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A dynamic liquid–liquid interfacial pressure detector for the rapid analysis of surfactants in a flowing organic liquid

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Abstract

Design and development of a dynamic interfacial pressure detector (DIPD) is reported. The DIPD measures the differential pressure as a function of time across the liquid–liquid interface of organic liquid drops (i.e., *n*-hexane) that repeatedly grow in water at the end of a capillary tip. Using a calibration technique based on the Young–Laplace equation, the differential pressure signal is converted, in real-time, to a relative interfacial pressure. This allows the DIPD to monitor the interfacial tension of surface active species at liquid–liquid interfaces in flow-based analytical techniques, such as flow injection analysis (FIA), sequential injection analysis (SIA) and high performance liquid chromatography (HPLC). The DIPD is similar in principle to the dynamic surface tension detector (DSTD), which monitors the surface tension at the air–liquid interface. In this report, the interfacial pressure at the hexane–water interface was monitored as analytes in the hexane phase diffused to and arranged at the hexane–water interface. The DIPD was combined with FIA to analytically measure the interfacial properties of cholesterol and Brij®30 at the hexane–water interface. Results show that both cholesterol and Brij®30 exhibit a dynamic interfacial pressure signal during hexane drop growth. A calibration curve demonstrates that the relative interfacial pressure of cholesterol in hexane increases as the cholesterol concentration increases from 100 to 10,000 $\mu g\,ml^{-1}$. An example of the utility of the DIPD as a selective detector for a chromatographic separation of interface-active species is also presented in the analysis of cholesterol in egg yolk by normal-phase HPLC-DIPD.

Keywords: DIPD; DSTD; Flow-based technique; Chromatography; FIA; Liquid–Liquid interface; Cholesterol; Brij®30

1. Introduction

Surfactants are chemical species that concentrate and align preferentially at an interface due to the presence of both hydrophilic and hydrophobic moieties within their structure. Alignment at the air–liquid surface (surface tension), solid–liquid and liquid–liquid interfaces (interface tension) often results in the lowering of surface tension at the given interface. Due to their unique properties (i.e., the ability to lower surface tension, foam, and create emulsions), surfactants and other interface-active species are widely used in many industries including food and beverage, personal care products, cosmetics, metal working, mining, agriculture, paper, and leather. Furthermore, surface-active chemicals are used in large quantities for many household applications.

Several methods have been developed for the measurement of surface tension. Most of these methods can be divided into three main groups: force methods (Du Noüy ring and Wilhelmy plate), shape methods (sessile drop, pendent drop, and spinning drop), and pressure methods (small bubble

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surfactometer) [1]. When a surfactant-containing solution forms a fresh interface, a finite time interval must elapse before equilibrium surfactant concentrations can be partitioned between the interface and the bulk solution. The aforementioned methods actually measure the static surface tension since the measurement is made after the interface-active analytes attain equilibrium at the air–liquid interface. The non-equilibrium interface tension is known as the dynamic interface tension. Most surfactant-driven processes, such as foaming, emulsification, coating flows and wetting, take place under non-equilibrium conditions, and therefore, dynamic interface tension information is an imperative parameter for understanding these processes. In principle, some of the surface tension measurement methods described above can be used to measure dynamic surface tension, however, most are not appropriate for high-throughput measurements. A few of the most common dynamic surface tension techniques are maximum bubble pressure [2], oscillating jet [3] and fast forming drop [4].

In the study of surfactants, another area of interest is the interfacial tension at the liquid–liquid interface. Specifically, adsorption at liquid–liquid interfaces plays a key role in the dynamics of multiphase systems. Though the principles are similar to adsorption at the air–liquid interface, measurements at the liquid–liquid interface require unique instrumentation. Ravera et al. reviewed the use of several techniques for liquid–liquid interfacial pressure measurements and found that while in theory many of the techniques commonly employed for air–liquid measurements can also be used for liquid–liquid interface studies, in practice, however, many obstacles, such as solvent miscibility, density, and viscosity came to the forefront [5]. Thus, relative to measurements at the air–liquid interface, few instruments are available for liquid–liquid interface studies and even fewer of these measure dynamic interfacial tension. The lack of methods to measure liquid–liquid interfaces is likely due to the difficulty of designing the instrumentation.

Most of the research in the area of liquid–liquid interfacial measurements focuses on the physics of adsorption at the interface, and the development and testing of theories to understand the kinetics and thermodynamics of adsorption at the interface [4–11]. Instead, we focus on the dynamic analytical signal resulting from adsorption at the liquid–liquid interface and have developed a simple and robust instrument to detect and quantify interface-active analytes in organic samples. The technique presented here offers a mechanically simple and inexpensive means to measure the liquid–liquid interface. This instrument can also be used to qualitatively describe the adsorption kinetics of interface-active analytes and the interactions of mixtures that affect the interfacial pressure [12,13].

Another important attribute of surface and interfacial tension measurement methodologies is the ability to carry out real-time analysis on dynamic systems containing complex and interacting surfactants. Previously, the dynamic surface tension detector (DSTD) was developed as a detection system for flow-based techniques, such as high performance liquid chromatography (HPLC), flow injection analysis (FIA), and sequential injection analysis (SIA). The DSTD responds to the surface activity of analytes at the air–liquid interface and offers a mechanically simple and inexpensive means to measure the air–liquid interface of a wide variety of samples, including samples that would foam using a bubble pressure technique [12–17]. The design and construction of the DSTD has previously been discussed in detail [12–19]. The DSTD has been used for the detection and study of species, such as common surfactants, polymers and proteins at the air–liquid interface. The most recent DSTD design is based on measuring the interior pressure throughout drop growth of repeatedly forming drops, which are pneumatically detached from a capillary tip.

Herein, we present an adaptation of the DSTD to form a dynamic interfacial pressure detector (DIPD) for the detection and analysis of species that are interface-active at the liquid–liquid interface. The DIPD complements the DSTD for evaluating chemical species that are either insufficiently soluble in aqueous media, or samples with unique liquid–liquid interface properties, or samples in which the dynamic interactions at the liquid–liquid interface are vital to their performance. In the presented experiments, the DIPD was coupled with FIA and HPLC for the study of the dynamic interface properties of typical surface-active species at the hexane–water interface.

2. Theory

The DIPD measurement follows principles similar to the DSTD [12]. In the DIPD, the internal pressure of repeatedly growing drops is measured relative to the pressure of the bulk solution phase in which the drops are grown, using a pressure transducer (see Figs. 1 and 2). Pressure is measured throughout the growth of the drop and the resulting drop pressure profiles are used to calculate the relative interfacial pressure.

The time-dependent Young–Laplace equation relates to the measured drop pressure, and was applied to calculate the

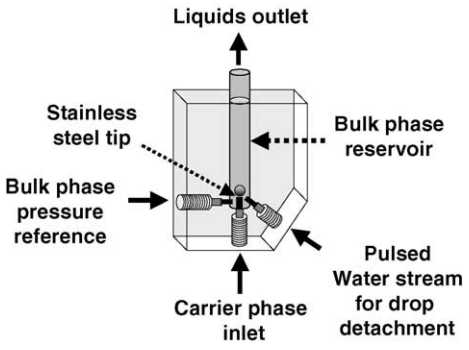

Fig. 1. A schematic diagram of the DIPD flow cell.

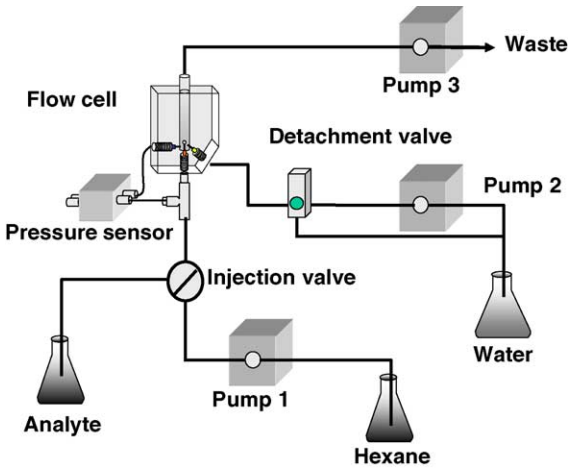

Fig. 2. A schematic diagram of FIA combined with DIPD for the liquid–liquid interfacial pressure measurement. Pump 1: piston pump at flow rate of 60 μl min^{-1} *n*-hexane, Pump 2: piston pump at flow rate of 4 ml min^{-1} water.

interfacial pressure from the pressure measurements:

$$P(t) = \frac{2\gamma(t)}{r(t)} + P_C \tag{1}$$

In Eq. (1), P is the drop pressure referenced to the water bulk phase pressure, γ is the liquid–liquid interfacial tension, r is the drop radius, and P_C accounts for viscous losses in the capillary tubing. The parameters, P, γ, and r, are functions of time, t, throughout the drop growth.

Drop pressure "profiles" are the raw signals obtained for the mobile phase ($P(t)_M$) (i.e., carrier stream), standard ($P(t)_S$), and analyte ($P(t)_A$). Combined with the dynamic interfacial pressure of the standard ($\pi(t)_S$), these three signals allow the dynamic interfacial tension of the analyte ($\pi(t)_A$) to be calculated using Eq. (2):

$$\pi(t)_A = \frac{\pi(t)_S \left[P(t)_M - P(t)_A\right]}{\left[P(t)_M - P(t)_S\right]} \tag{2}$$

The dynamic interfacial tension of the analyte ($\pi(t)_A$), calculated relative to the dynamic interfacial tension of the standard ($\pi(t)_S$), contains information regarding the diffusion and arrangement of analyte at the liquid–liquid interface.

It would be ideal to calibrate the DIPD sensor using a standard solution with known interfacial pressure $\pi(t)_S$. Likewise, it is advantageous to have a standard such that $\pi(t)_S$ is constant at a given standard concentration and independent of drop time, i.e, $\pi(t)_S$ is simply π_S. Unfortunately, due to the difficulty of liquid–liquid interfacial tension measurements, the liquid–liquid interfacial pressure of a suitable standard acting at the interface of *n*-hexane and water which has been confirmed by multiple techniques or multiple research groups could not be found in the literature [4,7,8,20–23]. For this reason, we report the relative interfacial pressure values, defined by Eq. (3):

$$\pi_R(t)_A = \frac{\pi(t)_A}{\pi_S} = \frac{\left[P(t)_M - P(t)_A\right]}{\left[P(t)_M - P(t)_S\right]} \tag{3}$$

Relative interfacial pressure is simply the interfacial pressure of the analyte divided by the interfacial pressure of the standard. Since the standard is consistent, the relative interfacial pressures of analytes can be compared to understand their interfacial properties and to analyze different analytes. In this work, isopropanol in *n*-hexane and *n*-octanol in *n*-hexane were evaluated, and applied, as standard solutions to achieve a relative interfacial pressure for analytes of interest from the DIPD, using Eq. (3). We found that both isopropanol and *n*-octanol align rapidly at the water–hexane interface thus providing a π_S independent of drop time.

3. Experimental

3.1. Materials

HPLC grade *n*-hexane, *n*-octanol and 2-propanol (isopropanol) were obtained from Fisher Scientific (Fisher Scientific international Co., Fair Lawn, NJ). Cholesterol (98% purity) and Brij®30 were purchased from Aldrich (Aldrich Chemical Co., Milwaukee, WI). All chemicals were used as received. Deionized (DI) water, demineralized to greater than 18 MΩ with a Millipore system (Millipore, Bedford, MA), was used as the bulk phase and in the preparation of all solutions. The water was degassed using an ultrasonic bath (Cole-Parmer Instrument Company, Vernon Hills, IL) with vacuum system for 15 min prior to use.

3.2. Dynamic interfacial pressure detector and its flow cell

A flow cell, fabricated from acrylic sheet, was designed in-house for this detector. A block of acrylic sheet was selected as the flow cell material because it is transparent, easily machined, and reasonably chemically hearty for demonstrating the detection principle. A schematic diagram of the DIPD flow cell is shown in Fig. 1. The flow cell is comprised of the following parts:

(1) Bulk phase reservoir
A 0.795 cm inside diameter (i.d.) × 2.22 cm long cylindrical hole was drilled into the block of acrylic sheet to serve as the bulk phase reservoir. In this work, water was used as the bulk phase and *n*-hexane was used as the carrier phase. To prevent *n*-hexane from adhering to the acrylic sheet flow cell wall, the bulk phase reservoir was lined with a 0.795 cm outside diameter (o.d.) × 4.40 cm long Teflon® tube (Brinkmann Instruments, Inc., Westbury, NY). This allowed the detached hexane drops to rise to the surface and be drawn to waste.

(2) Carrier inlet
The buoyant force operating on the carrier phase droplets must be sufficient to overcome the adhesive forces acting between the carrier phase and the tubing tip. The tubing tip material is chosen to minimize the adhesive forces, which consequently, minimizes droplet shape distortion. Since the DSTD uses an aqueous carrier phase, poly(etheretherketone), or PEEK™, is chosen as the tubing tip material. With the DIPD and a *n*-hexane carrier phase, however, it is more appropriate to use stainless steel as the tubing tip material. The stainless steel tip is situated inside a threaded inlet provided at the base of the flow cell. The stainless steel capillary sensing tip was made by cutting a syringe needle (Becton Drive 25 G × 11/2 in BD™, Franklin Lakes, NJ) to a length of 10 mm and polishing both ends of the tip until smooth. As can be seen in Fig. 2, a side-arm just outside the carrier inlet is connected to the pressure sensor (Validyne DP15-40-2430, Northridge, CA). With this design, most organic solvents with densities less than water and immiscible with water may be used as a carrier stream (mobile phase), such as *n*-hexane used herein.

(3) Hydraulic drop detachment
Hexane drops were hydraulically detached by degassed water flowing at a high flow rate and directed at the base of the forming hexane drop. The drop detachment stream, which flows water at 4 ml min^{-1} when briefly actuated, was regulated by an injection valve, and actuated at 2 s intervals. The hexane drop size can therefore be controlled by the detachment interval.

(4) Water pressure reference
The reference side of the pressure sensor was connected to the water reference inlet in the flow cell reservoir to measure the pressure in the bulk phase. The *n*-hexane carrier, under constant flow rate conditions, was delivered through the capillary sensing tip to form drops at the end of the tip. The drops were either detached with the hydraulic water stream or would ascend to the surface as buoyant forces overcome the forces of molecular attraction between the drop and the tip. When hydraulic detachment is employed, drops are shot with a pulsed stream of water and detached from the tip well before drops would have risen to the surface due to the buoyancy force. Hydraulic detachment is advantageous because it increases data density, improves drop time repeatability, and allows relative interfacial pressure to be calculated in real-time.

A pressure transducer continuously measured the pressure signal. The signal was conditioned with a high gain carrier demodulator (Validyne CD12, Northridge, CA). The raw pressure signal was collected at the rate of 100 Hz and instrument control was performed on a PC (1.4 GHz AMD Duron™ processor, Sunnyvale, CA) with a data acquisition board (AT-MIO-16XE-50, National Instruments, Austin, TX), using software written in-house (LabVIEW Version 6, National Instruments, Austin, TX). Data analysis was performed with MATLAB 6.0 (The MathWorks, Natick, MA).

3.3. Flow injection analysis (FIA)

A diagram of the FIA system coupled with the DIPD is shown in Fig. 2. A piston pump (Beckman, Model 114 M, Berkeley, CA) was used to introduce the carrier and sample to the flow cell at a flow rate of 60 μl min^{-1}. A second pump (Beckman, Model 114 M, Berkeley, CA) was required to control the flow of water into the flow cell chamber for hydraulic drop detachment. As the hexane drops are formed at the end of the stainless steel tip, they were detached with the pulse of water created by switching a valve in-line with the flow at a frequency of 0.5 Hz (2 s per drop). The detached hexane drops then rose to the surface and were aspirated out of the flow cell by a vacuum pump along with excess water from the hydraulic detachment process. This allowed the vacuum pump to maintain a stable bulk phase fluid level in the water reservoir.

The principles of FIA were utilized to introduce sample to the DIPD. A sample volume containing the interface-active analyte in *n*-hexane was injected into the *n*-hexane carrier using an injection valve (EC10W, Valco Instruments Co. Inc., Houston, TX) equipped with the appropriate length of PEEK™ tubing (Upchurch, Oak Harbor, WA) loop size. The sample plug then passed through PEEK™ tubing into the detector flow cell for analysis.

3.4. Normal phase high performance liquid chromatography

To demonstrate the operation of the DIPD as a selective HPLC detector, the FIA-DIPD instrument was modified to include a normal phase NP-HPLC cyano column (2.1 mm × 30 mm, Spheri-5, 5 μm, Brownlee Columns, Applied Biosystems, Inc., Foster City, CA) placed in between the DIPD flow cell and the injection valve. The *n*-hexane mobile phase was run at a flow rate of 60 μl min^{-1}. Cholesterol in egg yolk was separated by NP-HPLC and selectively detected by DIPD. To extract cholesterol from egg yolk, one egg yolk was mixed with 100 ml of *n*-hexane and the resulting biphasic mixture was shaken for two hours. Upon shaking, the cholesterol along with other organophilic compounds from the egg yolk partitioned into the *n*-hexane supernatant. Aliquots of the supernatant were injected into the NP-HPLC-DIPD system for chromatographic separation and detection.

4. Results and discussion

To test the response of the DIPD, and to ascertain the suitability of standards, a 100 μl plug of 0.5% isopropanol in hexane and a 100 μl plug of 0.1% octanol in hexane were injected separately into the water bulk phase of the DIPD

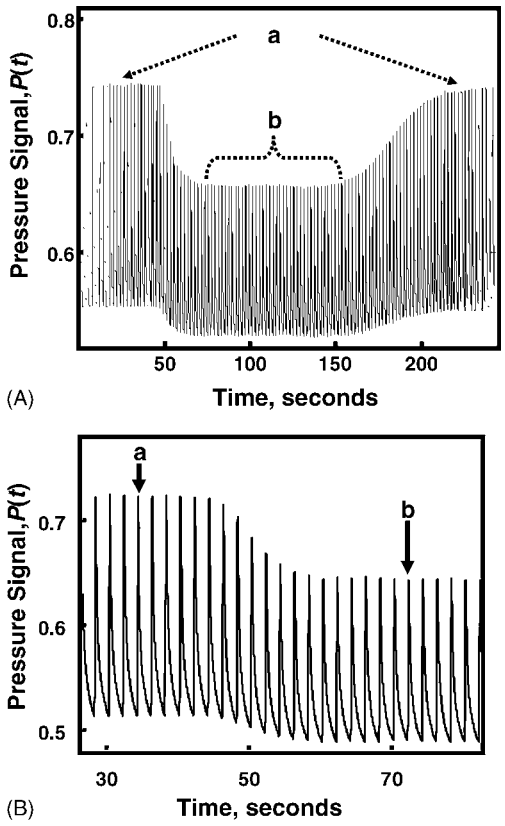

Fig. 3. (A) Raw pressure data, $P(t)$, obtained from the DIPD for a 100 μl injection of 0.1% *n*-octanol in *n*-hexane: (a) baseline signal of the *n*-hexane carrier phase; (b) steady-state concentration of 0.1% *n*-octanol in *n*-hexane. Each drop was detached from the end of the stainless steel tip at 0.5 Hz or every 2 s. (B) An enlargement of raw pressure data, $P(t)$, from Fig. 3(A). Each peak referred to the measured pressure of each drop that formed and detached at the end of the sensing tip every 2 s: (a) baseline signal of *n*-hexane; (b) steady-state concentration of 0.1% *n*-octanol in *n*-hexane.

system. Fig. 3(A) shows the raw drop pressure data, $P(t)$, of 0.1% *n*-octanol in *n*-hexane. During the first 50 s labeled "a", only *n*-hexane was present at the liquid–liquid interface. When the solution of 0.1% *n*-octanol in *n*-hexane reached the DIPD, labeled "b", the pressure signal, $P(t)$, decreased due to the lower interfacial tension (i.e., higher interfacial pressure) and achieved a steady-state signal that lasted for approximately 100 s.

In Fig. 3 (B), an enlargement of raw drop pressure data in Fig. 3 (A) is shown for clarity. The maximum of each of the sharp $P(t)$ profiles represents the maximum pressure of an individual drop. This maximum pressure, P_{max}, occurs when the liquid at the end of the sensing tip begins to form a new drop. Drops were detached from the capillary sensing tip every 2 s. Drop pressures decreased as the drop volume increased and were at their minimum values, P_{min}, just before detachment, according to Eq. (1).

Single drop pressure profiles of hexane, 0.5% isopropanol in hexane, and 0.1% octanol in hexane forming in the water bulk phase are shown in Fig. 4. These profiles were averaged from 10 raw $P(t)$ drop profiles. Comparing the pressure drop

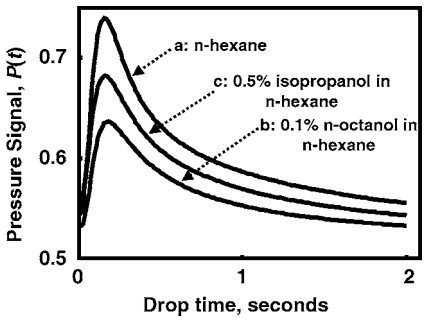

Fig. 4. Average drop pressure profiles of *n*-hexane (mobile phase carrier stream), 0.5% isopropanol in *n*-hexane, and 0.1% *n*-octanol in *n*-hexane from 10 drops under steady-state concentration conditions: (a) pure *n*-hexane drop; (b) 0.1% *n*-octanol in *n*-hexane; (c) 0.5% isopropanol in *n*-hexane.

profiles of *n*-hexane, 0.1% *n*-octanol in *n*-hexane, and 0.5% isopropanol in *n*-hexane illustrates that injecting small quantities of *n*-octanol or isopropanol into the *n*-hexane carrier dramatically lowers the interfacial tension (increases the interfacial pressure), and therefore, leads to a decrease in both P_{max} and P_{min}. The exquisite sensitivity of the DIPD is realized by noting that a volume of only 100 nl (equal to 0.1% of 100 μl) of *n*-octanol was injected into the *n*-hexane carrier, and since *n*-octanol achieved a steady-state concentration for a period of 100 s, *n*-octanol was measured with a high signal-to-noise ratio as it passed the DIPD at a rate of only 1 nl of *n*-octanol per second.

The drop pressure profiles of analytes that can be used as standards run parallel to the pure solvent drop pressure profiles from the pressure maximum to the drop detachment as shown in Fig. 4. Thus, *n*-octanol and isopropanol are good candidates as standards. Fig. 5 shows the relative interfacial pressure of 0.1% *n*-octanol and 0.5% isopropanol calculated using the drop pressure profiles shown in Fig. 4 and Eq. (3). The relative interfacial pressure of 0.1% *n*-octanol was calculated using 0.5% isopropanol as the standard and, likewise, the relative interfacial pressure of 0.5% isopropanol was calculated using 0.1% *n*-octanol as the standard. Fig. 5 shows that the relative interfacial pressure of both analytes is not

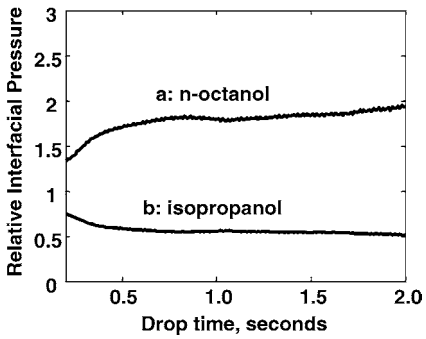

Fig. 5. Relative interfacial pressure of the analytes, after the drop pressure profile data in Fig. 4 were applied to Eq. (3): (a) the relative interfacial pressure of 0.1% *n*-octanol in *n*-hexane when 0.5% isopropanol in *n*-hexane was used as the standard; (b) the relative interfacial pressure of 0.5% isopropanol in *n*-hexane when 0.1% *n*-octanol in *n*-hexane was used as the standard.

dynamic over the measured drop growth (for drop time range of ~0.5–2 s) [16], therefore, both 0.1% *n*-octanol in *n*-hexane and 0.5% isopropanol in *n*-hexane were used as the standard solutions for sensor calibration, with 0.5% isopropanol used for calibration of the results reported herein. The calibration method defined in the Theory section (Eq. (3)) enables real-time calculation of relative interfacial pressure in a flowing system without the need of cumbersome optical measurements [16].

To demonstrate that the DIPD could be used to study the adsorption kinetics of interface-active molecules in *n*-hexane, the behavior of Brij®30 at the *n*-hexane–water interface was evaluated. The non-ionic surfactant, Brij®30 (Polyoxyethylene(4)lauryl ether), is a long chain polymer molecule that is soluble in both water and *n*-hexane. The Brij®30 hydrophobic chain is a lauryl group and the hydrophilic head group is polyoxyethylene ether. The characteristics of Brij®58 at the water/oil interface at constant drop size had been studied; although, in that work, the surfactant was dissolved in the aqueous phase [24]. The relative interfacial pressure of a 100 μl injection of 60 μg ml^{-1} Brij®30 in *n*-hexane is shown in Fig. 6(A) as a three-dimensional signal. Brij®30 shows

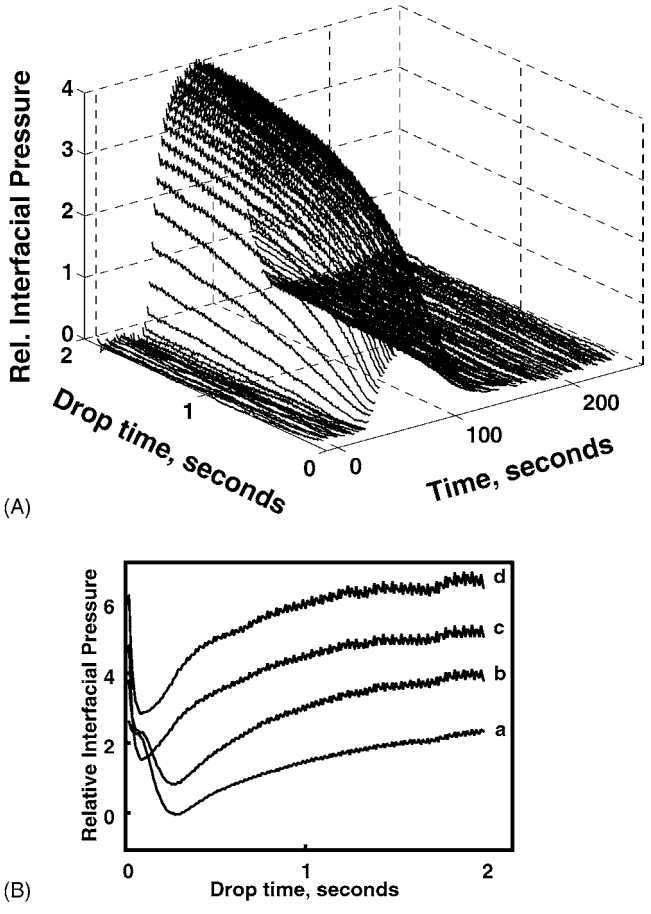

Fig. 6. (A) A three-dimensional relative interfacial pressure plot of 60 μg ml^{-1} Brij®30 in *n*-hexane using 0.5% isopropanol as standard. (B) A relative interfacial pressure plot of various concentrations of Brij®30 in *n*-hexane: (a) 20 μg ml^{-1}; (b) 40 μg ml^{-1}; (c) 60 μg ml^{-1}; and (d) 80 μg ml^{-1}.

a dynamic signal at this concentration and flow rate, with a relative interfacial pressure that changes by a factor of approximately four relative to 0.5% isopropanol in *n*-hexane as the standard, over the 2 s drop lifetime.

The DIPD was designed to detect interface-active analytes in a sample and give selective signals for analytes that do not quickly reach equilibrium at the interface. Due to the size of the Brij®30 molecule, as well as the concentrations studied, Brij®30 was in the process of diffusing and arranging at the interface during the DIPD measurement. Fig. 6 (B) shows the individual drop pressure profiles of Brij®30 at four concentrations. An increase in the Brij®30 concentration is accompanied by an increase in the relative interfacial pressure measured by the DIPD. The consistency in the shape of the last three-quarters of the interfacial pressure profile indicates that DIPD may be useful for analyte identification and to understand the dynamic processes at the liquid–liquid interface.

The kinetic effect in the relative interfacial pressure during the drop lifetime is similar to the phenomena observed at the air–liquid interface by the DSTD. When the relative interfacial pressure is calculated as shown in Eq. (3), subtracting the carrier phase pressure signal and dividing by the pressure signal of the standard accounts for the increase of the surface area during drop growth. Thus, the relative interfacial pressure as the drop grows is related to the concentration of the analyte at the interface. Factors that affect the shape of the drop time profile include the concentration of the analyte in solution, analyte diffusion constant, molecule arrangement at the interface, equilibrium concentration at the interface and transfer of analyte to the interface [4,7,8,20].

The DIPD and DSTD are complementary detectors in that the DSTD detects interface-active analytes that are soluble and present in aqueous samples, whereas the DIPD detects analytes that are soluble and present in organic solvents. Examples of organic-soluble analytes include petroleum products, cooking oils, hydrocarbons and high molecular weight alcohols. In this report, cholesterol was examined. Samples of cholesterol in *n*-hexane ranging in concentration from 100 to 10,000 μg ml^{-1} were evaluated with the FIA-DIPD system. A three-dimensional FIA-gram of a 100 μl injection of 1000 μg ml^{-1} cholesterol is shown in Fig. 7 as an example. The time axis shows the FIA injection profile. The drop time axis shows the relative interfacial pressure of cholesterol at the water–hexane interface using 0.5% isopropanol in *n*-hexane as the standard. The increase in relative interfacial pressure with drop time is the result of the dynamic transfer of analyte to the interface. Due to the size of the cholesterol molecule, it is suspected that much of the kinetic signal is due to the diffusion of molecules from the bulk phase (inside drop) to the interface. Fig. 7 indicates that the cholesterol response is indeed kinetically hindered possibly due to diffusion and rearrangement processes at the drop interface, and appears to then nearly reach interfacial equilibration. Future studies might involve investigation to determine whether the time required to reach a state near equilibrium can be used to

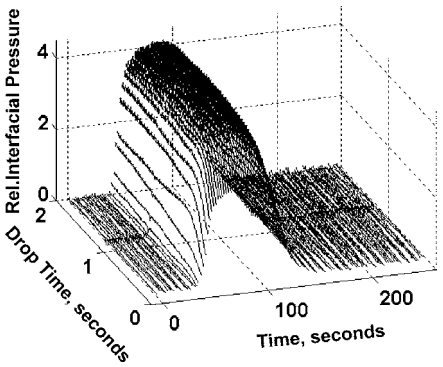

Fig. 7. A three-dimensional relative interfacial pressure plot of 1000 μg ml^{-1} cholesterol in *n*-hexane using 0.5% isopropanol as standard.

estimate the diffusion constant. Again, it is evident that the shape of an analyte interfacial pressure profile during drop growth might be useful for analyte identification.

The FIA profiles for multiple concentrations of cholesterol are given in Fig. 8(A). The interfacial pressure signals are an average of the relative interfacial pressure signals from 1.8 to 2.0 s in the drop profile (right before drop detachment, so the maximum relative interfacial pressure). The relative interfacial pressure increased with concentration of cholesterol. In Fig. 8 (B), the calibration curve of the concentration series of cholesterol from Fig. 8 (A) is shown. Fig. 8 demonstrates that the DIPD can be used to quantify the cholesterol concentration, and quantitative analysis for any surface-active analyte is possible in principle.

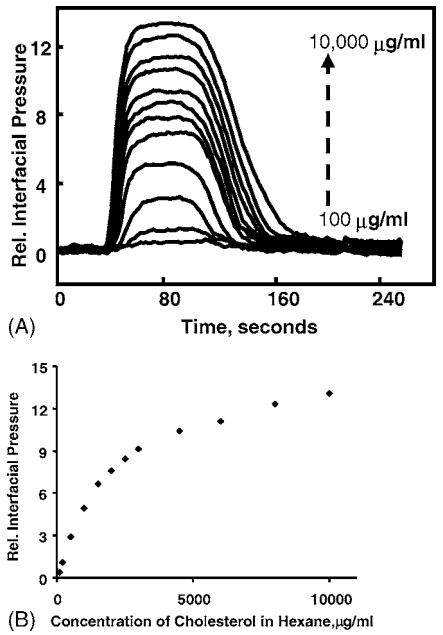

Fig. 8. (A) Overlay FIA-gram plots of relative interfacial pressure of various concentration of cholesterol over the range 100–10,000 μg ml^{-1} in *n*-hexane. The concentrations were 100, 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 6000, 8000 and 10,000 μg ml^{-1}. (B) A calibration curve of the cholesterol data is plotted against relative interfacial pressure and concentration of cholesterol in *n*-hexane.

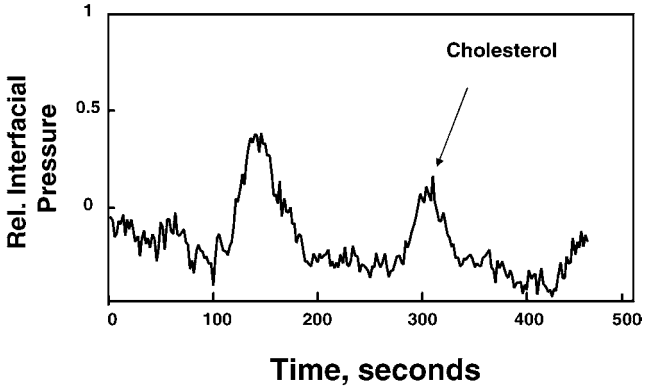

Fig. 9. Separation of cholesterol from egg yolk by NP-HPLC-DIPD. Experimental conditions are given in the text, and using 0.5% isopropanol as standard.

An example of the use of the DIPD as a selective detector for NP-HPLC was demonstrated with the extraction and normal phase liquid chromatographic separation of cholesterol from egg yolk. Because the DIPD selectively detects only interface-active compounds, only a few compounds in the sample must be separated to measure cholesterol in egg yolk. After the egg yolk was shaken with *n*-hexane, a 15 μl injection of the supernatant was injected into the *n*-hexane carrier phase of the NP-HPLC-DIPD system. The resulting chromatogram, given in Fig. 9, shows that cholesterol, with a retention time of approximately 300 s was separated from other organophilic egg yolks constituents, which eluted at approximately 150 s. The selectivity of the DIPD results in a relatively simple chromatogram, whereas use of another detector, such as absorbance or refractive index would be either not selective enough or not sensitive enough or both. The method of standard additions was used to confirm the retention time and concentration (250 μg ml^{-1}) of the cholesterol peak in the egg yolk extract, and the other organophilic egg yolk constituent remained unidentified.

5. Conclusions

An instrument known as the DIPD was designed and developed for making analytical measurements of surface-active species at the water–hexane interface. In principle, any combination of two immiscible liquids with different densities could be used with the DIPD. As a drop grows from a stainless steel capillary tip, the DIPD can be used to monitor the diffusion and arrangement of interface-active analytes at the liquid–liquid interface. The relative interfacial tension response can be used to identify and quantify surfactants and understand their behavior at liquid–liquid interfaces. The DIPD is a novel detector because it selectively measures the interfacial activity of analytes within drops eluting from a flowing stream into another immiscible liquid, and therefore, is a suitable detector for flow-based analytical techniques, such as FIA and NP-HPLC. Furthermore, the DIPD serves

as a detector for analyte quantification, as demonstrated by a calibration of cholesterol.

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