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Thermodynamic properties and photodegradation kinetics of indocyanine green in aqueous solution

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ABSTRACT

Aqueous solutions of indocyanine green (ICG) are spectrophotometrically and fluorimetrically studied as a function of the dye concentration. The integral absorbance concept is introduced to minimize uncertainties due to the use of only a point. This new method, provides a value of the equilibrium constant consistent with those obtained, by a different method, for safranine and thionine. The influence of ICG concentration on its fluorescence spectrum is determined. When ICG is exposed to white light degrades through the formation of free radicals. The overall kinetics of degradation is monitored spectrophotometrically and studied in an unconventional way. The results exhibit a power law, regardless of the initial concentration of dye. An apparent order of 1.5 suggests a complex mechanism where the collision between radicals is very important whilst a lifetime of 4 h it is indicative of a rapid degradation kinetics.

1. Introduction

Cyanine dyes are fluorescent compounds used for a wide range of applications from spectral sensitization of photographic emulsion [1] to wide band semiconductor [2] to nonlinear optical materials [3]. Due to their ability of absorbing light in the visible and near-infrared regions, cyanine dyes also have the capacity to color images of biological systems. Control of absorption properties of living tissues is a method widely utilized in diagnostics as well as and surgery [4]. Numerous clinical studies have shown that the removal of the inner limiting membrane (ILM) is a very effective procedure in many diseases involving the vitreoretinal interface [5]. ILM is a delicate and very thin structure so that its removal is a challenge for the surgeon. The microsurgical procedure has found benefit from the use of *vital* dyes, such as ICG [6]. Usually these dyes are able to penetrate living cells without inducing immediate degenerative changes.

ICG has been widely used not only as an optical imager for evolution of cardiac output, liver diseases and kidney functions, but also as an optical sensitizer in photo dynamic and photo-thermal therapy. ICG was approved by the Food and Drug Administration as an agent to evaluate hepatic function, cardiac output and for ophthalmic angiography. An important motivation for this large use of ICG is its strong absorption around 800 nm, near the isosbestic point of hemoglobin and oxyhemoglobin. At these wavelengths the blood and other tissues are relatively transparent and the penetration depth of light in biological tissue is the highest [7]. For these reasons ICG has to be administrated in the form of aqueous solutions. However, in aqueous solutions ICG undergoes transformations as aggregations and degradation [8]. Such changes result in discoloration decreased light absorption, decreased fluorescence and a shift in the wavelength of maximum absorption [9]. This means that it must be injected within hours as it otherwise rapidly loses its fluorescence. Despite its widespread use, little is known about the mechanism of degradation of ICG in aqueous solution and the effect of factors such as concentration, light exposure and temperature.

Recently we proved that an aqueous solution of ICG, comprising viscoelastic chondroitin sulfate and sodium hyaluronate, is an effective contrast medium in viscocanalostomy interventation [10,11]. In this paper our research is focused on dye-dye attractive forces which are generated in aqueous solutions. The purpose of the paper is to obtain chemical and physical information about ICG in order to design with less risk possible canalostomy intervention. Therefore the degradation kinetics of the dye when the solution is irradiated with white light has also been studied.

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2. Experimental section

2.1. Materials

ICG was purchased from Patheon Italia S.p.A., Italy. The commercial product, ICG-PULSION, was used without further purification. The absorption and fluorescence spectra of ICG gave no indication of dye impurity. For the solutions bi-distilled water was used.

2.2. Spectrophotometric and fluorimetric measurements

The absorption measurements were carried out with a Cary 100-Varian UV–Vis equipped with thermostatted cells. Aqueous samples were placed in rectangular quartz cells of 1 cm path length and absorption spectra were recorded at (30.0 ± 0.5) °C in the 200 – 800 nm wavelength region. The fluorescence measurements were carried out with a varian-fluorimeter at (30.0 ± 0.5) °C. The excitation and the emission slit widths were 5 mm and bandwidth of monochromator 20 nm. The excitation and the emissions wavelengths utilized for this study were 778 and 810 nm, respectively.

2.3. Photodegradation measurements

The irradiation experiments were performed by placing the samples in a self built light reactor. The photochemical activities of the samples were evaluated with a light source of 180 W biolux OSRAM fluorescent lamps (6500 K). The photo emission spectrum of the fluorescence lamps provides visible light in the range of 400–800 nm. The distance between light source and the bottom of the solution was about 15 cm and the reactor temperature was (30.0 ± 1) °C. At different time intervals small amounts of sample were taken and spectrophotometrically analyzed. For each sample the area under the curve of ICG spectrum was determined. The area measured vs. time allowed to monitor the progress of photodegradation reaction.

3. Results and discussion

3.1. Self-aggregation effect

ICG is a negatively charged, tricarbocyanine dye with strong absorbing properties in the near-infrared range and is only weakly fluorescent in its unbound state. It is composed of two polycyclic(benzoindotricarbocyanine) lipophilic moieties, linked with a polyene bridge. A linear alkyl chain terminated with a sulfonate group, bound to the nitrogens. As one can see in Fig. 1 the positive charge on a nitrogen atom is involved in resonance delocalization with a second nitrogen so that the charge is extensively delocalized between the two nitrogen atoms (resonance hybrides) giving a polar character to entire molecule. Herz showed how such polarity triggers strong intermolecular attractive van der Waals forces between planar polycyclic molecules resulting in aggregation, or self-assembly, both in aqueous solution and on solid surfaces [12,13]. The effect of ICG concentration in aqueous solutions on UV–Vis absorption was determined between ICG concentrations of 3.22 and 32.2 μM and spectra are shown in Fig. 2. The curves indicate the spectra to be made up by the superposition of two bands, the first with a maximum at 780 nm is more prominent in dilute solutions (M-band) the second, with a maximum at 715 nm, is stronger in concentrated solutions (D-band). We ascribe the M-band to the monomeric species and the D-band to the dimeric species. The absorbance, A, of a dye solution, in the spectral wavelength range $[\lambda_1,\lambda_2]$ is a function of both wavelength and dye molar concentration, C. Although the mathematical expression for this surface is unknown, one can always consider the experimental absorbance as the superimposition of monomer and dimer contributions. Then we write,

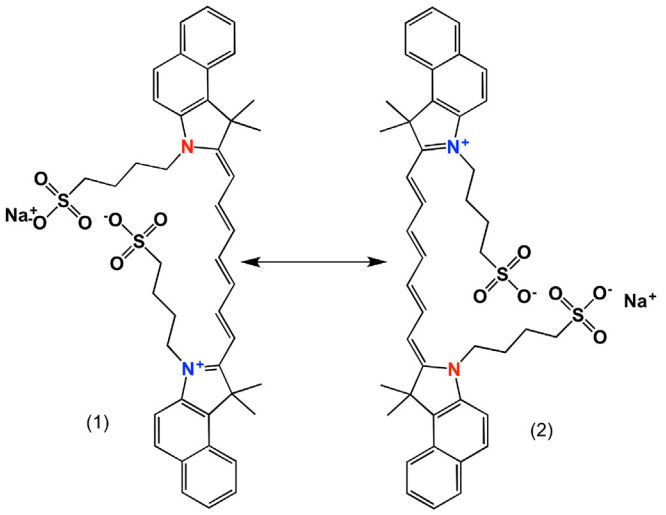

Fig. 1. Chemical structures of ICG. The two hybrid resonance delocalize the positive charge of a nitrogen atom over the entire molecule.

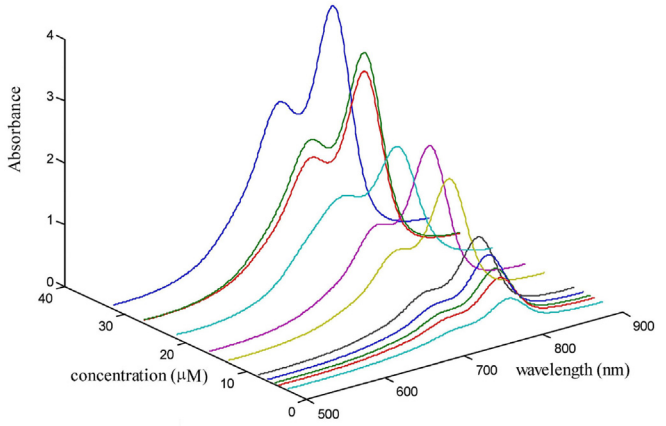

Fig. 2. 3D-plot depicting the complexity of the surface A(λ, C)for ICG in aqueous solution at 30 °C. The concentrations investigated are (3.22, 4.61, 5.38, 6.45, 8.06, 12.9, 21.5, 26.8, 26.9, 32.2)μM.

$$A(\lambda, C) = A_M(\lambda, C) + A_D(\lambda, C) \quad (1)$$

where λ is a wavelength (in nm) within the interval $[\lambda_1,\lambda_2]$, $A_M(\lambda,C)$ and $A_D(\lambda,C)$ are the partial absorption of monomeric and dimeric species in the dye solution at concentration C. Because of the mathematical complexity of the surface $A(\lambda,C)$ of Fig. 2, customarily the absorption is monitored at the wavelength of maximum absorption. However, a single wavelength poorly captures the magnitude of the absorption, the feature that is most directly structure-related and hence predictable. Herein we integrate the entire spectrum. In eq (1) the concentration dependence can be made explicit by using Beer's law,

$$A(\lambda, C) = \varepsilon_M(\lambda)\ell C_M + \varepsilon_D(\lambda)\ell C_D \quad (2)$$

where $\varepsilon(\lambda)$ is the molar extinction coefficient at wavelength λ and ℓ is the light path length. Since ℓ and C are independent of