ARCHIVES OF ACOUSTICS
9, 1-2, 113-116 (1984)

CAVITATION EFFECT IN SOME ERYTHROCYTE SUSPENSIONS

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The destructive effects of ultrasound on cell suspensions depend on the parameters of the ultrasonic field and the experimental conditions. Erythrocytes of three origins were investigated: frog, chicken and rat. The frequency of the ultrasound was 1 MHz, with the intensity ranging from 0.4 to 1.2 Wcm^{-2}. The volume concentrations of the samples were in the range 0.5-2 per cent. The investigation determined the volume concentration limit at which, at a sufficiently high field intensity, the destructive effect may be produced.

In well defined conditions (at sufficiently low concentration) cavitation occurs, which has destructive effects (haemolysis). Haemolysis is produced at a well defined concentration in each case, depending on the physical, chemical and biological properties of the erythrocytes. The threshold concentration varies for different erythrocytes; thus the following values were obtained at 0.4 Wcm^{-2}: chicken 0.5 per cent, frog 1 per cent, rat 2 per cent. At low intensities ($J = 0.4$ Wcm^{-2}), high frequency, short duration and diluted suspensions, cavitation plays a mayor role in the haemolysing action.

1. Introduction

Under well determined conditions, ultrasound produces destructive effects in biological cells. The destructive effects in diluted suspensions of erythrocytes are caused by cavitation [4]. The erythrocyte suspensions may be used as a model system in the study of the cavitation effect of ultrasound, because the destructive effect (haemolysis) may readily be evaluated by measuring the haemoglobin concentration in the supernatant obtained after exposure and centrifugation.

This paper presents a study of the parameters which determine the biological, and expecially the destructive, effects of ultrasound, aimed at elucidating the aspects of the ultrasound action mechanism on diluted erythrocyte suspensions, in vitro.

2. Material and method

Diluted erythrocyte suspensions from frog, chicken and rat were used. In the preparation of the suspension we needed a relatively considerable quantity of blood. The blood was collected in different ways, adopting the method to the species. The collected blood had to be very pure.

In order to prevent coagulation heparine was used. The fresh blood was centrifugated 10 min. to 3000 rotations. Then the erythrocytes were separated and washed three times in the centrifuge [7]. The ultrasonic source was a piezoelectric generator made by Tesla, operating in a continuous mode. Sonication to traveling wave of the 1 ml erythrocyte suspensions was performed at ambient pressure and at 18°C, the temperature being controlled by a thermocouple [2].

The parameters of the ultrasonic field were: frequency 1 MHz, intensity varying between 0.4 and 1.2 Wcm^{-2}. The volume concentration of the samples was varied between 0.5-2 %. The occurrence of cavitation was detected using chemical and luminiscence methods [1, 5, 6]. The intensity of the cavitation process was assayed as the time necessary for haemolysis to occur. The haemolysis degree was determined photocolorimetrically using the standard Drabkin reagent as the concentration of haemoglobin released in the supernatants.

3. Results and discussion

Threshold intensities and concentrations were determined, at which haemolysis is induced (Table 1, for chicken). At constant concentration, the time

Table 1. The sonication time (in s) necessary to produce haemolysis in erythrocyte suspensions (chicken, frog, rat) of different concentrations at three intensities of the ultrasonic field

Suspension	Intensity [Wcm^{-2}]	Volume concentration [%]				
		0.5	0.7	1.0	1.5	2.0
		Sonication time [s]				
Chicken	0.4	60	×	×	×	×
	0.6	20	180	×	×	×
	1.0	5	30	120	×	×
Frog	0.4	50	60	100	×	×
	0.6	20	30	60	×	×
	1.0	5	10	30	×	×
Rat	0.4	40	50	70	80	×
	0.6	5	10	20	30	100
	1.0			5	10	60

× = no haemolysis occurs.

required for haemolysis to occur is shorter for higher intensities. The threshold concentration for chicken erythrocytes is 1 per cent at 1.0 Wcm^{-2}. The corresponding concentrations for other suspensions vary as shown in Table 2, indicating the dependence on the biological properties of the cells membrane resistance, structure and dimensions [8-9]. The chicken cells are the most resistant.

Table 2. The sonication time (in s) necessary to produce haemolysis in chicken erythrocyte suspension of different concentrations at six intensities of the ultrasonic field

Intensity [Wcm^{-2}]	Volume concentration [%]					
	0.5	0.6	0.7	0.8	0.9	1.0
	Sonication time [s]					
0.4	60	×	×	×	×	×
0.5	40	180	×	×	×	×
0.6	20	70	180	×	×	×
0.8	10	30	90	×	×	×
1.0	5	10	30	70	100	×
2.2		5	10	30	70	120

× = no haemolysis occurs.

The correlation coefficients ($r_{c,t}$) were calculated between the concentration (c) and the time necessary to obtain haemolysis (t), at different constant values of intensity (J).

Similarly, the correlation coefficients ($r_{J,t}$) were calculated between the intensity (J) and the exposure time (t) at different constant values of the concentration (c).

The values of correlation coefficient ($r_{c,t}$) at various intensities show a positive and very close correlation between the intensity and the time necessary to obtain haemolysis.

The values of the correlation coefficient ($r_{J,t}$) show a negative correlation between the intensity and the time necessary to obtain haemolysis. The values are very significant.

Below the intensity threshold, the time becomes asymptotically longer, provided the concentration is above the threshold by a certain amount.

The cavitation threshold is the limiting intensity under which cavitation does not occur, while above which the formation of cavitation bubbles is possible. It varies as a function of the experimental conditions and the properties of a biological system. The intensity threshold increases with frequency [3].

The occurrence of cavitation is favoured in cell suspensions over that in pure liquids. This is because one must overcome only the adhesion forces in suspensions which are smaller than the cohesion one forces so that cavitation may

occur at intensities as low as 0.5 Wcm^{-2} (for given concentrations). The lethal effect in cells caused by cavitation is produced according to the "all or nothing" law, i.e. after the critical dose is exceeded haemolysis involves simultaneously all the cells [10]. The process involves the transient cavitation type.

4. Conclusions

1. At high frequency (1 MHz) erythrocytes diluted in saline solutions are haemolysed by cavitation, provided the concentration is below a critical value.

2. The cavitation threshold varies with the concentration and the type of cells.

3. The resistance of erythrocytes to ultrasound decreases from chicken to frog to rat.

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