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Pendant-drop method coupled to ultraviolet-visible spectroscopy: A useful tool to investigate interfacial phenomena

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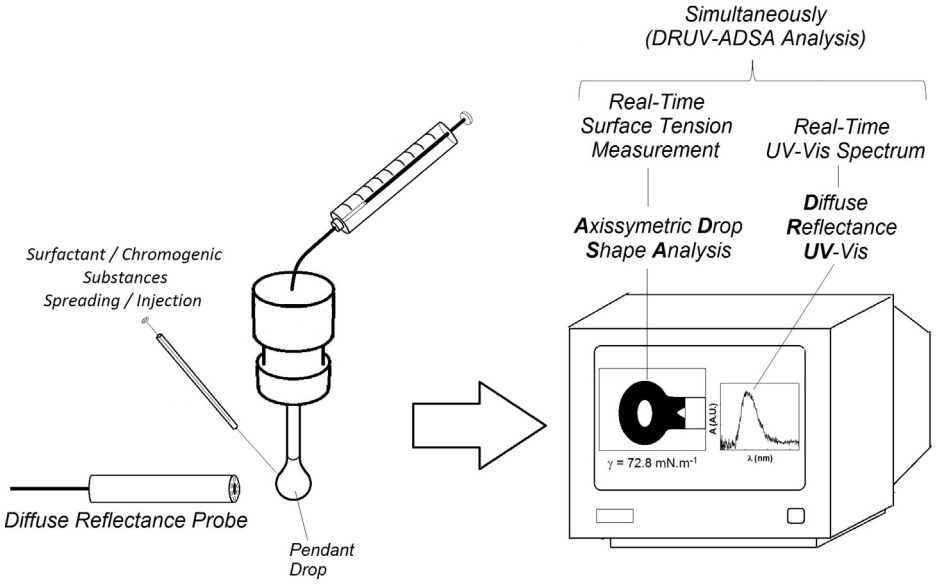
HIGHLIGHTS

- A new approach to simultaneously investigate surface tension and UV–vis spectra was developed.
- The axisymmetric drop shape analysis coupled to diffuse reflectance spectroscopy was used.
- The analysis requires low sample volumes and small analyte concentrations.
- The disaggregation of an organic dye driven by surfactants molecules was followed.
- The activity of an enzyme in the presence of phospholipid monolayers was investigated.

GRAPHICAL ABSTRACT



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ABSTRACT

UV–vis spectroscopy is a powerful tool to investigate surface phenomena. Surface tension measurements coupled to spectroscopic techniques can help to elucidate how the interface organization influences the electronic properties of molecules. However, appreciable sample volumes are usually necessary to achieve strong signals during conduction of experiments. This study reports on the simultaneous acquisition of surface tension data and UV–vis spectra by axisymmetric drop shape analysis (ADSA) coupled to

Abbreviations: ADSA, axisymmetric drop shape analysis; UV–vis, ultraviolet-visible; DRUV, diffuse reflectance at the UV–vis; PDT, photodynamic therapy; GPI, glycosylphosphatidylinositol; SDS, sodium dodecylsulfate; AO, acridine orange; TNAP, tissue-nonspecific alkaline phosphatase; DPPC, dipalmitoylphosphatidylcholine; AChol, cholestenone; SD, standard deviation; pNPP, *p*-nitrophenylphosphate; pNP^{-}, *p*-nitrophenolate.

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diffuse reflectance (DRUV) spectrophotometry using a pendant microliter-drop that requires small sample volumes and low analyte concentrations. Three example systems gave evidence of the applicability of this technique: (a) disaggregation of an organic dye driven by surfactant as a function of the surface tension and alterations in the UV–vis spectra, (b) activity of a glycosylphosphatidylinositol anchored enzyme estimated from formation of a colored product, and (c) interaction between this enzyme and biomimetic membrane systems consisting of dipalmitoylphosphaditylcholine and cholestenone. Apart from using smaller sample volume, this coupled technique allowed to investigate interfacial organization in the light of electronic spectra obtained *in loco* within a shorter acquisition time. This procedure provided precise interfacial information about static and dynamic systems. This has been the first study describing the kinetic activity of an enzyme in the presence of phospholipid monolayers through simultaneous determination of the surface tension and UV–vis spectra.

1. Introduction

The surface tension (γ) of pure liquids or solutions can be measured by many experimental approaches. The pendant drop method relies on axisymmetric drop shape analysis (ADSA) of γ measured at the liquid-air or liquid–liquid interface. Because ADSA uses a microliter sample, this technique is very useful when only a small amount of the target sample is available [1,2]. The traditional methods applied to determine γ usually needs the use of a plate or capillary in contact with the surfaces, thus causing a perturbation at the interface. However, by using ADSA it is possible a γ measurement based in the drop shape, without any external interference. Determination of γ is based on the Laplace equation of capillarity, which relates the pressure across the interface (ΔP) with γ and the principal curvature radii (R_1 and R_2):

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad (1)$$

This approach allows one to investigate the viscoelastic properties of the surfactant adsorption layer [3] and even to construct a surface pressure (π)–molecular area (A) isotherms from insoluble monolayers [4]. Construction of π-A isotherms by traditional techniques that employ Langmuir troughs requires at least milliliters of the liquid subphase and milligrams of the surfactants. However, the use of the pendant microliter-drop as the subphase demands reduced sample volumes and only a very small amount of the surface active compounds. The spread step is crucial for formation of a monolayer at the drop surface [5]: γ depends on the amount of surfactant adsorbed onto a specific drop surface area, so this step will provide specific γ or π values at a certain concentration. In turn, π corresponds to the decrease in γ of the pure aqueous solution (γ_0) in the presence of the surfactant:

$$\pi = \gamma_0 - \gamma \quad (2)$$

Apart from enabling γ determination, the use of reduced sample volumes is also interesting for other types of analyses, especially when an appreciable analyte concentration is necessary to achieve a considerable signal but small amounts of the analyte are available. The environment of a pendant microliter-drop requires low quantities of the sample because the volumes of the drops are in the order of dozens of microliters.

Another important feature of ADSA is the possibility of coupling simultaneous analysis. The drop can act as a microreactor in which hyphenated techniques aid monitoring of chemical reactions. UV–vis spectrophotometry is a convenient tool to quantify an analyte and to analyze the kinetics or even the dynamics of different systems. The traditional approach uses cuvettes and volumes in the order of milliliters. However, a diffuse reflectance UV–vis (DRUV) probe pointed at the pendant drop in an ADSA apparatus allows one to follow interfacial changes in the systems by spectroscopy. Similarly, McMillan et al. developed a patented technique of UV–vis analysis in the lifecycle of falling drops from the signal tensiotrace of an amplitude-modulated light, also comparing its experimental approach with the traditional UV–vis spectrophotometry [6], and even coupling it to a γ measurement that provides additional interfacial data of surfactant-containing systems [7]. Simultaneous γ and UV–vis analyses using sample volumes in the order of microliters provides an appreciable absorption signal for a low amount of sample. The increased area/volume ratio in a drop ($\sim$1.5 $m^2\,L^{-1}$) as compared to the area/volume ratio in a cuvette ($\sim$0.1 $m^2\,L^{-1}$) and in the Langmuir trough ($\sim$0.2 $m^2\,L^{-1}$) makes the interfacial phenomena become more relevant than the bulk phenomena.

DRUV-ADSA can be applied to study systems where interfacial processes result in spectroscopic changes. More than evidencing processes that occur at the drop microenvironment through UV–vis analysis, DRUV-ADSA also provides data about interfacial activity; allowing the elucidation of possible relations between bulk and surface. Monolayers of insoluble surfactants (such as phospholipids) and micelles of soluble surfactants constitute biomimetic systems. These model systems are widely employed to mimic the interaction of cell membranes with enzymes or other compounds, such as organic dyes used as photosensitizers in photodynamic therapy (PDT) [8,9]. DRUV-ADSA simultaneous analysis also aids investigation into the interaction between micelles and fluorescent dyes. This technique not only gives information about dye disaggregation, but it also reveals the interfacial features of the system through monitoring of the γ of the drop. DRUV-ADSA also allows for enzymatic kinetics monitoring. Spectrophotometric probing of product formation or reactant consumption is the traditional way to perform enzymatic activity analysis. This monitoring can be either discontinuous, by stopping the reaction after a time interval, or continuous, by following the reaction in real time. Enzymes attached to the lipid bilayer of the cell membrane via a glycosylphosphatidylinositol (GPI) anchor display surface activity [10,11]: GPI provides a hydrophobic moiety, whereas the polypeptide chain corresponds to the hydrophilic part. Because these enzymes act as surfactants, it is possible to verify how their interaction with lipid monolayers [12] affects their activity. One of the advantages of the DRUV-ADSA method is that it employs reduced amounts of expensive reactants like proteins and phospholipids. Moreover, this technique allows for simultaneous monitoring of the UV–vis and π or γ alterations in an increased surface area environment, making changes related to surface adsorption processes more important.

In the present study, three example systems provided evidence of the applicability of the DRUV-ADSA technique: (a) sodium dodecylsulfate (SDS)-induced disaggregation of the organic dye acridine orange (AO), used in photodynamic therapy [13], as a function of γ monitored by the DRUV-ADSA technique; (b) activity of a glycosylphosphatidylinositol anchored enzyme estimated from the formation of a colored product as com-