ANOMALOUS SORPTION IN THE INJECTION PORT OF THE GAS CHROMATOGRAPH

MILOŠ KREJČÍ, KAREL HÁNA AND MILENA ROUDNÁ

Institute of Instrumental Analytical Chemistry, Czechoslovak Academy of Science, Brno (Czechoslovakia)

SUMMARY

The origin of ghost chromatograms is explained and demonstrated experimentally. A method is suggested for the determination of the time constant for the desorption of material from the sample port rubber closure of the chromatograph. The effect of some parameters (*viz.* injection port design, carrier gas flow rate, and the kind of the rubber used for the septum) on the anomalous sorption is investigated experimentally and the potentiality of these parameters for producing ghost chromatograms is discussed.

INTRODUCTION

Chromatographic separation of the components of a mixture is feasible owing to their sorption in a stationary liquid or on a surface active adsorbent. Unfavourable sorption phenomena on the liquid phase support[1] or on the column wall sometimes significantly affect the chromatographic analysis. Extracolumn sorption, occurring most frequently in the sample port, leads to similar consequences. Sorption on the injection port rubber septum[2] can occur or material can be deposited in the injection port either by condensation of the sample owing to too low a temperature at the moment of injection, or by the decomposition of sample due to an unnecessarily high temperature, or eventually due to unsuitable material being used for construction of the sample port. The effect of the anomalous sorption is enhanced still further by the fact that the injection port temperature usually varies both within a single analysis and between the individual analyses; during the periods when no injection is carried out, it is usually lower by comparison with the column temperature.

Increasing demands on the separation efficiency of the gas chromatograph as well as on the precision of quantitative analysis also lead to increasing attention being devoted to the conception, design, and the proper function of the sample ports. Recently, three main problems have been investigated following the injection of mixtures of substances into a gas chromatograph. These are the questions concerning precise and reproducible introduction of very small samples (especially for capillary chromatography), questions connected with the separation efficiency of the gas chromatograph, and, finally, the questions associated with the anomalous sorption

of the injected substance prior to its entering the chromatographic column. The latter group of problems, concerning the questions of so-called ghost chromatograms, is studied in the present paper. In this work, the part of the pneumatic system as understood by the term "injection (sample) port" of the gas chromatograph is situated at the inlet of the chromatographic column, and is separated from the ambient atmosphere by a rubber septum (or by some other system of closure) through which a gaseous, liquid, or solid sample of the mixture of substances to be analysed may be introduced into the carrier gas stream.

THEORETICAL

The basic requirements that should be met by the injection port as far as possible, follow from the theory of elution chromatography[3]. In order to retain the column efficiency it is necessary for the solute volume being injected to enter the column, and to be eluted by the carrier gas from the injection port, in the form of a sharp input pulse with a maximum solute concentration in the carrier gas, c_0, in the shortest possible time. The limiting conditions (c_0, $t \rightarrow 0$) cannot be attained in practice. A deformation of the input pulse[4] always occurs; this manifests itself in the shape of the chromatographic zone recorded at the outlet of the separating column. The extent to which the input pulse is deformed can be affected by a number of factors, one of which is the anomalous sorption in the injection port (most frequently sorption on the rubber closure of the injection port). This is treated quantitatively in the present paper.

In this connection, we studied three basic factors which lead to the deformation of the input pulse emerging from the injection port: (1) the design of the sample port, (2) the operating conditions of the sample port, and (3) the phase equilibrium in the sample port. A procedure generally used for describing transient phenomena[5,6] has been employed to describe the process being studied. The influence of the injection port design and its respective working conditions is characterized by a time constant τ_c. The phase equilibrium in the injection port is characterized by a time constant τ_d. The course of the concentration pulse at the injection port outlet can be described by a general relation[7,8]

$$y(t) = f(0)Y(t) + \int_0^t f'(\xi)Y(t - \xi)d\xi \tag{1}$$

where $Y(t)$ and $y(t)$ are, respectively, the input and output time functions of the concentration pulse in the injection port, $f(t)$ is a function defining the dependence of the output signal on time for a unit step of the input signal. The course of the concentration pulse at the injection port outlet is a function of the effect of the design and working conditions of the injection port, characterized by the time constant τ_c as well as by the kinetics of setting up the phase equilibrium in the injection port, characterized by the time constant τ_d and

$$y(t) = y[y_c(t), y_d(t)] \tag{2}$$

Then, in equation (2), the time dependence of the input signal, for $y_c(t)$ is

$$f_c(t) = 1 - \exp\left(-t/\tau_c\right);$$

and for $y_d(t)$ is   (3)

$$f_d(t) = 1 - \exp\left(-t/\tau_d\right).$$

In solving equation (1) and/or (2) we presume $\tau_c \ll \tau_d$. Fig. 1 illustrates this; the courses of the output functions $y(t)$ are quoted for $\tau_c = 0.1$ and $\tau_d = 100$ along with a diagram of the output curve involving both factors (the input function is of a triangular shape[6,7]).

The deformation of the input concentration pulse can only be observed in a chromatogram if the concentration of the substance in the tailing part of the peak at the column outlet exceeds the threshold of the detector sensitivity. Because of the spreading of the pulse, and, thus, also of the peak tail on the column, the solute concentration in the carrier gas (relative to the peak maximum) at the column outlet
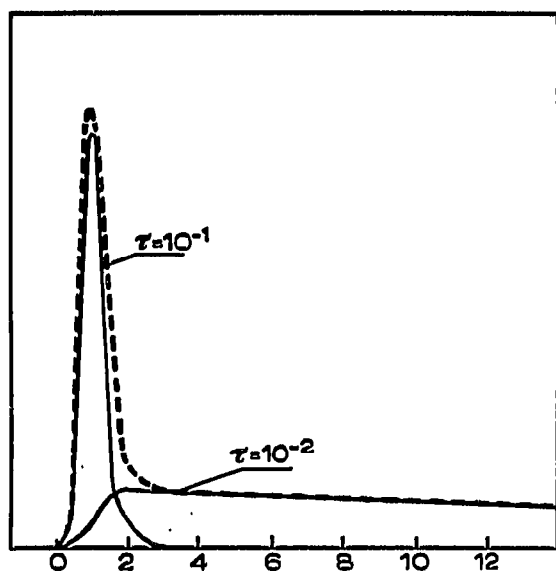

Fig. 1. Shape of the peak for various time constants.

is always lower than that at the injection port outlet. Therefore, it is usually difficult to measure directly the solute concentration in the tail of a zone on the chromatogram.

The sorption in the sample port of the material to be analysed proceeds only until the moment when the concentration pulse $y_c(t)$ leaves the sample port. The process of desorption depends only on the kinetics of the sorption equilibrium. This process is analogous to a chemical reaction of the first order. Schematically, it proceeds in such a way that one component is represented by the rubber (sorbent) + solute; the other component is then the solute released. At a time t_a, when the concentration pulse $y_c(t)$ leaves the injection port, there are a ml of solute sorbed in the rubber. The change of the solute concentration in the gas with time is given by

$$y_d(t) = \frac{a - x}{\tau_d} = \frac{dx}{dt}$$ (4)

where x is the solute volume in the gaseous phase, and $(a - x)$ is the solute volume

in the rubber at a time $t > t_a$. The solute concentration in the carrier gas (vol. % of the substance in the carrier gas) will be

$$c_t = 100(a - x)/\tau_a w \tag{5}$$

where w is the carrier gas volume flow rate (ml·sec^{-1}). In the case where c_t is less than the threshold of the detector sensitivity, errors come about in quantitative gas chromatography, especially when measuring or integrating the area of the chromatographic peak. In the case where the time constants τ_d for the individual components of the mixture analysed are equal, the relative values of the composition of the mixture suffer from a smaller error. However, the above time constants are very often different[9,10] for the various components of a single mixture, and, in such a case, the relative values may also suffer from considerable errors.

EXPERIMENTAL

The measurements were carried out on a W. Giede gas chromatograph model GCHF 18.3 F.I.D. (Berlin, Eastern Germany). The carrier gas, nitrogen in this case, was led at a known rate (about 1.0 ml/sec) from the source (Fig. 2) through a glass three-way stopcock (2), adjusted to the position A,B, into a 200 cm long brass chromatographic column of 0.3 cm I.D., packed with 8.1 g of 25% PEG 20M on-
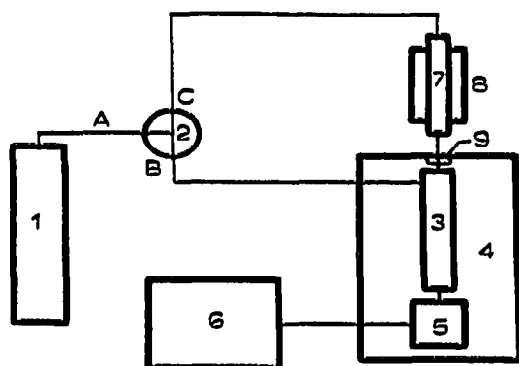

Fig. 2. Diagram of the apparatus. For the description see the text

Chromosorb P (Carlo Erba, Milan, Italy) (3). Then the effluent stream was led into the flame ionization detector (5) from which the signal was transferred on the scale of a recording millivoltmeter (6). It was possible, by turning the stopcock to the position A,C, to lead the carrier gas through a 22 cm long glass precolumn (7) of 0.5 cm O.D. The carrier gas was led into the precolumn via a metallic capillary terminating in an injection needle which passes through a silicone rubber septum that closes the upper part of the precolumn. The lower part of the precolumn is connected by a rubber tube with an injection needle by means of which the carrier gas was led, through the inlet block of the chromatograph (9), into the main column and detector. The precolumn was kept at a constant temperature of 79°, using a resistance heating oven (8) connected by means of an autotransformer. The main column was placed in a thermostat (4) and kept at a constant temperature of 70°.

Two experiments were carried out with the above arrangement. The principle of both consisted in introducing through the rubber closure a sample, diluted with the carrier gas, into the carrier gas stream passing through the stopcock (2) in position A,C, with the aid of an injection syringe (max. volume 1 ml). After allowing twice the retention time of the respective samples to elapse, the carrier gas stream passing through the column was interrupted for a certain time (from 10 to 300 sec) by turning the stopcock (2) into the position A,B. At the same time, the sensitivity was increased by a factor of 100 by means of a sensitivity attenuator. After the time determined, the stopcock was switched over again into the position A,C, and the sample fraction entrapped in the precolumn was eluted by the carrier gas flowing through.

In the first experiment, an empty precolumn was connected in the carrier gas conduit. The aim of this experiment was to determine the effect of the distances of the carrier gas intake and the tip of the sample charging needle from the rubber septum in the injection port on the amount of sample trapped in the precolumn (*cf.* Fig. 3). The experiment was performed in two parts. In the first, 200 µl of heptane
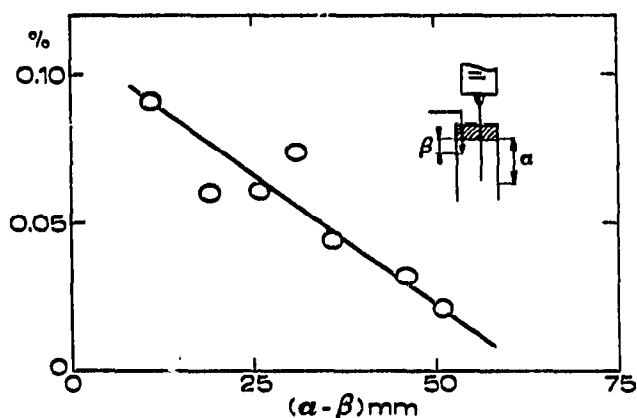

Fig. 3. Dependence of the heptane sorbed (as a percentage of the initial sample) on the sample port design.

vapour was introduced at a constant depth beneath the septum of the precolumn sample port and with various distances of the carrier gas inlet (3, 8, 18, 23, 33, and 44 mm) from the rubber closure. The carrier gas flow through the precolumn was interrupted for 300 sec. In the second case, the carrier gas intake was introduced 23 mm below the septum, and the sample inlet depth was varied (10 and 33 mm). The carrier gas flow through the precolumn was cut for 300 sec. This procedure was repeated several times without repeating the injection, and then the needle, feeding the carrier gas, was pulled up in such a way that the needle tip was just a bit below the rubber septum, and the carrier gas flow was again interrupted.

In order to determine the phase equilibrium (the second experiment) three kinds of rubber were used as packing for the glass precolumn. The rubber was cut to form small rolls of known diameters and heights. The procedure was the same as in the first experiment. The experiment was carried out, successively, with the precolumn packed with rolls of a transparent silicone rubber (I) of the type used for preparing inlet block septums (obtained from the firm of Giede — amount weighed

out 1.36 g, number of pieces 23, diameter 4 mm, height 5 mm); white opaque silicone rubber (II) (Rubena 3, made in Czechoslovakia — amount weighed out 1.45 g, number of pieces 34, diameter 4 mm, height 3.5 mm); and a red rubber from penicillin closures (amount weighed out 1.30 g, number of pieces 31, diameter 4 mm, height 4 mm). Vapours were introduced of acetone (60–500 μl as needed with respect to the rubber sample), butanol (200 μl), benzene (200 μl), and heptane (100–200 μl as needed). The carrier gas stream flowing through the precolumn was interrupted for 10–300 sec.

RESULTS AND DISCUSSION

The minimization of the time, τ_c, spent in the sample port by the substance introduced is a basic requirement in the lay-out of the sample port. As indicated above, the lowering of τ_c leads to less deformation of the input concentration pulse and as a consequence, also to the retention of the column efficiency. In connection with the study of anomalous sorption in the injection port, we shall have to determine how the above general requirement manifests itself with respect to the construction of the sample port. The gas volume between the gas flow controller and the sample port space proper has to be minimal. The injection of a certain volume of the sample into the carrier gas in the sample port leads to a rise in the pressure in the sample port, and, in the case where the pneumatic resistance of the column is sufficiently high, the back pressure may cause the gas to flow from the sample port towards the gas controller, *i.e.*, in the opposite direction to that desired. Thus, the substance injected remains in contact with the rubber closing the sample port, and the sample volume sorbed by the rubber increases. In spite of this, it is convenient to employ a constant gas flow. At the moment of injection, a quite pronounced increase in pressure (depending on the volume of the substance introduced) occurs with a consequent increase in the carrier gas flow rate into the column, so that the substance leaves the sample port more rapidly than in the case of keeping a constant pressure in the sample port. A reduction of the sample port volume also leads to a lowering of the time spent by the substance to be analysed in the sample port. The geometry of the sample port is not therefore of less importance in view of the time the sample spends in contact with the rubber closure. It is necessary to choose a design such that the forward gas velocity may be constant throughout the space of the sample port. If this requirement cannot be met, then the velocity of the carrier gas passing the rubber closure must be maximal.

In order to prove the effect of the sample port design on the amount of the analysed substance sorbed, we arranged the sample inlet system in such a way that the carrier gas could not pass directly through 1.5 ml of the sample port volume below the rubber septum. The sample was injected at various distances a from the rubber septum (*cf.* the scheme in Fig. 3). In spite of the carrier gas being led from a source of constant gas flow, a fraction of the sample had reached the rubber and became sorbed. We found that between 0.1–0.02% of the volume of the sample injected was entrapped in the rubber, depending on the distance at which the substance had been introduced (a charge of 200 μl of benzene was used). The sample introduced came in contact with the surface of the rubber closure by diffusion. The time for diffusion from the place of injection to the rubber surface was inversely proportional to the

carrier gas flow rate u. In our experimental set-up, there was an appreciable volume (approximately 5 ml) ahead of the sample port, where the gas might be compressed, the time of the solute contact with the rubber surface being thus prolonged. We have proved experimentally that the sorption of the sample occurs even when injected into the carrier gas stream at a distance of several centimeters below a rubber septum of 0.785 cm^2 in surface area. (For a more precise quantitative appreciation of the effect of the operating conditions of the sample port as well as of the phase equilibria on various rubbers, we enlarged both the contact surface and the weight of the rubber in the carrier gas, *cf.* the experimental section.)

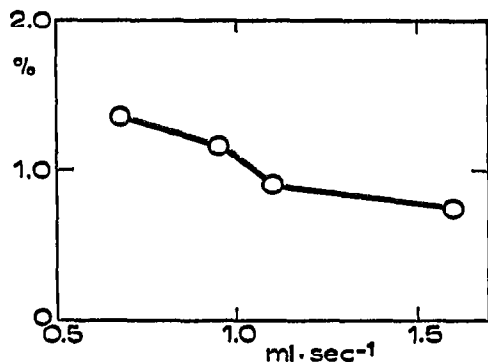The working conditions of the sample port are also important in view of the

Fig. 4. Dependence of the heptane sorbed (as a percentage of the initial sample) on the carrier gas velocity.

extent of anomalous sorption. In this connection, it is necessary first to appreciate the effect of the carrier gas velocity. The time constant τ_c is inversely proportional to the carrier gas velocity, $\tau_c = k/u$ (k is a proportionality constant). Therefore, an increase in the carrier gas velocity leads to a decrease in τ_c and thus to a shortening of the solute–rubber surface contact time. The dependence of the heptane volume released from the rubber on the carrier gas velocity is shown in Fig. 4.

At a constant carrier gas flow rate through the sample port, the solute concentration in the carrier gas falls uniformly due to the desorption of the material from the rubber. Under these circumstances, the occurrence of negative zones may be expected in the chromatogram, as described by the theory of vacantochromatography[11,12]. This phenomenon has been demonstrated by us by switching over the carrier gas stream to bypass the precolumn with the rubber at a time corresponding to double the retention time of the substance. Although the zero line was good (the asymmetry of the zone was ascribed to the dead space between the precolumn and the column proper of the chromatograph) a downward drift of the zero line occurred within the time corresponding to the retention time of the substance (Fig. 5, point E). It can so happen that the constancy of the carrier gas flow rate through the sample port is not retained. Calculation shows that the carrier gas flow rate falls by about 15% in the case of a sample port of 0.5 ml in volume and a 100 cm long column of 0.4 cm O.D. with temperature programs of 300° and 6°/min, for the sample port and the column, respectively. The drop in the carrier gas velocity occurs over about 30 sec; at the same time the solute concentration in the carrier gas also increases, as
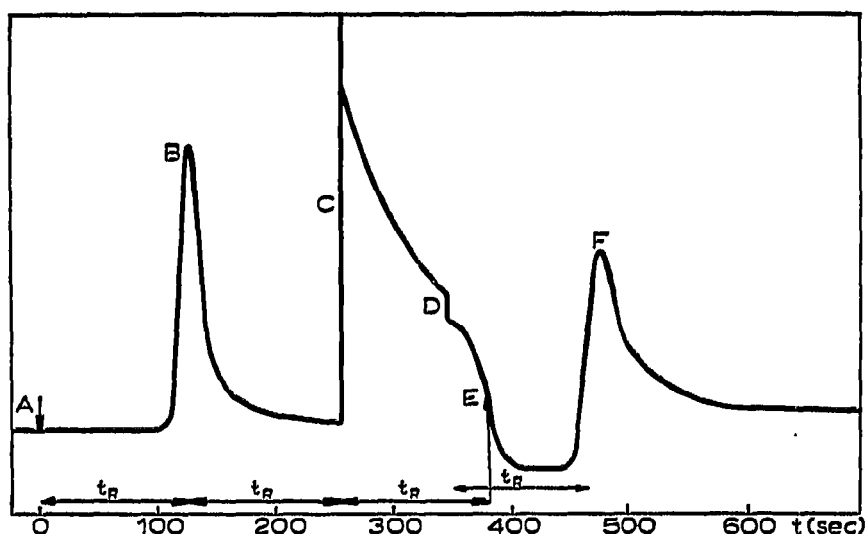
Fig. 5. The origin of a ghost chromatogram of benzene. For the description see the text.

the rate of desorption is independent of the carrier gas flow rate. A model of the process can be seen from Fig. 5. At point A, a sample was introduced into the chromatograph, the maximum of which appeared in the chromatogram at a retention time t_R. At a time $2t_R$ (point C), the carrier gas stream was switched to bypass the precolumn, and, at the same time, the sensitivity of detection was increased by a factor of 100. Thus, within the time between points C and D, the carrier gas was not flowing around the rubber surface. An inflection point (E) of the descending curve can be found again at a distance t_R from the point C, and the height corresponds to solute concentration in the carrier gas at the time $2t_R$ (point C). The substance desorbed from the rubber during the time between the points C and D is eluted out off the chromatograph, the maximum F being at a distance t_R from the point D, corresponding to the restoration of the gas flow through the precolumn. As soon as the concentration pulse has been eluted from the detector by the carrier gas, a very good base line is restored in the chromatograph, though the carrier gas contains the solute in a concentration that can be calculated according to equation (5). The volume of the material sorbed in the rubber will be higher for substances of higher boiling points, and this will cause more pronounced ghost chromatograms. This effect will be still further multiplied by any impurity sorbing in the sample port.

Associated with this phenomenon of ghost chromatograms is the case where a zone of a substance or substances which have not been present initially in the mixture analysed is found in the chromatogram. This was demonstrated under exactly the same conditions as quoted in the preceding case. However, the precolumn contained 0.253 g of 15% squalane-on-Sterchamol (Sterchamol Werke, Germany) instead of the rubber rolls. 0.05 μl of acetone were introduced on the column. After about twice the retention time of acetone, the same volume of butanol was injected. In the chromatogram of the second experiment both acetone and butanol zones were present. In this case, 0.25% of the acetone introduced in the first experiment was desorbed. Although it is the displacement of acetone by the more strongly sorbed butanol that takes place in the mechanism of the above process, the phenomenon can also be

observed in the case of the sorption of a component on the rubber closure or on the impurities in the injection port[2].

Along with the changes of the carrier gas flow through the injection port, one must also be aware of the complex of the number of causes for these changes. The effect of programming the temperature of the column and injection port has already been mentioned. In addition, changes in the carrier gas flow rate can be brought about by the different rates of evaporation of the sample in the injection port and/or by the time of introduction of a gaseous sample. These parameters affect the shape of the input concentration pulse, which influences both the volume of the component sorbed in the sample port and the resultant shape of the chromatographic zone[11]. The effect of the input concentration pulse on the chromatographic peak shape decreases with rising retention time. However, the potential for the origination of ghost chromatograms is not affected by this factor, as it depends solely on the kinetics of the sorption process.

A state of equilibrium between the component introduced and the rubber closure in the injection port is never reached because the concentration pulse is rather short in comparison with the rate of sorption, so that the time is insufficient for equilibration. Equilibrium is not attained even in cases when rubber closures are used for separating the components from the carrier gas[9]. In connection with the possible errors of the chromatographic analysis, we are primarily interested in the process of desorption, the rate of which is characterized by the time constant τ_d. The constants τ_d and a' for calculating the solute concentration in the carrier gas (eqn. (5)) are calculated from the relation $\log [a/(a - x)]$ vs. t (cf. the example in Fig. 6). The values of a' quoted in Table I are dependent, to a considerable extent,
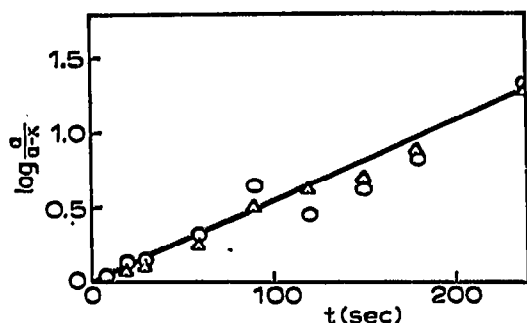

Fig. 6. Dependence of the volume sorbed on the time (t) of interrupting the carrier gas flow around the rubber surface (penicillin closures). (○) heptane; (△) acetone.

on the size of the contact surface area, while the time is dependent on the solute contact with the rubber at the moment when the concentration pulse passes through the sample port. In a real analysis, the solute concentration in the carrier gas will be lower than in our model experiment. If the ratio takes into account only the contact surfaces of the rubber septums in a conventional sample port and in the precolumn used by us, a solute concentration lower by an $f \sim 0.03$ could be expected ($f = t_s/t_{s,\text{real}}$), and $c_{t,\text{real}} = f c_t$. We can see on comparing the values in Fig. 3 with those in Table I that the value for the ratio of the sorption times $t_s/t_{s,\text{real}} < 0.1$. These concentrations are still sufficient for the genesis of ghost chromatograms. If we use eqn. (5) and the values from Table I, we can calculate the solute concentration in

TABLE I

VALUES OF τ_d AND a' FOR DIFFERENT KIND OF RUBBERS

	Silicone rubber				Penicillin closure	
	I		II			
	τ_d	a'	τ_d	a'	τ_d	a'
Heptane	49	1.4	66	2.9	83	0.35
Benzene	88	1.1	71	1.0		
Acetone	54	2.4	72	1.5	83	0.14

the carrier gas at a time longer than t_R. In our case, we have used a modified relation (5), $c_t = 100V(a' - x')/\tau_d w$, where V is the solute volume injected, a' and x' being expressed as percentages of the original area in the chromatogram. At a carrier gas flow rate of 1.02 ml/sec, there is 1% of acetone in the carrier gas (for the silicone rubber I) at a time of 1.3 t_R, and still 0.1% at 2.4 t_R; only at 3.5 t_R does the concentration drop to 0.01%. Better results were obtained in the experiment with the rubber used for penicillin closures, performed under identical conditions. At 1.2 t_R the acetone concentration in the carrier gas amounted to 0.04%, at 2.2 t_R 0.01%, and at 3.4 t_R the concentration fell to 0.002%. In analytical practice, one usually performs replicate analyses. In this case, the increase of the solute concentration in the carrier gas continues. As quoted above, the substances used in the present study are by no means those with which the contingency of sorption on the rubber closures is greatest. The values of the time constants in Table I show the sorption to be dependent not only on the type of solute, but also on the composition as well as manufacture of the rubber closures. From this viewpoint, a treatment promises better properties of the usual chromatographic septums.

Our experiments have shown the basic requirements for lowering the unfavourable effects brought about by anomalous sorption of the sample in the injection port. The requirements are as follows:

(1) The contact time of the substance introduced with the rubber septum of the sample port should be minimized by careful design of the injection block.

(2) The time of contact should be lowered by the use of a source of constant gas flow.

(3) A rubber containing a high quantity of filler, even if it is to the detriment of the mechanical properties of the closure, should be used for the septum.

LIST OF SYMBOLS

a maximum solute volume in the rubber (ml)
a' quantity a expressed as a percentage of the chromatogram of the initial sample
c_t solute concentration in the carrier gas at a time t (vol. %)
$c_{t,real}$ solute concentration in the carrier gas in a real arrangement (vol. %)
c_0 maximum input pulse concentration of the sample
f $= t_s/t_{s,real}$
$f(t)$ $= 1 - \exp(-t/\tau)$
k proportionality constant (cm)

V	volume of the solute introduced (ml)
t	time (sec)
t_a	time within which sorption proceeds (sec)
t_R	retention time (sec)
$t_{s,real}$	real time of sorption (sec)
u	carrier gas velocity (cm/sec)
w	carrier gas flow rate (ml/sec)
x	solute volume in the gaseous phase (ml)
x'	quantity x expressed as a percentage of the initial sample
$Y(t)$	input signal
$y(t)$	output signal
α	distance of the site of injection from the rubber closure
β	distance of the carrier gas intake from the rubber closure

REFERENCES

1 P. URONE AND J. F. PARCHER, *Anal. Chem.*, 38 (1966) 270.
2 A. TAMSMA, F. E. KURTZ, N. RAINEY AND M. J. PALLANSCH, *J. Gas Chromatog.*, 5 (1967) 271.
3 J. H. PURNELL, *Gas Chromatography*, John Wiley, New York, London, 1962.
4 J. C. STERNBERG, in J. C. GIDDINGS AND R. A. KELLER (Editors), *Advances in Chromatography*, 2nd Ed., M. Dekker, New York, 1966.
5 G. FODOR, *Laplace Transforms in Engineering*, Publ. House of Hungarian Academy of Sciences, Budapest, 1965.
6 M. KREJČÍ AND K. HÁNA, in C. L. A. HARBOURN AND R. STOCK (Editors), *Gas Chromatography*, Inst. of Petrol., Copenhagen, 1968.
7 K. HÁNA, *Collection Czech. Chem. Commun.*, 32 (1967) 968.
8 K. HÁNA, *Collection Czech. Chem. Commun.*, 32 (1967) 981.
9 J. A. VÖLLMIN, W. SIMON AND R. KAISER, *Z. Anal. Chem.*, 229 (1967) 1.
10 S. R. LIPSKY, C. G. HORVATH AND W. J. McMURRAY, *Anal. Chem.*, 38 (1966) 1585.
11 C. N. REILEY, G. P. HILDEBRAND AND J. W. ASHLEY, JR., *Anal. Chem.*, 34 (1962) 1198.
12 A. A. ZHUKHOVICKII, *All Union Sci. Conf., 2nd, Moscow, 1962, Gazovaya Chromatografiya*, Izd. Nauka, Moscow, 1964, p. 5.