

EDGAR BUCKINGHAM: FLUORESCENCE OF QUININE SALTS

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Malaria, an often-fatal disease, has been a worldwide plague for several thousand years. The discovery of the efficacy of substances present in the bark of various *cinchona* trees, native to the Andes, provided some relief. A real anti-malarial drug was not available until 1820, when Joseph Baptiste Caventou (1795-1877) and Josephe Pelletier (1788-1842) isolated *quinine* from the bark (1). Eighty years after their discovery, a statue honoring these chemists was erected in Paris (Fig. 1).

Other workers established the formula for quinine, showed that it acts as a diacid base, and that it is a methoxy derivative of a companion alkaloid, cinchonine. The elucidation of the structure of these compounds, largely due to the work of Wilhelm Königs (1851-1906) and Paul Rabe (1869-1952), was finally published in 1908 (2). More than three decades passed before the partial synthesis of quinine was achieved (3). The first completely stereoselective, total synthesis of this compound was reported in 2001 (4). However, despite the discovery of other antimalarial drugs, quinine is still manu-

factured from cinchona trees that are cultivated in South America and in the Far East.



Figure 1. Caventou and Pelletier

It must have been known since ancient times that certain substances appear to have one color when viewed by transmitted light and another when viewed obliquely. Mineralogists recognize a type of fluor-spar, pale green when viewed against the light, but appearing blue when viewed at an angle to the light. Unrefined petroleum shows the same kind of effect, as do certain substances when in solution. Fluorescein, used both in the laboratory as an indicator and industrially for the location of leaks in waste water systems, is a familiar example. Another is *quinine* or, because of its low solubility in water, one of its salts. The solution, colorless when viewed directly, appears blue when viewed at an angle to the incident light. The phenomenon exhibited by these various sys-

tems is termed *fluorescence*. With modern laser instrumentation and highly sensitive detectors, fluorescence has become a powerful analytical technique. For example, the laser-induced fluorescence detection of derivatized angiotensin peptides is applicable to quantities as small as a few hundred zetamoles (5).

The systematic study of fluorescence was initiated by Astronomer Royal, John Frederick Herschel (1792-1871) (Fig. 2). He named the phenomenon *epipolic dispersion*, derived from the Greek for "surface," because he believed that the effect originated in a layer adjacent to the entrance of light. For Herschel and his successors, the usual light source was the sun or daylight. Because the "detector" was the eye, the observations were not numerical but merely comparative. Colored glass or sometimes a prism was used to select spectral portions of the incident or emergent light. Observations had to be made in a dark room or enclosure, with the incident light entering through a hole or slit.



Figure 2. John F. W. Herschel

In 1845 Herschel described his experiments with solutions of quinine tartrate (6,7). He noted that when an approximately 1% solution is placed in a tall glass before an open window, the blue color can be seen by looking down into the glass. When the solution was trickled from one glass to another, the thin film seemed to be equally effective as the bulk solution. Herschel commented that light transmitted through a "quiniferous solution," thus producing a "dispersion," did not produce a dispersion in a second portion of solution.

In fact, the phenomenon described by Herschel had been noted earlier by David Brewster (1781-1868) (Fig. 3). He used a lens to focus sunlight and was able to demonstrate that the "dispersion was not confined to the surface layer, but extended well into the solution (8)."

George Gabriel Stokes (1819-1908) greatly extended the observations of Herschel and Brewster. With a box-like enclosure that enabled him to observe without darkening the room, Stokes examined quinine sulfate, solutions of various plant extracts, certain glasses, and even uranium compounds (9). He thus demonstrated that many systems exhibited phenomena similar to that shown by quinine salts. In a later report, Stokes used "fluorescence" to replace the older term (10). An apparently universal effect, that the fluorescence was emitted

at a wavelength longer than that of the incident beam, became known later as Stokes' Law.

Stokes noted that the quinine salts of numerous acids exhibited fluorescence, exceptions being the salts of HCl, HBr, and HI. In fact, the addition of one of these acids to a fluorescing quinine salt solution destroyed the effect. However, the fluorescence returned when the interferent, or quenching agent, was removed; e.g., by treatment with HgO. Mercury halides did not quench the fluorescence.

Other workers, notably Victor Pierre (1819-1886) (11), Jacob Edward Hagenbach (1833-1910) (12), and Cornelius Joseph Lommel (1837-1899) (13), extended the study of fluorescence. Pierre showed that a given substance does not fluoresce if the wavelength of the incident light is greater than a certain minimum. Hagenbach examined the fluorescence of numerous, mainly organic substances, including quinine sulfate. He found that the spectrum of a solution of this salt exhibited two maxima. One of Lommel's discoveries was that the fluorescence radiated by a volume element of a substance is proportional to the amount of the exciting light absorbed.

This was approximately the state of affairs when Edgar Buckingham (Fig. 4) began his work on fluorescence, particularly that of quinine salts. Born in Philadelphia on July 8, 1867 and graduated from Harvard in 1887, Buckingham spent a period in Stra_burg before moving to Leipzig in 1890, where he began the work mentioned. His aims were to extend the then-known facts and to interpret the results in terms of the Arrhenius ionic theory, which was strongly promoted in Ostwald's laboratory. Buckingham's optical equipment was simply the Stokes dark box, with colored glass filters for sunlight and, occasionally, artificial light (14). The availability of electrolytic conductance apparatus was a major asset, allowing him to assess the ionic state of his solutions.

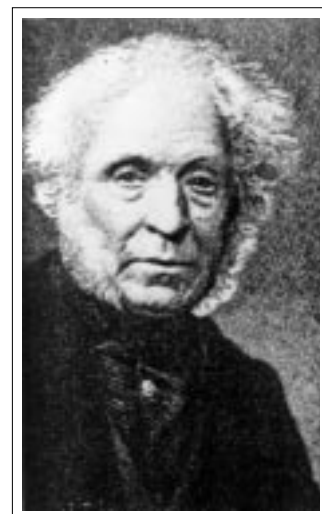


Figure 3. David Brewster

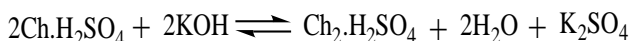
Preliminary experiments were carried out with eosin (tetrabromofluorescein), which behaves as a dibasic acid. Its red solution exhibits a green fluorescence. These experiments convinced Buckingham that the intensity of the effect was governed by the concentration of the *ions* of the solute. He then turned to quinine, known to contain two basic nitrogen atoms and thus capable of forming two series of salts. Because the basic properties of quinine are weak, however, Buckingham pointed out that, with respect to electrolytic dissociation, the salts formed with one equivalent of acid per molecule tend to act like binary neutral salts such as KCl.

Buckingham experimented with the hydrochloride, acetate, monochloroacetate, nitrate, and sulfate of quinine, as well as with the hydrogen sulfate, which he termed "bisulfate." With approximately millimolar solutions of each of the salts, he found that the hydrogen sulfate fluoresced more strongly, the acetate less strongly than the other salts, excluding the hydrochloride. As had been found by Stokes, the hydrochloride was nonfluorescent. From the German, "Chinin," Buckingham used the contraction "Ch" to indicate this compound. Quinine hydrogen sulfate was thus written $\text{Ch.H}_2\text{SO}_4$, or $(\text{Ch.H})\text{HSO}_4$. The fluorescence of a solution of this salt decreased as KOH was added, vanishing when the molar concentrations of this salt and of added KOH had become approximately equal. Because of the low solubility of free quinine in water, the experiments were conducted in approximately 64% alcohol.

In molecular terms, the reaction was presumed to be:



This implied that free quinine does not fluoresce. In a possible alternative reaction:

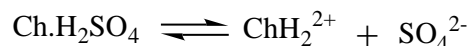


the (normal) sulfate, $(\text{Ch.H})_2\text{SO}_4$, would be formed; but the absence of fluorescence implied that the normal sulfate was also inactive. However, a 5mM 60% alcoholic solution containing both KOH and the normal sulfate produced a distinct, if weak, fluorescence, thus suggesting that the reaction indicated by equation (2) was unlikely.

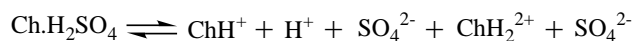
Because quinine acts as a univalent base in aqueous alcoholic solution, Buckingham assumed that the fluorescent species was the cation ChH^+ , formed by the reaction:



The addition of alkali destroyed this cation and also the fluorescence. However, the fluorescence was increased by the addition of HNO_3 . Another possibility considered was that the salt dissociated as follows:

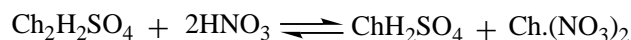


From an extensive series of measurements of electrolytic conductivity, Buckingham argued that, in solution, quinine hydrogen sulfate partially dissociates to give *both* univalent (ChH^+) and divalent (ChH_2^{2+}) cations:

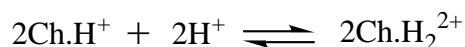


Buckingham came to the conclusion that the fluorescence was due to the quinine cations, and that the divalent species was the more effective. The addition of HNO_3 (*i.e.*, of hydrogen ion) to a solution of $\text{Ch.H}_2\text{SO}_4$ favors the conversion of the univalent to the divalent cation, with corresponding increase in fluorescence. Conductometric measurements of the quinine salts of strong acids (other than HCl, etc.) at millimolar concentration indicated almost complete dissociation. The stronger fluorescence of the hydrogen sulfate thus cannot be due to dissociation greater than that of the other salts.

If the above explanations are correct, the addition of excess strong acid to equimolar solutions of the various univalent quinine salts should cause the fluorescence to rise to the same maximum. Buckingham proved this experimentally (halides excluded) and found that even weaker acids in greater excess were also effective. He also found that if *small* amounts of HNO_3 were added to a millimolar quinine sulfate solution (cation, Ch.H^+), the conductance *decreased*. Because HNO_3 is an excellent conductor, this seems surprising. This result was attributed to the conversion of the univalent to the divalent quinine cation, with consequent removal of the highly conducting hydrogen ion:



If only the cations are considered, the equation becomes:



Obviously, the amount of hydrogen ion (*i.e.*, of HNO_3) added must be less than that implied by equa-

tions (6) and (7). Buckingham demonstrated this experimentally with amounts of HNO_3 that ranged from 1/5th to 1/50th of the amount of quinine nitrate. Through the common-ion effect, the addition of K_2SO_4 to a solution of quinine sulfate might be expected to depress the dissociation of the quinine salt, and thus diminish the fluorescence. In fact, the latter increased, a fact attributed to partial conversion of Ch.H^+ into divalent Ch.KH^+ ions.



Figure 4. Edgar Buckingham

Finally, Buckingham turned to the well-known quenching of fluorescence by halide ions. He repeated the experiments by Stokes, looking for possible causes of the effect. Halide solutions absorb active portions of the incident light or the fluorescent light itself. However, this light from a quinine salt solution was not extinguished when its container was surrounded by HCl solution. The presence of halides may have caused the formation of double or polymolecules of quinine. However, neither conductometric measurements nor freezing-point determinations supported this view. To obtain any quenching effect by HgCl_2 , which is only slightly dissociated in solution, a one hundred-fold excess is needed. Thus it is the chloride ion, and not merely a soluble chloride salt, that causes the quenching. Finally, when Ch.HCl is added to $\text{Ch.H}_2\text{SO}_4$, the fluorescence of the latter is strongly depressed. Thus the effect of halide ions does not depend upon their source.

Although Buckingham had examined and eliminated various possible causes of the quenching effect, he had to admit that he could not explain this effect. More than 30 years after he had finished this work, the following statement appeared in a paper by other workers: "This curious effect of halogen ions remains unexplained (15)."

In 1928 a double-beam photoelectric fluorimeter was used to make a careful study of the quenching of the fluorescence of 0.0025M quinine bisulfate solution by increasing concentrations of additives (16). The results are summarized in Fig. 6. These confirm that the quenching effect is essentially due to the additive anion; Ag^+ is the only cation with appreciable activity. The increasing order of quenching efficiency, indicated in

the Table below, is similar to that of the lyotropic series of ions that is relevant to various physicochemical phenomena such as the precipitation of colloids.

Refractive index measurements were used to obtain the numbers beneath the symbols. The numbers are measures of ionic deformability, *i.e.*, the looseness of the binding of the outer electrons. The authors suggested that the high deformability of the halide ions (CNS^- , known to be a powerful quencher, was not evaluated) enabled the excited quinine cations to return to normal conditions by radiationless transfer of energy through collision with the halide ions.

The work of Francis Perrin (1901-1992) was quoted in support of this collision theory. He showed that as the viscosity of the solvent is increased, a greater concentration of the fluorescing solute is needed to obtain maximum emission (17). Presumably the frequency of collision between the quinine ions, and hence their activation, is diminished in a more viscous medium. This decrease should also apply to collisions between the quinine ions and the quenching ions. The diminution of the quenching power of the halide ions is thus analogous to the increased concentration of quinine ions needed for maximum emission when the quencher is absent.

On his return to the U.S. Buckingham taught physics and physical chemistry at Bryn Mawr College from 1893 to 1899 and then was briefly affiliated with the University of Wisconsin. Apparently, he never returned to the quinine topic. He once remarked that he had studied harmony, not physical chemistry, under Ostwald (17). This is a reminder that Ostwald, by no means a regular attendant at lectures as a

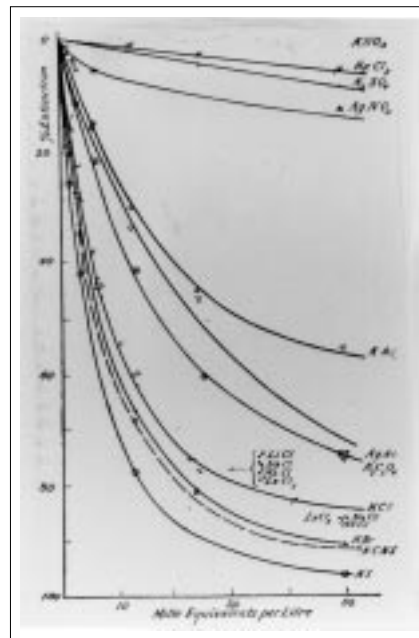


Figure 5. Quenching effect of salts on the fluorescence of quinine sulfate

Table. Quenching Efficiency of Various Ions

Quenching Ion:

F⁻ < NO₃⁻ < SO₄²⁻ < Acetate < Oxalate < Cl⁻ < Br⁻ < CNS⁻ < I⁻

Rel. Deformability:

2.5 3.66 3.65 8.7 12.2 18.5

university chemistry student, did manage to learn the viola parts of all of the 83 Haydn string quartets. This facet of Ostwald's interests must have appealed to Buckingham, who later took miniature scores to symphony concerts.

In 1902 Buckingham became a physicist in the U. S. Department of Agriculture and then transferred to the U.S. Bureau of Standards (now the National Institute of Standards and Technology). Here he published extensively on thermodynamics, hydraulics, fluid dynamics, and engineering physics. He retired in 1937 but remained scientifically active until his death in Washington, DC on April 29, 1940.

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