Bichromophoric sensor for the measurement of viscosity by fluorescence proportion

Patent Application: **MX/a/2015/016923**
Status: patent pending

Abstract: The present invention relates to novel fluorescent molecules sensitive to viscosity, for use in the study of heterogeneous systems. Also, the present invention relates generally to the design and synthesis of new bichromophoric molecules and its use as viscosity sensors.

Background

Fluorescent sensors are molecules whose emission of **fluorescence variates with respect to the properties of the sample** in which they are contained. These sensors allow the determination of physical and chemical properties in systems that cannot be analyzed by conventional techniques.

Spectroscopic and microscopic techniques used for the measurement of **fluorescence:**

- ✓ Have a high sensitivity,
- ✓ Are not very invasive and
- ✓ Allow the temporal resolution of changes observed in samples.

Their main impact, for example, is in biological sciences, where they help with the measurement of living cellular media. When analyzing samples of certain properties **through changes in the intensity of the observed fluorescence,** one of the determining factors is the **concentration** of the fluorescent sensor present on the sample.

The solution to this has been the use of concentration-independent properties: **lifetime of fluorescence** and **proportion of fluorescence**. Both can be used to measure a sample's viscosity, but there are some differences:

Lifetime of fluorescence	Fluorescence proportion
× Requires specialized microscopes	✓ May be applied in conventional fluorescence microscopes
× Requires more time for the data acquisition than the needed for measurement of fluorescence intensity (slow)	✓ The data acquisition time allows for real time resolution of processes (faster)

The fluorescence proportion is based on fluorescent sensors which emit light in **two different regions of the electromagnetic spectrum**, where the intensity of the emission on one side is influenced by a property of the media (**viscosity**, for example), while the other emission does not change and may be used as internal reference. Using proportion between fluorescence intensity in the two regions of emission, we obtain a measurement, which is **independent from the concentration** of the sensor.

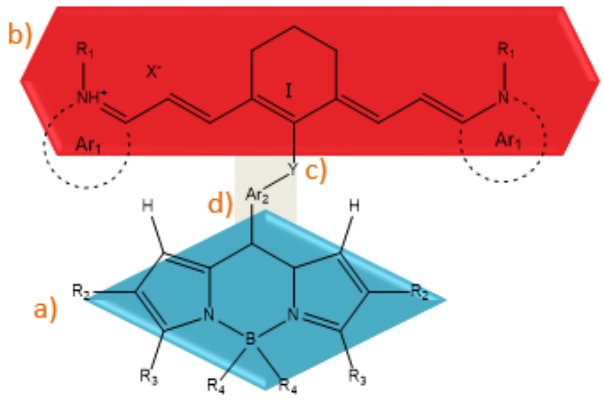
Description

The invention describes new fluorescent sensors with the general formula (I) for the microscale measurement of viscosity by the technique of fluorescence proportion.

The sensors are bichromophoric molecules formed as follows:

a) **BODIPY:** Good absorption in the visible spectrum (480-500 nm)
b) **Cyanine:** Emits in the close IR
c) **Bridge:** Ether or thioether group with simple sigma bounds. Keeps a) and b) electronically isolated
d) **Aryl group:** Donates electrons to form a Charge Transference State (CTS) (with a)

The process includes the stimulation of the BODIPY with a

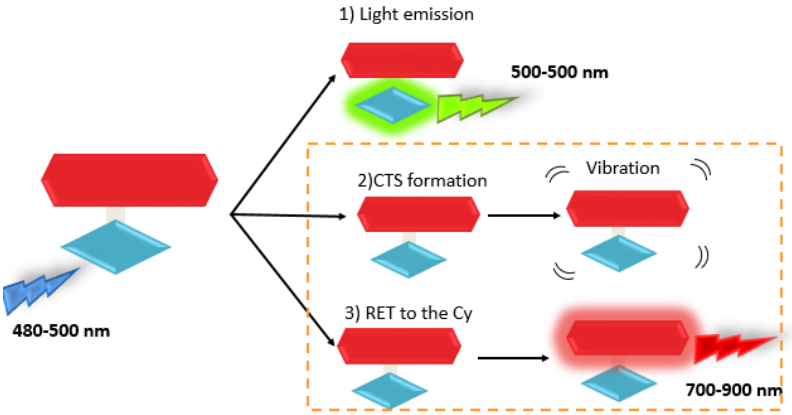


Formula (I)

wavelength between 480-500 nm. This brings the BODIPY to an excited state and has three ways to relax its state:

1) The BODIPY emits light in a wavelength of 500-550 nm (viscosity independent)
2) The BODIPY forms a Charge Transference State (CTS) with the electrons from the aryl group and subsequently has a vibrational relaxation (inversely proportional to the viscosity)

3) The BODIPY has a Resonance Energy Transference (RET) to the Cyanine, which emits light in a wavelength between 700-900nm. (directly proportional to the viscosity)



After making the measurements, it is possible to make a proportion between the BODIPY fluorescence (used as reference, due to its viscosity independence) and the Cyanine fluorescence (conditioned to the viscosity).

Stage of research

The research group made the design and synthesis of the bichromophoric sensor and its variants, as well as the verification of its chemical structure through NMR studies.

They have also proved the utility of these systems in the measurement of solvent viscosity with the fluorescence proportion method. For this purpose, high purity alcohols with known viscosity were used.

Application fields

In the technique of fluorescence proportion bichromophoric molecules are used to measure properties like temperature, polarity and viscosity. The measurement of viscosity in non-homogenous media is useful in:

- **Chemistry**: where it is used to measure the advancement of reactions such as polymerizations.
- **Biology**: where the viscosity of the media affect protein folding.
- **Medicine**: this method has been proposed as a method of diagnosis of illness.

The compounds described here can also be used for the formation of images with already known methods. For example, the formation of images can be done in biological samples like living or dead cells, or solid samples like polymers.

The cyanine and BODIPY used in the bichromophoric probe of this invention fulfill the requirements of absorption and fluorescence performance necessary for their use in **microscopy.** Their high biocompatibility also allows for its use in living systems.

Advantages

The proposed molecule in this invention presents several advantages in comparison with other known molecules:
The chromophores used in the synthesis:

- ✓ have **good optical properties** (fluorescence absorption and emission).
- ✓ are **easily introduced** inside living cells.
- ✓ are not very **toxic**.

The process has the following advantages:

- ✓ **Visible Light Irradiation:** the wavelength used to excite compounds of formula I is in the visible light region of the spectrum. This is less invasive to the sensor and the sample studies than UV light which is used with similar molecules. Besides, visible light reduces the autoflorescence of the sample improving the obtained signal.

- ✓ **Major spectral separation between emission maxima:** the two regions of fluorescence emission is larger and therefore exist less interference between the emissions, improving the quality of the measurement and allowing to isolate the two signals without losing fluorescence intensity by using optical elements such as filters.