

ORIGINAL ARTICLE



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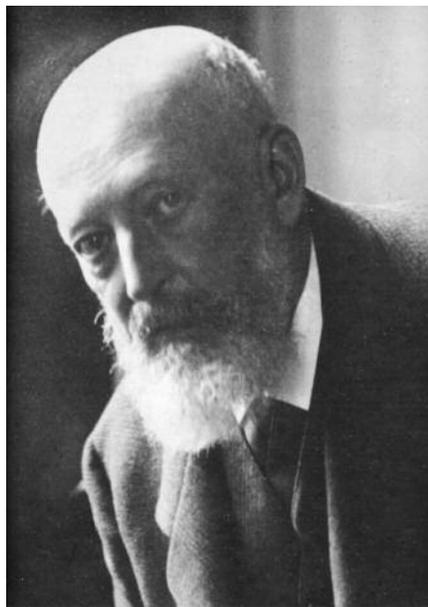
Sodium Fluorescein – Colourful Past, Bright Future

INTRODUCTION

Johann Friedrich Wilhelm Adolf von Baeyer (1835 – 1917), was awarded the Nobel Prize for Chemistry in 1905. He is remembered for his work with phthalein dyes, the barbiturate class of hypnotic drugs and for his “Strain” theory relating to carbon-carbon chemical ring formations such as Benzene.¹ The ophthalmic world however, remembers him for his work in the development of one of the most used diagnostic dyes in ophthalmic practice today – sodium fluorescein (or more correctly, di-sodium fluorescein).

First synthesised in 1871, fluorescein is commonly believed to be a “vegetable”, or plant-based dye, although it is actually completely artificial, being synthesised from the heating (known as “condensation”) of resorcinol and phthalic anhydride.² This compound is one of the most highly fluorescent substances known, being visible in water down to 0.02 parts-per-million under ultraviolet light.³ The “tar like” fluorescein base is insoluble in water. To reduce its toxicity and make it a soluble molecule it is converted to the alkaline di-sodium salt for aqueous formulation.⁴

In 1929, Hans Fischer observed the flow of sodium fluorescein in the iris blood vessels of rabbits. Similar observations were made in human subjects by Sorsby et al. during the late 1930s.⁴ It was not until the late 1950s, however, that Dr. Harold Novotny and Dr. David Alvis first used the technique of photographing the retinal vasculature using fluorescein injected intravenously. After performing a number of studies involving this technique on diabetic and hypertensive patients, the pair published their findings in 1961.^{5,6} This study, in conjunction with the development of the fundus camera by Zeiss between



Johann von Baeyer (1835 – 1917)

the 1920s and the 1950s, paved the way for the sodium fluorescein angiographic procedure as practiced today.⁴

CHEMISTRY

Fluorescein ($C_{20}H_{12}O_5$) is manufactured by the condensation or heating of resorcinol with phthalic acid anhydride at a temperature of approximately 200°C (392°F).⁴ The result of the reaction is a tar like solid which, when powdered, has a yellow-red to red fine grain, that is very insoluble in water.³ To allow for the formulation of an aqueous fluorescein injection, the molecule is first transformed to the di-sodium salt. This is usually achieved by either of two methods: (i) via reaction with concentrated sodium hydroxide or (ii) via a sodium acetate/acetic anhydride reaction to

produce diacetyl fluorescein, followed by the addition of sodium hydroxide to convert the diacetyl salt to di-sodium fluorescein.⁷

Di-sodium fluorescein ($C_{20}H_{10}Na_2O_5$, also known as uranine), is a highly hygroscopic orange-red powder, freely soluble in water with a deep, orange-red colour in concentrated solution, tending to a light yellowish-green on dilution. It exhibits an intense yellow-green fluorescence under ultraviolet light when in neutral to alkaline solution. The peak of absorption occurs at about 460–485nm, with emission around 530nm.^{3,4}

The intensity of fluorescence is dependent on pH, with the optimal fluorescence for the purpose of angiography occurring at a pH of about 7.5, ideal for *in-vivo* use.⁴ However, for stability purposes the di-sodium fluorescein injections used in ophthalmology are prepared at a pH of between 9.2 to 9.4, adjusted usually by the addition of sodium hydroxide or sodium bicarbonate.

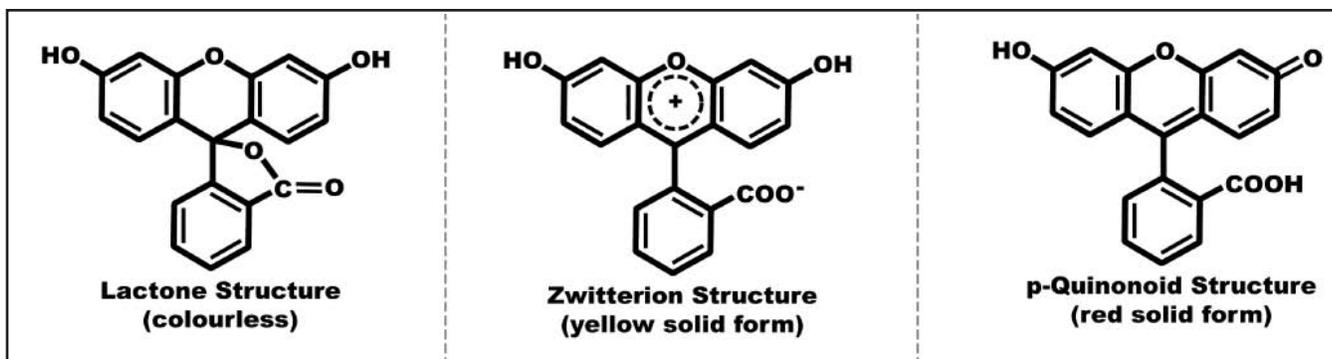


Figure 1: The three molecular forms of

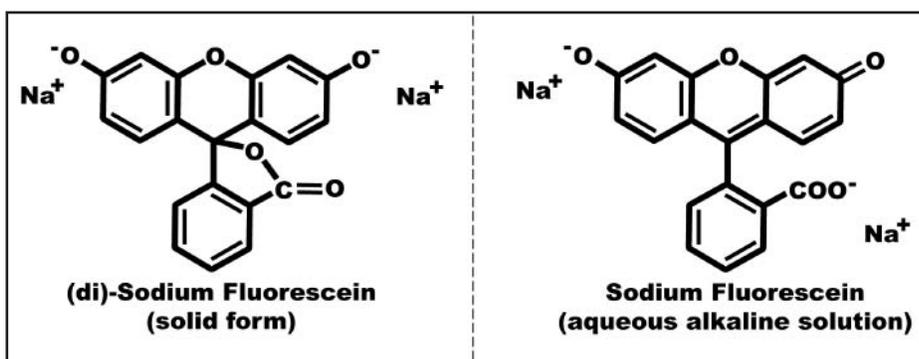


Figure 2: Sodium Fluorescein in solid & dissociated alkaline form.

Fluorescein 'base' exists in three molecular forms as a solid, as does the di-sodium salt.^{7,8} These comprise a lactone structure exhibiting no colour, a yellow-coloured 'Zwitterionic' structure, and a red-coloured p-Quinonoid form (Figure 1). The colourless form is difficult to isolate and only occurs in certain non-polar solvents, such as dioxan.⁸

Figure 2 illustrates the chemical structure of di-sodium fluorescein in solid form, and the alkaline dissociated form (note the p-Quinone nature), which occurs in typical ophthalmic injection formulations.

Typically, a concentrated alkaline di-sodium fluorescein solution is used in injections for angiography (up to 25%). At this concentration, the p-Quinone structure is present, giving the solution a strong red-orange colour.⁸

FLUORESCIEIN'S OTHER USES

There are a number of other important uses of fluorescein of which brief mention should be made.

Microbiological techniques utilise fluorescein attached to 'marker' antibodies specific for different types of microorganisms or other cell types. This allows for sorting, counting and statistical study of microbes using a flow cytometer – a machine that can separate cells (or other particles) depending on optical characteristics. Such techniques can also be used in the study of mammalian cell, to detect the prevalence of certain cell markers and characteristics, or to detect disease.⁹ A similar technique using fluorescein marked beads of defined sizes has been used to determine the movement and spread of fine sediment particles in river

and ocean systems and even residence times and fate of solids in sewerage treatment plants.

Water soluble fluorescein (the di-sodium salt) is also used to trace the path of liquids through geothermal reservoirs for geological study. Due to its thermal stability (tested to 300C) and its low detection limits (down to 0.02 parts-per-million), sodium fluorescein is considered the most suitable marker for such study.^{10,11} It has also been

used to trace the path of water through subterranean drainage systems, and very frequently by plumbers to trace water leaks in houses and factories.

SAFETY AND EFFICACY

In order to achieve a concentrated bolus of di-sodium fluorescein in the eye for angiographic examination, the sodium fluorescein is usually injected rapidly. Typically, this takes place via the antecubital vein. Since injection is swift, precautions must be taken to avoid extravasation of the solution, which is both painful and ineffective for angiographic purposes.^{12,13}

Intravenous di-sodium fluorescein is well tolerated in most patients, including young children, and exhibits relatively few side effects. Nausea and/or vomiting are the most common adverse reactions reported (usually transient). Other far less common mild to moderate adverse reactions include urticaria, rash, dizziness, dyspnea, syncope, pyrexia, thrombophlebitis, breathlessness and extravasation. Patients may also be warned that exposure to strong sunlight can cause sunburn, due to the photosensitive nature of the dye in the skin.⁴

The more severe adverse reactions are anaphylaxis, respiratory distress, cardiac arrest and death.⁷ It should be noted that these reactions are very rare (although they do occur). A 1986, study in the United States reported only one death across 221,781 angiographic procedures, with severe reactions – respiratory or cardiac events reported at a rate of 1 in 1900 patients.⁷ In the statistical studies of adverse reactions, it has not, to date, been possible to

dissociate the incidence of adverse reactions from the heterogeneity (non uniform structures) of products used since, in most instances, different brands and manufacturers supplied the market.

However, recently, the Australian manufacturer, Pharmed, was able to monitor adverse reactions to its product in the Australian market during a two year period when they were the sole supplier. Hence, the di-sodium fluorescein was from one manufacturer, with the same raw material source and same formulation and method of manufacture. During this period, with the use of over 70,000 vials of 10% and 25% material, the incidence of serious adverse events was 0.012% or about 1 in 10,000. This is also broadly in agreement with another recently published Australian study conducted by Kwan et al. (2006), where 132 (or 1.1%) mild to moderate adverse events were observed in 11,898 fluorescein angiograms. No serious adverse events were encountered in this study.²⁴

Kwiterovich et al. (1991) reported that patients who have had a previous adverse reaction to di-sodium fluorescein injection had a greater than 45% chance of having a similar or more severe reaction to subsequent injections.¹⁴ This is in contrast to a less than 2% chance for those patients that have had no previous adverse reactions. Patients with diabetes mellitus are often predisposed to cardiovascular disorders such as atherosclerosis and hypertension. These patients are regarded as members of a group at relatively high risk of acute cardiovascular complications during diagnostic procedures using di-sodium fluorescein.¹⁵

Kelly et al. (1979) found that orally administered di-sodium fluorescein produced fewer and less severe allergic reactions than did an equivalent intravenous injection.¹⁶ However, they concluded that oral administration was only useful when intravenous injection was overly difficult, such as in very young children, patients with diabetes, or those with no visible veins for injection. In a study by Razvi et al. (2001), 84 diabetic patients with macular oedema were given di-sodium fluorescein orally with a maximum dose of 2 grams. Nearly 5% of patients experienced mild adverse reactions.¹⁷ Although oral administration tends to be well tolerated, it should be noted that severe anaphylactic shock has been reported with oral administration, and this route appears to provide no guarantee of avoiding side effects.¹⁸

Studies have shown no significant difference in the incidence of moderate to severe adverse reactions between 10 and 25% solutions of di-sodium fluorescein when used for angiographic purposes.¹⁹ This is probably due to a similar absolute di-sodium fluorescein dose (in grams of sodium fluorescein per patient) being administered via the usual 3mL injection of 25% solution (0.75g), a 5–8mL (0.5–0.8g) injection of 10% solution, or other variations of volume and concentration favoured by different groups administering the procedure. Justice et al. (1977) reported that a 25% di-sodium Fluorescein solution was more effective in providing retinal contrast at the lower 3ml volume than a greater volume of the 10% solution.¹⁹

Certain factors that may predispose patients to adverse reactions have been identified in the literature. These include age, race, sex, disease states, concomitant medication, and known allergies, among others. Greene (1976) noted that adverse reactions are nearly twice as likely in males than in females.²⁰ Patients receiving pre-treatment or concomitant medication have a higher incidence of adverse reactions. Pre-treatment with anti-emetics and antihistamines show variable results. In some studies, pre-medication was associated with more adverse reactions than were procedures performed without premedication.¹⁴ However, premedication of patients with prior reactions lowered the incidence of reactions significantly in one study.²¹

Di-sodium fluorescein will typically stain the skin and urine yellow, usually lasting for no more than 6 to 12 hours. As the dye is excreted by the kidneys, this is considered a normal part of the drug's pharmacology rather than an adverse reaction, although caution is recommended in patients with renal failure. This reaction may adversely affect urine-based tests performed for a day or so after injection of di-sodium fluorescein (e.g. glucose).⁴

As for any intravenous medication, patient history should be reviewed and caution is required in patients at higher risk of allergy, in pregnancy, or in patients with renal failure, heart conditions, and peripheral vascular disease. Extreme care should be taken in administering di-sodium fluorescein injection to those patients having previously exhibited adverse reaction to the drug.

Due to the possibility of anaphylactic reactions, an emergency resuscitation kit containing suitable equipment and drugs should be readily available during any intravenous fluorescein procedure.

Kwan et al. (2006) noted that "Fatalities resulting from FFA (fundus fluorescein angiography)" are fortunately rare but severe adverse drug reactions are believed to be the third most frequent reason for malpractice suits in ophthalmology, behind cataract operation complications and the misdiagnosis of retinal detachments."²⁴ This article also provides a current survey of adverse reactions to intravenous fluorescein angiography.

FORMULATION OF THE INJECTION – REDUCTION OF ADVERSE REACTIONS

Often, the occurrence of adverse reactions to various medications is attributed to the nature of the drug itself, and di-sodium fluorescein is no exception. In the case of di-sodium fluorescein injections however, it may not be the only cause. The major use of fluorescein is not in medicine, but rather in engineering and microbiological or cellular studies. The grade and quality of di-sodium fluorescein depends upon the method of synthesis and the purification processes carried out. There appear to be only three manufacturers in the world supplying pharmaceutical grade material to the accepted standards of the United States Pharmacopeia (USP), British Pharmacopeia (BP) and the European Pharmacopeia (EP), although many more companies manufacture industrial grade

product. Producing an acceptable pharmaceutical grade is both expensive and difficult, and requires stringent quality control.

Di-sodium fluorescein has been used in medicine for more than 40 years, although high-grade material meeting pharmacopoeial specifications and standards has been developed only in the last 10-15 years. Tests for chemical impurities in manufacture including resorcinol, 1,2-dihydroxy benzoylbenzoic acid (DHBBA) (Figure 3), dimethylformamide, acetone and specific heavy metals have only been adopted relatively recently. The United States Pharmacopoeia (USP) and the European/British Pharmacopoeia (EP/BP) differ in their test standards for the active material and the injection. The USP does not have limit tests for the impurities that are known to occur in the starting material and is less stringent on limits for the impurities listed above. However, the EP/BP specifications do have limits within them and more are proposed for the future.

Yannuzzi et al. (1974) reported that the incidence of vomiting, nausea and allergic reactions differed between brands and batches of fluorescein solutions produced from different starting materials. There was a three-fold difference in adverse reactions depending on the starting material, each of which contained unknown quantities of impurities. The addition of sodium bicarbonate to the formulation may also have been a contributing factor to the variation in adverse reactions.²²

Jacob et al. (1982), in a U.K. study, observed a doubling of adverse reactions and an increase in severity of reactions over a period of six months. Dimethylformamide, sometimes used in raw material manufacture to solubilise the fluorescein base before the addition of the sodium ions, was found in the formulated injection under study. It was suggested the contamination by dimethylformamide was the cause of the dramatic increase in adverse reactions. The European and British Pharmacopoeias were modified in response to this study to include a limit test for the presence of dimethylformamide.²³

Small amounts of heavy metal contamination, eg. mercury, presumed to come from the catalysts used in the condensation reaction, have required recalls in the US (1992). Strict standards now apply to these substances in the current pharmacopoeial monographs. Tighter specifications and more precise manufacturing routes should reduce the occurrence of unacceptable impurities in formulated injections. As the various pharmacopoeia worldwide move to a consensus, the rate of adverse or allergic reactions to drugs like di-sodium fluorescein are expected to decline. Europe, through the EP and BP, is expected to adopt a new tighter standard specifically focusing on reducing the concentration of contaminants that are a by-product of the condensation reaction: resorcinol and DHBBA.

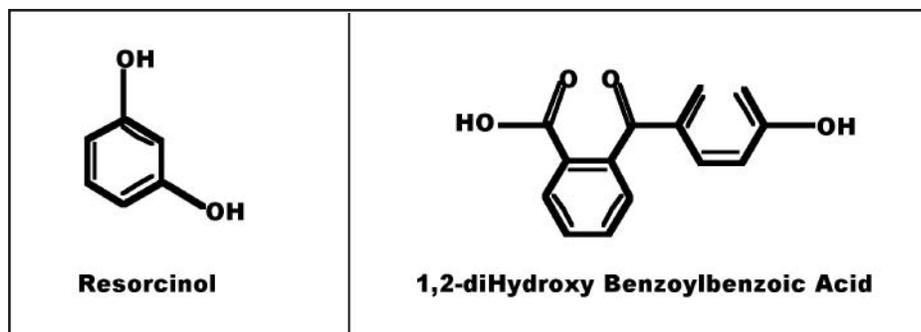


Figure 3: Manufacturing contaminants Resorcinol and DHBBA.

THE FUTURE

Since it was first synthesised by von Baeyer in 1871 through to the present day, di-sodium fluorescein has illuminated the fields of engineering, microbial and cellular study, and most importantly medicine and ophthalmology. The compound exhibits extraordinary fluorescence, visible down to concentrations in the parts-per-billion, and it is this characteristic that has ensured its continued use.

Although in common medical use for some time, adverse reactions to di-sodium fluorescein continue to occur, most commonly in certain high-risk patients. More often today, adverse reactions to medical drugs are discovered to be contingent on impurities in the manufacture and formulation of the drug itself. As manufacturing processes are increasingly refined, the occurrence of severe allergic and life-threatening reactions is expected to continue to drop from the current low rate of around 2%.

Already considered one of the safest dye substances for human intravenous use, di-sodium fluorescein will continue to be useful in the medical and ophthalmic fields. Recently, studies have shown it to be a useful diagnostic tool in a range of other processes in medicine, from the detection of brain stem injuries to marking some cancer types. As we move to tighter controls on manufacturers, more stringent requirements for purity and formulation, and the worldwide convergence of pharmaceutical standards, it is hoped that the risk to patients of fluorescein injection will continue to diminish.

Proprietary Statement: Mr. Mal Eutick is the managing Director of Pharmed Australia, a producer and supplier of sodium fluorescein, among other pharmaceuticals.

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On May 4, 2006, Earl Choromokos, CRA, FOPS, passed away.

Earl and I were friendly competitors from the day we met at the Bascom Palmer Eye Institute in the late 60s, and were good friends away from this business as well.

He was well known in ophthalmology for his original work in developing ICG angiography. Earl was a former president of the OPS, long time Chairman of the Board of Certification, and co-produced the COPRA examination with his friend, Terrance L. Tomer. I recommended him to Northwestern University, and over time he became a full time faculty member there. Later he moved on to the University of Cincinnati and earned his way to a full professorship. Among his proteges is his daughter, Nicole Choromokos Conley, CRA. Following his retirement, Earl became quite the professional in his woodworking hobby. He will be missed.



Lee Allen passed away on May 5, 2006. Lee was 95 years old. Lee was a founding member of the OPS and was the first elected president of our society. He truly was one of the

nicest and most honest men that I have ever met. Lee was well known in ophthalmology for his wonderful medical art renderings and for his early publications on basic fundus photography and especially for his work in adapting stereo to fundus photography. He also published an outstanding chapter on Slit-lamp Photography in the original book on Ophthalmic Photography. This very special person was a friend to many of us in the society and will be missed.

Johnny Justice, Jr.