discrimination between ions. Second, ratiometric and lifetime readouts can remove the effects of dye concentration and lamp intensity. Third, arrays of several dyes with chemometric analysis may identify mixtures rather than single ions. Fourth, portable optics with data-processing software should bring the method closer to field use. One further hope is that authors will report temperature and lifetime data along with mechanisms, so that the field moves from trial and error to real design rules.

CONCLUSION

Fluorescence quenching is a flexible, low-cost way of determining metal ions. To convert a dye–ion interaction into a reliable method, one must establish whether the quenching is dynamic, static or mixed and optimise pH, time, temperature and concentration. Paramagnetic ions such as Cu^{2+} and Co^{2+} generally respond most strongly, while heavy ions such as Pb^{2+} depend more on dye design. The literature up to 2026 shows steady progress in probe design, and nanomolar detection limits are now reported for porphyrin probes, but the old gaps remain: mechanisms are often

assumed, inner-filter effects are often ignored and detection limits are defined differently from paper to paper. Before these assays can be placed beside established instrumental methods, they need thorough validation against interferents and real samples, and the way constants and detection limits were obtained must be clearly stated.

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