

## COMMUNICATIONS

### The measurement of internal pressure of ampoules and its application to commercial products

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**Abstract**—This paper reports a method for determining the internal pressures of ampoules, from the head space and the change in volume on opening, as measured by displacement of water. Using this method, internal pressures of commercial ampoules were shown to be lower than atmospheric pressure. For example, the ratio of internal pressure to atmospheric pressure in a commercial ampoule of 5 mL distilled water was 0.884 at room temperature (23°C).

It is known that glass particles may be generated in snap-opening ampoules which could have serious consequences for material used for injection, and official standards regulate the levels of particulate contamination in parenteral solutions (USP, BP, JP).

The levels of contamination have been observed to be lower when ampoules were opened by a single flame (Alexander & Veltman 1985) or gas torch method (Tsuji & Lewis 1978; Gillies et al 1986), suggesting that one of the reasons for contamination by glass particles is that the head space in the ampoule is under reduced pressure. Allwood et al (1975) have reported on the internal pressures in bottles measured using a pressure transducer through a hole in the base of the container, and Beck (1985) has discussed pressure-temperature relationships in sealed containers. However, there have been few investigations which measured the internal pressure of the intact container.

In the present paper we report on a method to measure the internal pressures of ampoules and its application to commercial products.

#### Materials and methods

The glass device for determining the internal pressure of an ampoule is shown in Fig. 1. An ampoule and a stirring bar were placed in the glass vessel D which was filled with distilled water. With all stopcocks open distilled water was added through the column A until the level of water was on the line L, and stopcock A1 was closed. Ampoules were previously scratched on the constriction to reduce the force required to open them and the neck portion was broken by collision of ampoule head with the stirring bar. The water level at L changed as the internal pressure of the ampoule returned to atmospheric pressure. Stopcocks B1 and B2 were then closed and the whole device was inverted with gentle shaking if necessary to ensure the gas bubbles were freed from the neck of the ampoule. On re-inversion the gas moved out of the ampoule and into A. The stopcock A2 was then closed and A and B were removed from C and weighed. The result was compared with the weights of A and B filled with distilled water to the level L. The values obtained corresponded with the head space and the change in volume when the ampoule was broken.

As the head space volume of mass-produced ampoules seemed to have more uniformity than manually sealed ampoules, commercial products were investigated (Tables 1-3).

Experiments were carried out at 20-23°C, or at 5 or 40°C. Distilled water to fill the glass device was degassed by sonication to prevent bubbles generated by stirring.

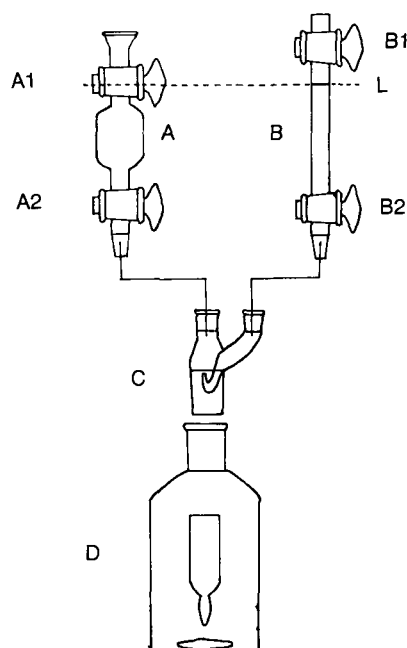


FIG. 1. Apparatus for the measurement of internal pressure of ampoules.

In preliminary investigations, the effect of stirring on the water level was found to be negligible and the error of measuring weights of A and B filled with water was within the limit of measurement.

The internal pressure of the ampoule ( $P$ ) is the sum of the partial pressures of the gas and water vapour, and applying the Ideal Gas Law,

$$P = P_{\text{gas}} + P_{\text{vp}} \quad (1)$$

$$P' = P'_{\text{gas}} + P'_{\text{vp}} \quad (2)$$

$$P_{\text{gas}} V_{\text{gas}} = P'_{\text{gas}} V'_{\text{gas}} \quad (3)$$

where  $P_{\text{gas}}$  is the pressure of gas,  $P_{\text{vp}}$  is the vapour pressure,  $V_{\text{gas}}$  is the head space volume in ampoule, and  $P'$  and  $V'$  are the corresponding parameters after opening the ampoule;  $P'$  is equal to atmospheric pressure. Equations 1 and 2 can be rearranged to give equation 4.

$$\frac{P}{P'} = \frac{P_{\text{gas}} + P_{\text{vp}}}{P'_{\text{gas}} + P_{\text{vp}}} \quad (4)$$

If  $P_{vp}$  and  $P'_{vp}$  is small compared with  $P_{gas}$  and  $P'_{gas}$ , equation 4 may be approximated as follows;

$$\frac{P}{P'} = \frac{P_{gas}}{P'_{gas}} \quad (5)$$

From equation 3:

$$\frac{P_{gas}}{P'_{gas}} = \frac{V'_{gas}}{V_{gas}} = \frac{V_{gas} + \Delta V_{gas}}{V_{gas}} \quad (6)$$

where  $\Delta V_{gas}$  is difference of volume of head space on opening the ampoule and equation 6 becomes;

$$\frac{P_{gas}}{P'_{gas}} = \frac{V_{gas} + \Delta V_{gas}}{(V_{gas} + \Delta V_{gas}) - \Delta V_{gas}} = \frac{W/d_{liq}}{(W - \Delta W)/d_{liq}} \quad (7)$$

where  $d_{liq}$  is the density of water,  $W$  is the weight of water corresponding to the volume of head space after opening the ampoule, and  $\Delta W$  is the weight of water corresponding to the change in volume of head space on opening the ampoule.

According to equations 5 and 7;

$$P_r = \frac{P}{P'} = \frac{W}{W - \Delta W} \quad (8)$$

where  $P_r$  is the ratio of internal pressure to atmospheric pressure.

## Results

The ratio ( $P_r$ ) of internal pressure to atmospheric pressure of 5 mL distilled water ampoules is shown in Table 1. The mean value of  $P_r$  was  $0.884 \pm 0.013$  at 23 °C, and the standard deviation was less than 1.5%.

The  $P_r$  values of various commercial 5 mL ampoules are shown in Table 2 indicating diminished pressure in the head space of ampoules at 20 °C.

The effect of temperature on internal pressures of three batches of ampoules is shown in Table 3.

## Discussion

It has been shown possible to estimate internal pressure from the

Table 1. Ratios of internal pressure of ampoules to atmospheric pressure (23 °C).

Sample	W(g)	$\Delta W$ (g)	$P_r$
1	2.67	-0.38	0.877
2	2.53	-0.36	0.875
3	2.63	-0.35	0.884
4	2.57	-0.39	0.869
5	2.75	-0.32	0.895
6	2.58	-0.28	0.902
Mean	$2.62 \pm 0.08$	$-0.35 \pm 0.04$	$0.884 \pm 0.013$

Sample: distilled water for inj. (Fuso, Japan, 5 mL).

Table 2. Ratio of internal pressure of commercial ampoules to atmospheric pressure (20 °C,  $n = 10$ ).

Sample	W(g)	$\Delta W$ (g)	$P_r$
A	$3.179 \pm 0.056$	$-0.555 \pm 0.026$	$0.851 \pm 0.004$
B	$2.489 \pm 0.051$	$-0.494 \pm 0.017$	$0.834 \pm 0.006$
C	$3.190 \pm 0.101$	$-0.421 \pm 0.032$	$0.883 \pm 0.004$
D	$3.144 \pm 0.076$	$-0.248 \pm 0.015$	$0.927 \pm 0.004$
E	$3.568 \pm 0.067$	$-0.293 \pm 0.016$	$0.924 \pm 0.003$
F	$3.043 \pm 0.050$	$-0.394 \pm 0.012$	$0.851 \pm 0.005$

Sample A: distilled water for inj. (Mect, Japan, 5 mL), B: distilled water for inj. (Hishiyama, Japan, 5 mL), C: distilled water for inj. (Toyo-Jozo, Japan, 5 mL), D: distilled water for inj. (Dainihon, Japan, 5 mL), E: distilled water for inj. (Kobayashi, Japan, 5 mL) F: isotonic sodium chloride inj. (Mochida, Japan, 5 mL).

Table 3. Effect of temperature on internal pressure of ampoules.

Sample	Temp. (°C)	n	W (g)	$\Delta W$ (g)	$P_r$
A	5	9	$0.75 \pm 0.03$	$-0.21 \pm 0.02$	$0.784 \pm 0.015$
	20	6	$0.78 \pm 0.04$	$-0.14 \pm 0.03$	$0.848 \pm 0.025$
	40	10	$0.99 \pm 0.03$	$-0.05 \pm 0.03$	$0.952 \pm 0.024$
B	5	9	$2.32 \pm 0.08$	$-0.60 \pm 0.02$	$0.795 \pm 0.006$
	20	6	$2.48 \pm 0.07$	$-0.34 \pm 0.02$	$0.879 \pm 0.008$
	40	8	$2.86 \pm 0.07$	$-0.10 \pm 0.04$	$0.967 \pm 0.011$
C	5	9	$2.46 \pm 0.15$	$-0.43 \pm 0.02$	$0.852 \pm 0.006$
	20	10	$2.61 \pm 0.10$	$-0.26 \pm 0.04$	$0.911 \pm 0.013$
	40	9	$2.99 \pm 0.11$	$0.18 \pm 0.02$	$1.065 \pm 0.006$

Sample A: nyclin inj. (Toa Eiyo, Japan, 1 mL), B: distilled water for inj. (Fuso, Japan, 5 mL), C: neophyllin inj. (Eisai, Japan, 10 mL).

volume of gas in ampoules before and after opening. The volumes could be measured by displacing a weight of water.

The internal pressures in commercial ampoules determined by the present method were lower than atmospheric pressure (Table 2), and increased with rising temperature (Table 3).

Diminished pressure may be caused in the sealing procedure. The head space gases will expand as the upper portion of the ampoule is heat sealed and is cooled to room temperature after sealing. Diminished pressure in the ampoule may cause glass particles generated in snap-opening to fall into the ampoule and therefore, should be avoided.

As measured values of  $P_r$  could be affected by gas in the ampoule being partly dissolved in water, in a preliminary test, about 3 mL of air in A was moved to D, and again returned to A. The difference of weights before and after moving the air was negligible.

The internal pressures of ampoules are affected by temperature, through changes in density of water, solubility of the gas in water, and expansion of the glass. As the temperature rises, the liquid phase expands and compresses the head space; but the solubility of the gas in water decreases and the gas in the head space will increase. The effect of expansion of glass on the volume of gas in the head space, however, will be small.

Equation 1 may be written as

$$P = \frac{nRT}{V_{gas}} + P_{vp} \quad (9)$$

where  $n$  is the moles of gas in the head space at temperature  $T$  and  $R$  is the universal gas constant. As the temperature increases from  $T_1$  to  $T_2$ ,  $V_{gas2}$  and  $n_2$  are given by equation 10 and 11.

$$n_2 = n_1 + [s_1(P_1 - P_{vp1})V_{liq1} - s_2(P_2 - P_{vp2})V_{liq2}] \frac{d_{gas2}}{MW_{gas}} \quad (10)$$

$$V_{gas2} = V_{gas1} + \frac{d_{liq2} - d_{liq1}}{d_{liq2}} V_{liq} \quad (11)$$

where  $V_{liq}$  is the volume of liquid in the ampoule,  $s$  is the solubility of the gas in water,  $d_{gas}$  is the density of gas, and  $MW_{gas}$  is the molecular weight of the gas.

In the present study,  $V_{gas}$  is estimated from observed value of  $W - \Delta W$ :

$$V_{gas} = (W - \Delta W)/d_{liq} \quad (12)$$

Applying values of  $P_r$  and  $V_{gas}$  from equation 12 to neophyllin injection results obtained at 20 °C ( $V_{liq} = 10$  mL, head space gas is  $N_2$ ), the internal pressure at 40 °C can be calculated from equation 9 by substituting the calculated values from equations 10 and 11. The calculated value of 1.049 at 40 °C from 0.911 at 20 °C was similar to the observed value, 1.065 (Table 3).

**Conclusion.** A method of measuring internal pressures of ampoules has been described. The internal pressure of ampoules can be measured by displacement of water. Internal pressures in ampoules are generally lower than atmospheric pressure and may be one of the reasons for contamination by glass particles when the ampoule is opened.

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## Dose-dependent decrease in rat plasma amino acids after acute administration of ethanol

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**Abstract**—Male rats were given three different doses of ethanol in i.p. injections (0.66, 1.33 and 2.00 g kg<sup>-1</sup>). A dose-dependent decrease in the concentrations of most plasma amino acids was observed. For the total amino acid concentration this decrease was 5, 16 and 22%, respectively, compared with a saline-treated control group. It has previously been suggested that the oxidation of ethanol plays an important role in the amino acid decreasing effect of ethanol. In this study the lowest dose used (0.66 g kg<sup>-1</sup>) was calculated to be high enough to keep the enzyme systems involved in ethanol oxidation saturated during the 60 min course of the experiment. The observation that the ethanol-induced decrease in plasma amino acid levels was more pronounced with higher ethanol doses indicates that not only the oxidation of ethanol but also ethanol itself is important in the effect of ethanol on plasma amino acid concentrations.

We have previously reported that ethanol, when given in acute doses, induces a rapid decrease in the concentrations of most plasma amino acids in rat (Eriksson et al 1980) and in man (Eriksson et al 1983). The mechanisms underlying this effect of ethanol is complicated and not well understood. Both effects of ethanol itself (Eriksson et al 1981) and effects of its metabolites (Hagman & Jagenburg 1989) have been proposed.

The ethanol elimination rate in rats is virtually constant down to the rather low saturation level of hepatic alcohol dehydrogenase (Makar & Mannering 1970; Braggins & Crow 1982). Furthermore, this rate is also, within a wide range, independent of the administered dose of ethanol (Braggins & Crow 1981). These observations offer an opportunity to distinguish between effects caused by ethanol itself and effects caused by its metabolites. At ethanol concentrations above the saturation level the rats can only metabolize a specific amount of ethanol per unit time. This means that all further effects of ethanol above this level must be due to ethanol itself, and that they are independent of the metabolism of ethanol.

The present study is an attempt to distinguish between direct

and indirect effects of ethanol on plasma amino acid concentrations, using this approach.

#### Materials and methods

Male Sprague Dawley rats, about 200 g (ALAB, Sollentuna, Sweden), were used. They were housed for at least one week in a room maintained on a 12 h light/dark cycle and had free access to food and water. During the experiment no food or water was given. Ethanol was diluted in saline and injected intraperitoneally in doses of 0.66, 1.33 or 2.00 g kg<sup>-1</sup>. Control rats received an equivalent volume of saline (10 mL kg<sup>-1</sup>). Sixty min after the injections the animals were killed by decapitation. About 5 mL blood was collected in EDTA tubes and immediately centrifuged at 10 000 g for 10 min. The plasma samples were stored at -70°C until amino acid analysis.

Amino acids were analyzed by ion-exchange chromatography after deproteinization with sulphosalicylic acid as described elsewhere (Eriksson et al 1980).

Statistical significances were assessed by linear regression analysis.

#### Results

Ethanol exerts a decreasing effect on the total concentration of amino acids in rat plasma and the magnitude of this effect is dependent on the administered dose of ethanol (Table 1). The lowest ethanol dose used (0.66 g kg<sup>-1</sup>) induced a decrease of 5%, whereas the highest dose (2.00 g kg<sup>-1</sup>) induced a decrease of 22%.

The concentrations of most of the individual amino acids also declined in a dose-dependent manner after acute administration of ethanol (Table 1). This correlation was statistically significant for all measured amino acids except glutamine and lysine. Alanine was the amino acid the concentration of which showed the largest decrease (17, 34 and 44%, respectively) after administration of the three ethanol doses.

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