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(54) Title: METHOD FOR DETERMINING INTERFACIAL TENSION

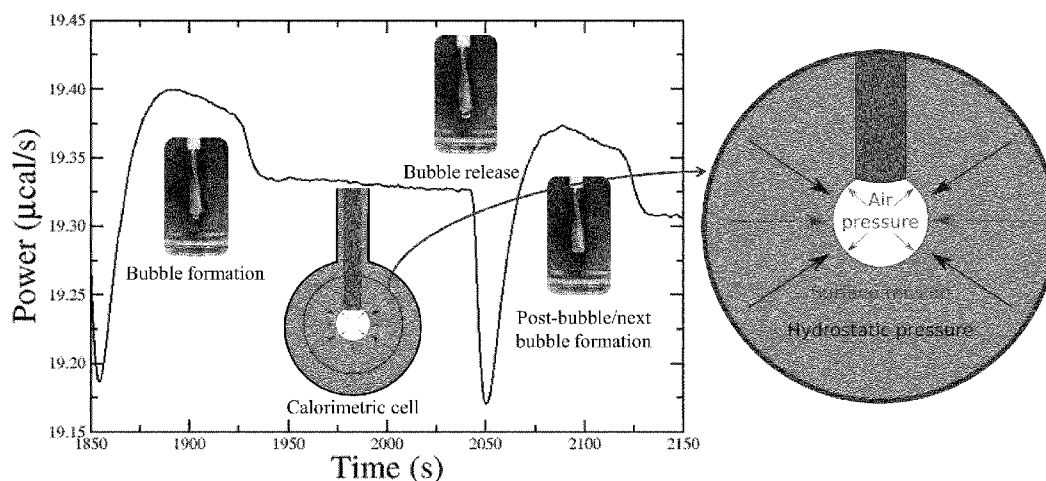


Figure 1

(57) **Abstract:** The present invention relates generally to methods for characterization of fluids, and, particularly, to methods of determining an interfacial tension between a first liquid and a second fluid, the method comprising the steps of: (a) introducing the second fluid into the first liquid at a constant flow-rate so as to cause the second fluid to periodically form successive droplets or bubbles; (b) recording a heat change of step (a) as a function of time, obtaining a periodic heat versus time profile; (c) obtaining the period of the heat change recorded in step (b); (d) providing a calibrated relationship of the interfacial tension as a function of period for said constant flow-rate and said second fluid; and (e) correlating the period obtained in step (c) with the calibrated relationship, and determining the interfacial tension between the first liquid and the second fluid for said constant flow-rate.



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DESCRIPTION

METHOD FOR DETERMINING INTERFACIAL TENSION

5 TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to methods for characterization of fluids, and, more particularly, to methods for determination of interfacial tension of fluids.

10 BACKGROUND OF THE INVENTION

A number of techniques are available to assess structural, kinetic and energetic properties of molecules at interfaces (e.g. interfacial tension), such as ellipsometry and different microscopy and spectroscopy approaches [Javadi, A. et al. "Characterization
15 Methods for Liquid Interfacial Layers". Eur. Phys. J. Spec. Top. 2013, 222 (1), 7–29; Dukhin, S. S. et al. "Dynamics of Adsorption at Liquid Interfaces: Theory, Experiment, Application; Studies in Interface Science". Elsevier Science: Amsterdam, Netherlands, 1995; Vol. 1; and "Surface Science Techniques". Bracco, G.; Holst, B., Eds. Springer-Verlag: Berlin, 2013].

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Interfacial tension is the most basic of these properties and different devices are available to determine its value for a given liquid sample in contact with another fluid, for example pendant drop tensiometers (that determine interfacial tension from the shape of a droplet), maximum drop volume tensiometers (that use the force balance
25 between gravity and surface tension to get the value of the latter), bubble pressure tensiometers (that use the maximum pressure of a bubble to get the surface tension of a liquid), spinning drop tensiometers (that determine interfacial tension from the shape of a droplet under an imposed forcing), and Wilhelmy plate tension meters (that determine interfacial tension from a force exerted on a thin wet plate).

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These instruments are specifically designed to get surface or interfacial tension of standard liquids in contact with different fluids and no additional information is supplied by them. Furthermore, they consume quite a lot of sample, every solution has to be independently prepared and, since surface and interfacial tension are extremely
35 sensitive to the concentration, the instrument has to be intensively cleaned between

measurements. Given the importance of this fundamental property, new methods able to provide additional information while measuring interfacial tension (regarding liquid-fluid interaction, the fluid being either gas or liquid) or surface tension (regarding liquid-gas interaction) , with decreased consumption of sample and human effort in the
5 preparation of solutions and cleaning would clearly present advantages over current methods.

BRIEF DESCRIPTION OF THE INVENTION

10 The inventors of the present invention have observed that the kinetic calorimetric profile of droplet or bubble formation heat in different liquids upon injection of a fluid at a constant flow-rate and measured by use of a standard calorimeter exhibited a fully reproducible periodic signal. This signal data in the form of heat vs. time profile allows determining the interfacial tension value for a given liquid sample with high precision.

15 Therefore, the present invention provides a solution for the aforementioned problems based on the use of a standard calorimeter (or any other device for calorimetry measurement) for the determination of the interfacial tension of fluids. To this end, a method based on the measurement and recording of heat change of successive
20 droplets or bubbles as a function of time is provided. The method of the invention allows obtaining data in shorter time periods and using less resources when compared to methods conventionally used for measuring interfacial tension.

Additionally, standard calorimeters already have an injection system that can be
25 employed to automatically vary the concentration without need to clean the instrument or to manually prepare solutions when doing a series of experiments of the same solvent with different concentrations of a solute. This, together with the small volume of the sample cell in modern calorimeters, contributes to save a significant amount of sample. Finally, calorimeters can provide additional information other than interfacial
30 tension measurements, the kinetic heat signal can be connected to different process including condensation, and/or evaporation, and/or diffusion between the fluids involved, compression or expansion of the fluid injected, as well as drop/bubble formation, growing and delivery.

35 Thus, in a first inventive aspect, the invention provides a method of determining an

interfacial tension between a first liquid and a second fluid, the method comprising the steps of:

- 5 a. introducing the second fluid into the first liquid at a constant flow-rate so as to cause the second fluid to periodically form successive droplets or bubbles;
- b. recording a heat change of step (a) as a function of time, obtaining a periodic heat versus time profile;
- c. obtaining the period of the heat change recorded in step (b);
- 10 d. providing a calibrated relationship of the interfacial tension as a function of period for said constant flow-rate and said second fluid; and
- e. correlating the period obtained in step (c) with the calibrated relationship, and determining the interfacial tension between the first liquid and the second fluid for said constant flow-rate.

15 Thus, the method of the present invention allows obtaining highly reproducible periodic heat change vs. time profile which can be easily used to determine interfacial tension values of different fluids by appropriate calibration of interfacial tension vs. period for bubble or droplet formation.

20 In a second inventive aspect, the invention provides a method for establishing the calibrated relationship provided in step (d) of the method according to first inventive aspect, wherein this method for establishing the calibrated relationship comprises the steps of:

- 25 i. applying steps (a) to (c) of the method according to any of the embodiments of the first inventive aspect to at least two different pure first liquids of which the respective interfacial tension with the second fluid is known upon being introduced in step (a) at a given flow-rate;
- ii. plotting their respective interfacial tensions as a function of their respective
30 obtained periods; and
- iii. fitting the plotted data to a function thus providing a relationship between the interfacial tension to be determined and the period for said given flow-rate.

The kinetic calorimetric profile obtained by the method according to the first inventive
35 aspect of the present invention exhibits a fully reproducible periodic signal of heat as a

function of time. This signal data is then processed in order to retrieve its period which will be afterwards correlated with the calibrated relationship so as to obtain the interfacial tension value of this first liquid.

- 5 A direct proportional relationship has been surprisingly found between period and interfacial tension and, therefore, a calibration suffices to perform the correlation between both.

10 In an embodiment the calibrated relationship is obtainable from the method according to the second inventive aspect of the invention. Preferably, the relationship between the interfacial tension and period is fitted to a linear function.

15 In a preferred embodiment, the calibrated relationship is established by taking two pure liquids of known interfacial tension, one of high interfacial tension (water, for instance) and another one of low interfacial tension (such as cyclohexane, ethanol or the lowest point that will be measured with a calorimeter). The bubble formation period for each injection flow-rate that is going to be used is measured for each pure liquid according to the method of the first inventive aspect of the present invention. As a result, with these two points the calibrated relationship from which the interfacial tension value is to be
20 determined is thus established.

Further, in a third inventive aspect, the invention provides a data processing apparatus comprising means for carrying out the steps (c) to (e) of any of the embodiments of the method of determining an interfacial tension according to the first inventive aspect.

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In a fourth inventive aspect, the invention provides a computer program comprising instructions which, when the program is executed by a computer, cause the computer to carry out the steps (c) to (e) of any of the embodiments of the method of determining an interfacial tension according to the first inventive aspect.

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In a fifth inventive aspect, the invention provides a computer-readable medium comprising instructions stored thereon that, when executed, cause the computer to carry out the steps (c) to (e) of any of the embodiments of the method of determining an interfacial tension according to the first inventive aspect.

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In addition, any of the embodiments of the methods of the present invention can be performed using commercially available devices for calorimetry measurements with no modifications at all. Thus, standard calorimeters can be used to determine interfacial tension of first liquid samples and the second fluid with high precision.

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The heat change vs. time profile recorded with commercially available calorimeters or any other device for calorimetry measurement may carry unwanted noise produced during capture, storage, transmission, processing, or either conversion of the same. Said non-useful information or noise of the recorded heat change vs. time profile is

10 reduced or mitigated by particular embodiments of the first inventive aspect.

In another inventive aspect, the invention is directed to the use of a calorimeter for the determination of the interfacial tension between a first liquid with a second fluid.

15 In a final inventive aspect, the invention is directed to the use of a calorimeter for carrying out steps (a) and (b) of any of the embodiments of the method of determining an interfacial tension according to the first inventive aspect, the calorimeter comprising:

- a container cell configured to house separately a first liquid from a second fluid;
- 20 - a first capillary configured to introduce the second fluid into the first liquid;
- first conveying means in fluid communication with the first capillary, the first conveying means configured to convey the second fluid at a constant flow-rate into the first liquid in such a way that said second fluid is introduced into the first liquid as droplets or bubbles;
- 25 - sensing means in thermal contact with the first liquid, and configured to detect heat change associated to the periodical formation of successive droplets or bubbles; and
- a recording unit associated to the sensing means and configured to record the heat change detected by the sensing means as a function of time.

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DESCRIPTION OF THE DRAWINGS

These and other characteristics and advantages of the invention will become clearly understood in view of the detailed description of the invention which becomes apparent

35 from a preferred embodiment of the invention, given just as an example and not being

limited thereto, with reference to the drawings.

- Figure 1 Schematic representation of bubble formation and heat change (power) versus time recorded during bubble formation upon injection of air at a constant flow-rate in a liquid for determination of interfacial tension of said liquid.
- Figure 2 Heat change (power) vs. time profile corresponding to an experiment of air injection into water at a flow-rate 0.022 $\mu\text{L/s}$.
- Figure 3 Signals corresponding to the injection of air into three liquids (water, surfactant solution and ethanol) at different flow-rates (0.111, 0.055, 0.028, and 0.022 $\mu\text{L/s}$, as indicated on the right of each row).
- Figure 4 Power vs time profiles corresponding to five independent experiments of air injection into water at 0.022 $\mu\text{L/s}$.
- Figure 5 Power vs time profile corresponding to an almost complete experiment of air injection into a 0.55 mM solution of C_{12}G_2 in water at 0.022 $\mu\text{L/s}$.
- Figure 6 Interfacial tension of three liquids (71 mN/m for water; 35 mN/m for the C_{12}G_2 solution and 22 mN/m for ethanol) vs the average period between minima obtained in the calorimetric signal of 20 different bubbles for each liquid at flow-rates of 0.111 ($\bullet$), 0.055 ($\blacktriangle$), 0.028 ($\blacksquare$) and 0.022 ($\blacklozenge$) $\mu\text{L/s}$.
- Figure 7 Power vs time profiles corresponding to an almost complete experiment of air injection into ethanol at 0.022 s/ μL .
- Figure 8 Integral of the negative and positive contributions to the signals corresponding to several bubbles injected in pure water at 0.022 $\mu\text{L/s}$.
- Figure 9a,b Four heat change (power) vs. time continuous profiles recorded whilst introducing a particular second fluid into a first liquid at a constant flow-rate. In particular, air has been injected at 0.037 $\mu\text{L/s}$ into a 2.96, 0.7 and 0.0 mM solution of C_{10}G_2 in water, and at 0.028 $\mu\text{L/s}$ into a 2.41 mM solution of C_{10}G_2 in water.
- Figure 10a,b Discretization and correction of the profiles shown in Figure 9a, b based on respective baseline curves for each one, in such a way that the corrected heat change profiles oscillates about a constant value.
- Figure 11a,b The corrected heat change profiles which oscillate about a constant value (i.e. 0) shortened in time-axis to a particular time window which has an acceptable variance.
- Figure 12 Different examples of applying any of the Fourier transform-type techniques

to either recorded or corrected heat vs time profile.

Figure 13 The result of apply Fourier transform on the corrected and shortened heat change profiles of Figures 11a,b, wherein more than one period peaks can be seen.

5 Figure 14 Set of periods for a given fluid and liquid combination which varies the solute concentration in the first liquid adjusted to a mathematical function for different flow-rates.

Figure 15 The Fourier-transformed profile corresponding to air injected at 0.037 $\mu\text{L/s}$ into a 0.7 mM solution of C_{10}G_2 in water shown in figure 13, decomposed into a number N of Gaussian functions.

Figure 16 A comparison of interfacial tension obtained by the method of the present invention and data coming from the literature for other standard methods is shown.

Figure 17a-c A sampled heat vs. time profile recorded while introducing air at a flow-rate of 0.028 $\mu\text{L/s}$ into a 2.96 mM solution of C_{10}G_2 in water. The sampled profile of figure 17a is up-sampled in figure 17b, and averaged based on a 2D histogram in figure 17c.

Figure 18a, b The profile of figure 17c after applying a Savitzky-Golay filter (figure 18a) and the profile of figure 18a after applying a moving average.

20 Figure 19 A corrected heat change as the difference between the recorded heat change and the obtained baseline curve of figure 18b.

Figure 20a-c Moving Fast Fourier Transform applied to figure 19, Area-Normalized M-FFT, and final FFT obtained from the average of the 'Area-Normalized M-FFT' of figure 20b.

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DETAILED DESCRIPTION OF THE INVENTION

All the features described in this specification (including the claims, description and drawings) and/or all the steps of the described methods can be combined in any combination, with the exception of combinations of such mutually exclusive features and/or steps.

In the context of the present invention, the term "interfacial tension" or "IFT" refers to the cohesive forces at the interface between a liquid and a fluid (gas or liquid). The molecules at the interface do not have an isotropic distribution of neighboring atoms

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and thus they interact more strongly with those directly associated with them on the interface. This forms an interface “film” with specific structural, mechanic, kinetic and energetic properties. In the case of a liquid and a gas, the term “surface tension” (SFT) is also used. There are several different units for interfacial and surface tension; typically mN/m (which is equivalent to dynes/ cm) is used. This property is of primary importance for the characterization of molecules in a number of chemical industries including cleaning, cosmetics, pharmaceutical and food, as well as for fundamental research, because the interfacial properties are connected with the ability of molecules to self-assemble in solution.

10

The inventors of the present invention have observed that the heat released or absorbed in the creation of liquid droplets or gas bubbles upon injection of a fluid in a liquid sample allows determining the interfacial tension of liquid samples with high precision. Simultaneously, the heat change signal by itself contains useful information for the characterization of the system at molecular level.

15

Therefore, in the first inventive aspect, the present invention is directed to a method of determining an interfacial tension between a first liquid and a second fluid, the method comprising the steps of:

20

- a. introducing the second fluid into the first liquid at a constant flow-rate so as to cause the second fluid to periodically form successive droplets or bubbles;
- b. recording a heat change of step (a) as a function of time, obtaining a periodic heat versus time profile;
- c. obtaining the period of the heat change recorded in step (b);
- 25 d. providing a calibrated relationship of the interfacial tension as a function of period for said constant flow-rate and said second fluid; and
- e. correlating the period obtained in step (c) with the calibrated relationship, and determining the interfacial tension between the first liquid and the second fluid for said constant flow-rate.

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As defined above, the method of the present invention comprises a step (a) of introducing the second fluid into the first liquid at a constant flow-rate so as to cause the second fluid to periodically form successive droplets or bubbles.

35 The term “fluid” refers to a substance that continually deforms (flows) under an applied

shear stress. Fluids are a subset of the phases of matter and include liquids, gases, plasmas, and to some extent, plastic solids. Fluids are substances that have zero shear modulus, or, in simpler terms, a fluid is a substance which cannot resist any shear force applied to it. In the context of the present invention, the term “fluids”
5 includes single liquids, mixtures of liquids, gases, mixtures of gases and mixtures of liquids and gases.

The term “liquid” refers to a fluid that conforms to the shape of its container but retains a constant volume independent of pressure. As such, it is one of the four fundamental
10 states of matter (the others being solid, gas, and plasma), and is the only state with a definite volume but no fixed shape. In the context of the present invention, the term “liquid” includes pure liquids (for example water and alcohols) but also miscible and immiscible mixtures of two or more liquids, for example, a mixture of water and different organic solutes (alcohols, etc), water and biological molecules including sugar, lipids or
15 proteins, or mixtures of other solvents with different solutes.

Non-limiting examples of first liquids suitable for the method of the present invention are water, alcohols, alkanes acids, etc., as well as mixtures thereof.

20 In a preferred embodiment, the first liquid is water, ethanol or aqueous solutions of different solutes (generally surfactant molecules).

In the method of the present invention, the second fluid is introduced into the first liquid to cause the second fluid to periodically form successive droplets, if the second fluid is
25 liquid, or bubbles, if the second fluid is a gas.

Non-limiting examples of second fluids suitable for the method of the present invention are liquids such as organic solvents insoluble in the first liquid (for instance oil, cyclohexane or alkane molecules if the first liquid is water) and gases such as air,
30 nitrogen, oxygen, hydrogen, methane, etc.

In a preferred embodiment, the second fluid is a gas, more preferably air.

In the context of the present invention, the term “air” refers to a mixture of about 78%
35 nitrogen, 21% oxygen, water vapor, argon, carbon dioxide, and very small amounts of

other gases.

In step (a) of the method of the present invention, the second fluid is introduced into the first liquid at a constant flow-rate.

5

The term “flow-rate” (also known as volumetric flow-rate, volume flow-rate, rate of fluid flow or volume velocity) is the volume of fluid which passes per unit time, usually represented by the symbol Q (sometimes V), being herein represented by variable r throughout the entire description. There are several different units for flow-rate; typically $\mu\text{L/s}$, m^3/s or L/s are used. In the context of the present invention, the “flow-rate” refers to volume of the second fluid introduced into the first liquid per unit time. In the method of the present invention, the flow-rate of the second fluid is constant.

10 In a preferred embodiment, the constant flow-rate of the second fluid ranges between 200 microliters in 9000 seconds and 200 microliters in 900 seconds.

Upon injection of the second fluid at a constant flow-rate into the first liquid, the second fluid periodically forms successive droplets or bubbles. The formation of droplets or bubbles within the first liquid causes a heat change therein.

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In the context of the present invention, the term “heat change” refers to the thermal disturbance caused within the first liquid by the creation of the successive droplets or bubbles. In particular, and without being bound to any theory in particular, the inventors of the present invention believe that the heat change corresponds to: (i) the sudden formation of the droplet or bubble in the liquid sample once a critical pressure is reached in the second fluid; and (ii) the growth of the droplet or bubble till it reaches a maximum volume and is released towards the first liquid (see Figure 1).

25 Assuming that either a calorimeter or another device for calorimetry measurement is used within the method of the present invention, the following applies.

Briefly, the conditions to get a periodic kinetic heat signal upon injection of a fluid into a liquid are as follows:

- The injection flow-rate should be slow enough to allow the device to follow the heat signal (i.e. heat change), considering the response time of said device.

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This means that the whole process should be significantly slower than the response time of the device.

- The injection flow-rate should be fast enough to produce a significant heat signal in the device. If the flow rate is too slow the power might be under the detection threshold of the device, even though the total energy of the process is large.
- The bubble/drop should be large enough to be detectable by the device. This means that the heat exchanged during the bubble/drop formation and delivery process is large enough to be detected by the device.
- The bubble/drop should be small enough to avoid contacts with the cell walls during its formation and delivery.
- The pressure after the delivery of each bubble/drop should be the same in order to get a periodic signal. This means that closed cells do not produce a true periodic signal due to the increment of pressure in the cell upon continuous injection of the second fluid. Small variations of pressure are observed during the formation of each bubble/drop but it should be recovered upon releasing.

A heat sensor should be in close contact with the calorimetric cell. In an embodiment, the heat sensor is a Peltier device implemented in the device.

Within a calorimeter or another device for calorimetry measurement, the heat change recording is performed through the first liquid, preferably by use of a Peltier device in close contact with the external walls of the sample cell where the first liquid is contained.

With all of that, and as it can be derived from the figures, the period retrieved depends on the following factors:

- Diameter of the capillary from which the fluid is injected.
The diameter of the capillaries used is normally less than 1 mm but it can be modified provided that it is smaller than the width of the sample cell.
- Hydrostatic pressure in the tip of the capillary.
Preferably, slightly above the atmospheric pressure, that is, ~5-10 mb at the tip of the capillary, where the bubbles or drops are formed.
- Injection flow rate.

The optimal value depends on the response time and on the sensitivity of the

calorimeter or device for calorimetry measurement. In an embodiment, the flow-rate is from 0.022 to 0.22 $\mu\text{L/s}$.

- Temperature of the sample cell and of the injected fluid.

Experiments were done with the temperature of the sample cell between 10 and 60 °C, while external temperature ranged between 20 and 25 °C.

- Interfacial tension between the two fluids (composition or chemical features of the two fluids). Values were tested from 22 to 72 mN/m.

In a preferred embodiment, the temperature is maintained constant throughout the steps (a) and (b) of the method of the present invention. In that case, the heat change is measured as the power applied to keep the temperature constant within the sample cell where the first liquid is contained. Typically, $\mu\text{cal/s}$ are used as units for the heat change which is equivalent to the applied power.

The inventors of the present invention have observed that the profile of the heat change versus time obtained by the method of the present invention exhibits a fully reproducible periodic signal that depends on the molecular composition of the first liquid. Examples of these fully reproducible periodic signals can be seen in figures 2 to 5, 7 and 9 (a and b).

In figure 1, a schematic representation of bubble formation and the associated heat change (this is, power consumed by the calorimetry device used in the measurement) versus time recorded during bubble formation is shown.

In figure 2, the heat change (power) versus time profile corresponding to an experiment of air injection into water at a flow-rate of 0.022 $\mu\text{L/s}$ is shown. The signal corresponding to 16 bubbles is clearly seen. The profile portion corresponding to each bubble formed is perfectly reproducible and the signals are equally separated, implying that all bubbles have the same volume and the involved heat is similar.

Figure 3 shows the signals correspondent to the injection of air into three different liquids (water, surfactant solution and ethanol) and at different flow-rates (0.111, 0.055, 0.028 and 0.022 $\mu\text{L/s}$, as indicated on the right of each row). The experiment corresponding to ethanol at 0.111 $\mu\text{L/s}$ is not shown since the bubbles are overlapped, i.e. the bubbles are formed so quickly that several ones can be present in the sample

cell at the same time. The x-axis of all plots is on the same scale to facilitate the comparison between experiments.

Figure 4 shows the power versus time profiles corresponding to five experiments of air injection into water at a flow-rate of 0.022 $\mu\text{L/s}$. In figure 4, the signals corresponding to the formation of 3 bubbles are clearly seen in each experiment. As shown in figure 2, the heat change profile caused by each bubble formation upon air injection is perfectly reproducible and thus the signals are equally separated. This entails that all the bubbles caused have the same volume and that the involved heat in each one is equivalent.

Figure 5 shows the power vs time profile corresponding to an almost complete experiment of air injection into a 0.55 mM solution of C_{12}G_2 in water at a flow-rate of 0.022 $\mu\text{L/s}$. In figure 5 the signals corresponding to 29 bubbles are clearly seen. The heat change profile caused by each bubble formation upon air injection is perfectly reproducible and the signals are equally separated, so all the bubbles have the same volume and that the involved heat is equivalent.

Figure 6 shows the interfacial tension of three samples liquids, this is, 71 mN/m for water, 35 mN/m for a C_{12}G_2 solution and 22 mN/m for ethanol vs. the average period between minima obtained in the calorimetric signal of 20 different bubbles for each sample liquid at flow-rates of 0.111 ($\bullet$), 0.055 ($\blacktriangle$), 0.028 ($\blacksquare$) and 0.022 ($\blacklozenge$) $\mu\text{L/s}$. The lines are the linear fitting of the results at each flow- rate. All data correspond to a temperature of 298 K.

Figure 7 shows a heat change (power) vs. time profile corresponding to an almost complete experiment of air injection into ethanol at a flow-rate of 0.022 $\mu\text{L/s}$. A clear evolution of the signal profile is observed. This is probably due to the overlapping and coalescence of bubbles that remain attached to the capillary used for air injection while more bubbles are being formed. Processing steps of the recorded data, as well as modifications in the design of the injection system as described hereinafter are desirable to avoid or mitigate this effect.

In figure 8, the integral of the negative and positive contributions to the signals corresponding to several bubbles injected in pure water at a flow-rate of 0.022 $\mu\text{L/s}$ is

shown.

Thus, upon injection of the second fluid as defined above, the method of the present invention comprises a step (b) of recording a heat change of step (a) as a function of
5 time, obtaining a periodic heat versus time profile.

The method of the present invention can be performed using commercially available calorimetry devices with no modifications at all.

10 In a preferred embodiment, steps (a) and (b) of the method of the present invention are performed by the use of a calorimeter, more preferably by use of an isothermal titration calorimetry (ITC).

Hereinafter, different techniques will be described regarding step (c) of the method
15 according to the present invention for obtaining the period of the heat change profile recorded in step (b). All of these techniques aim to retrieve accurately the period of the periodic heat change profile.

After the profile is obtained in step (b), the period between droplets or bubbles is
20 determined. In the context of the present invention, the term "period" refers to time elapsed between two consecutive droplets or bubbles.

The inventors of the present invention have observed that the period, as for the periodic heat change as a function of time representing the subsequent drop or bubble
25 creation, also depends significantly on the molecular composition of the first liquid.

Figures 9a,b show four heat change (power) vs. time continuous profiles recorded whilst introducing a particular second fluid into a first liquid at a constant flow-rate. In these experiments, air has been injected at a flow-rate of 0.037 $\mu\text{L/s}$ into a 2.96, 0.7
30 and 0.0 mM solution of C_{10}G_2 in water, and at a flow-rate of 0.028 $\mu\text{L/s}$ into a 2.41 mM solution of C_{10}G_2 in water, respectively.

It is to be noted that these recorded periodic profiles tilt or offset as time increases. The causes may be varied: change of the heat capacity of the medium when altering its
35 properties (incorporation of air in the cell of the calorimetry device), initial drift of the

baseline, foam formation, appearance of microbubbles, etc.

As defined above, the method of the present invention also comprises a step (c) of obtaining the period of the heat change profile recorded in step (b). The recorded
5 periodic profiles may be stable oscillating around a constant value, or may be affected by disturbances as those explained before. For those cases where the recorded periodic profile tilts, offsets or is altered by outstanding noise as time increases, the following techniques are of special application.

10 With most common techniques to retrieve the period of a periodic function, it is necessary to eliminate or mitigate the drifting of the recorded signal and uphold it to oscillate about a constant value. To this end, a general corrector algorithm has been designed that, independently of the alterations suffered in the experiment, is able to find deviation of the data due to noise or offset with respect to a constant value and
15 correct them.

The most extended technique to retrieve the period of a periodic function is any of the Fourier transforms type. Any of these techniques allows converting a periodic signal in time-domain (evolution of a magnitude, e.g. heat change, over time) to a frequency
20 spectrum, that is, into frequency-domain. On this spectrum, the characteristic frequency (the one with highest amplitude) can be identified, being the calculation of its period immediate by the associate equation ($T = 1 / f$, wherein 'T' denotes period and 'f' denotes frequency). Nevertheless, to correctly perform a Fourier transform, the signal must be oscillatory with respect to a constant value, which is not always fulfilled, as can
25 be seen in the tilt or offset of any the periodic profiles of figure 9a,b as time increases.

Accordingly, in a particular embodiment of the method according to the present invention, step (c) further comprises correcting the heat change recorded as a function of time based on a baseline curve of said heat change, in such a way that the corrected
30 heat change oscillates about a constant value.

In a particular embodiment, the above mentioned correction of the heat change based on the baseline curve comprises the following steps:

35 c1'. obtaining the heat change as a set of data in such a way that:

if heat change is recorded continuously, the heat change is discretized into such set of data, or

if heat change is recorded by sampling, performing step c2';

5 c2'. selecting a given percentage of data, those data being preferably chosen randomly;

c3'. fitting the selected data to a smooth curve, preferably by a cubic spline function;

c4'. repeating steps c2' to c3' for a plurality of different sets of data and fitting each set of data by the corresponding smooth curve;

10 c5'. obtaining the mean value among those smooth curves corresponding to each set of data, a baseline curve being obtained;

c6'. generating a corrected heat change as the difference between the original heat change and the obtained curve being plotted as a function of time in such a way that the corrected heat change generated oscillates around value 0 over time.

15

In a preferred embodiment, the discretization of the heat change in step c1' of the method as defined above is performed by Monte Carlo method.

20 Figures 10a,b shows an example of the discretization and correction of the profiles shown in Figure 9a,b based on respective baseline curves for each one, in such a way that the corrected heat change profiles oscillates about a constant value.

As it was mentioned, the signals recorded and shown in figure 9a,b can be considered as analog signals, i.e. continuous signal for which the time-varying feature is, in this particular case, the 'heat change' produced by bubble or droplets creation.

25

Left side plots of figures 10a,b show the heat change discretized into a given set of data. A 1% of this data is selected randomly by a Monte Carlo method, and it is interpolated by a smooth curve, which will be a cubic spline function by preference.

30

The latter is repeated a high number of times (preferably 1000 times or more) for a plurality of different sets of data (also chosen by Monte Carlo method, 1% each), the resulting sets of data being fitted afterwards by the same smooth curve (e.g. cubic spline function). Once those smooth curves corresponding to each set of data are

35 obtained, the mean value among all is calculated, this mean value corresponding to the

continuous baseline curve which can be seen in figures 10a,b (left side plots).

Right side plots of figures 10a,b show the corrected heat change, which has been generated as the difference between the original heat change (i.e. the profiles of figure 9a,b) and the obtained baseline curve. As it can be seen, the corrected heat change profile oscillates around a constant value over time (0 in this case), being thus eligible for applying any of the Fourier transform techniques to retrieve the period.

In a particular embodiment, the step (c) of the method of the present invention further comprises determining a particular time window of either the heat change or the corrected heat change which has an acceptable variance with regards to the full time range.

In a preferred embodiment, said determination of the particular time window of either the recorded or the corrected heat change comprises the following steps:

- selecting at least one interim time window of either the recorded or the corrected heat change with a default size, preferably a quarter of the full time range;
- calculating the variance of each of the constant interim time windows and identifying the interim time window having the lowest variance;
- increasing sequentially by a predefined time-size the size of the identified interim time window, and calculating for each iteration the associated variance within such increased time window;
- continuing increasing the size of the interim time window by the same predefined time-size until:
 - the associated variance reaches an unacceptable value, preferably until the associated variance of the last iteration doubles the variance of the preceding iteration, or until
 - the associated variance in each iteration converges, this convergence being achieved when a truncation error is less than a predetermined value;
- designating the last increased interim time window with an acceptable variance as the particular time window of either the recorded or the corrected heat change which has an acceptable variance with regards to the full time range.

Figures 11a,b shows an example of a corrected heat change profile which oscillates about a constant value (i.e. 0) being shortened in time-axis to a particular time window which has an acceptable variance. The shortened size is represented starting at the beginning of recording by the respective continuous line.

5

Upon correction of the heat change vs. time profile to obtain an oscillatory signal around a constant value, the inventors of the present invention have observed that, in some cases, not the entire signal is susceptible to be analysed through the Fourier transform.

10

Normally, from 2000 s on the recording of the signal, it undergoes amplification in the amplitude of the oscillations, which may affect the baseline and the periodicity of the signal; that is, altering the result in a way that may be difficult to quantify.

15 To avoid this type of issue, in this embodiment the corrected heat change vs. time profile is shortened to a selected time interval which undergoes less alteration of the signal. To measure this alteration, the variance of the signal will be used by preference.

20 The following protocol has been used to obtain the plots of figures 11a,b. An interim time window of $\frac{1}{4}$ of the total signal of the corrected heat change was selected, and it was moved along the time-axis while calculating the variance of each interim time window shifted. The interim time window having the lowest variance was therefore identified, and this identified interim time window was then sequentially increased by a time-size of 100 s, the associated variance being also calculated at the same time.

25

As a stop condition, the size of the interim time window (later on corresponding to the shortened heat change vs. time profile) was further increased until the associated variance reached an unacceptable value, being in this particular case until the associated variance of the last iteration doubles the variance of the preceding iteration.

30

Regarding the retrieving of the period from a periodic function provided that it is affected by disturbances as mentioned before, figure 12 shows different examples of applying any of the Fourier transform-type techniques to either recorded or corrected heat vs. time profile in order to stress the importance of corrected the signal in order to
35 get an identifiable period. The three examples are applied to a particular heat vs. time

profile recorded whilst injecting air at a flow-rate of 0.028 $\mu\text{L/s}$ into a 2.96 mM solution of C_{10}G_2 in water. The Fourier transform-type techniques applied being, respectively:

- Global Fast Fourier Transform (the so-called G-FFT) applied to the entire recorded
5 heat change with no baseline subtraction (this is, not corrected);
Moving Fast Fourier Transform (the so-called M-FFT) applied to the recorded heat
change with no baseline subtraction, using a time window of 20%; and
G-FFT applied to recorded heat change upon subtraction of the baseline, that is, to the
corrected heat change vs. time profile.

10

As it can be observed from these three plots transformed, only the last one (the one
applied to the corrected profile) has an identifiable period among all the others, whilst
the first and second ones do not allow to identify a period correctly.

- 15 In a preferred embodiment, the period of the heat change recorded as a function of
time in the step (c) of the method as defined above is retrieved by applying any of the
following techniques to the corrected heat change:
- global Fourier transform over the entire recorded heat change profile; or
 - moving Fast Fourier transform, preferably over a time-window of 20%.

20

In this sense, figure 13 shows the results of applying Global Fourier transform to the
corrected and shortened heat change profiles of Figures 11a,b, wherein more than one
period peaks can be seen.

- 25 For this type of profiles with different periodic components, the Fourier transform may
not reveal a single peak and, in addition, its width may be variable. Then, not a
characteristic frequency is obtained, but a frequency distribution. In these cases it is
important to be able to discern which is the period corresponding to the bubbles or
drops formation, and which corresponds to other –unwanted– components of the
30 signal.

- A person skilled in the art would take the period corresponding to the main peak since
it is clearly differentiable among all the others, thus discouraging the pursuit of other
periods. Nevertheless, this is not always correct, and to solve this indeterminacy, two
35 techniques are proposed herein, depending on whether the experiment is being

analysed individually or within set of measurements.

In the first case, an autocorrelation of the data is applied prior to the Fourier transform and the Fourier transform is applied to the autocorrelated data. Thus, in a particular
5 embodiment, step (c) of the method of the present invention further comprises prior to obtaining the period of the recorded heat change, the application of autocorrelation to either the recorded or the corrected heat change in time-domain.

In the second case, the experiment is being analyzed within a set of experiments. In a
10 particular embodiment, the first liquid has a given concentration of a solute with surface activity, and steps (a) to (b) are repeated at least once changing said given concentration of solute, at least two different heat changes in time-domain being recorded for different solute concentration. In this case after obtaining the period according to the method as defined above for each of these recorded heat changes,
15 the method further comprises applying the following steps to each of the transformed heat changes in frequency-domain:

- determining the period with the highest amplitude for each of the at least two transformed heat changes and plotting the periods versus solute concentration;
- adjusting the set of periods to a mathematical function;
- 20 • identifying the outliers among these periods, and:
 - discarding at least one of them, and/or
 - refitting the period for at least one of these outliers by another period that falls on the adjusted mathematical function.

25 In a preferred embodiment, the mathematical function to which the set of periods are adjusted is either of a Langmuir isotherm-type or a logarithmic-polynomic function.

Figure 14 shows an example of a set of periods obtained for a set of experiments in which a given fluid was injected at several flow-rates into a liquid (solution of $C_{10}G_2$ in
30 water) having a solute concentration. In the figure the periods are plotted as a function of the solute concentration and are adjusted to a mathematical function for each flow-rate. In these experiments, the same solute, fluid and liquid have been used and the experiments have been performed at the same temperature, thus only varying the solute concentration and the injection flow-rate.

The expected correlation between the data has been used in order to minimize the ambiguity due to the appearance of several peaks and therefore to optimize the identification of the correct period. Within the behaviour of the curves represented (which is obtained through the adjustment to a Langmuir isotherm-type or a logarithmic-polynomic function), it can be observed if a point has been calculated correctly. In this figure, the points corresponding to atypical data (this is, outliers) in the set of the results have been already adjusted.

In figure 14, the Extended Langmuir Isotherm function has been used for this aim. The steps performed to obtain figure 14 are as follows:

- i. all the data from experiments are fitted to such mathematical function using the peak with the largest amplitude in the FFT;
- ii. outliers are identified; and
- iii. since the period corresponding to other peaks are also identified, the period that is closest to the period predicted by the Extended Langmuir Isotherm is taken as a reference, if it does not coincides with that with maximum amplitude

With the adjustment to the data represented, it can be established around what period it would be expected to obtain a value according to the trend of the rest of the experiments. A wide range is therefore taken around said value and it is sought in it (not in the total range) the largest peak resulting from the FTT.

The characteristic period for each concentration and flow-rate is therefore determined, but not the final value since other in the proximity may affect it. Due to the presence of other peaks in the Fourier transform close to the characteristic period, a further treatment on each of them is preferably applied to determine and correct their uncertainty.

In an embodiment where the transformed heat change in frequency-domain after applying any of the techniques as defined above comprises more than one period, identified as a main period corresponding to the period with the highest amplitude and at least one secondary period corresponding to periods with smaller amplitudes, the method comprises in step (c) the following steps applied to the function of amplitude versus period resulting from applying any of the techniques as defined above:

- decomposing the function into a number N of Gaussian functions over the main

period of the function;

- evaluating the amplitude-width rate for each Gaussian function, the one with the highest rate being identified as the main Gaussian function;
- calculating the intersection area of each remainder Gaussian function with the main Gaussian function;
- setting a weighting value for each Gaussian function based on such intersection area; and
- sizing the period value taking into consideration the contribution of each Gaussian function with their weighting value.

10

In a preferred embodiment, the steps (a) to (b) are repeated at least once at same conditions for a first liquid having different solute concentrations, at least two different heat changes in time-domain being recorded, one corresponding to each solute concentration. In this embodiment the main Gaussian function is identified with the one with the closest period to the mathematical function expected according to the method as defined above.

Figure 15 shows a particular example of the Fourier transform of the profile shown in figure 13, corresponding to air injected at a flow-rate of 0.037 $\mu\text{L/s}$ into a 0.7 mM solution of C_{10}G_2 in water shown in figure 13 but decomposed into a number N of Gaussian functions over the main period of the function.

The profile of figure 15 comprises 4 Gaussian functions around the main period (≈ 64 sec.), of which the main Gaussian function is identified as the one with the highest amplitude-width rate, and the other three secondary periods corresponding to periods with smaller amplitudes. In other words, over an interval around the main period, an adjustment is made to the number of Gaussian functions (four in this example) and the one with the highest ratio amplitude / width as the main one is selected.

The main peak is identified with the main period and three other peaks, which add uncertainty to the determination of the defining period of the measure, can be seen.

The normalized area of intersection of the remainder Gaussian functions with the main one is determined and a weighted average is made with a statistical weight proportional to said area (being maximized for the main Gaussian since its intersection

with itself is 100%). Upon this, the period value is sized taking into consideration the contribution of each Gaussian function with their weighting value.

In other words, the final period is determined as the average of all the periods (those
5 corresponding to the different Gaussian functions identified), weighted by the intersection of each Gaussian with that corresponding to the main period.

It is to be noted that, in case a number of experiments are performed, the reference
peak is preferably identified as the closest to the period predicted by the Extended
10 Langmuir Isotherm fitting, for instance. On the contrary, for individual measurements, including the calibration points, the reference period is that which preferably corresponds to the Gaussian with highest amplitude.

Figure 16 shows a comparison of interfacial tension obtained by the method of the
15 present invention and data coming from the literature for other standard methods.

The inventors of the present invention have found that uncertainties within the method have the following contributions:

- Standard error obtained from covariance matrix of the sum of Gaussian
20 function; and/or
- FFT inherent uncertainty.

In this figure, the interfacial tension determined using the present method according to the invention for ethanol and decyl-maltoside ($C_{10}G_2$) in aqueous solution for different
25 concentrations (■), together with data coming from the literature for other standard methods to measure interfacial tension for the same systems (●), are plotted. The shadow indicates the uncertainty calculated of the data. As a conclusion, it can be seen clearly observed that the accuracy of the present invention in regards of the known literature is high.

30

In the following, other data treatment processes which improve the correct determination of the period are explained.

As it was already mentioned, in a particular embodiment, the step (c) of the method as
35 defined above further comprises correcting the heat change recorded as a function of

time based on a baseline curve of said heat change, in such a way that the corrected heat change oscillates about a constant value. This entailed the proviso that any of the Fourier Transforms techniques can be applied with good expectation in determining a differentiable period.

5

Alternative to methods explained hereinbefore, in a particular embodiment, the above mentioned correction of the heat change based on the baseline curve comprises the following steps:

- c1. obtaining the heat change as a set of data in such a way that:
 - 10 if heat change is recorded continuously, the heat change is discretized into such set of data, or
 - if heat change is recorded by sampling, performing step c2;
- c2. up-sampling said set of data with data chosen randomly, and distributing it over the recorded set of data,
- 15 c3. plotting the data from step c2 into a 2-D histogram, preferably each bin of the 2-D histogram having a width equal to the square root of the size (L) of the original set of data;
- c4. calculating the mean value of heat change for each bin of the 2-D histogram, and plotting each calculated mean value as a function of time;
- 20 c5. interpolating the data resulting from the previous step into a smooth curve, preferably by a cubic spline function, a baseline curve being obtained; and
- c6. generating a corrected heat change as the difference between the recorded heat change and the obtained baseline curve being plotted as a function of time in such a way that the corrected heat change generated oscillates around value 0
- 25 over time.

In a preferred embodiment, the discretization of the heat change in step c1 of the method as defined above is performed by Monte Carlo method.

- 30 Figure 17a shows a sampled heat vs. time profile recorded while introducing air at a flow-rate of 0.028 $\mu\text{L/s}$ into a 2.96 mM solution of C_{10}G_2 in water. In this figure, a set of data of the heat change has been obtained by sampling each second the recorded heat change. Since the overall recording lasted 1 hour, 3600 dots form said set of data in the array.

35

In figure 17b, this sampled profile has been up-sampled ten times, that is, now the number of dots forming such up-sampled set of data consists of 36000 dots in the array. In particular, the data to produce the up-sampling is randomly selected by a Monte-Carlo method. Due to the up-sampling, many of the original recorded set of data
5 are repeated, thus creating a new Z-axis to measure the repetition of data as can be seen in figure 17b.

This process lowers the resolution of the original recorded set of data, thus highlighting the most densely populated regions in the heat change-time plot, which corresponds to
10 the most representative data for subsequently calculating the baseline. On the other hand, the noisy regions are more diluted, since it is less likely that up-sampled data falls on said regions.

This information after up-sampling is plotted into a 2-D histogram, where the bins of the
15 2-D histogram will have a constant width. In an embodiment, each bin of the 2-D histogram has a width equal to the square root of the size of the original set of data. That is, for the example of figure 17 each bin width of the 2-D histogram would be of 60 s or 1 min., thus gathering all the dots (including the dots of the up-sampling) which falls within that minute of recording. The mean value of heat change for these bins is
20 calculated, thus obtaining the mean heat change for each minute of recording, and being decreased the number of data in time domain to 60 (one each minute, during the original hour of overall recording).

In figure 17c, average of the heat change based on the previous 2D histogram is
25 plotted in time-axis.

In another particular embodiment, after step c4 and prior to step c5, the method of the present invention further comprises applying a finite impulse response filter of the type of moving average, preferably using a time-window having a size correspondent to
30 10% of the size of the set of data discretized from the recorded heat change.

In a preferred embodiment, after step c4 and prior to step c5, the method according to the present invention further comprises the following steps:

- providing at least one time-window;

- applying a Savitzky-Golay filter to the plot resulting from step c3 using a mathematical function and the provided time-window, obtaining as a result a filtered plot; and
- in case that more than one time-windows be provided, averaging the obtained filtered plots into a single function.

Even in a more preferred embodiment, the three time-windows have sizes of 20%, 30%, and 40% of the time scale.

- 10 Figure 18a and b show a particular example of the profile of figure 17c after applying a Savitzky-Golay filter. Particularly, the Savitzky-Golay filter is used with a parabolic fitting in this example. In addition, the filter is independently applied using different three time windows of 20%, 30% and 40% of the total time scale of the experiment. These time windows are wide enough so as to assure that all the fluctuation types that may arise in
- 15 the recorded profile are mitigated.

- Since the filter is independently applied three times, in this example the final result is taken as the average of the three resulting functions. Then, an interpolation using cubic-splines has been applied in order to get a full baseline array for all the time
- 20 values.

It is to be noted that, for the first and last points of this array, since they cannot be interpolated, the original experimental heat change-time points are taken.

- 25 In figure 18b, a moving average has been applied to the filtered profile of figure 18a. In particular, a time window of 10% has been used. Advantageously, this is to decrease unlikely residual sharp changes in the previous estimated baseline.

- Figure 19 shows a corrected heat change as the difference between the recorded heat
- 30 change and the obtained baseline curve of figure 18b.

A further method for retrieving the period of the heat change recorded as a function of time in the step (c) of the method according to the present invention is shown in figures 20a to c and it is explained as follows:

Figure 20a shows figure 19 after applying Moving Fast Fourier Transform when a time window of 20% is used. As it can be observed, the FFT starting at each time is represented in the y-axis with a gradient for the amplitude of each peak.

- 5 In figure 20b, the FFT shown in the y-axis of figure 20a are normalized by area. Thus, the FFTs with several periods, i.e., with several peaks, will have lower amplitudes. Advantageously, this diffuses the regions of the signal with less periodicity (or, which is the same, more noise).
- 10 As a result, figure 20c shows a final FFT obtained from the average of the 'Area-Normalized M-FFT' of figure 20b. Notably, in the majority of cases, it provides a single peak that corresponds to the main period without needing further refinement of data treatment to retrieve the period.
- 15 In a preferred embodiment, steps (a) to (e) of the method of the present invention are repeated for a set of different flow-rates while maintaining the same second fluid.

As define above, the method of the present invention also comprises a step (d) of providing a calibrated relationship of the interfacial tension as a function of period for
20 said constant flow-rate and said second fluid.

In this sense, another aspect of the present invention refers to a method for establishing the calibrated relationship provided in step (d) of the method as defined above wherein this method comprises the steps of:

- 25 i. applying steps (a) to (c) of the method of the present invention to at least two different pure first liquids of which the respective interfacial tension with the second fluid is known upon introduced in step (a) at a given flow-rate;
- ii. plotting their respective interfacial tensions as a function of their respective obtained periods; and
- 30 iii. fitting the plotted data to a function thus providing a relationship between the interfacial tension to be determined and the period for said given flow-rate.

As a result of this method, the calibrated relationship of figure 6 shall be obtained, for instance.

In a preferred embodiment, steps (i) to (iii) are repeated for the same different pure first liquids at a set of different flow-rates, wherein for each of these flow-rates a different function is obtained.

- 5 Another aspect of the present invention refers to a data processing apparatus comprising means for carrying out the steps (c) to (e) of the method of the present invention.

The present invention also refers to a computer program comprising instructions which,
10 when the program is executed by a computer, cause the computer to carry out the steps (c) to (e) of the method as defined above.

Another aspect of the present invention refers to a computer-readable medium comprising instructions stored thereon that, when executed, cause the computer to
15 carry out the steps (c) to (e) of the method as defined above.

As previously mentioned, the method of the present invention can be performed using commercially available calorimetry devices with no modifications at all.

- 20 Therefore, another aspect of the present invention refers to the use of a calorimeter for the determination of the interfacial tension between a first liquid fluid with a second fluid.

In a preferred embodiment, the calorimeter is an isothermal titration calorimeter (ITC).
25

Minor modifications in the calorimetry or device for calorimetry measurement would allow determining bubble formation processes with more resolution. This would allow quantifying both the thermodynamics and kinetics of the process. Therefore, the signal recorded presents less noise and, consequently, needs less data treatment.

30 In a particular embodiment, the calorimeter used for carrying out the steps (a) and (b) of the method of the present invention comprises:

- a container cell configured to house separately a first liquid from a second fluid;
- a first capillary configured to introduce the second fluid into the first liquid;

- first conveying means in fluid communication with the first capillary, the first conveying means configured to convey the second fluid at a constant flow-rate into the first liquid in such a way that said second fluid is introduced into the first liquid as droplets or bubbles;
- 5 - sensing means in thermal contact with the first liquid, and configured to detect heat change associated to the periodical formation of successive droplets or bubbles; and
- a recording unit associated to the sensing means and configured to record the heat change detected by the sensing means as a function of time.

10

It has been surprisingly observed by the inventors of the present invention that wider capillaries result in different data recorded and therefore different accuracy obtained, being the wider the capillary, the higher the accuracy of recorded heat change.

- 15 In a particular embodiment, the calorimeter used for carrying out the steps (a) and (b) of the method of the present invention further comprises a second capillary configured to introduce a different second fluid into the first liquid, and a second conveying means in fluid communication with said second capillary, wherein the second conveying means is configured to convey said different second fluid at a constant flow-rate into
- 20 the first liquid.

Therefore, according to this embodiment it is possible to modify the solute concentration or solvent nature of the first liquid in a single experiment.

- 25 Advantageously, with two independent injection systems (one intended to change composition of the first liquid in the sample cell and the other for introducing the second fluid) avoids the need to open the system to change the composition. This allows speeding up the overall experiment time since the time needed to span a variety of solvent mixtures is significantly reduced. Thus, measurements of liquid/fluid interfacial
- 30 properties are extensively facilitated. By using two independent injectors, the heat change in bubble formation for each solute concentration could be measured, as well as the heat involved in the concentration change in the bulk phase.

- Advantageously, an injection system with enhanced pressure control achieves a dual
- 35 effect since, on one hand, the pressure control allows better control over the heat

change measurement and, on the other hand, the pressure control comes with a pressure indication so that not only information of the bubble frequency and the associated heat is obtained by measuring the heat change but also by the pressure variation that the capillary suffers when the bubble is released.

5

In a preferred embodiment, the sensing means of the calorimeter are either a thermistor or a thermoelectric device, such as a peltier module.

EXAMPLES*Interfacial tension measurements*

- 5 Within figure 3, the interfacial tension of the three samples was measured at 298 ± 0.1 K by using a Lauda drop volume tensiometer (TVT 2 model, Germany) with the measurement cell connected to an external temperature bath. A capillary with inner radius of 1.70 mm and a 2.5 mL syringe were employed in all cases.
- 10 In all experiments, ultrapure water (Elix 3 purification system, Milipore Corp.), ethanol (99.8 % min. purity from Panreac) and a 0.55 mM dodecyl- β -D-maltopyranoside ($C_{12}G_2$) aqueous solution were used. The surfactant (from Anatrace) and the ethanol were used as received.
- 15 The measurements were performed using the “standard mode” as programmed in the LAUDA software, that is, the flow- rate is sequentially halved 4 times starting from $0.81 \text{ s}^{-1} \cdot \mu\text{L}$ for water and surfactant solution and from $0.40 \text{ s}^{-1} \cdot \mu\text{L}$ for ethanol, registering the maximum volume of the drop when it detaches from the capillary. The obtained interfacial tension values were $70.95 \pm 0.11 \text{ mN/m}$, $21.85 \pm 0.10 \text{ mN/m}$ and $34.81 \pm$
- 20 0.20 mN/m for water, ethanol and the surfactant solution, respectively.

The calorimetric experiments were performed with an Isothermal Titration Calorimeter instrument (VP-ITC from MicroCal, Inc). The injection syringe was carefully cleaned and filled with normal air at room pressure. Air from the syringe was continuously

25 injected in the sample cell containing one of the liquids at fixed constant rates of 0.111, 0.055, 0.028 and $0.022 \mu\text{L/s}$, depending on the experiment. Rates slower than $0.022 \mu\text{L/s}$ are not allowed in the used injection system. Thus, a series of bubbles were formed and released in the tip of the capillary. The stirrer was switched off in all measurements in order to prevent mechanical perturbation of the bubbles and

30 therefore ensure that they are spontaneously released when they reach a critical volume. No system to remove the released bubbles was introduced, thus they can travel to the top of the coin-shaped sample cell or higher up through the connected outlet tube, eventually reaching the air gap at the top of the tube, remaining there till the end of the experiment. Parallel air-injection experiments were performed out of the

35 calorimeter in a simple pyrex flask to observe the bubble injection.

The ITC raw data (power vs time plots) showed a highly reproducible periodic profile for the experiments performed at moderate flow-rates. The formation of each bubble was clearly identified by a sharp negative peak (exothermic process) followed by a positive plateau, before the signal comes back to the baseline. This behavior was clear for the experiments performed with water and the surfactant solution (Figs. 2, 3, 4 and 5) and it reveals the presence of at least two kinetic processes for each bubble. For ethanol the signal seems to evolve with time within the same experiment, and the period between bubbles is significantly shorter than those of water and the C₁₂G₂ solution (see Figs 6 and 7). Additionally, the signal corresponding to different bubbles in ethanol seems to overlap at 18 and 36 s/ μ L and, to a lesser extent, also at 45 s/ μ L and thus no baseline between bubbles is detected (Fig. 3). If the flow rate is too high, then it may be not possible to distinguish the bubble formation, and therefore it was not possible to obtain a clear signal at 9 s/ μ L for this solvent due to the extremely high overlap of the calorimetric signal between bubbles at that flow-rate.

As expected, the faster the injection, the shorter the period of the bubble formation. For each flow-rate, the interfacial tension of the different samples showed to be perfectly proportional to this period (see Fig. 4). This allows performing interface tension measurements using ITC with no modifications in the instrument, upon obtaining a calibration curve for a variety of samples/mixtures, as in other methods for interfacial tension determination (for instance the maximum drop volume method). The area of the signal corresponding to each bubble was integrated, separating the positive and the negative contributions. A polynomial baseline correction was applied in order to perform this integration. Both contributions were well reproducible for different injections within the same experiment and also for different independent experiments (see Fig. 8). As mentioned above, these signals should contain information on at least two coupled processes. Using the instrument experimental setup with no modifications, the volume of the bubbles is expected to be between 3 and 9 μ L, depending on the liquid used.

CLAIMS

1.- A method of determining an interfacial tension between a first liquid and a second fluid, the method comprising the steps of:

5

a. introducing the second fluid into the first liquid at a constant flow-rate so as to cause the second fluid to periodically form successive droplets or bubbles;

10

b. recording a heat change of step (a) as a function of time, obtaining a periodic heat versus time profile;

c. obtaining the period of the heat change recorded in step (b);

d. providing a calibrated relationship of the interfacial tension as a function of period for said constant flow-rate and said second fluid; and

15

e. correlating the period obtained in step (c) with the calibrated relationship, and determining the interfacial tension between the first liquid and the second fluid for said constant flow-rate.

2.- The method according to claim 1, wherein step (c) further comprises correcting the heat change recorded as a function of time based on a baseline curve of said heat change, in such a way that the corrected heat change oscillates about a constant value.

3.- The method according to claim 2, wherein correcting the heat change based on the baseline curve comprises the following steps:

25

c1. obtaining the heat change as a set of data in such a way that:

if heat change is recorded continuously, the heat change is discretized into such set of data, or

if heat change is recorded by sampling, performing step c2;

30

c2. up-sampling said set of data with data chosen randomly, and distributing it over the recorded set of data,

c3. plotting the data from step c2 into a 2-D histogram, preferably each bin of the 2-D histogram having a width equal to the square root of the size (L) of the original

35

set of data;

c4. calculating the mean value of heat change for each bin of the 2-D histogram, and plotting each calculated mean value as a function of time;

5

c5. interpolating the data resulting from the previous step into a smooth curve, preferably by a cubic spline function, a baseline curve being obtained; and

10 c6. generating a corrected heat change as the difference between the recorded heat change and the obtained baseline curve being plotted as a function of time in such a way that the corrected heat change generated oscillates around value 0 over time.

15 4.- The method according to claim 3, wherein after step c4 and prior to step c5 the method further comprises applying a finite impulse response filter of the type of moving average, preferably using a time-window having a size correspondent to 10% of the size of the set of data discretized from the recorded heat change.

20 5.- The method according to any of claims 3 or 4, wherein after step c4 and prior to step c5 the method further comprises the following steps:

- providing at least one time-window;
- applying a Savitzky-Golay filter to the plot resulting from step c3 using a mathematical function and the provided time-window, obtaining as a result a filtered plot; and
- 25 • in case that more than one time-windows be provided, averaging the obtained filtered plots into a single function.

30 6.- The method according to claim 5, wherein three time-windows are provided having sizes of 20%, 30%, and 40% of the time scale.

7.- The method according to claim 2, wherein correcting the heat change based on the baseline curve comprises the following steps:

35 c1'. obtaining the heat change as a set of data in such a way that:
if heat change is recorded continuously, the heat change is

discretized into such set of data, or

if heat change is recorded by sampling, performing step c2;

- c2'. selecting a given percentage of data, those data being preferably chosen randomly;
- 5 c3'. fitting the constant data to a smooth curve, preferably by a cubic spline function;
- c4'. repeating steps c2' to c3' for a plurality of different sets of data and fitting each set of data by the corresponding smooth curve;
- 10 c5'. obtaining the mean value among those smooth curves corresponding to each set of data, a baseline curve being obtained;
- c6'. generating a corrected heat change as the difference between the original heat change and the obtained curve being plotted as a function of time in such a way that the corrected heat change generated oscillates around value 0 over time.

15

8.- The method according to any of claims 3 to 7, wherein discretization of the heat change is performed by Monte Carlo method.

- 20 9.- The method according to any of claims 1 to 8, wherein in step (c) the period of the heat change recorded as a function of time is retrieved by applying any of the following techniques to the corrected heat change:

- global Fourier transform over the entire recorded heat change profile; or
- moving Fast Fourier transform, preferably over a time-window of 20%.

- 25 10.- The method according to claim 9, wherein the first liquid has a given concentration of a solute with surface activity, and steps (a) to (b) are repeated at least once changing said given concentration of solute, at least two different heat changes in time-domain being recorded for different solute concentration;

- 30 wherein after obtaining the period according to claim 8 for each of these recorded heat changes, the method further comprises applying the following steps to each of the transformed heat changes in frequency-domain:

- determining the period with the highest amplitude for each of the at least two transformed heat changes and plotting the periods versus solute concentration;
 - 35 • adjusting the set of periods to a mathematical function;

- identifying the outliers among these periods, and:
 - discarding at least one of them, and/or
 - refitting the period for at least one of these outliers by another period that falls on the adjusted mathematical function.

5

11.- The method according to claim 10, wherein the mathematical function to which the set of periods are adjusted is either of a Langmuir isotherm-type or a logarithmic-polynomic function.

- 10 12.- The method according to any of claims 10 or 11, wherein the transformed heat change in frequency-domain after applying any of the techniques defined in claim 8 comprises more than one period identified as a main period corresponding to the period with the highest amplitude, and at least one secondary period corresponding to periods with smaller amplitudes;

15

wherein the method comprises in step (c) the following steps applied to the function of amplitude versus period resulting from applying any of the techniques defined in claim 9:

- 20
- decomposing the function into a number N of Gaussian functions over the main period of the function;
 - evaluating the amplitude-width rate for each Gaussian function, the one with the highest rate being identified as the main Gaussian function;
 - calculating the intersection area of each remainder Gaussian function with the
- 25
- main Gaussian function;
 - setting a weighting value for each Gaussian function based on such intersection area; and
 - sizing the period value taking into consideration the contribution of each Gaussian function with their weighting value.

30

13.- The method according to claim 12, wherein steps (a) to (b) are repeated at least once at same conditions, at least two different heat changes in time-domain being recorded corresponding to different solute concentrations;

- 35 wherein the main Gaussian function is identified with the one with the closest period to

the mathematical function expected according to any of claims 10 or 11.

14.- The method according to claim 2 and any of claims 1 to 13, wherein step (c) further comprises determining a particular time window of either the heat change or the
5 corrected heat change which has an acceptable variance with regards to the full time range.

15.- The method according to claim 2 and any of claims 1 to 14, wherein determination of the particular time window of either the recorded or the corrected heat change
10 comprises the following steps:

- selecting at least one interim time window of either the recorded or the corrected heat change with a default size, preferably a quarter of the full time range;
- calculating the variance of each of the constant interim time windows and
15 identifying the interim time window having the lowest variance;
- increasing sequentially by a predefined time-size the size of the identified interim time window, and calculating for each iteration the associated variance within such increased time window;
- continuing increasing the size of the interim time window by the same
20 predefined time-size until:
 - the associated variance reaches an unacceptable value, preferably until the associated variance of the last iteration doubles the variance of the preceding iteration, or until
 - the associated variance in each iteration converges, this convergence
25 being achieved when a truncation error is less than a predetermined value;
- designating the last increased interim time window with an acceptable variance as the particular time window of the corrected heat change which has an acceptable variance with regards to the full time range.

30 16.- The method according to any of claims 1 to 15, wherein step (c) further comprises prior to obtaining the period of the recorded heat change, the application of autocorrelation to the recorded heat change in time-domain.

35 17.- The method according to any of claims 1 to 16, wherein the steps (a) to (e) are

repeated for a set of different flow-rates while maintaining the same second fluid.

18.- The method according to any of claims 1 to 17, wherein the second fluid is a gas, and preferably air.

5

19.- The method according to any of claims 1 to 18, wherein the constant flow-rate at which the second fluid is introduced into the first liquid ranges between 200 microliters in 9000 seconds and 200 microliters in 900 seconds.

10 20.- The method according to any of claims 1 to 19, wherein the temperature is maintained constant throughout the steps (a) and (b).

21.- The method according to any of claims 1 to 20, wherein the steps (a) and (b) are performed by the use of a calorimeter.

15

22.- A method for establishing the calibrated relationship provided in step (d) of the method according to any of claims 1 to 21, wherein this method comprises the steps of:

- 20
- i. applying steps (a) to (c) of the method according to any of claims 1 to 21 to at least two different pure first liquids of which the respective interfacial tension with the second fluid is known upon introduced in step (a) at a given flow-rate;
 - ii. plotting their respective interfacial tensions as a function of their respective obtained periods; and
 - iii. fitting the plotted data to a function thus providing a relationship between the interfacial tension to be determined and the period for said given flow-rate.

25

23.- The method for establishing the calibrated plot according to claim 21, wherein the method comprises repeating steps (i) to (iii) for the same different pure first liquids at a set of different flow-rates, wherein for each of these flow-rates a different function is obtained.

30

24.- A data processing apparatus comprising means for carrying out the steps (c) to (e) of the method according to any of claims 1 to 21.

25.- A computer program comprising instructions which, when the program is executed
35 by a computer, cause the computer to carry out the steps (c) to (e) of the method

according to any of claims 1 to 21.

26.- A computer-readable medium comprising instructions stored thereon that, when executed, cause the computer to carry out the steps (c) to (e) of the method according
5 to any of claims 1 to 21.

27.- Use of a calorimeter for the determination of the interfacial tension between a first liquid fluid with a second fluid.

10 28.- Use of a calorimeter in the method according to any of claims 1 to 21 for carrying out the steps (a) and (b), the calorimeter comprising:

a container cell configured to house separately a first liquid from a second fluid;

a first capillary configured to introduce the second fluid into the first liquid;

15 first conveying means in fluid communication with the first capillary, the first conveying means configured to convey the second fluid at a constant flow-rate into the first liquid in such a way that said second fluid is introduced into the first liquid as droplets or bubbles;

sensing means in thermal contact with the first liquid, and configured to detect heat change associated to the periodical formation of successive droplets or bubbles;
20 and

a recording unit associated to the sensing means and configured to record the heat change detected by the sensing means as a function of time.

29.- Use of a calorimeter according to claim 28, wherein the calorimeter further
25 comprises a second capillary configured to introduce a different second fluid into the first liquid, and a second conveying means in fluid communication with said second capillary, wherein the second conveying means is configured to convey said different second fluid at said constant flow-rate into the first liquid.

30 30.- Use of a calorimeter according to any of claims 28 or 29, wherein the first conveying means of the calorimeter comprises a pressure control sensor.

31.- The calorimeter according to any of claims 28 to 30, wherein the sensing means are either a thermistor or a thermoelectric device, such as a peltier module.

32.- The calorimeter according to any of claims 27 to 31, wherein the calorimeter is an isothermal titration calorimeter.

FIGURES

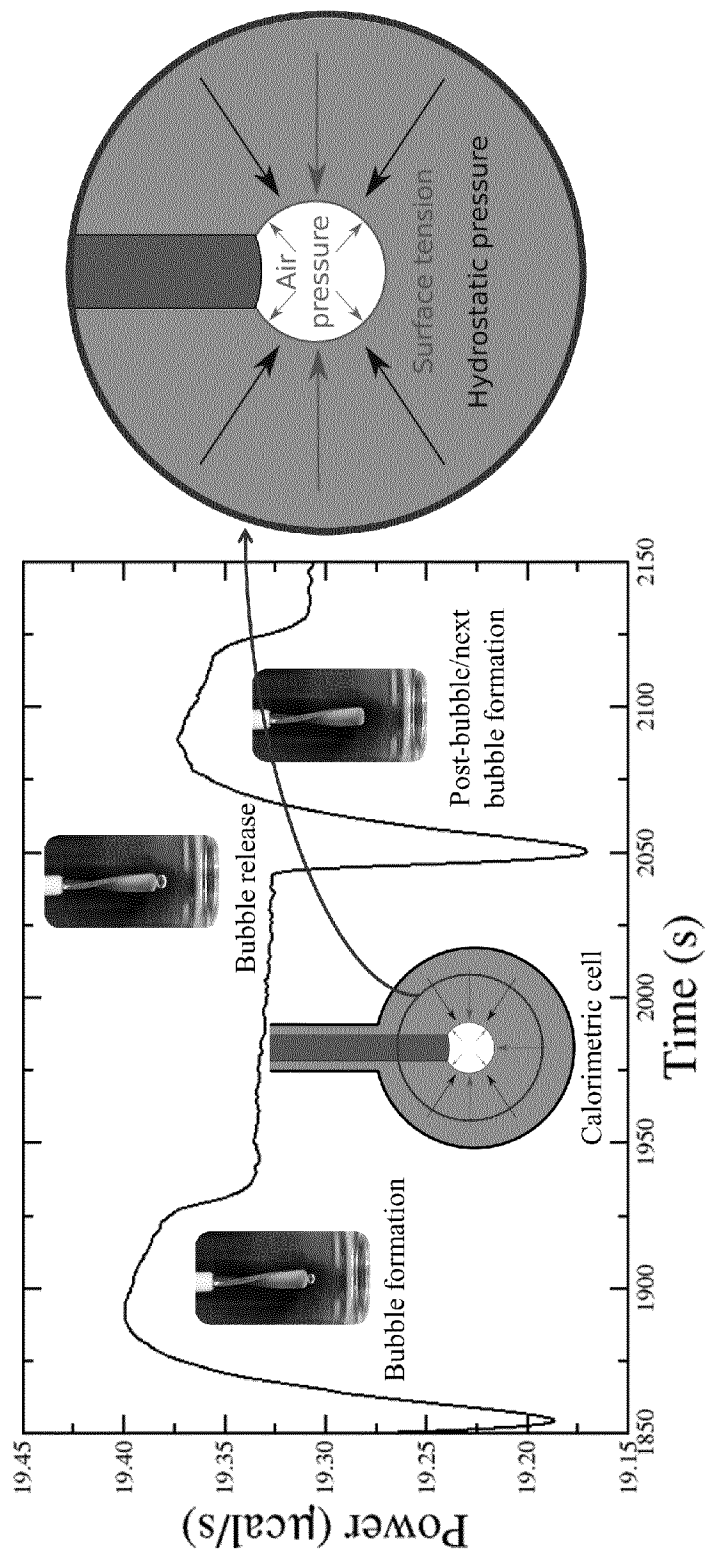


Figure 1

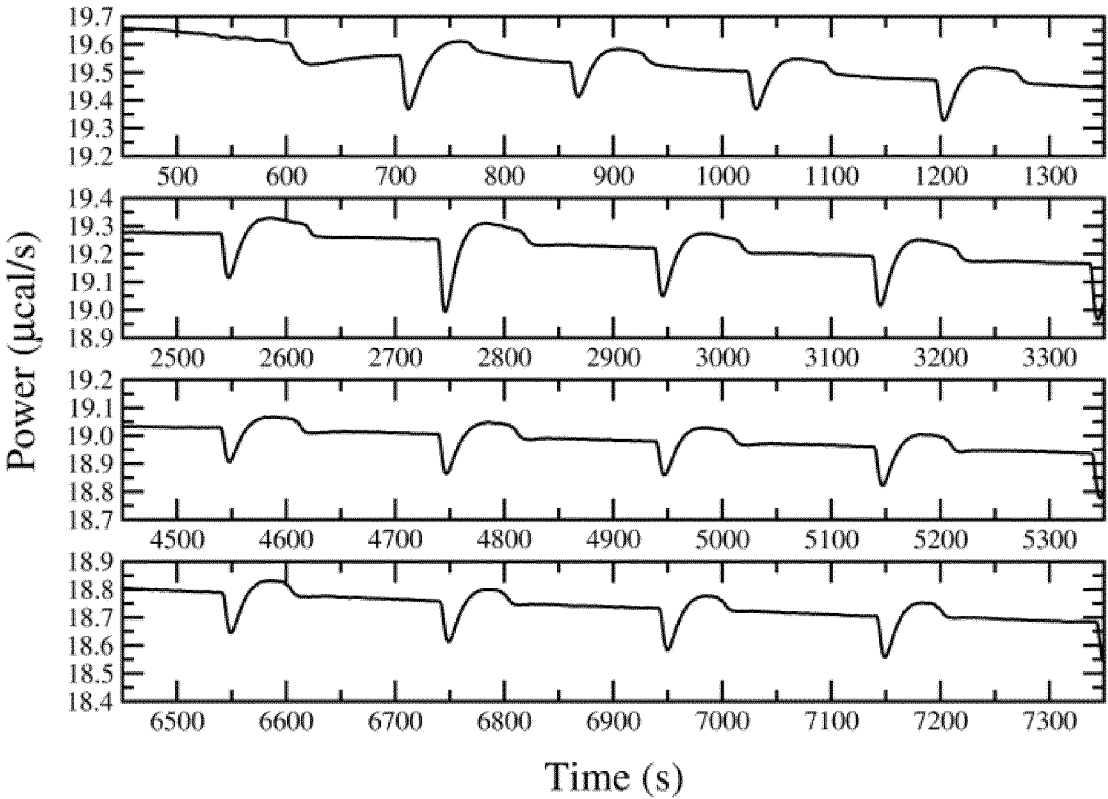


Figure 2

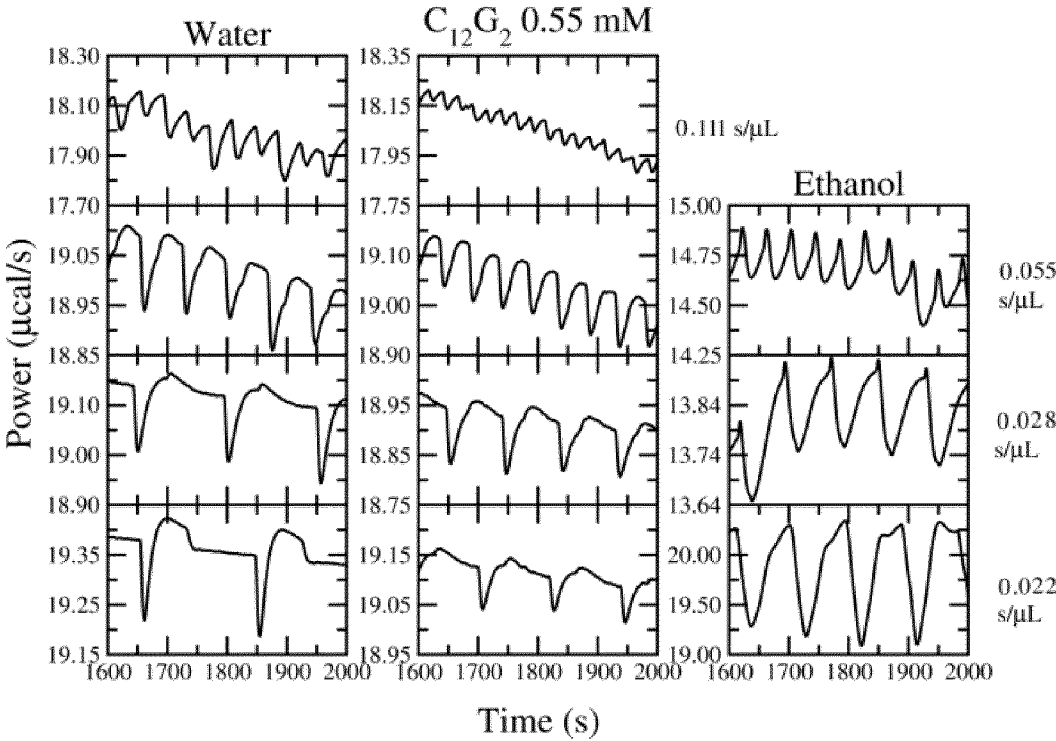
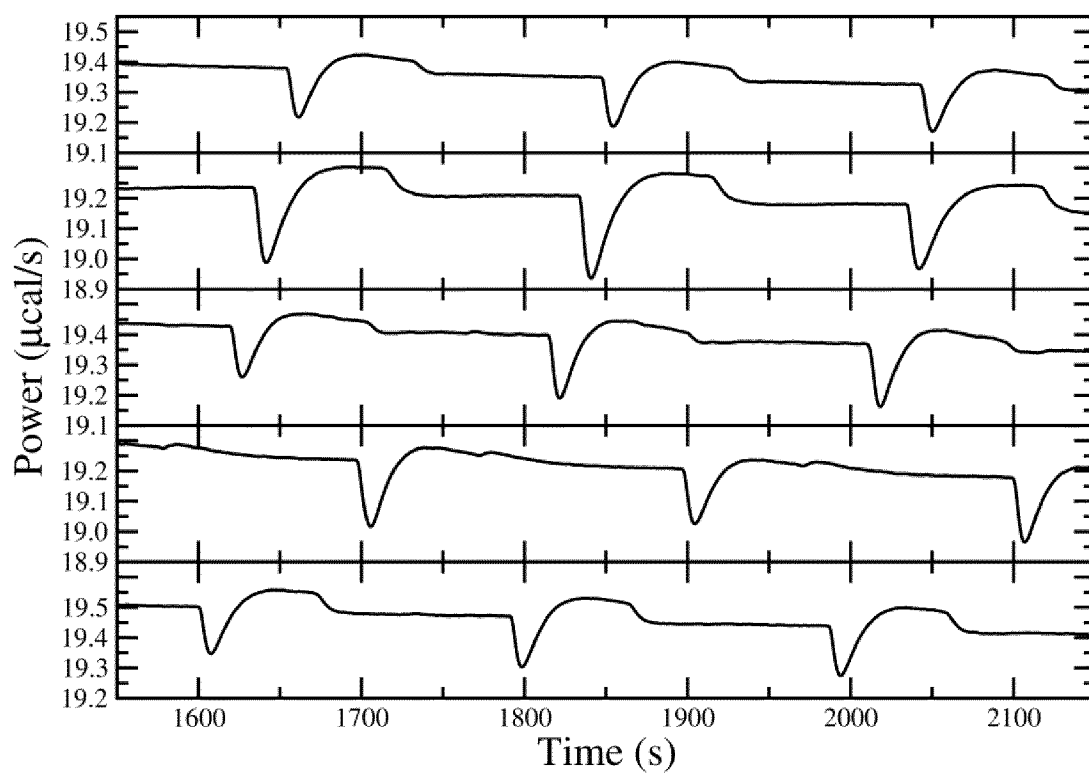
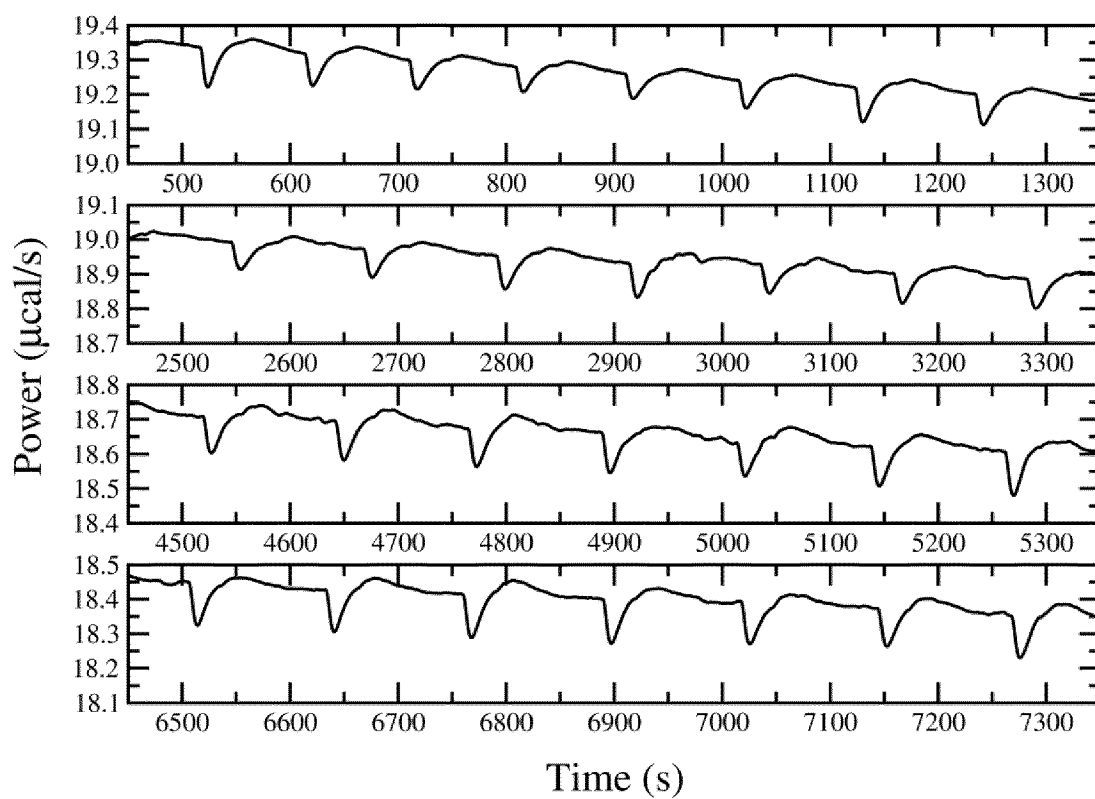


Figure 3

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**Figure 4****Figure 5**

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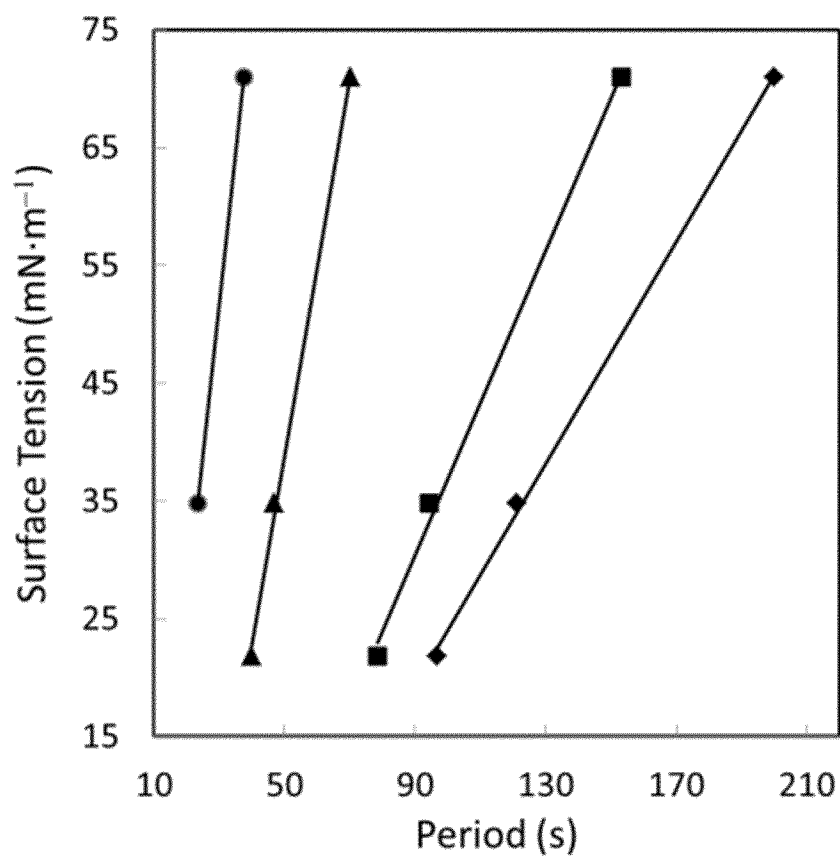


Figure 6

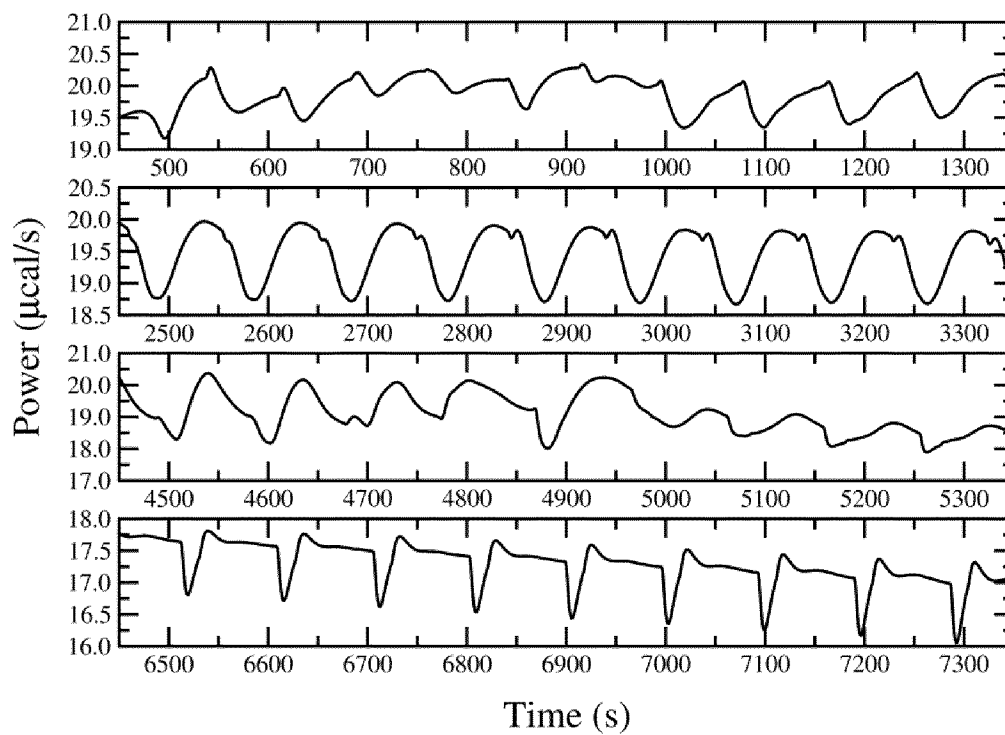


Figure 7

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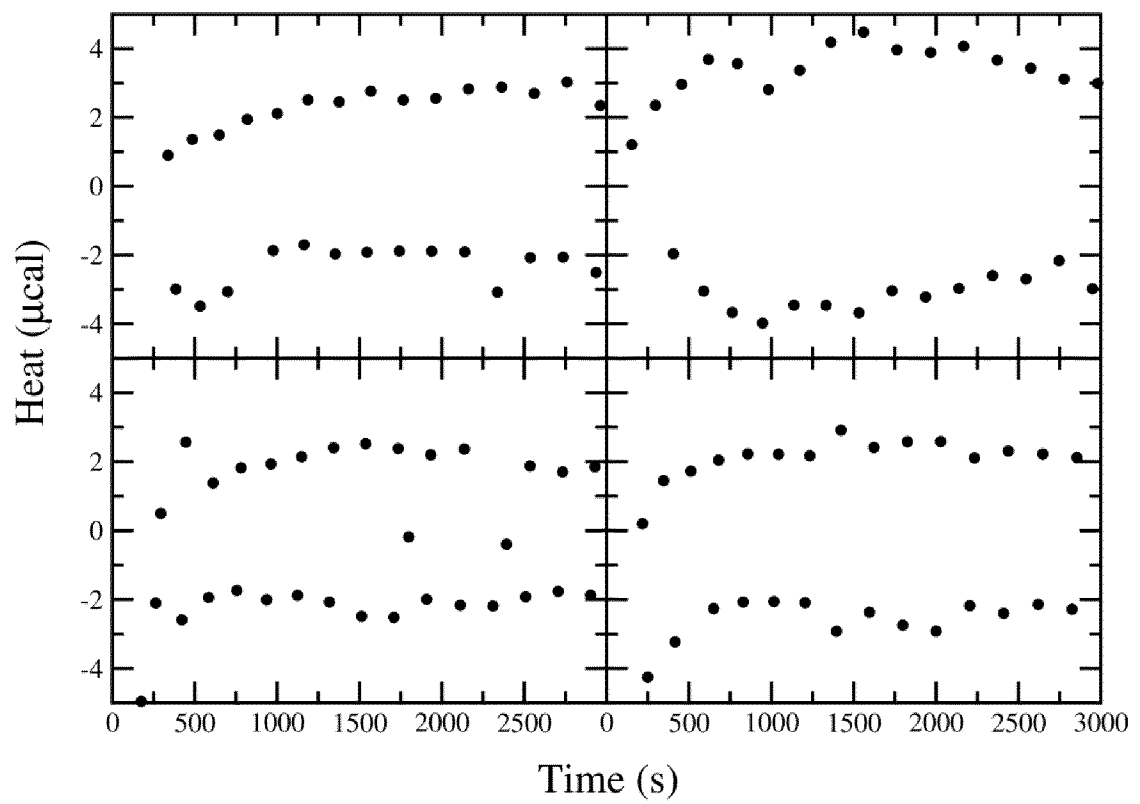


Figure 8

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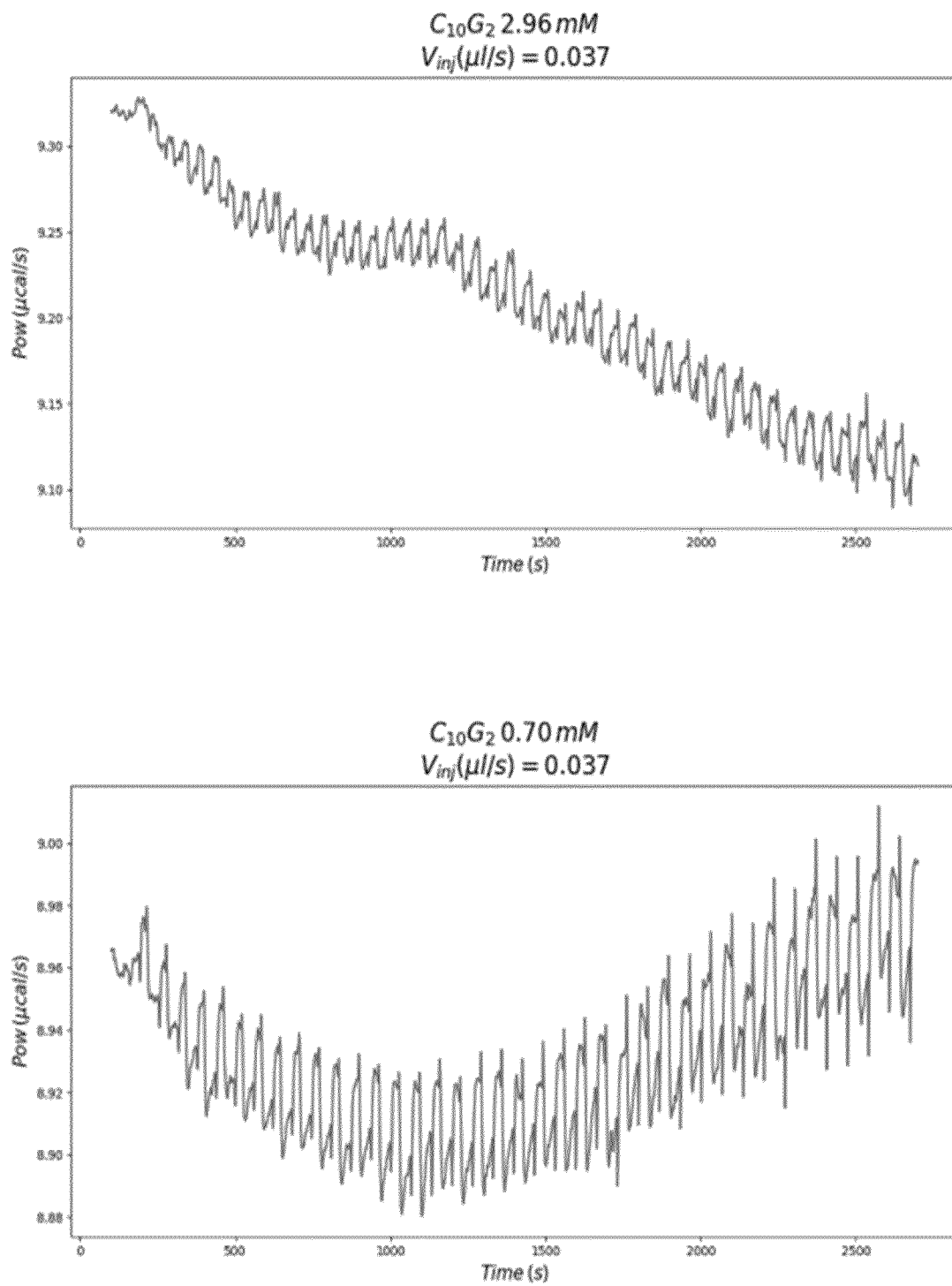


Figure 9a

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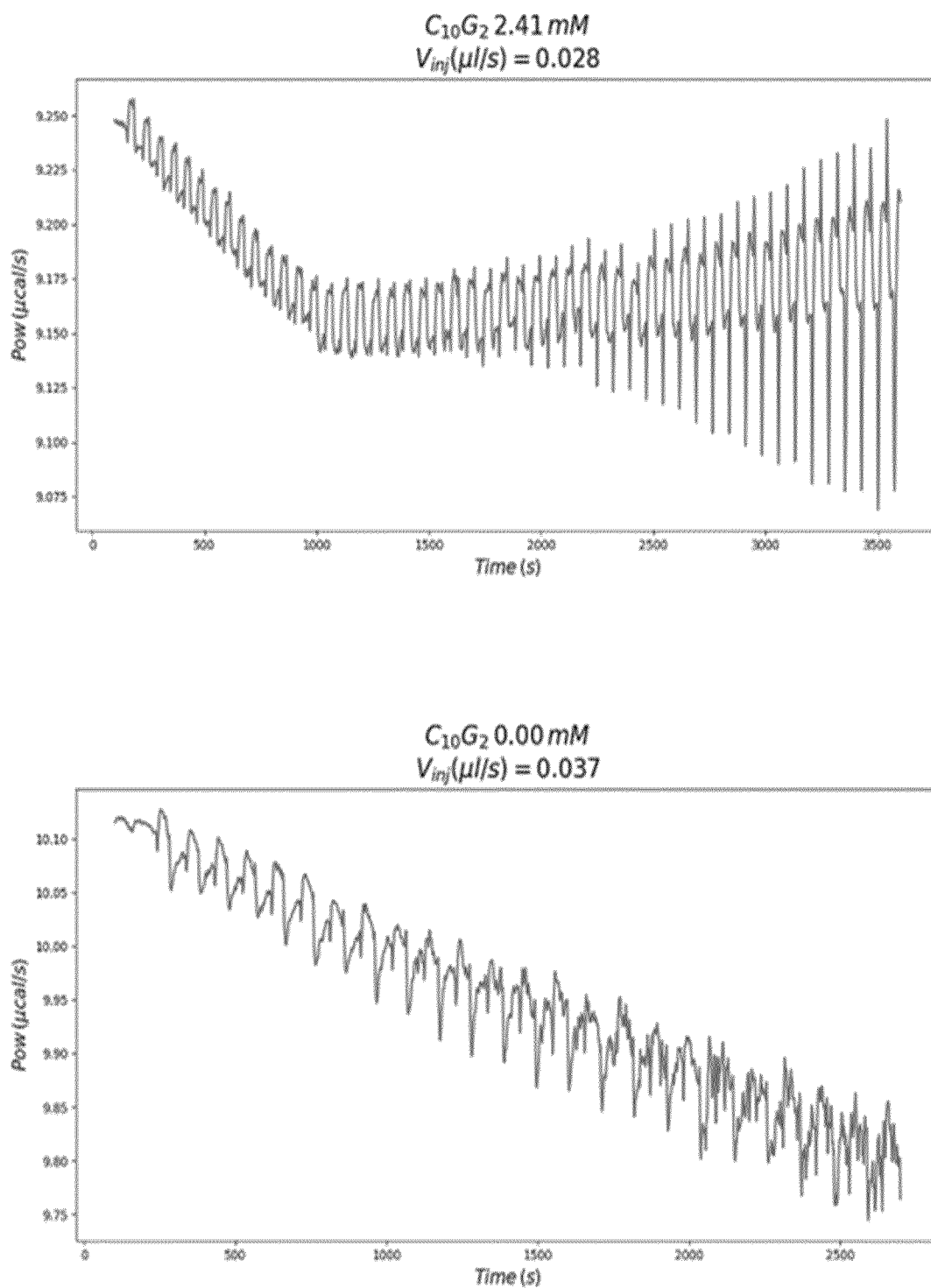


Figure 9b

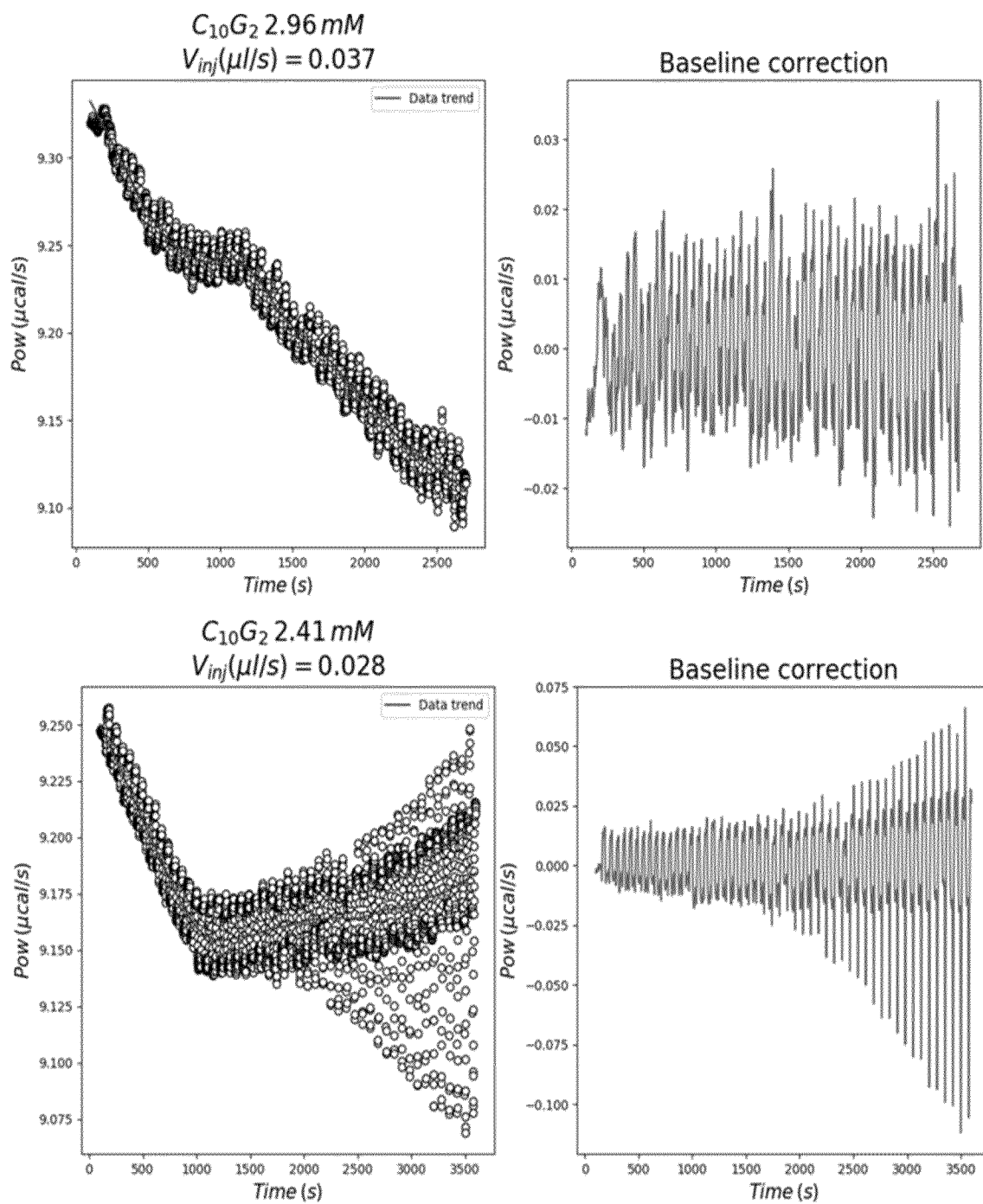


Figure 10a

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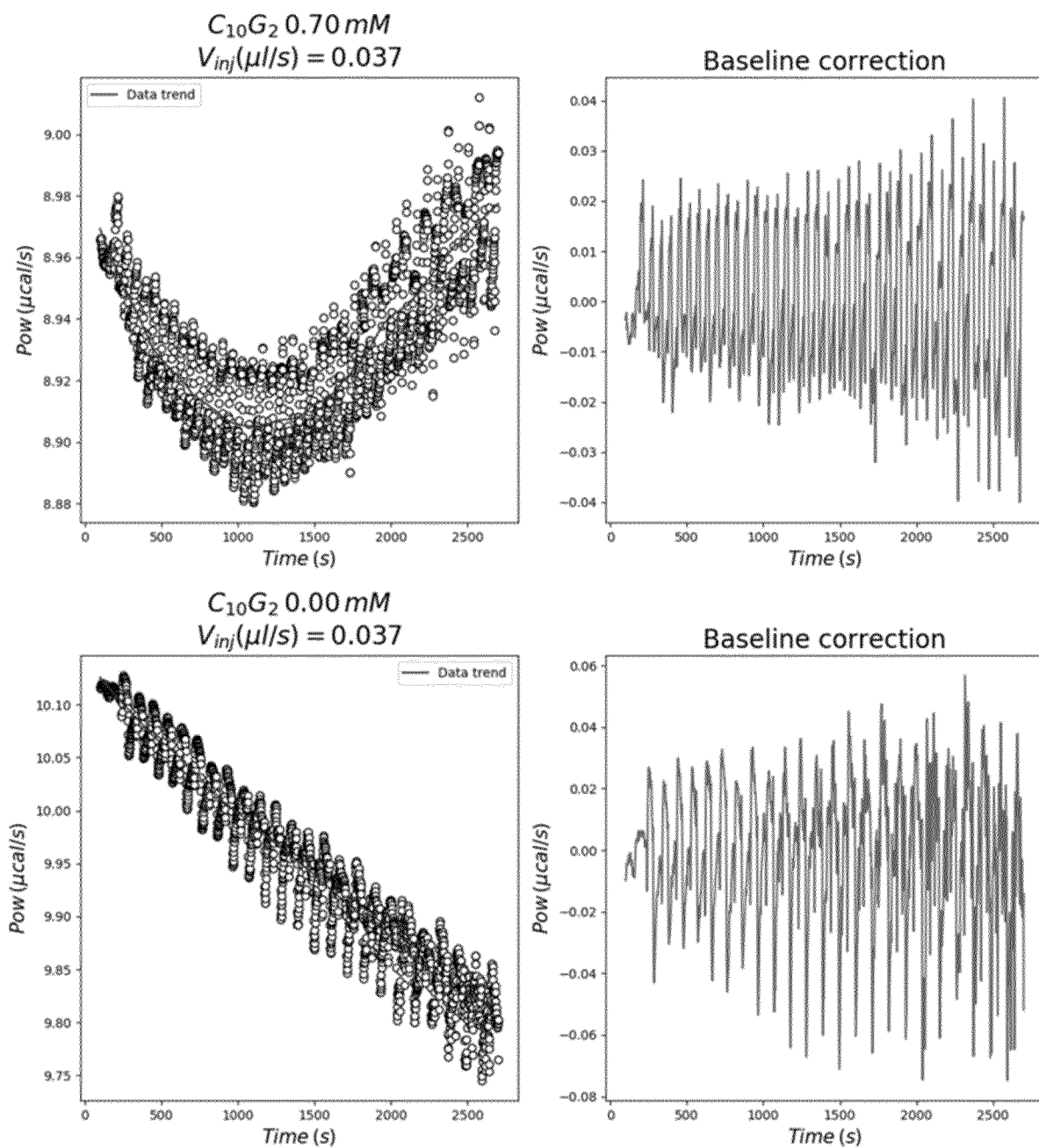


Figure 10b

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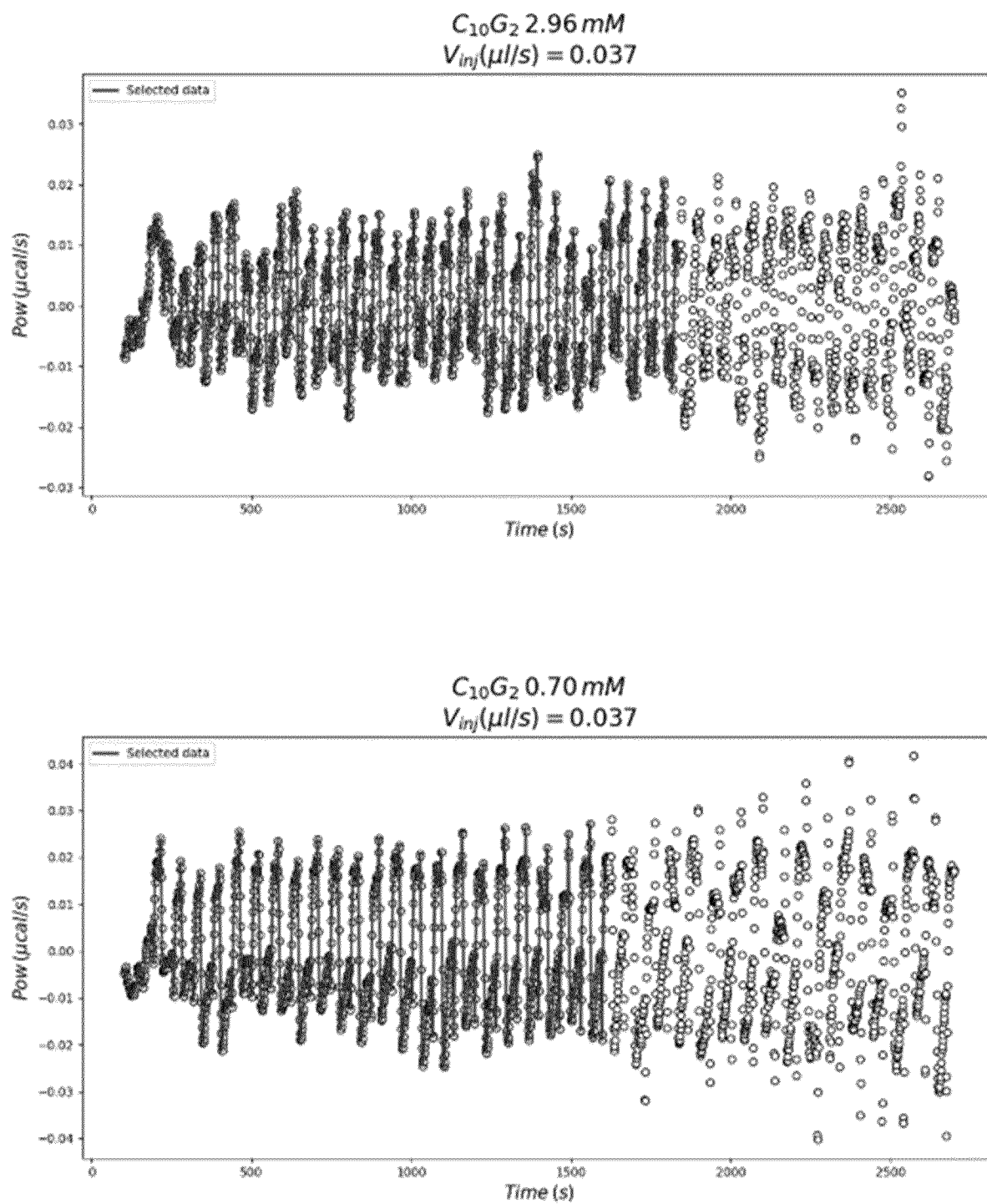


Figure 11a

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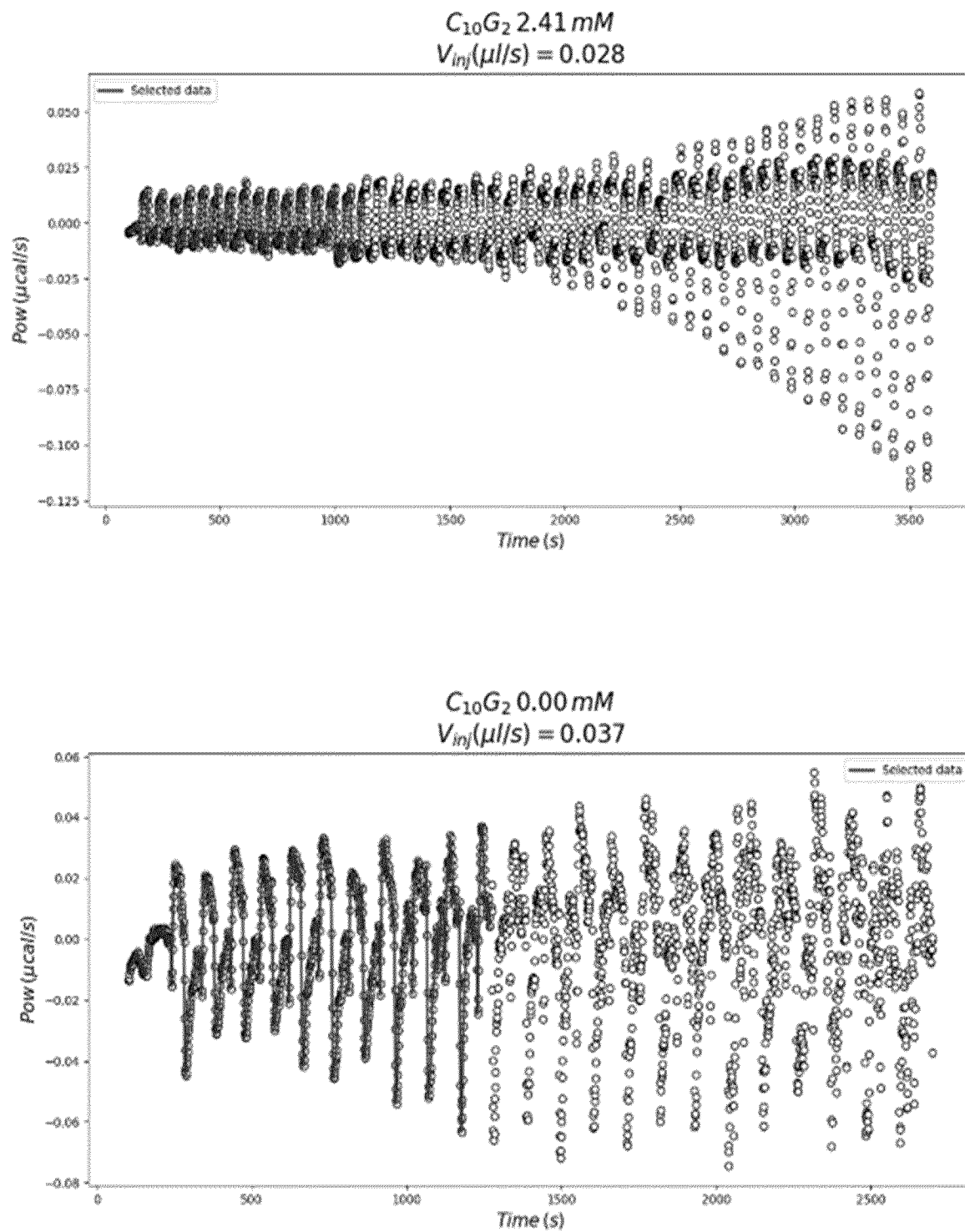


Figure 11b

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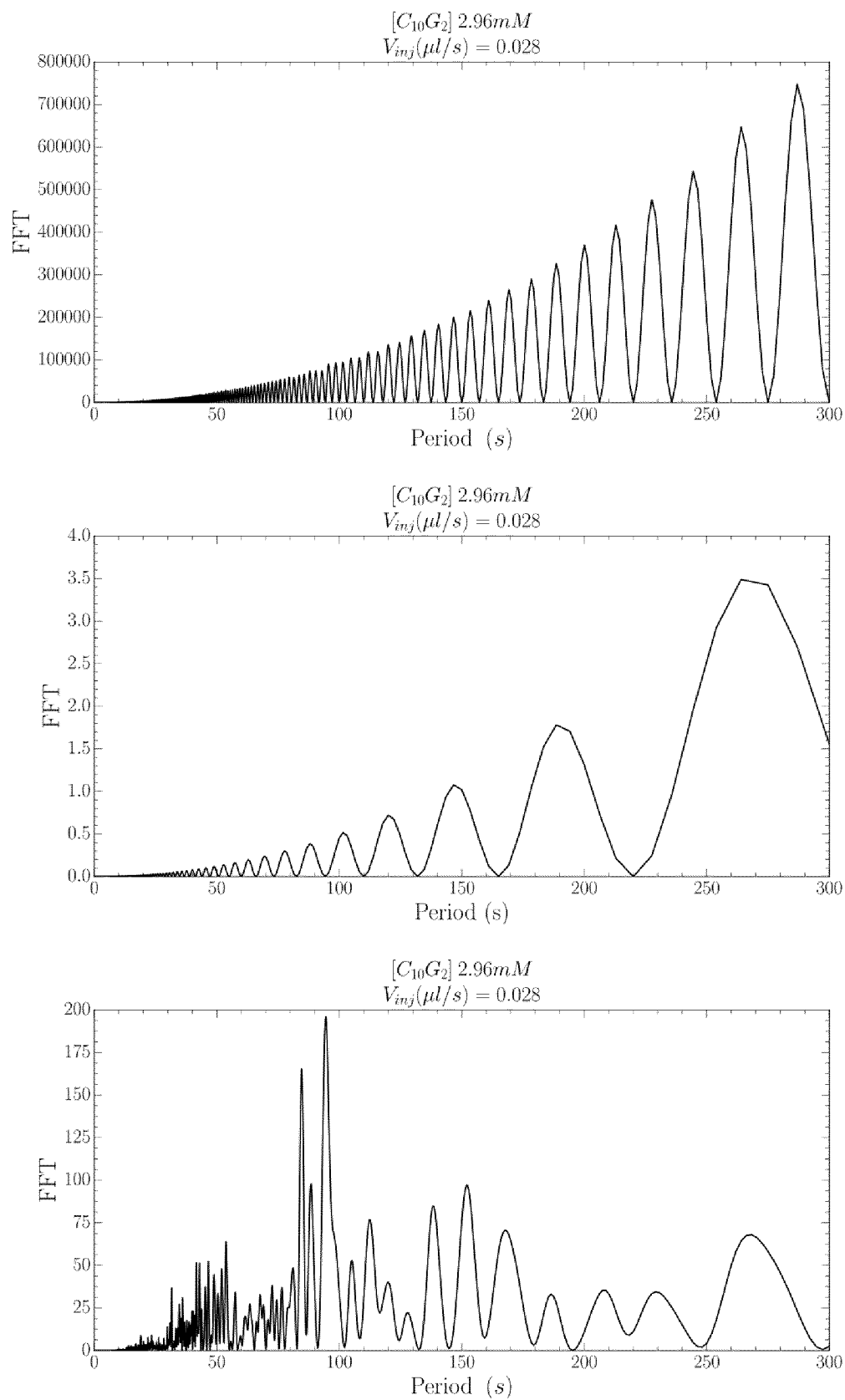


Figure 12

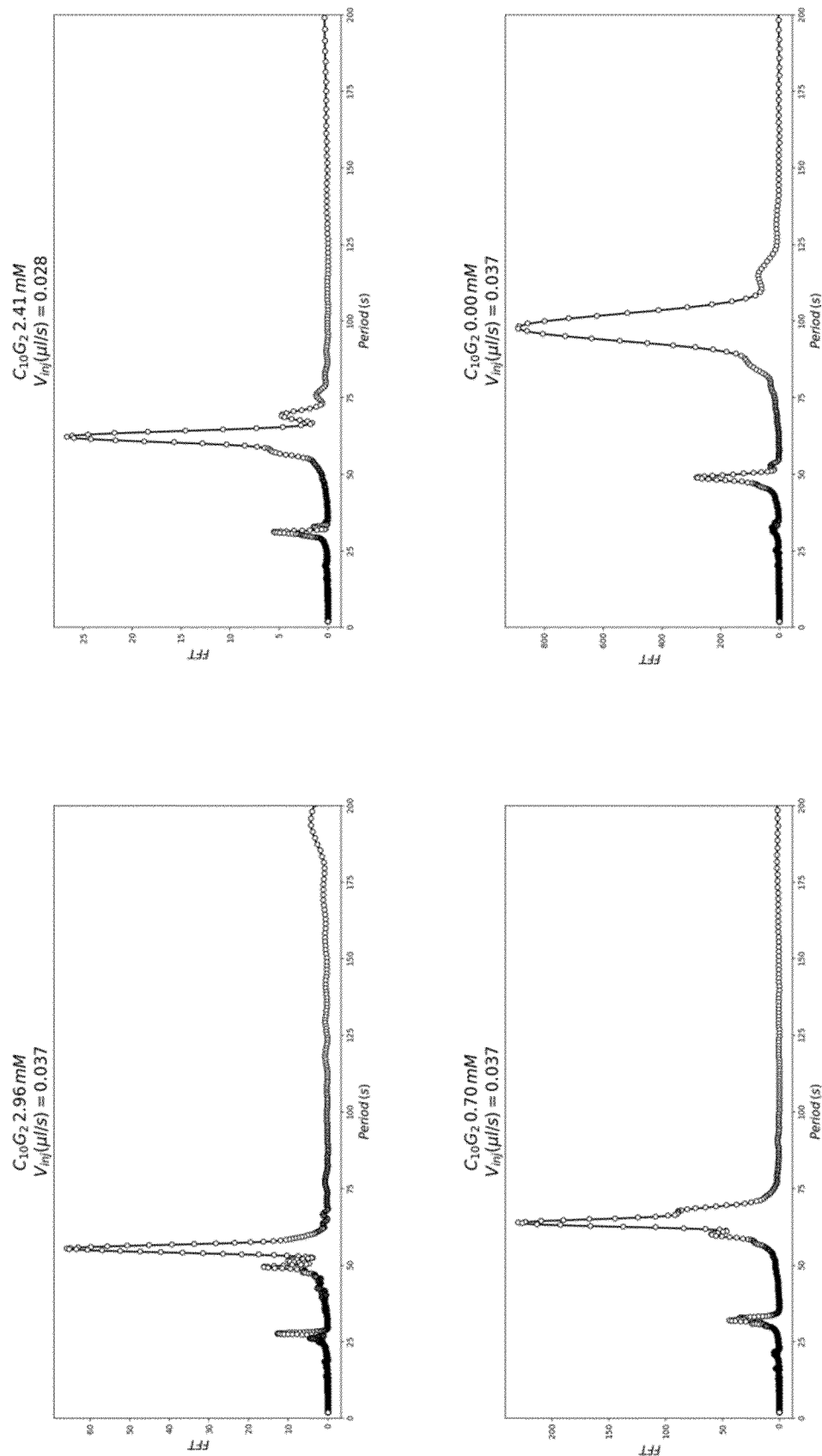


Figure 13

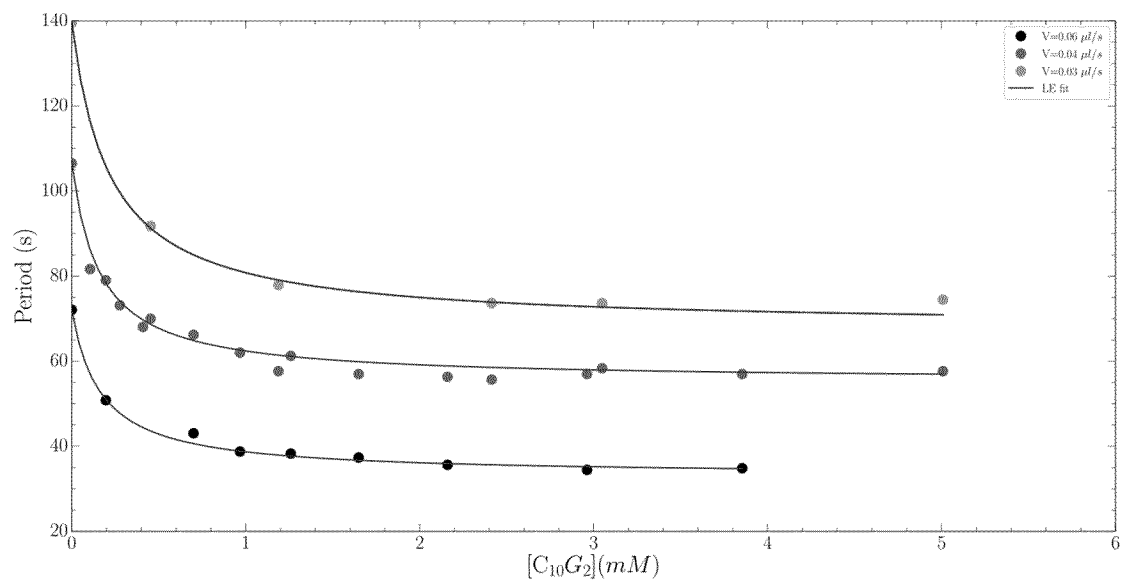


Figure 14

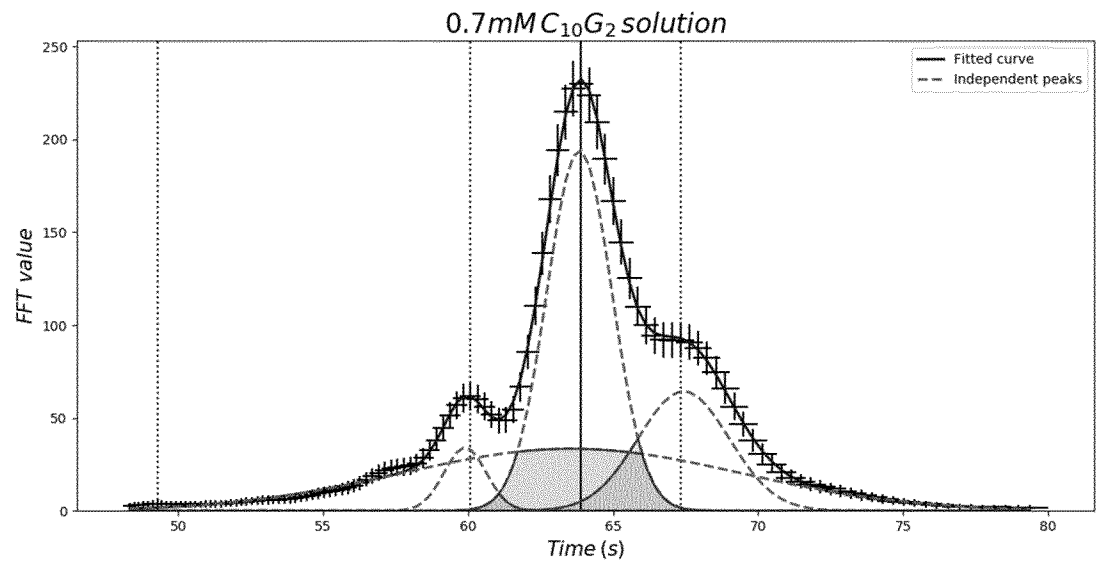


Figure 15

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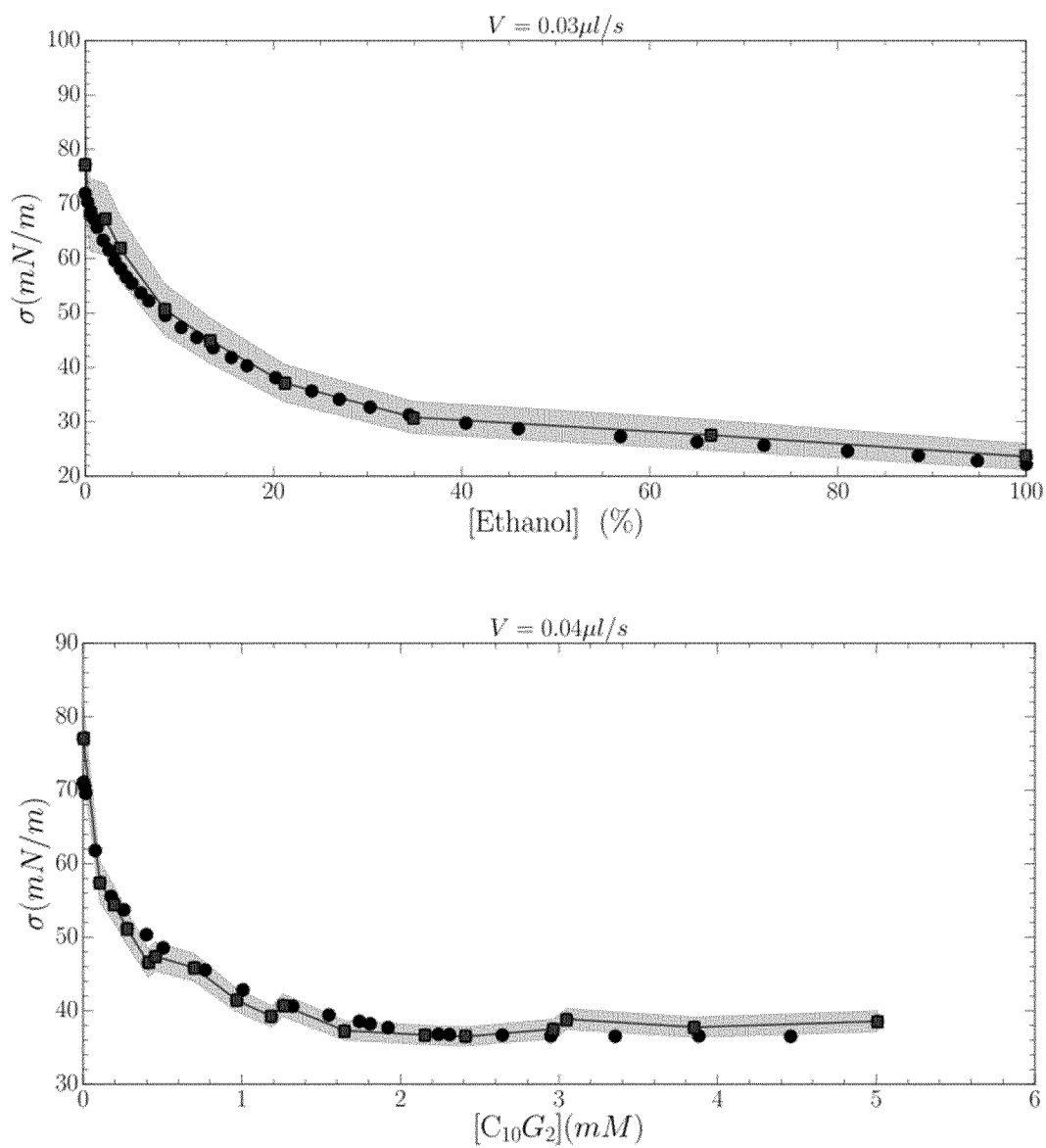


Figure 16

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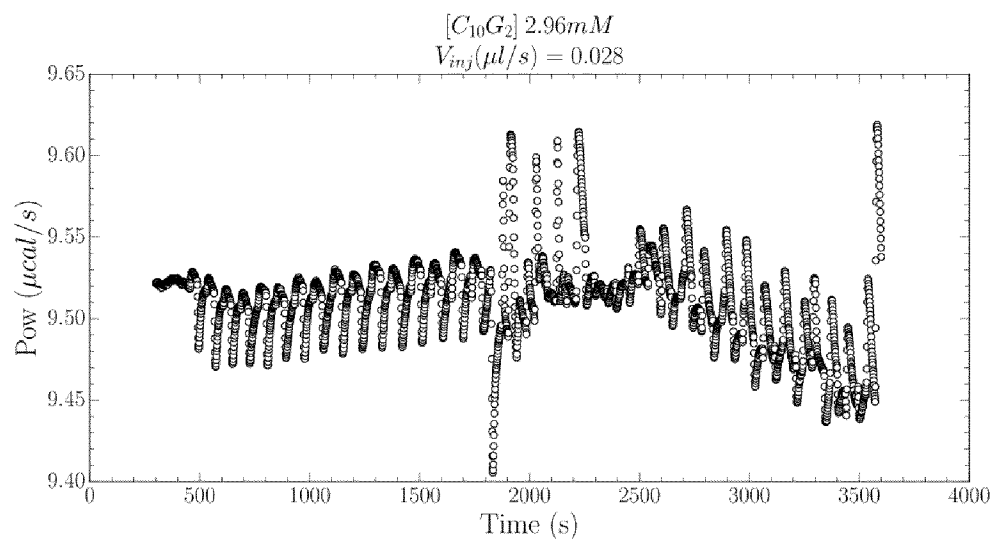


Figure 17a

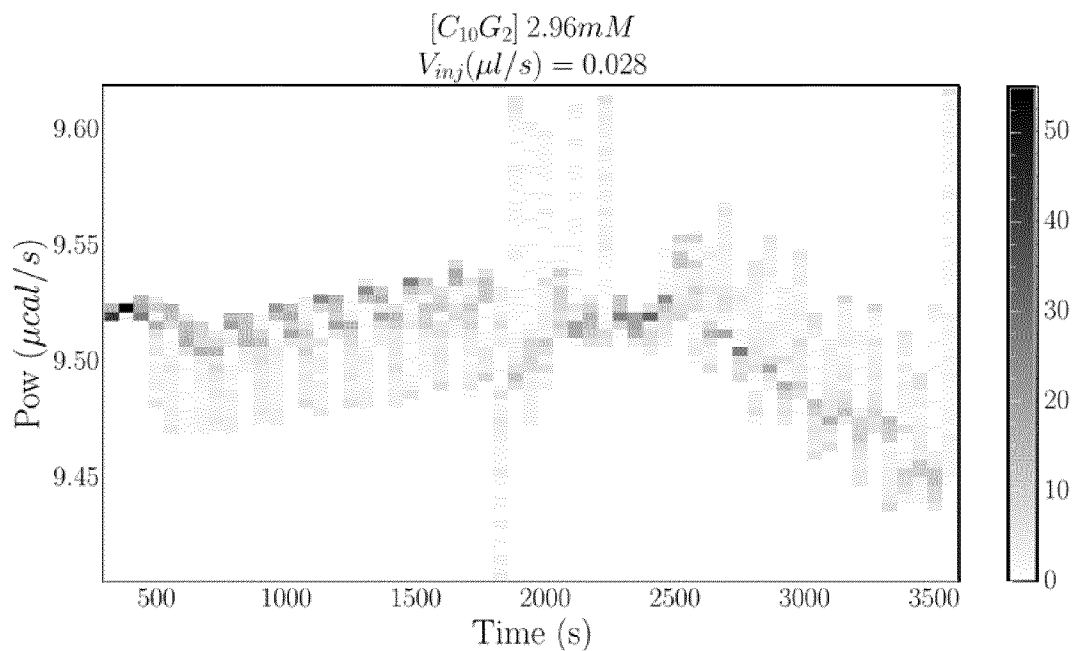
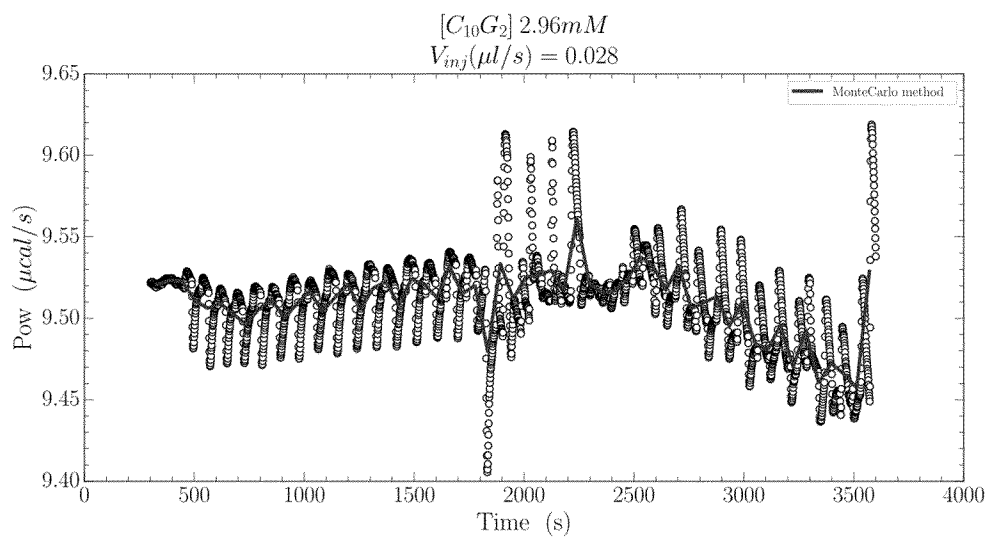
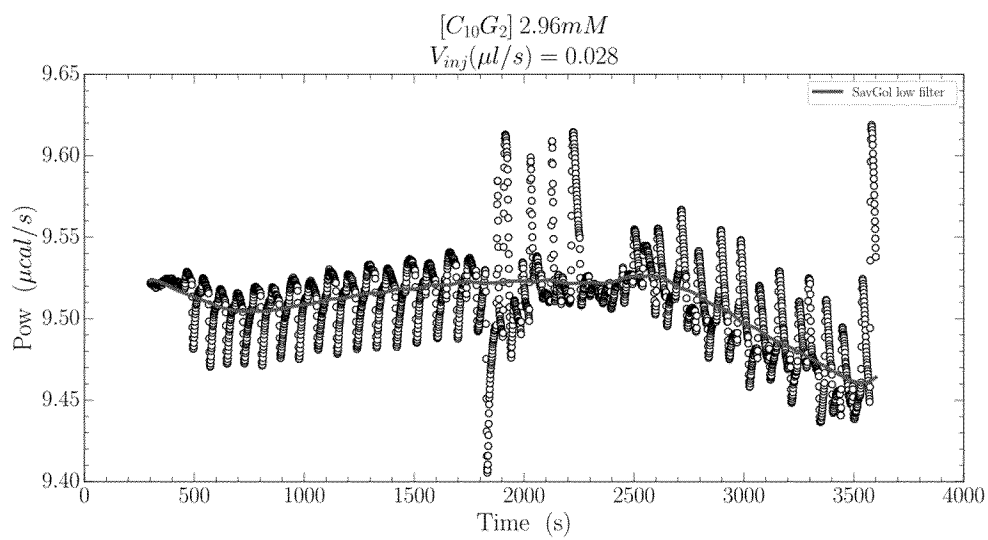
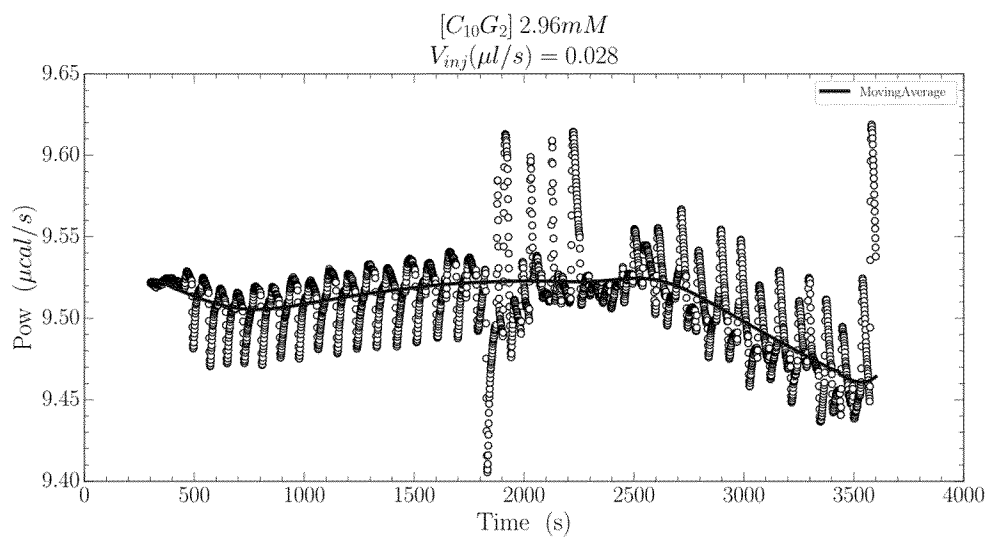
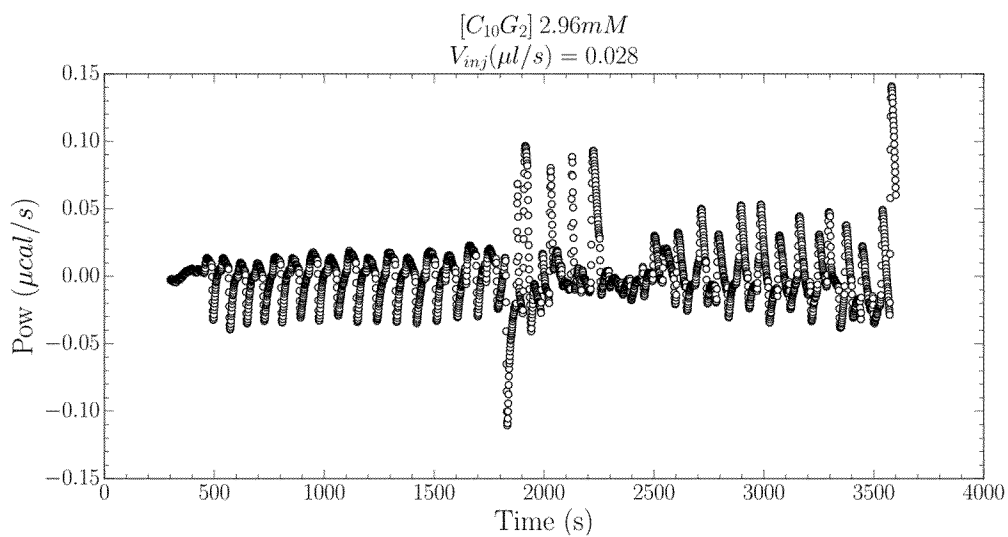


Figure 17b

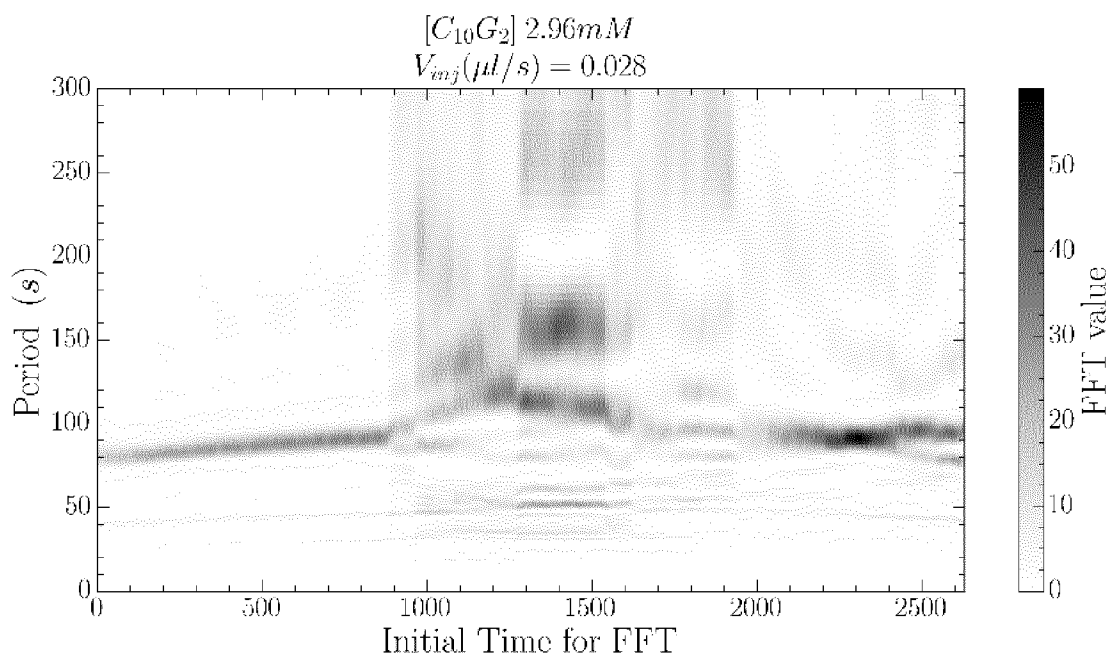
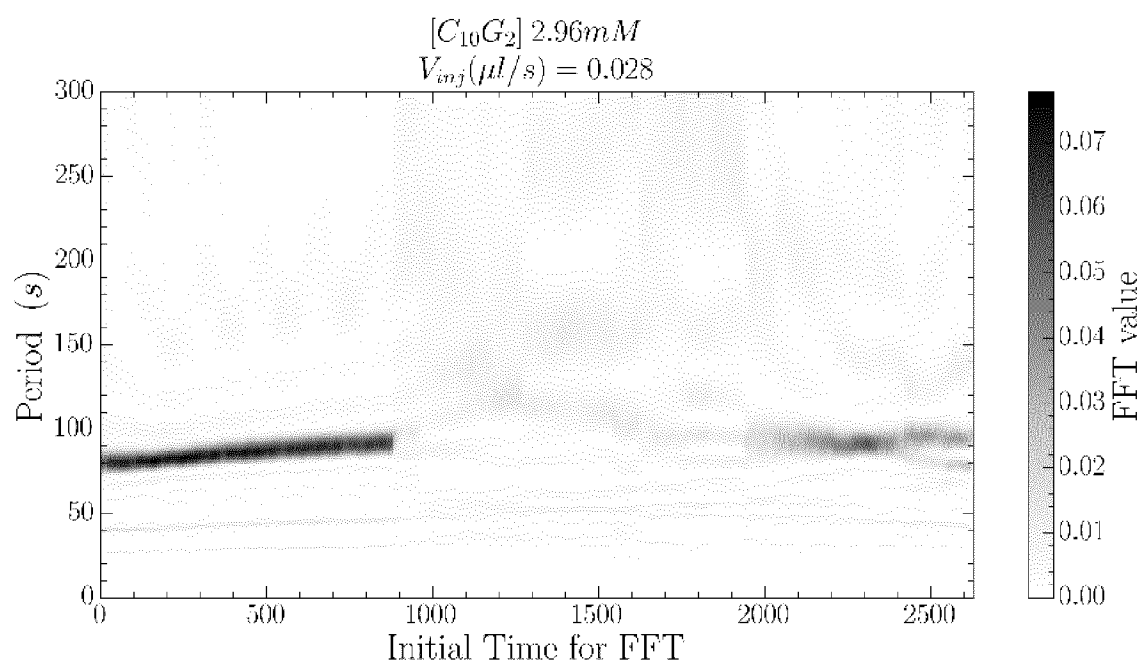
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**Figure 17c****Figure 18a**

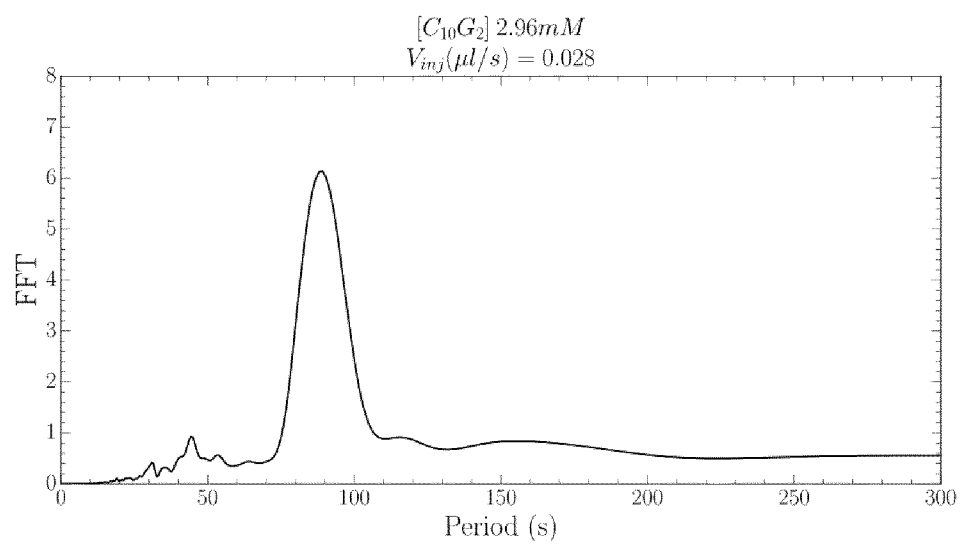
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**Figure 18b****Figure 19**

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**Figure 20a****Figure 20b**

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**Figure 20c**

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/073001

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N13/02 G01N25/20
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MICHAEL B ROBINSON ET AL: "High-temperature containerless calorimeter", REVIEW OF SCIENTIFIC INSTRUMENTS, AIP, MELVILLE, NY, US, vol. 56, no. 3, 31 March 1985 (1985-03-31) , pages 430-436, XP001409855, ISSN: 0034-6748	24,27
A	Setions Introduction, I. Apparatus and V. Surface Tension Measurements -----	1-23,25, 26,28-32
X	US 5 269 176 A (H00L KEVIN O [US]) 14 December 1993 (1993-12-14)	24
A	column 1, line 12 - column 1, line 18; figures 1,2 column 1, line 66 - column 2, line 54 column 3, line 22 - column 5, line 47 -----	1-23, 25-32



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

6 November 2019

Date of mailing of the international search report

13/11/2019

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Authorized officer

Foster, Keir

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2019/073001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5269176	A	14-12-1993	NONE
