HEAD SPACE ANALYSIS

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SUMMARY

Additional analytical information is given about the method of head space analysis. From the data presented it can be concluded that this technique may be advantageous for enzyme kinetic studies in turbid solutions, provided a volatile organic substance is involved in the chemical reaction. Also some qualitative aspects of a new mammalian enzyme: acetoacetate decarboxylase (acetoacetate carboxylyase: E.C. 4.1.1.4) are presented.

INTRODUCTION

Nowadays, gas chromatography has become a familiar analytical technique for the separation and the subsequent quantitation of a great variety of complex mixtures.

The purpose of this paper is to demonstrate that using head space analysis, gas chromatography can also be used for some other purposes, the determination of the heat of vaporization or the detection of enzymes catalyzing reactions in which volatile substances are involved.

The head space is the vapour, which is in equilibrium with its liquid phase. Provided the dissolved organic substances to be analyzed are sufficiently volatile, the determination of the concentration of these substances in the vapour phase can be used as a measure of the concentration of these substances in the liquid phase. This technique is especially useful for the determination of *low* concentrations of *highly* volatile substances, when dissolved in *aqueous* solutions. This is even more true when the aqueous solution also contains proteins and other solid materials. Hence, head space sampling can be considered to be an "off-line" technique to obtain preliminary separation of the substances under investigation, from other interfering materials.

METHOD

In a foregoing publication[1] the method of head space analysis was proposed for the determination of acetone and β-ketobutyric acid in blood serum. Except for the nitrogen gas flow (from 40 ml to about 70 ml/min) no changes have been applied in the gas-chromatographic conditions.

The technique of head space sampling is very simple. In brief: with an air-tight syringe, 1 ml of air is injected into a glass, serum-capped vial, the latter containing the aqueous solution to be analyzed. In our case the volume of the vial is constantly 40 ml and the volume of the aqueous solution 0.2 ml or more. Provided equilibrium between the vapour phase and the aqueous phase is obtained, the plunger is moved several times to and fro and 1 ml of head space vapour is removed and *directly* injected into the gas chromatograph.

RESULTS

Analytical details (I)

Fig. 1 demonstrates the separation of a mixture of some volatile substances in a head space sample obtained from an aqueous solution. As can be seen, clear separation is obtained between acetic aldehyde, ethyl ether, acetone, methyl- and ethyl alcohol.

Fig. 2 demonstrates the calibration curves of acetic aldehyde, acetone, methyl- and ethylalcohol. As can be seen, straight lines are obtained when the peak height is used as a measure for the concentration of these substances in the aqueous phase. It can be seen from the attenuation positions of the detector, that the sensitivity of the method for the different substances changes significantly, as could have been expected.

It has already been mentioned[1] that for calibration the standard solutions must have approximately the same osmolality as the unknown sample. Hence, analyzing serum samples, physiological saline must be used as the solvent calibration.

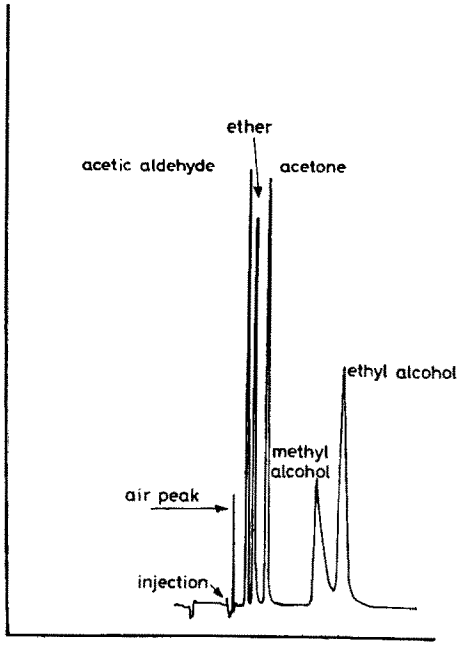


Fig. 1. Gas-chromatographic separation of acetic aldehyde, ethyl ether, acetone, methyl-, and ethyl alcohol. Sample: 1 ml head space vapour.

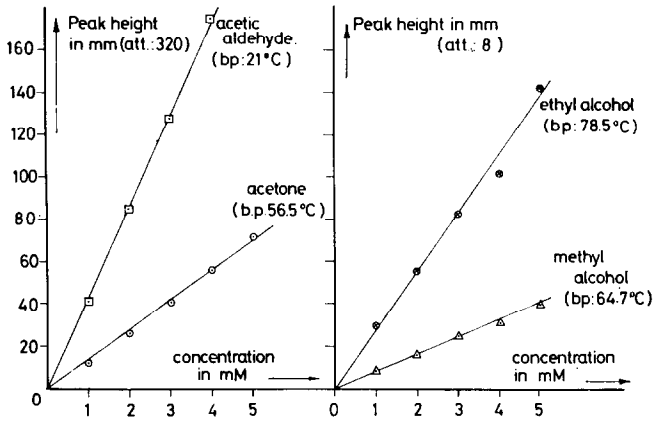


Fig. 2. Calibration curves of: acetic aldehyde, acetone, methyl- and ethyl alcohol. Sample: 1 ml head space vapour (att. = attenuation G.C.).

The accuracy of the head space sampling technique is *very* satisfactory: using 0.2 ml of a 0.2 mM acetone solution we calculated identical standard deviations: 1.2% of the original peak height, at 20° and 37°. In order to obtain this accuracy the following points are important: (1) avoid contamination of the air which is injected into the serum-capped vial: (2) keep the time between sampling and injection as short as possible (normally within 5 sec), otherwise the peak height will decrease, due to adhesion of the substances to the glass wall of the syringe; (3) always inject the head space sample in the same manner.

It must be mentioned that, even after 50 determinations, commercially available "air-tight" syringes may start leaking.

Another advantage of this technique is that repeated sampling from the same vial does not decrease the peak height. When 0.2 ml of an aqueous solution, containing 0.2 mmoles acetone per litre, was used, even after eight head space samples of 1 ml, no decrease could be observed. Hence, with this technique, reaction kinetic studies can easily be performed.

In a previous publication[1] some variables influencing concentration in the vapour phase were given. For instance: the volume of the aqueous phase and the osmolality of the aqueous solution. Therefore, in this paper only some additional information about this subject will be given.

Fig. 3 shows that increasing the volume of the head space sample up to 2 ml gives a linear increase of the peak height (vol. aqueous phase: 0.2 ml; concentration acetone: 0.6 mM). Increasing the head space volume over 2 ml, this relationship is no longer linear. This is probably due to an increase of the injection time of the head space sample, because, when the volume of the sample is related to the peak *area*, the linear relationship is restored.

We reinvestigated more extensively the influence of the temperature of the serum-capped vial upon the peak height (vol. aqueous phase: 0.2 ml; concentration acetone: 0.6 mM). The results are shown in Fig. 4. From this figure it can be seen that the logarithm of the peak height is linearly related to the reciprocal value of the absolute temperature. The thermodynamic explanation is given by the law of Clapeyron–

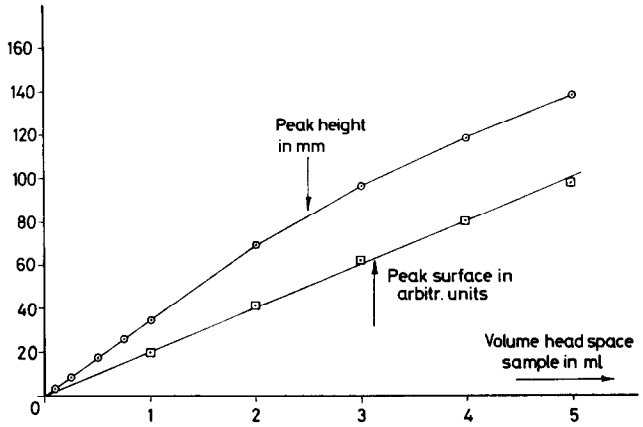
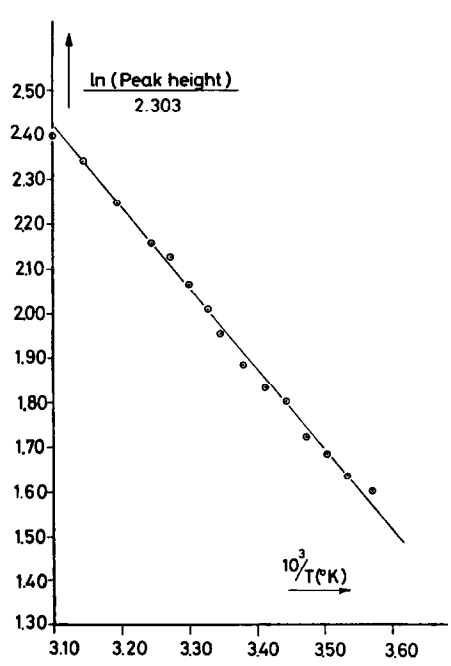


Fig. 3. Influence of increasing volume (ml) of the head space vapour upon the peak height (in mm) and peak surface (arbitr. units).



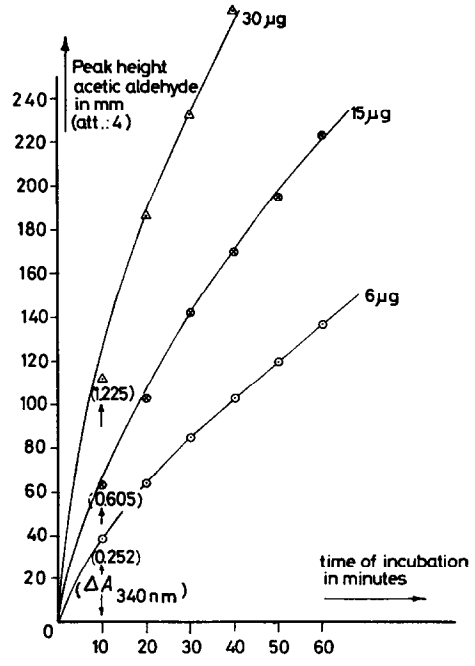


Fig. 4. Relation between the temperature of the serum-capped vial, expressed in $10^3/°K$, and the acetone concentration in the head space vapour, expressed in log (mm peak height).

Fig. 5. Increase of acetic aldehyde concentration in the head space vapour caused by the oxidation of ethyl alcohol and catalysed by different amounts (5,15 and 30 µg protein) of purified alcohol dehydrogenase (Boehringer). The values in brackets represent the changes of the absorbance after 10 min of incubation (UV method).

Clausius[2] which, approximately, says that if plotting the logarithm of the vapour pressure against the reciprocal value of the absolute temperature, the slope of the curve at any point, multiplied by $-R$ yields the value of the heat of vaporization. For this physical constant we calculated from our curve a value of 7.56 kcal/mole at 15°, while by calorimetry[3], determined with pure acetone, a corresponding value of 7.22 kcal/mole is found. In future the head space sampling technique may be valuable for more fundamental studies of this kind.

Biochemical applications (II)

Aldehydes. Fig. 5 demonstrates that for the determination of the activity of alcohol dehydrogenase (E.C. 1.1.1.1) head space analysis can be used. In this experiment we used purified enzyme (Boehringer) and compared, under identical experimental conditions, the increase of the acetic aldehyde concentration in the head space vapour with the increase of NADH, by means of a spectrophotometric method[4]. The sensitivities of both methods have the same order of magnitude. However, the head space analysis is preferable to the spectrophotometric method in cases, where enzyme kinetic studies have to be performed in turbid solutions, such as tissue homogenates.

Head space analysis has also been applied to investigations into the origin and mechanism of different types of diarrhoea. Pretreatment of faeces, including mild oxidation and subsequent head space analysis may result in the presence of different aldehydes in the head space vapour.

Fig. 6 demonstrates the different aldehydes which, after mild oxydation at 37°, with this technique, may be found in the case of a patient with a putrefactive diarrhoea. The C_2 peak originates from L+D-lactic acid, while the C_3, iso-C_4, and iso-C_5 peak (a+b) may originate from metabolites (the α-hydroxy acids) of glutamic acid (via α-aminobutyric acid), valine, leucine plus isoleucine, respectively. According to Van de Kamer, these α-hydroxy acids are produced by the action of the putrefactive flora in the large intestine.

Acetone and acetoacetate. The determination of acetone and acetoacetate in blood has already been reported on[1]. In that publication we also mentioned the exist-

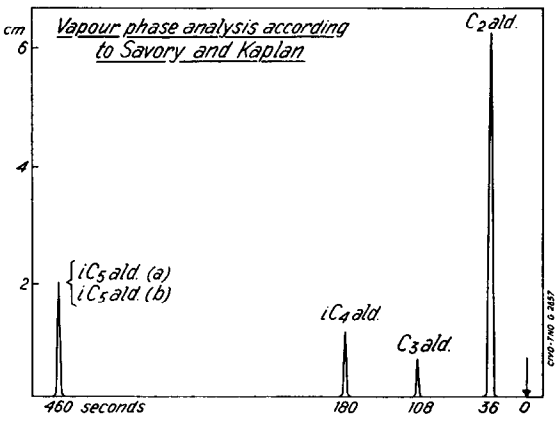


Fig. 6. Chart tracing of the gas-chromatographic separation of different aldehydes (kindly supplied by Dr. J. H. van de Kamer; CIVO-TNO).

TABLE I

QUALITATIVE ASPECTS OF MAMMALIAN ACETOACETATE DECARBOXYLASE, IN COMPARISON WITH THOSE OF THE BACTERIAL ENZYME (E.C. 4.1.1.4)

Characteristics	*Mammalian*	*Bacterial*
Present in:	blood & tissue (liver)	*Clostridium acetobutylicum* (By.)
Ultrafiltrable	not	not
Type of protein	albumin fraction	8 sub-units (M = $30–35 \cdot 10^3$ each)
pH optimum	yes	yes
Michaelis-Menten constant	yes	yes
Q_{10} (in the normal range of enzymes)	yes	yes
Heat-stable	not	not
Activity is influenced by:		
Urea	yes	yes
Hg^{2+}	yes	yes
CN^-	not	yes
Iodoacetate	yes	yes
Vit. B_2 (riboflavin)	yes	?
Pyruvic acid	not	not

ence of a mammalian enzyme: acetoacetate decarboxylase (E.C. 4.1.1.4). The characteristics of this enzyme were investigated by means of the acetone concentration in the head space vapour as a result of the degradation of acetoacetate. Only some qualitative aspects of this enzyme will be presented here (Table I), together with those of the bacterial enzyme[5–7].

The most striking observation presented in this Table may be the probable albumin-like nature of the mammalian enzyme, because, as far as we know, albumin has never been mentioned to possess enzyme activity. Full proof of the existence of the acetoacetate carboxylase can only be accepted by the demonstration of the quantitative results.

Finally, considering human breath as the head space of blood *in vivo*, some interesting biochemical substances have been mentioned in the literature. Jansson and Larsson[8] mentioned the presence of: methane, methanol, ethanol and isoprene; while Chen[9] reported acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and after methionine ingestion, methane-thiol, and dimethyl-sulfide to be present in human breath. The former were mostly found under pathological conditions.

The applicability of head spaces analysis will increase by further investigation into the optimal experimental conditions, and last but not least, by the construction of new detectors with increased sensitivity.

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