Optica Applicata, Vol. XXXIX, No. 2, 2009

Measurements of solution fluorescence – a new concept

STEFAN GĘBALA

Gdynia Maritime University, Department of Physics, ul. Morska 83, 81-225 Gdynia, Poland;
e-mail: stgebala@am.gdynia.pl

The presented studies are based on the predictions of the behavior of certain types of fluorescence pigments in water solutions. Their asymmetric structure causes spontaneous surface concentration of the investigated sample. As a result, a densely packed, organized surface layer is created. This layer facilitates much better conditions for fluorescence than the rest of a sample. A set-up for solution surface fluorescence measurements has been assembled. This paper presents the results of the studies which confirm the occurrence of this phenomenon. The studies involved testing several biological samples. The described concept of fluorescence measurement has significant practical aspect.

Keywords: fluorescence, amphophility, surface self concentration, liquid crystal.

1. Introduction

Investigations into solution fluorescence are difficult [1]. Single molecules with fluorescent properties are surrounded by a great number of solution molecules which are insensitive to light. This is a reason for their relatively low efficiency. Besides, pigment molecules are subject to chaotic thermal movements. Components of these oscillations towards exciting light and towards emission cause the Doppler broadening of the emitted fluorescence lines. The instantaneous changes in these molecule concentrations in the measurement area also cause fluctuations of the registered light. Additionally, the part of inducing light can penetrate deep in the solution and excite the pigment energetic levels there. Fluorescence light, which is generated in the depth of a sample may be absorbed and then emitted several times on its way to the surface. The results of solution fluorescence investigation are difficult to interpret due to reemission and reabsorption phenomena. They require complicated recalculations based on not always reliable and clear assumptions. In comparison with these phenomena, the bathochromic effect of fluorescence, resulting from the pigment molecules shift at the moment of light absorption and their recoil at the moment of fluorescence quant emission, seems easy to interpret. Summarizing, the objective

difficulties during the solution fluorescence investigation are so significant that their spectra are not too specific and their analyses provide very limited possibilities of practical applications [2].

2. Amphophilic molecules' behavior in solutions

Molecules of fluorescent pigments are not symmetric [3]. Some of them have amphophylic structures, i.e. just like molecules of surface active compounds, they have the endings with different water affinity. One end is more hydrophilic and the other is more hydrophobic (lypophylic). A good example comes with flavons, specific substances of plant origin, which are common in nature. Figure 1 presents a molecule of chrysine, typical flavon. At the upper end of this molecule hydrophobic phenyl ring appears while at the bottom hydrophilic hydroxyl groups are observed. Molecules with such structures should spontaneously concentrate at a free surface of a water solution, *i.e.* at the border between water and air. The hydrophilic endings are pulled towards the water solution, while the hydrophobic ones are pushed above. When amphophylic molecules occur in the sample, they have a minimum potential on its free surface. They migrate from the depth to the surface. Finally, the pigment molecules are concentrated and generate a compact layer on the free surface. Amphophylic properties cause their uniform orientation in a perpendicular to surface layer direction. Small asymmetries

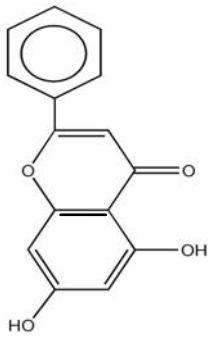

Fig. 1. Amphophyle molecule of chrysine.

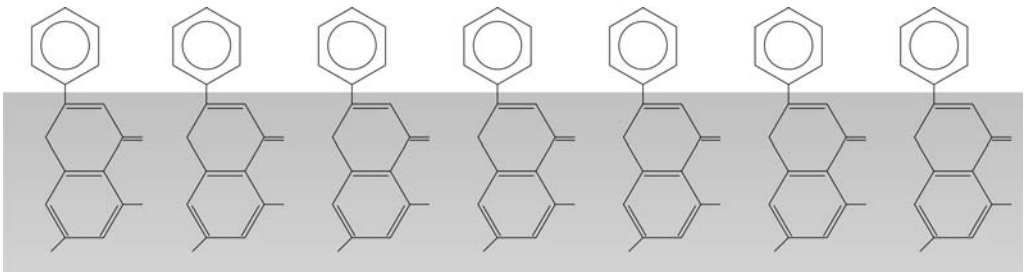

Fig. 2. Amphophyle molecules on the surface of water solution.

repeated in the molecular structure cause smaller interactions which put these molecules in order in the layer.

Therefore, it is possible that concentration of amphophylic pigments on the free surface of water solution creates an ordered fluorescent structure. This structure is similar to a singular layer of liquid, smectic crystal [4]. Fluorescent pigments may create a dense layer of stabilized and uniformly oriented molecules (Fig. 2). Concentration of fluorescent pigments in such structured monolayer should improve the properties of the registered fluorescence so that it should be similar to solid state fluorescence properties. Long-range forces stabilize pigment molecules in this structure. So a decrease in fluctuations of measured fluorescence intensity is observed. There are no any conditions for the Doppler disarray of fluorescent spectra to appear in such layer. Additionally, the bathochromic effect should not be registered. Like in crystals, the momentum of light quanta interacts with the mass of the whole of the pigment surface structure, not with the mass of a single molecule. Moreover, dense packing of fluorescent molecules on the sample surface (almost beyond the solution) should increase fluorescence efficiency and eliminate its disturbances with secondary effects, such as reabsorption and reemission.

3. Apparatus

A traditional measurement of solution fluorescence does not employ the concentration of pigments near the surface (Fig. 3). The illuminating light does not reach there. The part of the sample, where pigment molecules may not occur, is illuminated and all molecules move towards the surface. The traditional instruments for liquid sample fluorescence measurements are based on similar procedures. Therefore, there was a need for instrument modification. The geometry of the measurement had to be changed so that the excitement of the horizontal layer of pigments was possible as well as the registration of the fluorescence light created in this layer. Both basic elements

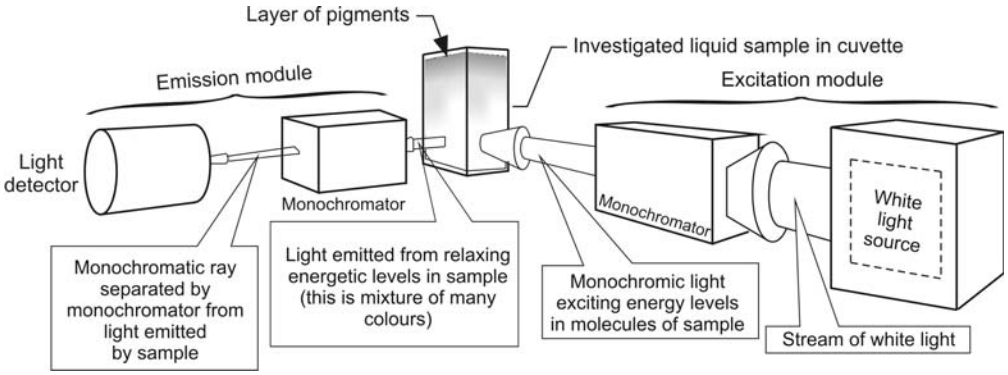

Fig. 3. Fluorescence measurement in traditional way.

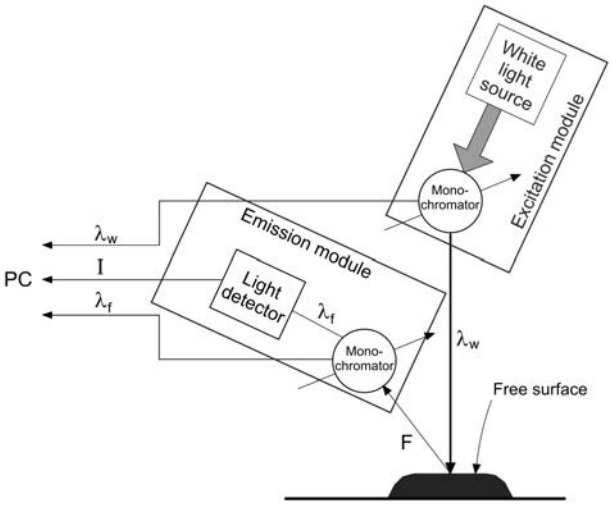

Fig. 4. Concept of surface fluorescence measurement.

of fluorescence measurement, *i.e.,* excitement and registration, had to be made from the top (Fig. 4).

4. Materials and methods

The concept must be verified. Natural polar solutions with lots of asymmetric pigment molecules were looked for. Honey, wine and potato mash were chosen for the studies.

One of the criterions for classification of honey available on the market is its botanical origin, *i.e*. its type. Different floral types of honey contain different kinds of fluorescent pigments from nectar of different types of plants. In favorable conditions, bees make clean types of honey. Besides, the market price structure is such that beekeepers tend to produce clean honey types. Thus samples of various types of honey are easily available, they are diverse and classified by the beekeepers. Moreover, there is an economically justified need for the preparation of an instrumental method for honey floral types identification. Thus the bee keepers were ready to cooperate in this matter.

The choice of wine was also based on common availability of samples. The need for an instrumental method, which would confirm wine authenticity was an additional reason.

Flavon chrysine (Fig. 1) is common in potatoes. Thus their consistence was changed in order to be able to seek the fluorescence from the surface structure of these model amphophilic pigment molecules. Also easily available samples supported the choice of potatoes too.

The studies were carried out using a set-up based on the Fluorat-02-Panorama spectrofluorymeter. A special adapter was built for it in order to change its traditional

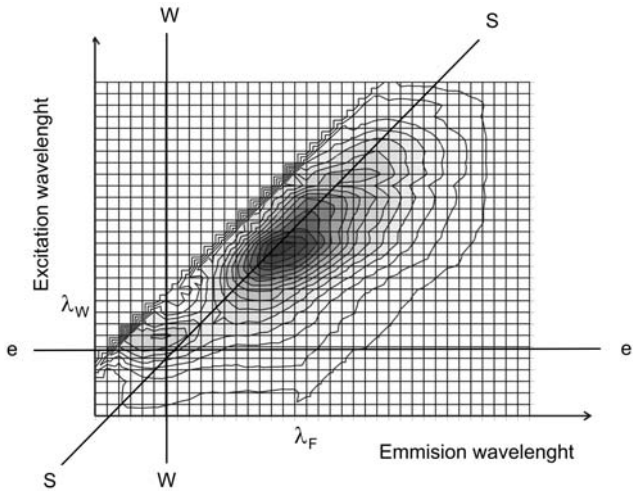

Fig. 5. Total fluorescent spectrum of buckwheat honey.

measurement range. Both the excitation and fluorescent light were transmitted using fiber-optic technology. Methodology was adjusted having in mind the tasks and the study potential. Traditionally, in order to determine the study area, light absorption is measured. Then in order to determine the energy levels, excitation and emission spectra are measured. A method for the measurement of light absorption in a pigment monolayer on a surface has not been fixed yet. The necessary range of measurements was impossible to determine on this way. Preliminary fluorescence measurements in the entire range of the set-up possibilities were made. In this way the available areas of surface fluorescence measurements have been determined. In order to study the fluorescence from surfaces of honey, wine or mashed potatoes, the set-up proved to have been appropriate. The complete spectrum is a 3-D surface and the excitation, emission and synchronous spectra are the transects: *ww*, *ee* and *ss* (Fig. 5). The synchronous spectra were placed along the direction with the highest fluorescence diversity, and those were analyzed.

5. Experimental verification

Solutions of natural origin may contain different fluorescent pigments. The presented concept is based on an assumption that the asymmetric structure of certain molecules contents in solution may cause their spontaneous concentration on its free surface. These pigments cover the surface of a sample with a thick fluorescent layer, while the other pigments remain in the solution. In such case, the excitation and registration of fluorescence from the top involves several processes (Fig. 6). Monochromatic light flux (1) reaches the layer from the top, penetrates the layer and is partly absorbed (2). The rest of light is absorbed in the solution (3), until it completely

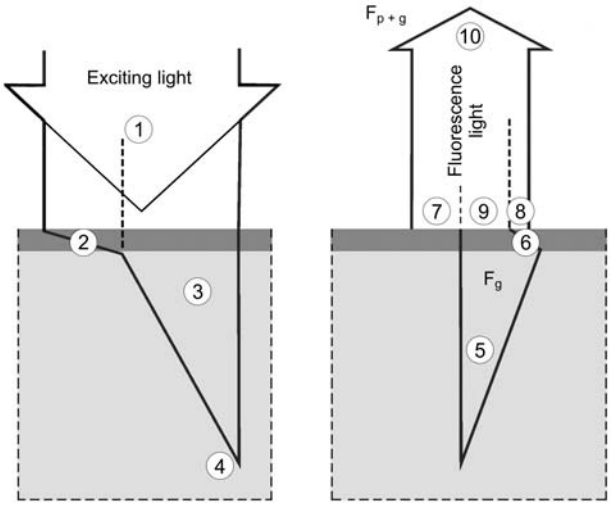

Fig. 6. Reabsorption and reemission inside arrangement of liquid sample with pigment layer on surface. 1 – fotoexcitation of sample surface, 2 – light absorption in pigment layer, 3– light absorption in volume of the solution, 4 – range of excitation light penetration, 5 – light emitted by pigment content in volume of solution, 6 – reabsorption in pigment layer, 7 – fluorescence from the pigment layer, 8 – reemission from the pigment layer, 9 – fluorescence from volume of the solution not absorbed in the pigment layer, 10 – light flux emitted over the sample, F_{p+g} – fluorescence registered above the sample free surface, F_g – fluorescence registered under the surface.

disappears (4). The excited energetic levels in the solution become the sources of fluorescence F_g (5), and on its way outside the sample it is partly absorbed in the surface layer (6). The part that is not absorbed (9), adds to fluorescence from the surface layer: primary (7) and secondary (8). This creates emitted light flux over the sample surface F_{p+g} (10). With properly weak excitation light flux, secondary processes (6 and 8) may be disregarded. Thus the surface fluorescence will be a difference between fluorescence registered above the sample surface (F_{p+g}) and below it (F_g). This is very clear for different wine types (Fig. 7) [5]. Similar results were also obtained for samples of different honey types (Fig. 8) [6, 7] and potato mash (Fig. 9) [8]. It can be observed that the surface fluorescence (F_p) has got much greater impact on total fluorescence than it can be deduced from the contribution of the volume of this layer in the entire sample volume. This means that on the surface of these solutions there are layers which produce significant part of fluorescence registered above.

6. Conclusions

Although the presented plots of solution fluorescence spectra have not been smoothed, they seem to be exceptionally appropriate. The liquid sample fluorescence measured

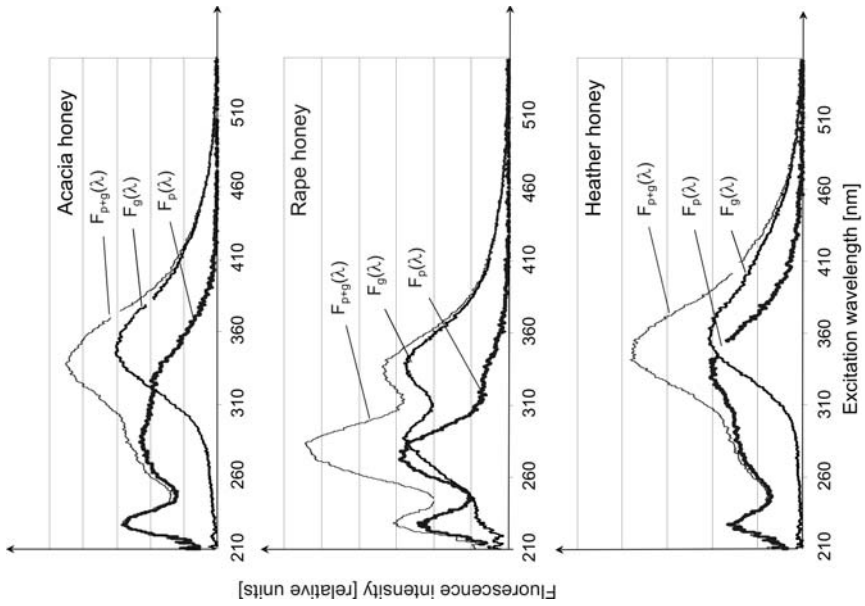

Fig. 8. Different fluorescence spectra of different types of honey registered above the sample surface $F_{p+g}(\lambda)$, under the surface $F_g(\lambda)$ and the calculated surface fluorescence spectrum $F_p(\lambda)$.

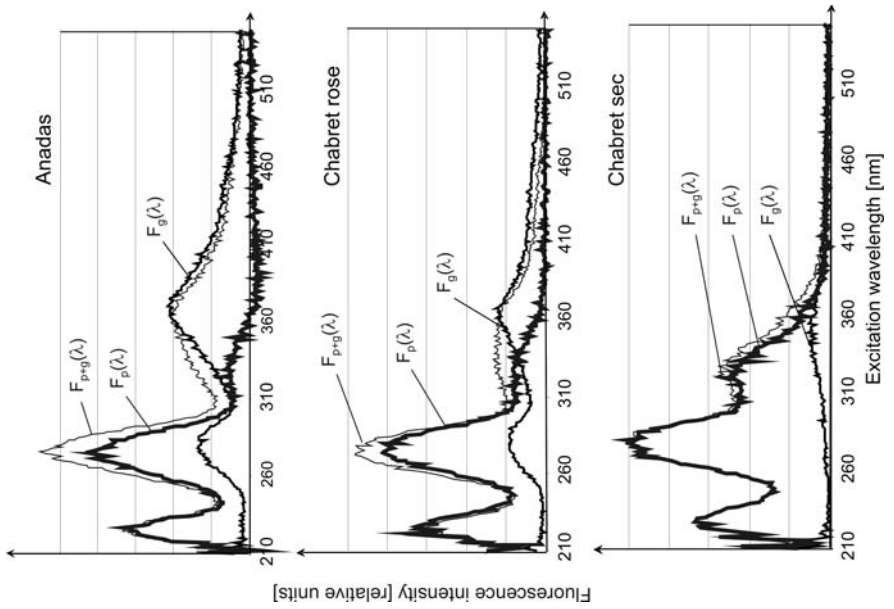

Fig. 7. Fluorescence spectra of several types of wine registered above the sample surface $F_{p+g}(\lambda)$, under the surface $F_g(\lambda)$ and the calculated surface fluorescence spectrum $F_p(\lambda)$.

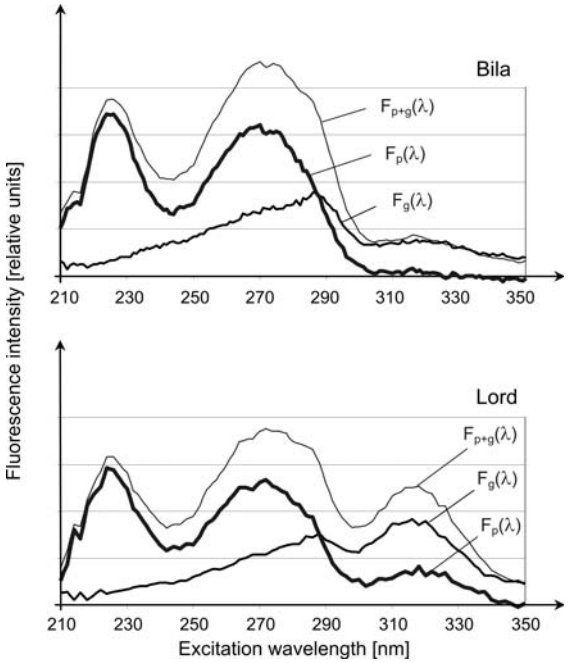

Fig. 9. Fluorescence spectra of two potato kinds.

in new geometry appears to be more efficient, stable and repeatable than the traditional one [2, 7].

1. It has been proved that fluorescence layer may appear on the surface of certain water solutions.

2. The phenomenon may occur in other water solutions which contain pigments with amphophylic molecules.

3. The properties of the registered fluorescence indicate potential ordering of liquid crystal in the pigment monolayer.

4. Spontaneous surface concentration and stabilization of pigment molecules dissolved in the solution facilitates easier fluorescence application in natural sample investigations.

References

[1] KAWSKI A., *Fotoluminescencja roztworów*, PWN, Warszawa, 1992 (in Polish).

[2] RUDOFF K., KAROUI R., DUFOUR E., LUGINBÜHL W., BOSSET J.-O., BOGDANOV S., AMADO R., *Autentication of the botanical origin of honey by front-face fluorescence spectroscopy. A preliminare study*, Journal of Agricultural and Food Chemistry **53**(5), 2005, pp. 1343–1347.

[3] BARLTROP J.A., COYLE J.D., *Principles of Photohemistry*, Wiley, 1978.

[4] ADAMCZYK A., *Ciekłe kryształy*, [In] *Encyklopedia Fizyki Współczesnej*, PWN, Warszawa, 1983, pp. 489–499 (in Polish).

[5] GĘBALA S., PRZYBYŁOWSKI P., *Possibilities of using the fluorescence in wine research*, Polish Journal of Food and Nutrition Sciences **57**(3), 2007, pp. 363–366.

[6] GĘBALA S., PRZYBYŁOWSKI P., TARGOWSKI W., *Conception of measurement and numerical analysis of surface fluorescent spectra of honey*, Bromatologia i chemia toksykologiczna, Vol. 37, Warszawa 2005, pp. 133–139 (in Polish).

[7] GĘBALA S., PRZYBYŁOWSKI P., BORAWSKA M., PIEKUT J., *The classification of natura honeys based on the surface spectrofluorimetry shapes analysis*, Bromatologia i chemia toksykologiczna, Vol. 37, Warszawa 2005, pp. 627–631 (in Polish).

[8] GĘBALA S., *Zastosowanie metody SSSA do pomiaru fluorescencji soku ziemniaczanego*, [In] *Materiały IV Konferencji Naukowej "Ziemniak spożywczy i przemysłowy oraz jego przetwarzanie"*, Wrocław 2006, pp. 88–89 (in Polish).

Received December 28, 2007
in revised form June 2, 2008