

Fluorescence spectroscopy

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FLUORESCENCE SPECTROSCOPY

Introduction

Fluorescence spectroscopy, also called fluorometry or spectrofluorescopy, is a type of spectroscopy, used to MEASURE IDENTIFY AND MEASURE CONCENTRATIONS IN a sample. The process implicate the excitation of the electrons in molecules of specific compounds by a beam of light (typically, ultraviolet Light) and induces them to emit Light (usually, visible Light). To measure fluorescence is used devices called fluorometers. The main use of this process is in research fields for analyzing organic compounds in areas, such as medicine, biochemistry and chemistry.

Importance in clinical medicine

Due to the high sensitivity and specificity of Fluorescence spectroscopy, this is an important diagnostic and research tool in medicine. Nowadays, Fluorescence spectroscopy is being used in medical microbiology field with a lot of aims, such as pseudomonad taxonomic purpose at species and genus level, diagnosis of fungal infections and in the detection of virus.

According to many studies, this process is also considered a promising diagnostic technique for microorganisms associated diseases diagnosis when combined with spectroscopic fingerprints. Furthermore, it can be used to study the pathophysiological steps of several microorganisms.

Another common use of this technique is in the identification of hormones, alkaloids and vitamins in formulations and biological fluids. And, also, in low dosage drug formulations (containing less than 1mg per dose unit) and biological samples with low concentration of drugs, since it is necessary the high sensitivity of spectrofluorimetry.

Advantages and Disadvantages

As it was already pointed, one of the most important advantages of this technique is due to its high sensitivity and specificity. Another is its fast and rapid diagnosis ability.

The main disadvantage is that not all compounds fluoresce.

How does it work?

Instrumentation

There are two types of devices for this technique: Filter fluorometers and spectrofluorometers.

The main difference between them is the way that they isolate the incident Light and fluorescent light. In the Filter fluorometers it is used filters to do that, while in the Spectrofluorometers it is used diffraction monochromators.

Process

The process itself is the same for both of the instruments. Basically, there is an excitation source which provides Light. The Light passes through a filter or monochromator and reaches the sample. Then, there will be a proportion of the incident Light which will be absorbed by the sample, making some of the molecules in the sample fluoresce. Since, the fluorescent Light is emitted in all direction, some will pass through a second filter or monochromator and will reach the detector, placed at 90° to the incident Light beam to reduce the probability of transmitted or reflected incident Light reach the detector.

Conclusion

Currently, almost every diagnostic procedure used for the diagnosis of microorganisms have some barriers that should be overcome. Studies states that Fluorescence spectroscopy already overcomes some of those limitations and, also, that this technique has the potential to improve even more in the near future. For example, with the integration of fiber optic systems, in order to dignose microorganisms in vivo.

Another future improvement that should be done is related to the automatization of this technique that will make possible the diagnostic many samples at the same time.

References

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