Measuring instruments and Services for Interface Science

FOCUS ON SURFACE TENSION IN FORMULATION FOR DRUG DELIVERY

Designing drug carriers that specifically deliver a drug to the tumor site is of particular interest, as this would significantly improve the drug's selectivity and reduce the severe side-effects associated with chemotherapy. For this purpose, exploiting the pH near tumors to trigger drug release is a promising targeting strategy. Therefore, understanding the behavior of pH sensitive lipids is crucial to optimize the efficiency of these drug delivery systems.

Scientific publications have recently highlighted the use of the TRACKER™ for the study of insoluble monolayers, compression experiments, and interfacial rheology in formulation for drug delivery.

In their work, **The pH-Induced Specific Area Changes of Unsaturated Lipids Deposited onto a Bubble [2],** the authors propose an original and simple approach, using the TRACKER™, for studying the behavior of pH-sensitive lipids monolayer undergoing gradual changes in its environment (Fig1).

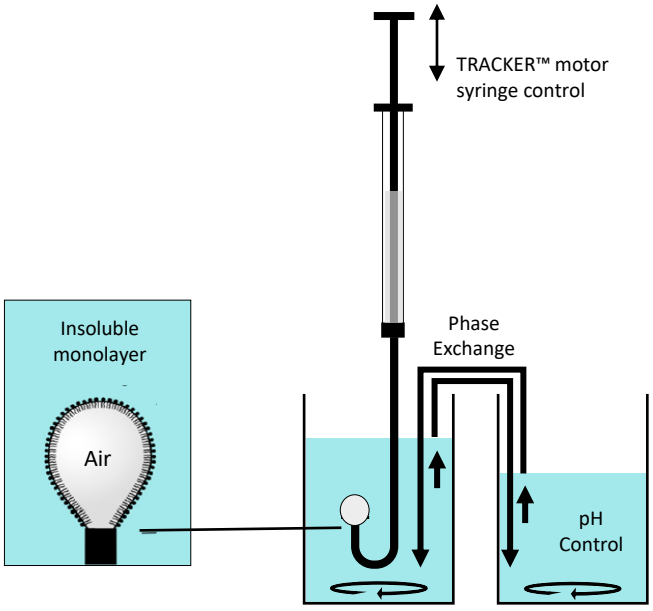

Fig1: Experimental setup for the study of insoluble monolayers, compression experiments, and interfacial rheology using TRACKER™. Thanks to the Phase exchange option, the Bubble is setup in the cuvette and the water circulation allows modifying the bulk phase composition and real-time pH measurement.

The results rely on the deposition of insoluble lipids onto the water/air interface of a bubble [3] maintained at a constant volume, controlled and monitored by TRACKER™.

This experimental approach, in contrast to the classical ones for studying Langmuir monolayers have several significant advantages like the easy control of the surrounding bulk composition, the fast experimental time for a monolayer to be ready, the small bulk volume, and mostly the simple way to carry out dilatational rheology.

This work highlights the performances of the TRACKER™ and the benefits of the experiment setup :

- The TRACKER™ easily allows to perform interfacial rheology and accessing the viscoelastic properties of the monolayer.
- The TRACKER™ allows to perform successive isothermal compressions of the same monolayer, with a gradual modification of the bulk composition (pH), through a very simple protocol. During monolayer compression, the Bond number (Bo) retained a suitable value Bo > 0.15, confirming the applicability of the algorithm used in the TRACKER.
- The TRACKER™ can perform long time measurement including a sequence of regulations fully controlled by the software.
- The phase exchange option of the TRACKER™ (Fig1&2) enables to modify the composition of the aqueous phase during measurement and very fine-tuning in the bulk phase pH without replacement of the monolayer.

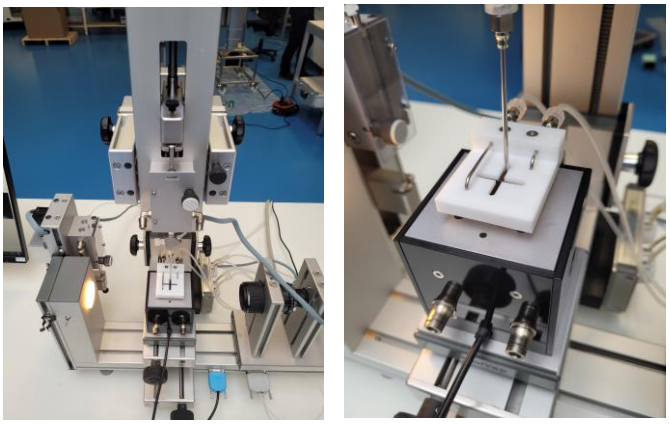

Fig2: TRACKER™ equipped with the Dense phase exchange module

Dr. Nicolas Anton [1] , currently Associate Professor at the Faculty of Pharmacy in Strasbourg, France, is one of the authors of this work. He kindly accepted to discuss with us about his research and experience using the TRACKER™ in formulation for drug delivery.

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Interview with Dr. Nicolas Anton [1], May 2022

Teclis: Dr. Nicolas Anton, related to your experience what is the contribution of interface science in the field of pharmaceutical formulation?

Dr. Nicolas Anton: In fact, it is a science that is difficult to grasp, unless you have been introduced to the technique in a physics-chemistry laboratory and then being able to understand its interest. An organic chemist will not have any vision on surface tension measurement, what can be done with it, what is the dilatational interfacial rheology... Personally, I had the chance to discover this science at the beginning of my career at the University of Pau and then in Angers where I used the TRACKER™. In 2008, when I arrived at Strasbourg University, there was no surface tension measuring instrument in the Lab. I thought the technic was of the most importance for pharma applications that I decided to buy one.

Teclis: why did you chose TRACKER™?

Dr. Nicolas Anton: First, I had already used the TRACKER™ during my PhD, I knew its capabilities. Of course, I gathered my colleagues' opinion on other instruments they have been using… At the end, I decided to buy the TRACKER™, because it is the most reliable drop shape analysis instrument to precisely control the volume of the drop on a very long time, 24 hours or more, which was very important in my research. In addition, many parameters can be modified in the TRACKER™ software, which is very helpful to fine-tune an experiment. This was also what motivated my decision.

Teclis: what do you think are the main advantages provided by TRACKER™?

Dr. Nicolas Anton:

As I said, the control of the volume of the drop / bubble over a long time with the TRACKER™ is the most efficient. Once you've ensured there no leak on the syringe you can run measurements that last 24 hours or even more. Second, the software offers a lot of experiment settings that you can modify to fine-tune an experiment protocol. Third, the scenario function that allows to program the sequence of regulations you want to perform in advance. This feature is a real asset to save time and ensure repeatability.

I recently discovered that the density parameters, in the user interface, can be programmed as a function of temperature: a useful feature for temperature sweep experiments I am carrying out, and time won! I don't think other instruments offer this kind of specific function for researchers.

It's typically TECLIS to offer options for researchers who want to dig a little deeper.

Teclis: And what about the limits of the instrument ?

Dr. Nicolas Anton: Cleanliness is a constraint obviously, but this is a problem for all surface tension measuring technics.

The real limit comes from the studied system itself. Generally, you study isolated molecules, because when you measure complex systems, you get results, but it is complicated to interpret them.

Teclis: In practice, what do you use Surface science for?

Dr. Nicolas Anton: Our core activity is formulation for drug delivery. Equipment such as spectrometers, particle size analyzers and surface charge analyzers are the most widely used. The rheometer is also used for parallel research such as biomaterials or the characterization of gels.

The TRACKER™ is used more for fundamental questions. In formulation, we usually work with non-miscible fluids, and we use stabilizers, such as surfactants or polymers. Surface tension is used to determine the stabilizers' interfacial properties, to characterize and compare oils, to know the stability of molecules at interfaces. If we modify the temperature, if we modify the composition by adding additives, do the properties change? Particularly for non-ionic surfactants which are sensitive to temperature.

For example, I carried out a series of measurements to characterize temperature sensitive polymers. Their hydrophilic/lipophilic behavior changes depending on the temperature with a boundary characterized by a V in the surface tension curve. The idea is to vary the parameters of interfacial rheology and temperature to make the link with the volume rheology of this polymer.

The TRACKER™ is also used to create model interfaces. For example, we are currently working on emulsion formulations that aim to increase the effect of antibiotics on multi-resistant bacteria. In vitro, we observe that the antibiotic in the presence of other compounds has a tenfold effect on the bacteria. The idea is to build a model interface and link it to the formulation. The bacterial compound is supposed to be mechanically destabilized by the antibiotic, by adding the antimicrobial compounds and/or emulsion, we will emphasize, with the TRACKER™, perturbations of the monolayer such as modifications of its dilatational rheological properties.

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As another illustration, we want to measure the interface modifications when we graft ligands (polymers, nanoparticles, etc.) onto a formulated drop. To do so, we use a study protocol in which a surfactant is deposited on the surface of the drop, the equilibrium interfacial tension and the viscoelastic modulus are measured. In a 2nd step, other compounds are added to study either the interactions with the monolayer or to modify the monolayer. For this we use the TRACKER™ Phase Exchange option...

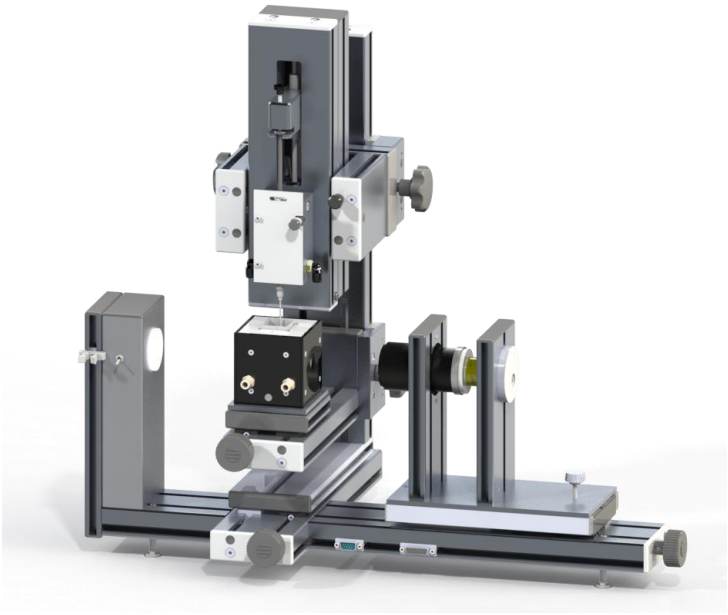
Teclis: This is the approach you described in your paper [2]

Dr. Nicolas Anton: The general idea is to make a monolayer, change the environmental conditions and measure the changes at the interface. To do so, we have developed an experimental approach, alternative to Langmuir trough [3]. First, you deposit lipids that are not soluble in water on the surface of an air bubble with a second syringe filled with these lipids in chloroform. Once lipids are deposited, you "wash" the solution to evacuate the chloroform, using the TRACKER™ phase exchange. In fact, it's like the Langmuir trough technique but the experiment is simpler to handle, quicker to clean with the TRACKER™ and it gives access to other information such as the dilatational rheology and the composition of the external phase can be changed.

Teclis: what kind of experiments are you using the TRACKER™ phase exchange for?

Dr. Nicolas Anton: Usually, I use the TRACKER™ phase exchange for washing. After depositing molecules at the interface of a bubble with chloroform, which is partially soluble in water. Or when I add molecules in successive stages to modify the interface chemically like a polymerization. I change the external phase considering that this will not modify the surface because the molecules are not soluble in water [3], or that the modified surface becomes insoluble in water, or considering that the adsorbed molecules do not desorb.

I can also use the TRACKER™ phase exchange in a closed loop, for example to gradually modify the pH [2]. This is very convenient, it is a multi-step process, and the concentration must be carefully controlled.



TRACKER™ Standard

TRACKER™ determines the dynamic surface / interfacial tension between two immiscible fluids by performing a numerical analysis of the shape of a rising or pendant Drop/Bubble.

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References

[1] Nicolas Anton – PhD University of Angers, France - Associate Professor at University of Strasbourg, CNRS, CAMB UMR 7199 and INSERM (French National Institute of Health and Medical Research), UMR 1260, Regenerative Nanomedicine (RNM), FMTS, F-67000 Strasbourg, France - contact: nanton@unistra.fr

[2] The pH-Induced Specific Area Changes of Unsaturated Lipids Deposited onto a Bubble Interface. Nicolas Anton, Philippe Pierrat, Germain A. Brou, Gildas K. Gbassi, Ziad Omran, Luc Lebeau, Thierry F. Vandamme, and Patrick Bouriat. Langmuir 2021, 37, 2586−2595

[3] A study of insoluble monolayers by deposition at a bubble interface. Nicolas Anton, Philippe Pierrat, Luc Lebeau, Thierry F. Vandamme and Patrick Bouriat. Soft Matter 2013.

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How to control the surface pressure

TRACKER™ Phase Exchange Application Note

The presence of surface- active molecules at an interface changes its physico-chemical properties. The amplitude of these changes, that can be characterized by surface pressure, depends strongly on the surface concentration.

The surface pressure (Π) is defined as the difference of interfacial tension between a pure interface and an interface in the presence of surface-active molecules.

$$\Pi = \gamma_0 - \gamma$$

Where γ_0 corresponds to the interfacial tension between the two pure phases and γ the measured surface tension.

A good understanding of surface-active laden interfaces as a function of surface pressure requires varying the surface concentration. The control of this concentration can be complex. Indeed, the surface pressure at the equilibrium is governed by the adsorption kinetics of the molecules and their initial concentration.

Phospholipids are major components of lipid droplet monolayers and biological membranes and play a significant role in their structuring and stabilization.

An oil/water interface coated with phospholipids has been used to produce interfaces with different surface pressures, as shown in Figure 1.

The drop tensiometer Tracker™ allows precise real-time control and modulation of the surface pressure of the interfaces.

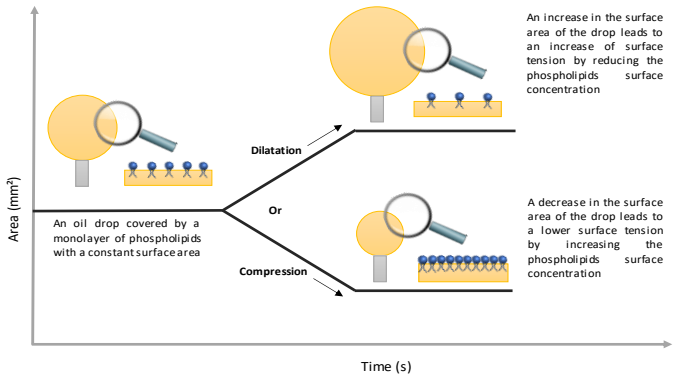

Figure 1 : Drop area as function of the time of a phospholipid-coated oil/water interface during an experiment

The **Experimental Protocol** consists in 4 steps:

1. An oil drop (triolein) is formed in a buffer solution.
2. At t = 100 sec, a preparation of unilamellar (100 nm) large vesicles of phospholipids is injected to reach a concentration of 0.005% (w/w) in the buffer solution
3. After an adsorption time of 1500 sec, **the aqueous phase is replaced by a fresh buffer solution** to remove non-adsorbed phospholipids.
4. The surface pressure is then simply controlled by increasing or decreasing the interface surface area.

Figure 2 shows the surface tension as a function of time for an oil/water interface. The initial tension is 32 mN/m and is consistent with the literature [1-4].

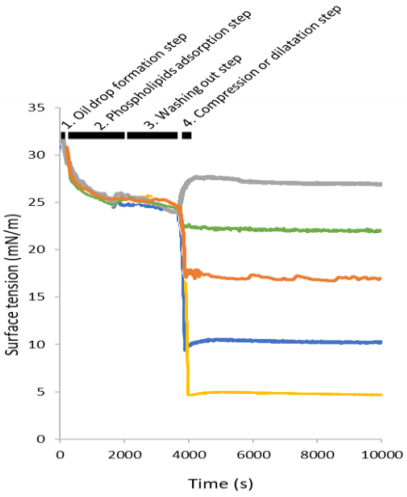

Figure 2: Variation of the interfacial tension at the triolein/water interface as a function of time. Surface pressure reaches final values of (grey) 5 mN/m, (green) 9 mN/m, (orange) 16 mN/m, (blue) 22 mN/m and (yellow) 28 mM/m .

After injection of the phospholipids, the surface tension decreases slowly over time; the phospholipids adsorb at the interface. Exchanging the aqueous phase stops the phospholipids adsorption and only the variation of the drop surface area allows to modify the surface concentration and thus the surface pressure of the phospholipid monolayer. In this example, one expansion of the drop area was performed to decrease the surface pressure (i.e. increase the tension); and four compressions were performed to increase the surface pressure (i.e. decrease the tension).

CONCLUSION

The surface pressure of an interface can be controlled using the drop tensiometer Tracker. Specific or custom-made interfaces can be made to mimic different interfacial systems and to study rheological properties at different interfacial pressures. Thus, it is possible to figure out the building-blocks of an interface and to study the adsorption of one or several molecules sequentially added or to determine the exclusion pressure of molecules.

References

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