

## **A method for measurement of the bubble formation threshold in biological liquids**

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Bjørnø, L., L. O. Kornum, P. Krag, C. H. Nielsen, and P.-E. Paulev. 1977. A method for measurement of the bubble formation threshold in biological liquids. *Undersea Biomed. Res.* 4(2): 97–102.—Liquid under pressure is saturated with a given gas, such as argon, nitrogen, or air, by circulation through a column of gas exchangers. A sample of the gas-saturated liquid is isolated in a test chamber, the volume of which can be increased by means of a moving piston. The piston motion is cyclical with a variable frequency. Pressure in the test chamber is measured by means of a capacitive pressure pick-up. When the volume increase of the gas-saturated liquid in the test chamber is compensated for by the development of gas phase bubbles, the pressure decrease will stop; the recording device will show a pressure plateau, or a dip in the pressure-time course, depending on the velocity of the growth of the bubbles. Bubble formation threshold was independent of the frequency of the piston movement within frequency limits from 1 Hz down to  $10^{-3}$  Hz. Most experiments were carried out at a single frequency of 0.5 Hz. This new method appears to have advantages over previous ones.

### **decompression sickness**

The mechanisms for initiation of bubble formation and the factors determining their development in the blood of subjects with decompression sickness is insufficiently understood at present. Although immediate recompression treatment of decompression sickness is accepted as the treatment of choice, prophylactic measures to avoid bubble formation would be of fundamental importance. To study bubble formation in biological liquids with these aspects in mind, we developed a method based on the premises described below.

The threshold for bubble formation in a gas-saturated liquid system depends on the following factors: 1) the liquid itself, which may have weak spots where it breaks and forms bubbles; and 2) the interface between the liquid and its environment. Regardless of the character of the external stimulus, bubble formation in a liquid system must be caused by alteration of pressure and/or temperature, either locally or globally. Bubble formation in diving is almost always caused by pressure alterations; an exception to this rule can be found in a study by Graves, Idicula, Lambertsen, and Quinn (1973). The formation of bubbles is temperature-dependent, and the tendency to develop decompression sickness can be reduced by the use of warm water immersion (Balldin and Lundgren 1972).

This study concentrates on bubbles formed in extracellular fluid. The following liquids were analyzed separately: deionized water, physiological saline (0.9% NaCl), and human blood plasma. The test liquid sample was placed in a chamber with solid walls. A fraction of one of these walls was formed by the top of a cyclically movable piston. The internal surface of the chamber should ideally have no influence on the bubble formation threshold.

In this study, bubble formation threshold means the minimal pressure measured during the first pressure reduction produced by the traction of the piston. This limit value is not synonymous with the pressure at which bubbles were first observed. The liquids were saturated with gases at different hydrostatic pressures. The gases studied were air, nitrogen, and argon. Temperature of the system was room temperature. Hydrostatic pressure means the pressure at which the liquid was saturated; hydrostatic pressure is thus the pressure in the chamber before the start of the piston movement.

## METHODS AND MATERIALS

### Chamber

The chamber (Fig. 1) was milled from a brass block and was built as a box to allow the mounting of glass windows, a brass piston, and a measuring device with plane, smooth surfaces. To reduce the influence of the internal walls of the test chamber on bubble formation threshold, the walls were polished with very fine-grained flint paper so as not to leave any chemical substances except the smooth brass. After cleaning, the chamber was soaked in the liquid later used for examination, bubble formation was provoked by a few decompression/recompression cycles, and the bubble containing liquid was expelled. This preparation allowed exact reproduction of the pressure-time curves in the following experiments (up to six reproduction series were obtained). To our knowledge, no other preparation has yielded similar reproducibility, including chemical substances such as Harvey used (1951). Chamber pressure was reduced because the volume of the chamber increased as a result of the motion of a piston which formed part of the chamber's wall. The piston was made cyclic by an eccentric (Fig. 1). A 2-mm eccentric would create a traction of approximately  $2 \cdot 10^7$  Pa(N/m<sup>2</sup>), i.e.,

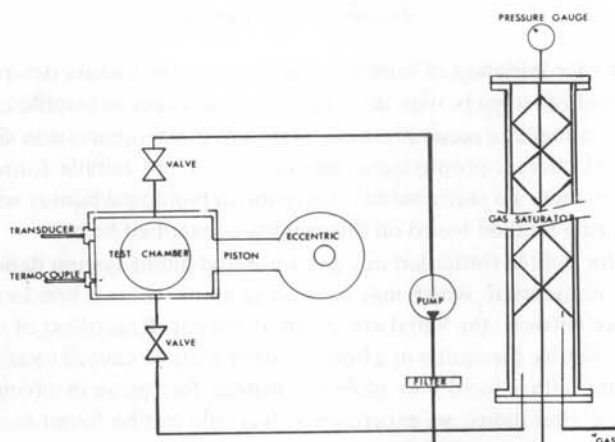


Fig. 1. Block diagram of experimental equipment.

corresponding to a decompression from a depth of 2 km of seawater to surface if the walls were infinitely rigid and the liquid unable to form bubbles. The chamber formed part of a continuous flow system with the gas saturation, allowing liquid in the chamber to be gas-saturated at a given hydrostatic pressure. The diffusion column and the chamber were placed under pressure with the gas to saturate the liquid.

### Gas saturation

The diffusion column was built as a row of 25 cones and funnels (Fig. 1) which allowed the liquid to flow down along the column as a thin liquid film. The diffusion time for obtaining more than 97% saturation was determined mathematically by considering the liquid film as a wall isolated on one side. Relevant parameters were determined by pilot experiments and by means of diffusion coefficients derived from Landholdt and Börnstein (1969); the necessary diffusion time was determined to be less than 30 min for all circumstances. The saturation procedure was continued for the time necessary to allow equilibrium between the gas phase and the liquid phase, so the final hydrostatic saturation pressure,  $P$ , of the liquid corresponded to the hydrostatic pressure above.

### Temperature

The temperature of the liquid was measured with a chromium-aluminum thermocouple using a water bath as a reference.

### Pressure

Two types of pressure measuring system were used. The hydrostatic pressure in the total system was measured by means of a Bourdon manometer (Klaus Fischer, type 4M 07.1 AR 160) with an accuracy  $\pm 5.0 \times 10^3 \text{ N/m}^2$ . To obtain both static and dynamic registration, a capacitive transducer (DISA No. 9051 F-1771H) was chosen for our measurements. The transducer was connected to an ultraviolet paper recorder. The circuit consisted of transducer, tuning plug, oscillator, and converter, and is described in the 1971 DISA Manual. The capacitive transducer was statically calibrated for a pressure range of approximately zero to 29 ATA.

### Procedure

The system was filled with approximately 0.5 liter of liquid. With the two valves open, both test chamber and diffusion column were pressurized to a certain hydrostatic pressure which allowed total saturation. The pump was started and the saturation procedure began with the hydrostatic pressure kept constant. At the end of the saturation procedure, the pump was stopped and the two valves closed. The piston started from the lowest point with the smallest volume each time. The cyclic motion of the piston was initiated. Pressure alterations are illustrated in Fig. 2.

At the beginning of the decompression, bubbles were formed in the liquid bulk by the outward piston movement which increased the total test chamber volume. However, some bubbles became smaller in volume when pressurized. Since the motion was cyclic, the bubbles oscillated in the bulk liquid and at the walls.

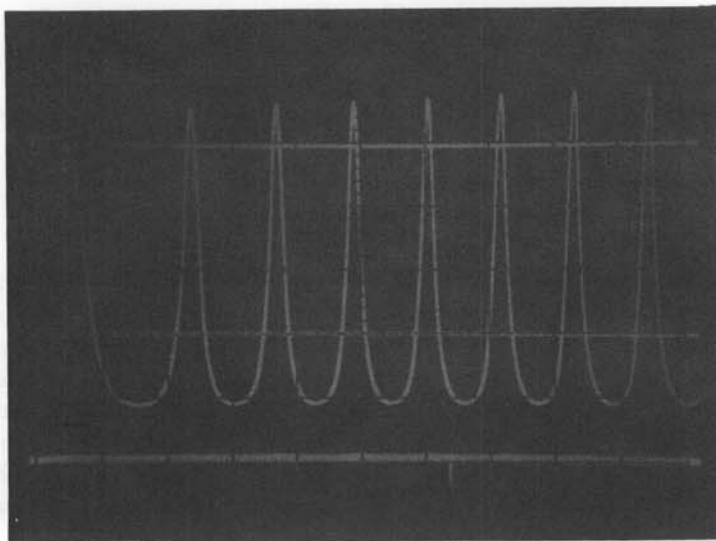


Fig. 2. Photograph of a typical pressure record; abscissa indicates 2 s per time division; ordinate is hydrostatic saturation pressure,  $P$ , equal to 3 ATA.

## RESULTS

Measurements with deionized water, i.e., ordinary tap water filtered through a resin ion exchanger, were performed to elucidate the importance of the following parameter on the bubble formation threshold quantitatively.

### Frequency of piston motion

The outward piston motion increased the test chamber volume, causing the chamber pressure to decrease. As soon as the volume increase resulted in the formation of a gas phase in the liquid, the pressure decrease stopped and the pressure record showed a plateau or a dip, depending upon the velocity of bubble growth (Fig. 2). The bubble formation threshold of deionized water showed no dependence on the frequency of piston movement within the range from 1/1000 cycles per second (cps or Hz) to 1 Hz (Bjørnø, Kornum, Krag, and Paulev 1975).

To facilitate the experimental procedure, it was decided to carry out all subsequent experimental procedures at one frequency (0.5 Hz) because of the lack of dependence noted above.

### Hydrostatic pressure

The number of bubbles of a given size per volume unit of deionized water increased with increasing hydrostatic pressure.

### Repeated decompressions

The maximum pressure measured in the test chamber during the periodic motion of the piston increased with increasing numbers of periods and approached an asymptotic value (Fig.

2). The maximum pressure in the test chamber (peak values in Fig. 2) was reached when the bubble volume was sufficiently large to allow equilibrium between diffusion in the gas phase during pressure increment and out of the gas phase during pressure decrement.

The minimal pressure measured in the test chamber (bottom of the curve in Fig. 2) during the periodic piston movement was constant for successive periods. This result may validate the following statement: repeated decompressions of gas-saturated liquid do not change the bubble formation threshold.

The bubble formation threshold of water, saline, and human blood plasma was a function of the hydrostatic saturation pressure (Fig. 3). The bubble formation threshold of water and saline increased with increasing hydrostatic saturation pressure (Fig. 3). These observations are based upon few measurements and their importance may be limited. Saturation of each of the liquids with pure nitrogen, pure argon, or atmospheric air produced the same bubble formation threshold (Fig. 3).

## DISCUSSION

Kenrick, Wismer, and Wyatt (1924) reduced the pressure in liquids to one atmosphere and measured the time interval between the reduction of pressure and the appearance of bubbles in a microscopic field. The interval "varied between wide limits even under apparently identical

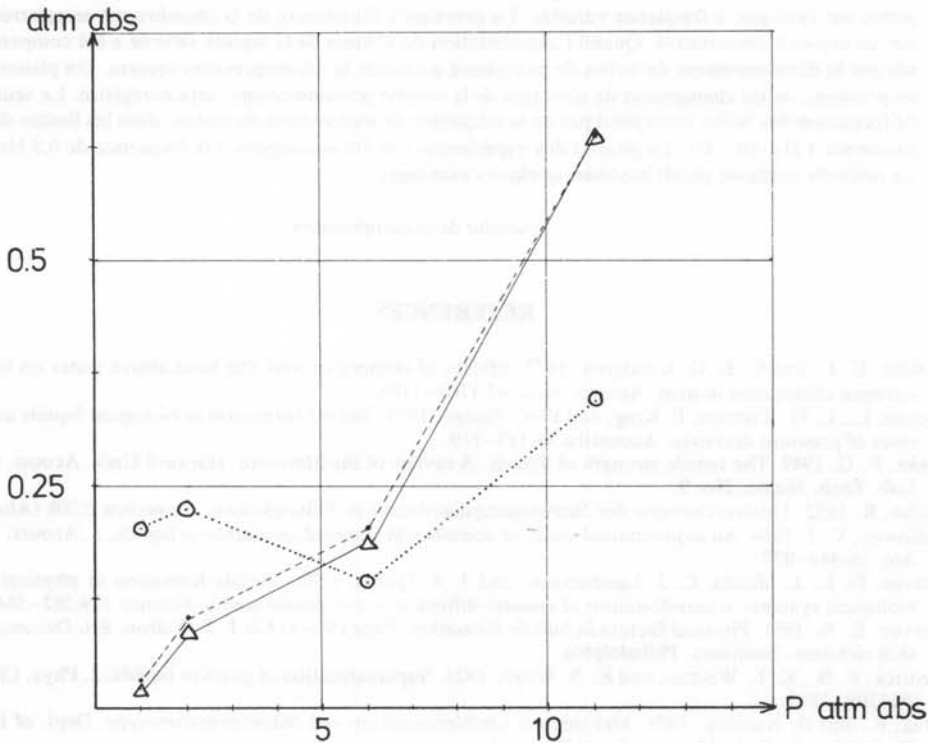


Fig. 3. Hydrostatic saturation pressure,  $P$ , of liquid is on abscissa; pressure measured during decompression of liquid, i.e., bubble formation threshold, is on ordinate. Saturation of deionized water (triangles) and physiological saline (dots) with atmospheric air, pure nitrogen, and pure argon (in different exposures) all showed same bubble formation threshold. Human blood plasma saturated with atmospheric air symbolized by circles.

conditions." The lack of reproducibility may be explained by the fact that the probability of the occurrence of bubbles outside the microscopic field of observation is high (Krag and Kornum 1974).

Bubble formation thresholds obtained by our method on deionized water were in principal agreement with the results obtained by Blake (1949), Esche (1952), and Galloway (1954) using ultrasound. However, Galloway (1954) obtained a somewhat lower threshold. This is to be expected, since higher frequency ultrasound must result in rectified diffusion where the ultrasonic energy facilitates bubble formation and growth. We have therefore avoided the ultrasonic method, and the technique described in this study may be preferable for studies of the bubble formation threshold in liquids. Further information may be obtained in the work of Krag and Kornum (1974).

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Bjørnø, L., L. O. Kornum, P. Krag, C. H. Nielsen, and P.-E. Paulev. 1977 Détermination du seuil de formation de bulles dans des liquides biologiques. *Undersea Biomed. Res.* 4(2): 97-102.—Une liquide sous pression est saturée d'un gaz choisi (argon, azote, ou air) par circulation à travers une colonne d'échangeurs de gaz. Un échantillon de la liquide saturée est ensuite isolé dans une chambre dont le volume peut être augmenté ou diminué à l'aide d'un piston. Le mouvement du piston est cyclique, à fréquence variable. La pression à l'intérieur de la chambre est enregistrée par un appareil capacitive. Quand l'augmentation de volume de la liquide saturée a été compensée par le développement de bulles de gaz (phase gazeuse), la décompression cessera. Un plateau de pression, ou un changement de direction de la courbe pression-temps, sera enregistré. Le seuil de formation des bulles ne dépend pas de la fréquence de mouvement du piston, dans les limites de fréquence 1 Hz -  $10^{-3}$  Hz. La plupart des expériences ont été accomplies à la fréquence de 0,5 Hz. La nouvelle méthode paraît posséder quelques avantages.

maladie de décompression

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