



Low-cost equipment for electroformation of Giant Unilamellar Vesicles

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ABSTRACT

Giant Unilamellar Vesicles (GUVs) constitute a very useful model for biomembranes. They are usually generated by electroformation using sinusoidal potential waves applied between two platinum electrodes or electrodes composed of glasses covered by indium tin oxide (ITO).

Here we present the design of a programmable, compact and low cost wave generator that allows applying different waveforms, even with initial non-periodical shapes. Thus, it allows to optimize the potential waveform for the particular GUV' composition.

We also describe the design of a low cost, reusable chamber with stainless steel electrodes that allows using very small volumes of solution (0.3 mL). With this chamber, a larger number of GUVs were generated in less electroformation times compared to ITO electrodes. The general characteristics of the obtained GUVs are comparable to those electroformed in platinum or ITO surfaces.

The chamber, combined with the wave generator comprises a simple, reusable, programmable and low cost system for the formation of GUVs.

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Specifications table

Hardware name	GUVs generator
Subject area	<ul style="list-style-type: none"> • Chemistry and Biochemistry • Medical (e.g. Pharmaceutical Science) • Biological Sciences (e.g. Microbiology and Biochemistry) • Educational Tools and Open Source Alternatives to Existing Infrastructure
Hardware type	<ul style="list-style-type: none"> • Biological sample handling and preparation
Cost of Hardware	75 dollars

1. Hardware in context

Giant Unilamellar Vesicles (GUVs) are micrometer-sized lipid vesicles (liposomes) widely used in membrane biophysics studies. Their main advantage over other model membrane systems is that, having sizes comparable to that of cells, they can be observed with optical microscopy.

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GUVs can be generated by lipid emulsification, swelling, microfluidic-based methods or electroformation [1,2]. This last technique was pioneered by Angelova and Dimitrov in 1986 [3], it involves modulating the spontaneous swelling of lipids within an aqueous solution using an externally applied electric field. Electroformation is the most used method for producing GUVs, as it enables modulation of the lipid hydration process to form relatively monodisperse, defect-free vesicles [4]. For example, from a set of 274 papers randomly selected that deals with topics related to membrane biophysics, and that name “GUV”, 131 (~50%) also name “electroformation”.

For electroformation, lipids are dissolved in an organic solvent, and deposited on two electrodes usually composed of indium tin oxide (ITO)-coated glasses or platinum. For example, from the 131 papers in which the words “GUV” and “electroformation” appear, 54 (~40%) also named “ITO” whilst 25 (~20%) also named “Platinum”.

Lipids self-assemble into a stack of bilayers on a substrate when the organic solvent is removed by evaporation [2]. Following solvent evaporation, the electrodes are placed in contact with an aqueous solution. On hydration, the bilayer stacks separate out very slowly. If the bilayer edges are allowed to merge at a faster rate, multi-lamellar vesicles (MLVs) are formed. Increasing the rate of bilayer separation by application of electric fields, one can obtain uni-lamellar vesicles [2]. Therefore, an alternating potential difference is applied across the electrodes, stimulating the swelling process of the hydrated lipid layer. For GUV’s electroformation, a wave generator is thus required. The optimal potential field for a high yield of large vesicles has been an active research field, and several cell geometries, wave amplitudes, frequencies and modulations have been tested. For instance, Bi et al studied GUV generation using interdigitated ITO electrodes and different lipid mixtures [5]. They found that smaller widths generated bigger GUVs under the same conditions, and that a solution height above 600 mm has no influence on the GUV formation. Furthermore, the diameters of the GUVs decreased with increasing frequency at a constant amplitude, and increased with increasing amplitude.

Li et al. used two plasma cleaned ITO electrodes in a face-to-face layout in order to successfully generate GUVs from zwitterionic lipids as well as charged lipids in high concentration saline solutions [6]. Drabik et al. used platinum electrodes in a polytetrafluoroethylene (PTFE) cell and analyzed the effect of duration of electroformation, usage of electrodes and frequency of applied AC field and its voltage on the properties of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine GUVs. According to their results, long times does not influence outcome whilst voltage and frequency did significantly influence GUV’s properties [7].

All these studies indicated that the optimal waveform depends on the lipid composition and solution conditions. Thus, in order to use different vesicle compositions and conditions, a wave generator with modifiable wave characteristics is necessary.

For GUV formation, a fluid lipid membrane is required, meaning that the temperature has to be higher than the membrane melting temperature. Therefore, depending on the lipid composition and experimental conditions, electroformation has to be performed in a stove at temperatures in the range 30–60 °C. Thus, a compact wave generator is preferred.

Regarding the electrode materials, platinum and ITO-coated glasses are expensive. Furthermore, ITO-coated glasses easily break. ITO-coverages prepared in the laboratory requires the sputtering equipment and are instable (the surface coverage eventually dissolve with consecutive cleanings). Additionally, the conductivity of the surface is not always optimal and is not reproducible. Therefore, alternatives to platinum and ITO-coated glasses have been reported. Cao et al. proposed the usage of glyoxylic acid modified aluminum electrodes for GUV generation. Using eggPC they obtained high yield of vesicles with diameters >0.1 mm, which were not produced on platinum and indium tin oxide at the same experimental conditions [8].

Carbon fiber electrodes have also been tested, and the authors found that fast GUV formation can be achieved due to the small size of the (and thus, fast diffusion toward) electrode surface [9]. On this electrode material, hydration of the lipid layer due to the application of a negative potential has previously been reported [10].

The usage of stainless steel as electrode material has also been reported. GUVs of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine with similar properties to those formed with platinum were successfully generated [4]. Stainless steel is reusable, unlike ITO-coated glasses, that eventually loses de covering or aging occurs during their repeated use, which is reflected in their surface topography on the nanoscale [11]. Compared to platinum, stainless steel electrodes have the advantage of being rigid, reducing the risk of bending and improving control over the electrode-separation distance, thus leading to a uniform electric field. Furthermore, stainless steel is cheaper than platinum and ITO-coated glasses.

When ITO-coated glasses are used, the chamber is usually prepared between the two glasses in each experiment, thus the volume is not always the same and furthermore, sometimes the lipid suspension leaks from the chamber.

Regarding the chamber material, the use of 3-D printed laboratory tools has boosted in the last years, and become an important source of accessible tools [12]. Here we propose using a 3D printed chamber with stainless steel electrodes with the main advantage of low manufacturing costs and fast production. Different cell geometries can be tested with new chambers, which are easily and economically prepared with a 3D printer.

This chamber, combined with the home-made wave generator described here have the following advantages:

- Compact and programmable wave generator
- Reusable chamber
- Low cost and easily set up

2. Hardware description

The wave generator was assembled with easily obtaining materials and components. The more complex parts are the microcontroller and the digital potentiometers; being the rest of the components voltage regulators, operational amplifiers, resistances, capacitors and discrete semiconductors.

The hardware points to achieve two main goals:

- Reducing the equipment cost (compared to a commercial equipment). It is important to point here that, depending on the country, high shipping and customs costs have to be paid when an equipment is acquired from a foreign country. This increases the gap between the accessibility of researcher of different countries to equipment and also between local developments and commercial equipment.
- To generate a modulated signal, non-periodical, unlike most wave generators. Some lipid compositions generate more and larger GUVs when the amplitude of applied potential wave increases progressively from zero to the desire amplitude. This is a non-periodical modulation at the start of the signal application. With the proposed wave generator design, different signal shapes can easily be programmed for each experiment, depending on the specific requirements of the lipid mixture.

The wave generator allows applying waves with the following characteristics:

Frequencies in the range [2 Hz; 500 Hz]

Amplitudes in the range [0 V; 5 V], maximum peak-to-peak potential (V_{pp}) of 10 V

Shapes: Sine, tangent, square, triangular, pulses, sawtooth or personalized functions

Possible offset: $\pm 2.5V$ (not implemented in the present set-up)

Power Supply: $\pm 7.5VDC$

Duty Cycle: 1–99% (square and pulse only)

Modulation period (one-time at start, or periodical): 1–3600 s

Modulation functions: Welch/Barlett/Hanning/user defined

This wave generator is used in combination with a chamber that consists of a 3D printed plastic cell with stainless steel electrodes. The wave generator together with the chamber are potentially useful to researchers in order to prepare GUVs due to the following reasons:

- Low cost and compact wave generator.
- Easy to use and easy to program wave generator.
- High variety of waveforms, with possibility of non-periodical signals.
- Low cost and reusable chamber of low volume.
- Different cell geometries easily constructed.
- High conductive, easily cleanable and low cost electrodes.

3. Design files

Design files summary

Design file name	File type	Open source license	Location of the file
chamber.stl	3D printing file	Sketchup Make	Available with the article
CabinetTop.stl and CabinetBase.stl	3D printing file	Sketchup Make	Available with the article
AcrylicCovers.PDF	PDF	Adobe Reader	Available with the article
Schematics.PDF	Circuit and PCB	Proteus Demo	Available with the article
PCB_Gerber.zip			
Vs2010Code.7z	Software	Visual Studio 2010	Available with the article
Wavegen.7z		Free version at https://www.visualstudio.com/free-developer-offers	
Firmware.7z	Firmware	CCS Pic-C Demo at http://www.ccsinfo.com/ccsfreedemo.php	Available with the article
PCB_Pinout.PDF	PDF	Adobe reader	Available with the article

3.1. 3D printing

chamber.stl: design of the electroformation cell.

CabinetTop.stl and CabinetBase.stl: design of the wave generator cabinet. AcrylicCovers.PDF is the design for a lower cost alternative.

3.2. Electronics

Schematics.PDF: Complete circuit schematic (pdf export from Proteus demo).

PCB_Gerber.zip: File in open ASCII vector format for 2D binary images for manufacturing the printed circuit board.

3.3. Software and firmware

- Wavegen.7z: Binaries for Win ×86 of the configuration interface developed in MS Visual Studio 2010. Free version can be found at <https://www.visualstudio.com/free-developer-offers>.
- Vs2010Code.7z: Visual Basic project and source code of the configuration interface.
- Firmware.7z: source code and HEX file for direct programming.

4. Bill of materials

Designator	Component	Number	Cost per unit-currency	Total cost-currency	Source of materials	Material type
Chamber	3D printing plastic filament.	A volume of 4176 mm ³	\$5 (US Dollars)	\$5 (US Dollars)	Printing company	Polymer
Electrodes	Stainless steel	Sheet (10 mm × 10 mm × 1 mm): 2 units	\$0,5 (US Dollars)	\$1 (US Dollars)	Local hardware store	Metal
PCB	Printed Circuit Board	1	\$2 (US Dollars) + shipping	\$2 (US Dollars) + shipping	www.pcbgogo.com	2-Layer FR4 1.6 mm
Cabinet	3D printed case	Bottom: 19019 mm ³ Top: 48676 mm ³	\$35 (US Dollars)	\$35 (US Dollars)	Printing company	Polymer
Electronic components	Electronic components	51	Depends on the component (see "BillOfMaterials_PCB.PDF")	31.68	www.digikey.com and www.aliexpress.com	Depends on the component (see "BillOfMaterials_PCB.PDF")

5. Build instructions

5.1. Hardware assembly

Download the file "PCB_Gerber.zip" and send it to a PCB manufacturing company.

Import the files "CabinetTop.stl" and "CabinetBase.stl" and send it to a 3D printing company. An acrylic case may also be used, following the plan drawn in "AcrylicCovers.PDF"

Order the electronic components detailed in "BillOfMaterials_PCB.PDF"

Once all the components are received (cabinet, electronic components and printed circuit), weld the electronic components on the printed circuit according to the scheme detailed in "Schematics.PDF".

Connect the programmer to the board and write the file "Prueba_18F2550_R2 R_timer.HEX" (in Firmware.7z) into the PIC microcontroller (we used "Microchip ICD2").

Wire the connectors according to the image shown in Fig. 1 (a higher resolution image can be found in the file "PCB_Pinout.PDF")

Assemble the board and components into the printed cabinet as shown in Fig. 2. WARNING! Be careful when connecting the electric transformer to the AC line input (electric shock danger).

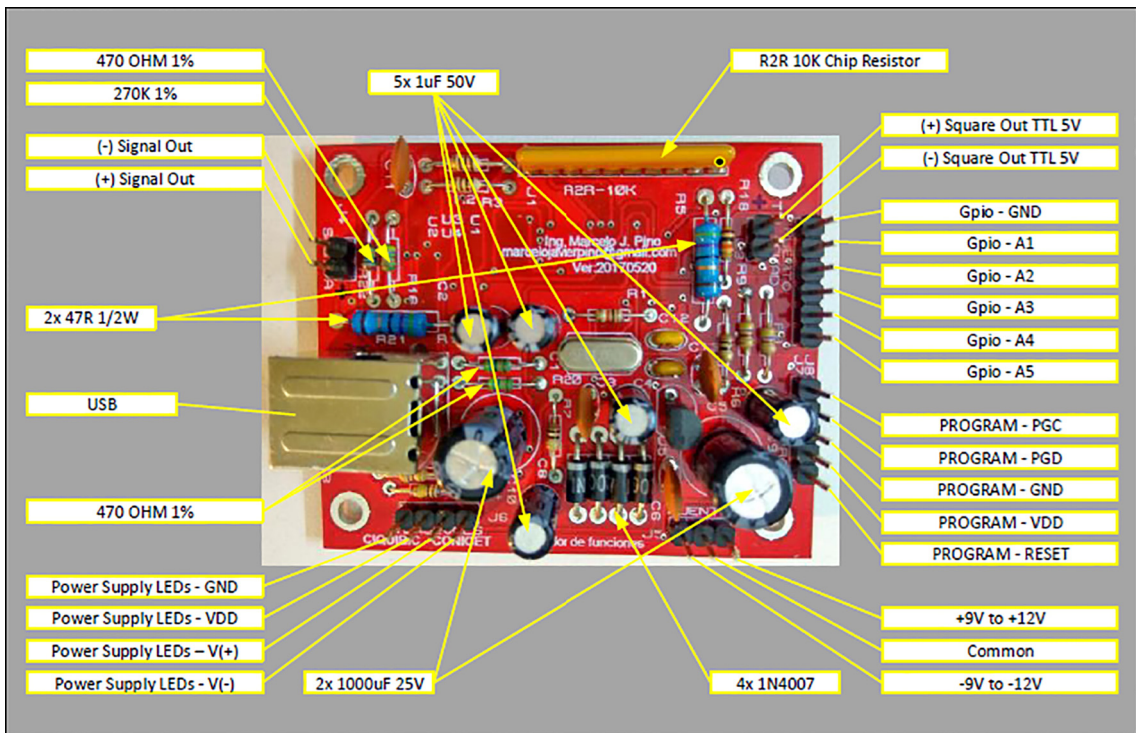


Fig. 1. Image of the board, detailing the electronic components and external connections (power supply and signal output). A higher resolution image can be found in the file “PCB_Pinout.PDF”.

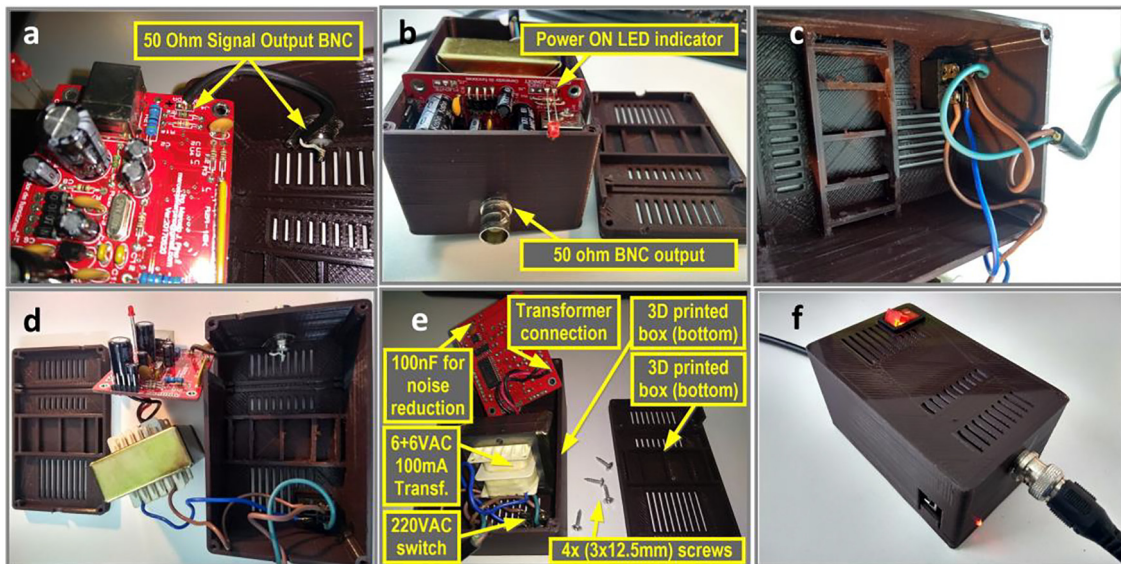


Fig. 2. Device assembly: images showing the location of the components inside the cabinet. a. Connection from board to 50 Ohm BNC output. b. Welding of power LED. c. Connection of the external power supply and ON/OFF switch. d. and e. image showing the interior of the device. f. assembled device.

5.2. Hardware install

Connect the device to power supply and to USB port of the computer. Turn on the device and wait until windows report uninstalled software. Then, follow the procedure described Fig. 3:

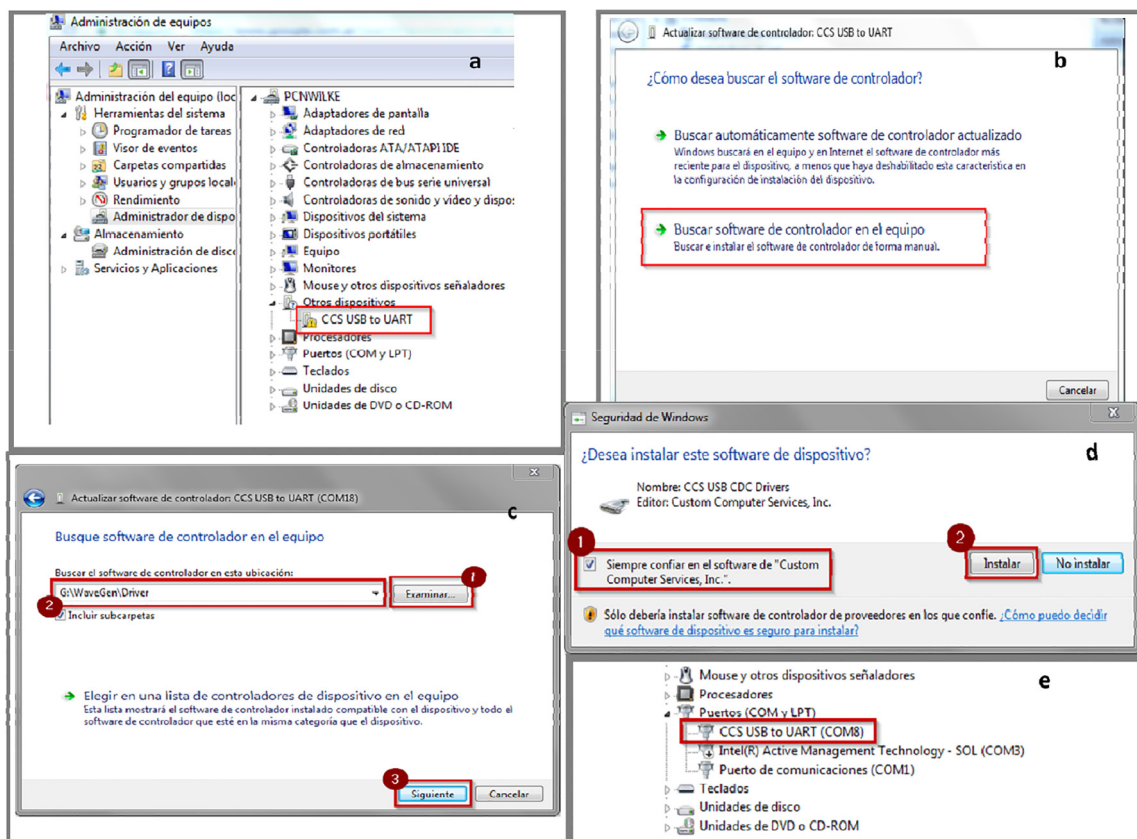


Fig. 3. Hardware Install, steps a. to e. See [Supplementary Material](#) for an English translated version of this figure.

- Open the device manager, and localize CCS USB to UART. Right-click and select “update drivers”
- Select “Browse my computer for driver software”
- Click on “Browse...” (1). Find and select the “wavegen/driver” directory (2). Click “next” (3)
- A warning message appears; select “always trust in Custom Computer Services Inc software” (1). Then, click “Install” (2)
- Verify the assigned serial port number (COM#), COM8 in the example of [Fig. 3](#).

5.3. Chamber assembly

For assembling the chamber, download the file chamber.stl and send it to the 3D printer software. Use the stainless steel electrodes with the dimensions given in the table of [Section 4](#) (Bill of Materials).

6. Operation instructions

6.1. Chamber use instructions (see [Fig. 4](#))

- Over the previously cleaned electrodes (ethanol is preferred for reasons of safety and low cost) apply about 5 μL (the amount needed to spread a single layer on each electrode) of the lipid mixture from a chloroformic 0.50 mg/mL solution. Tip: use a Hamilton syringe with a blunt needle point (Point style 3). The electrodes should be at the temperature required for the particular lipid mixture (above the lipid melting temperature).
- Dry the lipids with a N_2 stream, and eliminate the residual solvent putting for 1 h the electrodes in a vacuum chamber.
- Insert the electrodes in the cell cavities.
- Fill the cell with the desired aqueous solution.
- Insert the lid on top, going through the electrodes, until it makes contact with the top of the cell.
- Seal the lid and the cell with laboratory film (Parafilm). If electroformation is performed at high temperatures (50–60 $^{\circ}\text{C}$), add a Parafilm film between the lid and the cell, and seal the chamber with special care to avoid evaporation.

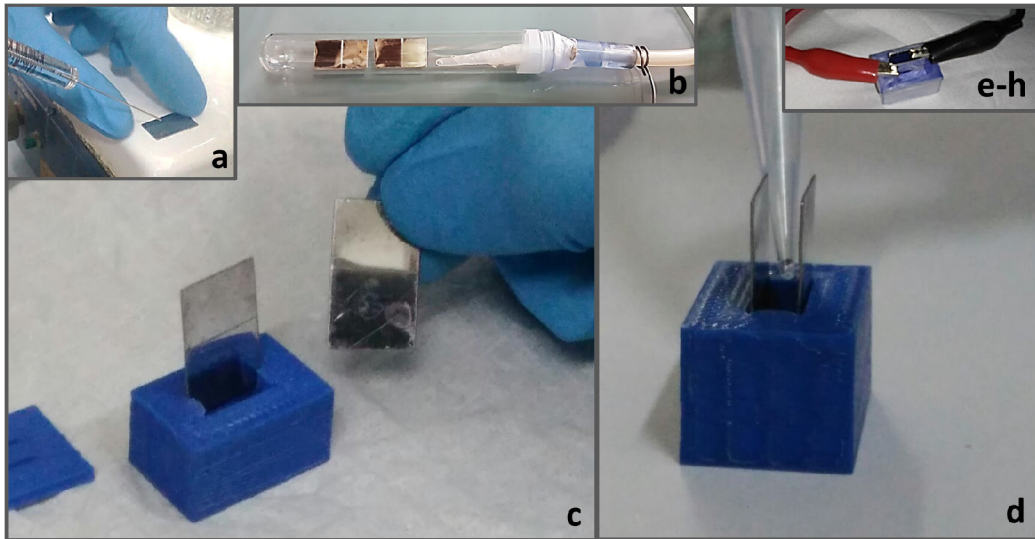


Fig. 4. Chamber assembly process, steps a. to h.

- g. Put the cell on the stove at the required temperature (above the melting temperature of the lipid mixture)
- h. Connect the electrodes to the wave generator to begin the electroformation.

6.2. Software use instructions

Open “GeneradorDeFunciones.exe”, a window will appear, such as that shown in Fig. 5. The buttons, text-box and plot are detailed in the legend.

Fig. 6 shows the steps for setting the waveform parameters. Select the serial port number (COM#) from the list (COM8 in the example of Fig. 3), and click “conectar” in order to connect with the device. Then, read the current configuration in device by clicking “leer señal”; the window will show the current parameters (waveform, frequency, amplitude and type and length of modulation). Item 6 in Fig. 5 shows the serial data without processing. This helps for debugging (for technical use).

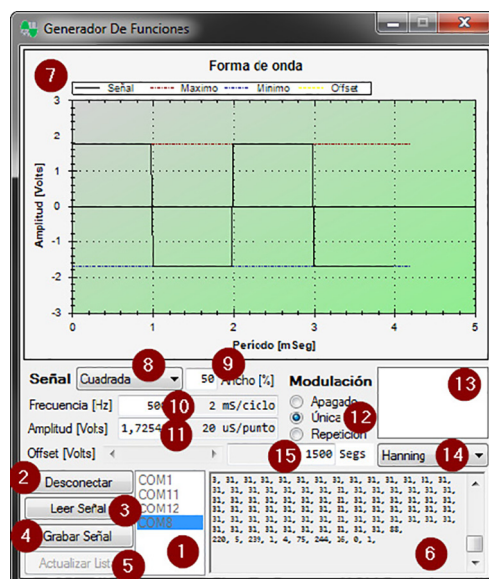


Fig. 5. Window of the wave generator software. 1. List of all available serial ports; 2. Connect/Disconnect button; 3. Read data from wave generator hardware; 4. Write data to the device; 5. Update serial port list (only enabled in offline mode); 6. Serial port data as is; 7. Wave display; 8. Waveform selector: Sine, Square, Triangle, etc.; 9. Duty-Cycle 1–99%; 10. Frequency in Hertz; 11. Amplitude in Volts ($V_{pp}/2$); 12. Modulation (“Off”; “just once at the beginning”; or “repeated”); 13. Modulation function display; 14. Amplitude modulation function selector (Welch, Bartlett, Hanning, Custom); 15. Modulation period in seconds. See [Supplementary Material](#) for an English translated version of this window.

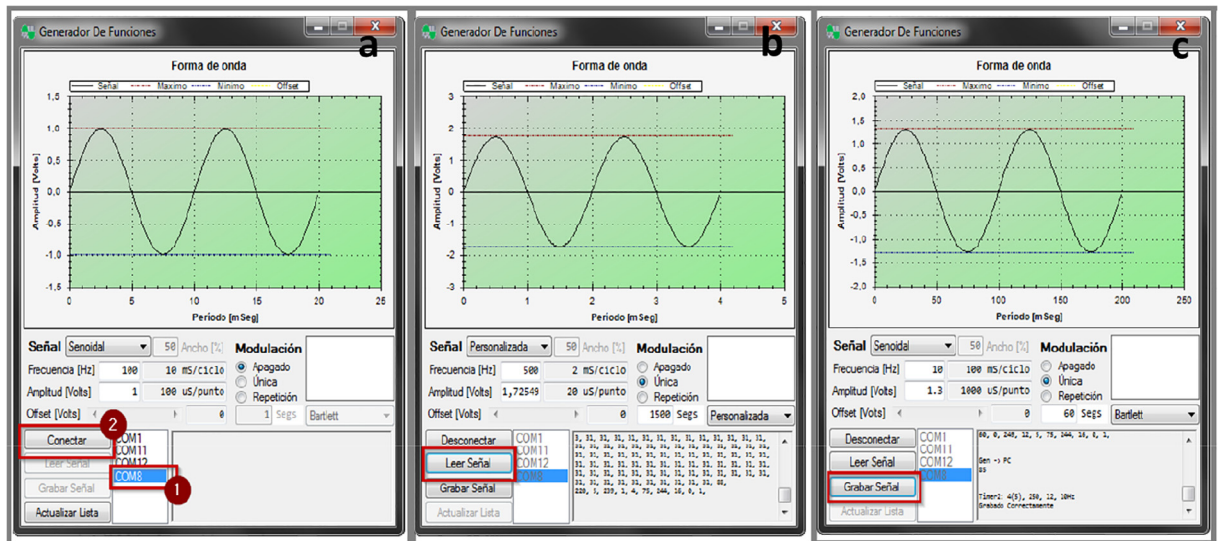


Fig. 6. Software windows showing the user steps. a. select the serial port number shown in Fig. 3 e (1) and click “conectar” in order to connecting with the device (2). b. Click “leer señal” for reading the last used signal. c. Once the desired signal is configured, click “grabar señal” for writing the signal into the device. See [Supplementary Material](#) for an English translated version of the window.

Once loaded the last used parameters, they can be modify according to the new requirements using the buttons described in Fig. 5. Finally, write the configuration to the device by clicking “Grabar señal”. Now the device is ready to use.

7. Validation and characterization

The potential applied by the wave generator was registered with an oscilloscope, and a good response was obtained. Figs. 7 and 8 shows two examples:

Example 1 (Fig. 7):

Initially, a sine wave potential of 10 Hz is applied, with amplitude from 0 to 2.6 V_{Vpp} increasing linearly in 60 s (Bartlett modulation). After that, the amplitude is maintained at 2.6 V_{Vpp}.

Example 2 (Fig. 8):

This is a personalized waveform modulation, detailed in Table 1. A sine waveform is used, with larger amplitude value of 1.74 V. The initial wave modulation is defined by a unidimensional array of 100 elements (column “Normalized amplitude” of “Example2.PDF”, see details in the legend of Fig. 7).

Using our wave generator and the chamber with stainless steel electrodes, GUVs composed of dimyristoyl phosphatidylcholine (DMPC, from Avanti Polar Lipids) were generated applying the potential wave detailed in example 1. The behavior of the DMPC GUVs upon increasing temperature was tested. Fig. 9a show a representative GUV (from 50 observed vesicles). The general behavior is similar to those previously reported for DMPC vesicles electroformed on platinum electrodes, vesicles show regions with sharp edges below 23 °C due to the solid-liquid crystalline phase coexistence, at the melting temperature vesicles deform, and adopt spherical shapes at higher temperatures [13].

We also tested the ability of our system to generate GUVs composed of a complex lipid mixture. Fig. 9b shows a representative GUV prepared with E. Coli extract total (from Avanti Polar Lipids, Inc). In this case, the waveform of example 2 (in three steps) was applied, as proposed by Kubiak et al. [14]. The observed vesicles resembled those formed with the polar lipid extract of E. Coli [14].

Finally, we prepared GUVs of DMPC and stearic acid (3:1) at basic pHs using as electrodes both, ITO coated glass and stainless steel. The wave shape was the same than those for electroforming DMPC vesicles. The chamber with the glasses was assembled before the electroformation and the stainless steel were used with the 3D printed chamber. We found that our chamber generate a higher number of GUVs in half of the time of electroformation. The GUVs showed in Fig. 9c were generated by 1 h of electroformation in ITO-covered glass electrodes (left) and by 0.5 h electroformation in stainless steel electrodes (right). Fig. 10 shows the average size of 20 individual GUVs followed along 30 s (4 frames/s), the error bars correspond to the standard deviation of the parameters along the 30 s, and thus they measure the time-fluctuation. There are no significant differences in the GUV population generated with each electrode; both kinds of electroformed GUVs have similar size and temporal fluctuations.

In summary, the proposed equipment is able to generate GUVs of simple and complex compositions, with similar characteristics to those previously described [13,14]. The main advantage of the equipment proposed here are the low cost and

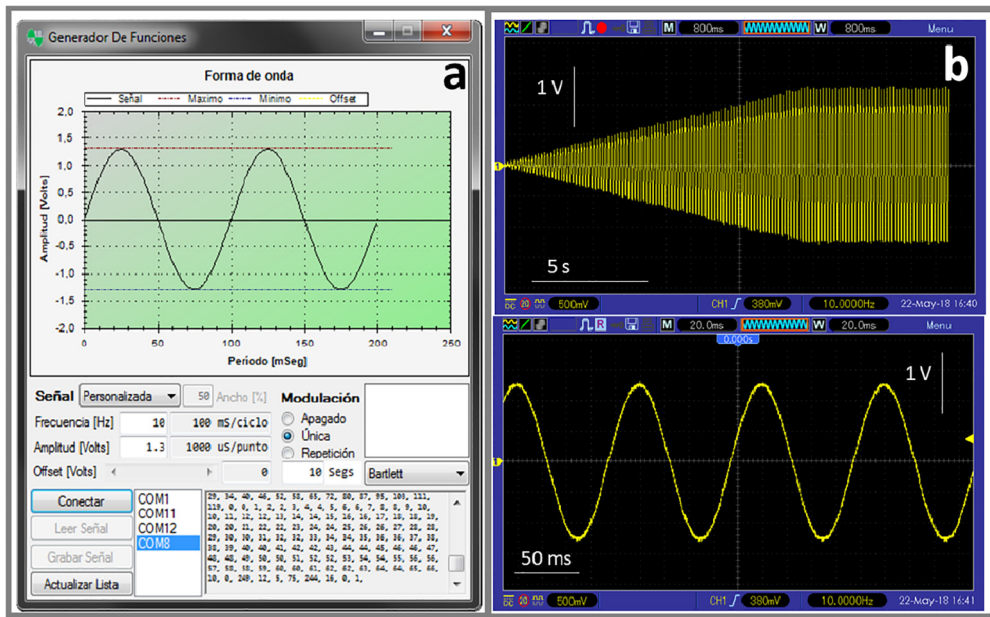


Fig. 7. a. Software window showing the parameters of example 1. b. recorded signal (different scales). See Supplementary Material for an English translated version of the window.

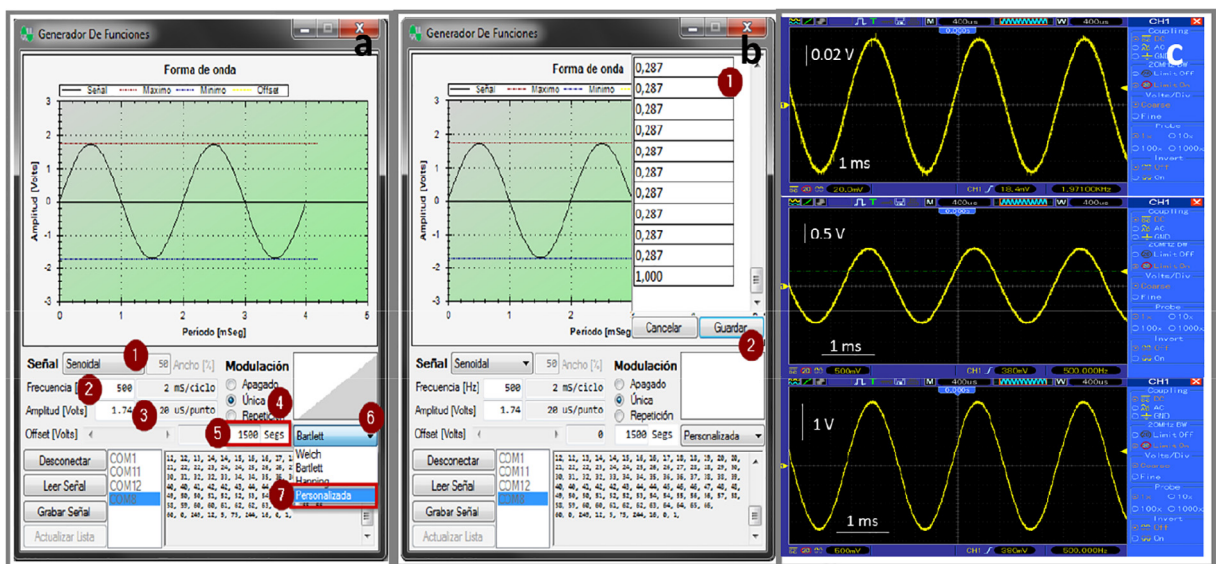


Fig. 8. Software windows showing the parameters of example 2. a. For the application of this waveform, a sine waveform is selected (1) with a frequency of 500 Hz (2) and an amplitude of 1.74 V (3). A single step modulation (4) of 1500 s (5) of the type "personalized" (6) is selected. b. The column "Normalized amplitude" of "Example2.PDF" has to be copied in the textbox (1), and saved (2). c. Recorded signals. See Supplementary Material for an English translated version of the window.

Table 1
Waveform parameters for example 2.

Frequency/Hz	Time/min	Vpp/V	Amplitude/V	Normalized amplitude*
500	5	0.14	0.07	0.04
500	20	1.00	0.5	0.32
500	90	3.48	1.74	1

* Each amplitude was normalized by the larger value.

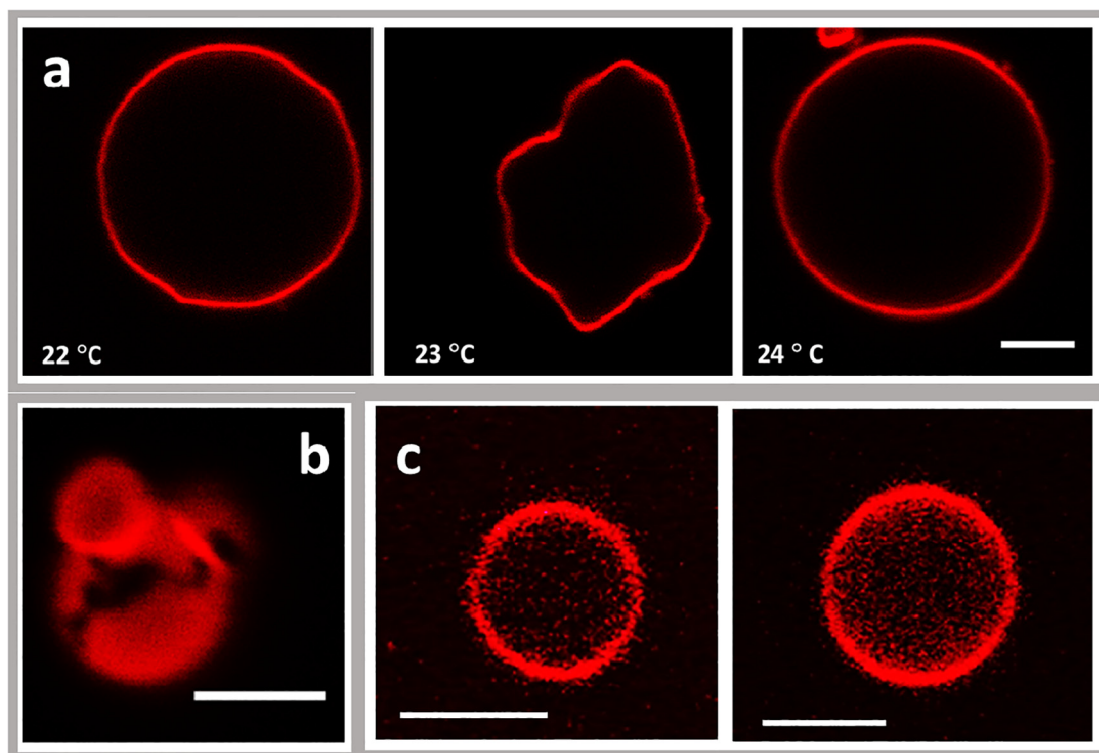


Fig. 9. Representative GUVs of different compositions. a. Vesicle composed of DMPC at the indicated temperatures, courtesy from ML Fanani and Y. Zulueta. b. Vesicle composed of the total lipid extract of *E. Coli*, courtesy of L. Cancelarich. c. GUVs composed of DMPC: Stearic acid generated on ITO-coated glass (left) and with stainless steel (right). Bars: 5 μm .

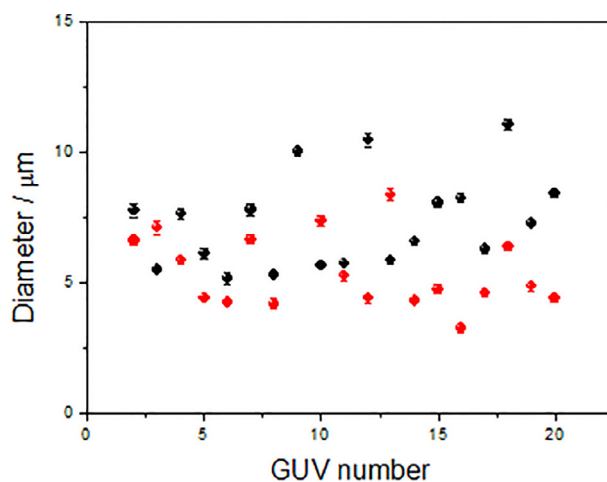


Fig. 10. Temporal average of the size of 20 GUVs, and their temporal fluctuations in a time lapse of 30 s (error bars). GUVs were formed on ITO coated glasses (red) or on stainless Steel (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

easily fabrication. The wave generator is compact an programmable, while the electrodes are reusable and the chamber is easily and inexpensibile renovated.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ohx.2018.e00037>.

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