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TESTING OF CAPILLARY COLUMNS FOR QUANTITATIVE MEASUREMENT OF CATALYTIC AND ADSORPTIVE ACTIVITY

M. AHNOFF* and L. JOHANSSON
Analytical Chemistry, AB Hässle, S-431 83 Mölndal (Sweden)

SUMMARY

Measurement of the time dependence and concentration dependence of losses of injected solutes permitted distinction between (1) adsorptive activity; (2) catalytic activity and (3) chemisorptive activity with non-zero activation energy.

Columns with different stationary phases were tested for their catalytic activity against propoxyphene, isosorbide 5-mononitrate and bis(trifluoroacetyl)metoprolol. For each test substance, the measured decomposition rate constants differed by a factor of 20 or more between the most and least active column. For each column, the activities towards the three test substances were not related to each other.

INTRODUCTION

Methods for testing column activity have to be developed still further to meet the requirements for truly quantitative test results, arising from both the development and application of capillary columns. An increasing number of the applications deal with quantitative analysis and, consequently, more attention should be paid to whether the analytes are quantitatively eluted from the column.

In favourable cases, losses of injected solutes are revealed by the visual appearance of the chromatogram, *e.g.*, when reversible adsorption causes badly shaped peaks, or when decomposition is extensive and the degradation products are eluted close to the parent peak, giving a plateau on one side of the peak. An example of the latter type is given in Fig. 1. In such a case, the shape and area of the plateau can be used for measuring decomposition[1]. Under more realistic conditions (lower temperature, other peaks present in the chromatogram), the plateau may be difficult to detect. Instead, systematic measurements of the area of the parent peak may reveal losses. For example, the elution of nitrate esters at 100°C shown in Fig. 2 was found to be incomplete by measuring peak areas at different flow-rates.

Today, tests are usually based on a single injection of a "polarity mixture"[2]. Of these, the procedure proposed by Grob *et al.*[3] is the most comprehensive. Despite all the useful information obtained from such a test, it has severe limitations: (1) owing to the selection of test substances, it is a low-temperature test; at the temperatures at which these substances are eluted, adsorptive effects are predominant while catalytic effects are small; (2) the results are dependent on the amount injected and

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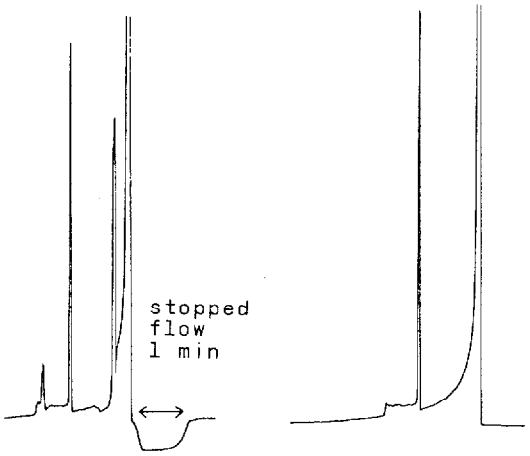

Fig. 1. Chromatograms showing the decomposition of bis(trifluoroacetyl)metoprolol on a 25-m SP-2100 fused-silica column at 200°C. Left: the flow was stopped 30 sec after injection and restored 1.0 min later.

other experimental conditions; (3) the test solution must be very carefully calibrated, if losses below 5% are to be considered; (4) the test does not differentiate between losses of different origin and nature, such as adsorption/catalysis or column/injector effects.

A better understanding of the phenomena causing incomplete elution will lead to better procedures for column evaluation.

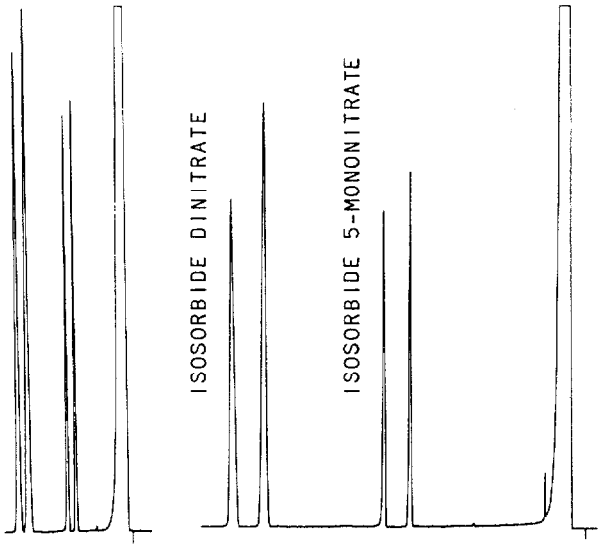

Fig. 2. Isosorbide dinitrate, isosorbide 5-mononitrate, *n*-tetradecane and *n*-pentadecane, eluted from a 25-m CP™-Sil-5 Pyrex column at 2.0 bar (left) and 0.6 bar (right) carrier gas pressure; column at 100°C.

EXPERIMENTAL

Measurement of time-dependent losses

Method 1. A test mixture containing the analyte [methadone, propoxyphene, isosorbide dinitrate, isosorbide 5-mononitrate or bis(trifluoroacetyl)metoprolol] and a suitable inert reference substance is injected at two or more different carrier gas flow-rates at constant temperature. The test mixture need not be calibrated. The decomposition rate constant, k, is measured from the change in relative peak area, A, according to:

$$k = \frac{\ln (A_1/A_2)}{t'_{R2} - t'_{R1}} \tag{1}$$

or

$$k = \frac{\mathrm{d} (\ln A)}{\mathrm{d}t'_R} \tag{2}$$

the half-life is $t_{1/2} = \ln 2/k$. The peak area corresponding to zero loss is calculated from:

$$A_0 = A \cdot \exp \left[\frac{\mathrm{d}(\ln A)}{\mathrm{d}t'_R} \cdot t'_R \right] \tag{3}$$

Method 2. With a calibrated test mixture, where A_0 is accurately known, decomposition rates may be measured from a single injection:

$$k = \frac{\ln (A_0/A)}{t'_R} \tag{4}$$

This method should only be used when losses are extensive and are known to be concentration-independent.

Measurement of concentration-dependent losses

These are carried out by injecting a number of dilutions of the same test mixture, containing the analyte and a reference substance. If necessary, detectors more sensitive than the flame ionization detector have to be used to cover the concentration range of interest. The amount injected is calculated from the peak area of the inert reference substance. The amount of solute per unit area of the solute peak is plotted against the amount.[4]

RESULTS AND DISCUSSION

Injector effects

The column can only be tested as a part of a chromatographic system. Losses may appear in the injector or during the sample transfer from syringe to injector;

TABLE I

DECOMPOSITION RATES AT 180°C FOR DIFFERENT COLUMN LOADINGS OF ISOSORBIDE DINITRATE

Column: 25 m × 0.3 mm siloxane-deactivated fused silica, coated with immobilized SE-54, 0.5 μm.

Amount (pg)	$k \cdot 10^3$ *(sec^{-1})*	$t_{1/2}$ *(min)*
15	21.3	0.54
45	21.5	0.54
60	20.3	0.57
200	20.9	0.55

those in the injector may be of the same type as in the column, *i.e.*, due to adsorption and decomposition. In addition, discrimination effects may appear. With splitless or on-column injectors, incomplete solvent trapping may occur.

Injector effects may be investigated by (1) changing the injector or instrument, (2) changing the working parameters, such as temperature, total flow through the injector and amount of solvent injected. These effects should not be underestimated and must be under control for succesful evaluation of column effects.

Column effects

From an experimental point of view, losses are of two types: time-dependent losses and concentration-dependent losses. Time-dependent losses may be independent of the amount injected over several orders of magnitude. One example was given in an earlier paper[5]; another is given in Table I. Similarly, concentration-dependent

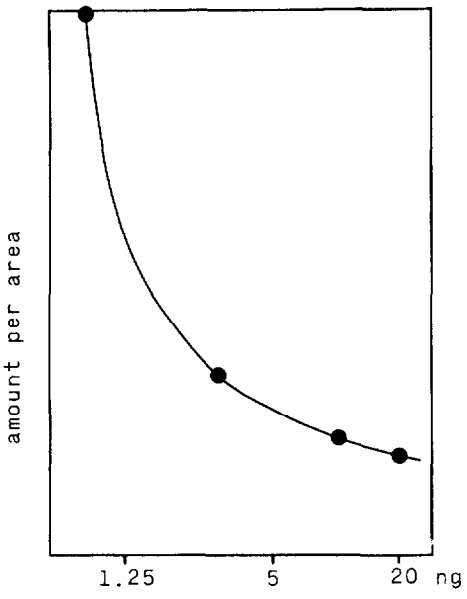

Fig. 3. Response factors obtained for different amounts of methadone. Column: CP™-Sil-5, 25 m, Pyrex; 200°C isothermal.

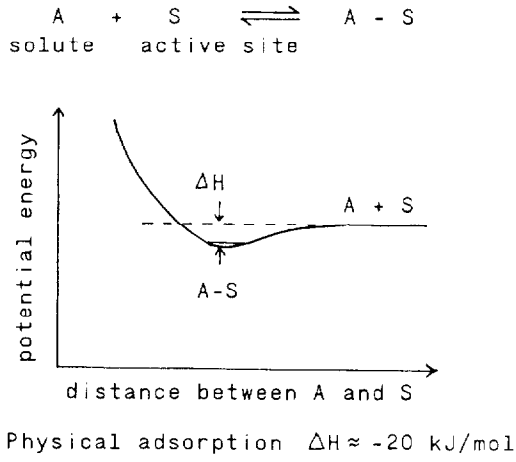

Fig. 4. Adsorption.

losses, as in Fig. 3, may be independent of the column flow-rate, provided that the temperature is kept constant.

Column activity causing time-dependent, concentration-independent losses may be regarded as catalytic activity, while activity causing concentration-dependent losses should be regarded as adsorptive activity. Simple theoretical models are given below to justify this view.

Theoretical distinction between adsorptive, catalytic and chemisorptive activity

The interaction between a solute and an active site in the column can be described by the rections in Figs. 4–6. The solute, A, attaches itself to the active site,

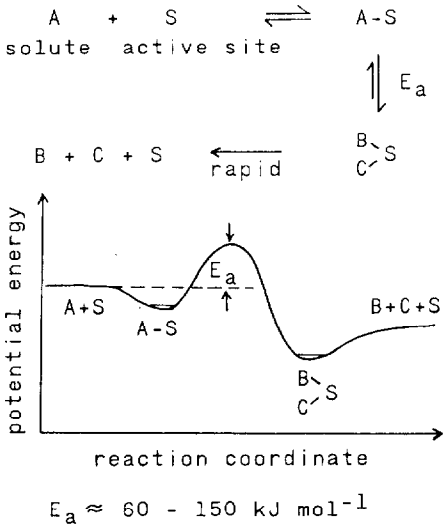

Fig. 5. Catalytic decomposition.

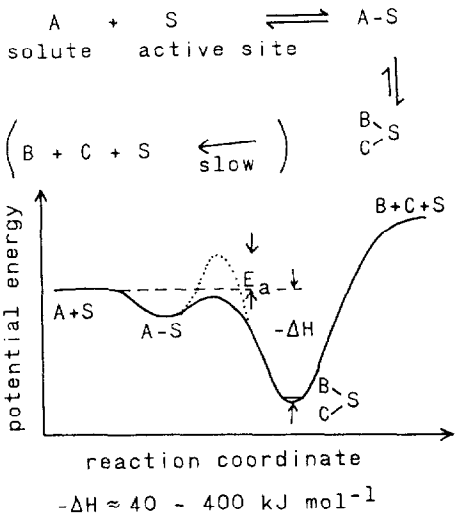

Fig. 6. Chemisorption.

S. This may be followed by a reaction, here leading to the adsorbed species B and C. These may then be desorbed, leaving a free active site S.

The case described in Fig. 4 is that of physical adsorption. No chemical reaction is involved. The amount of free active sites is not constant, but dependent on the amount of solute adsorbed, which in turn is dependent on —among other factors— the amount of solute present. Therefore, the adsorptive activity will show a concentration dependence.

The case described in Fig. 5 is that of a catalytic decomposition. Desorption is so fast that only a small fraction of all active sites exists as complexes with the solute or its degradation products. The number of active sites is little affected by the amount of solute present. The existence of an activation energy for the decomposition reaction makes the loss time-dependent: longer retention times result in larger losses. Losses also increase with increasing temperature.

Finally, the case described in Fig. 6, is that of chemisorption. The enthalpy change is much larger than in the case of physical adsorption, making desorption of the solute or its degradation products very slow. Often chemisorption proceeds with zero activation energy[6]. The situation is then similar to that of physical adsorption, the main difference being that $-\Delta H$ is much greater. If chemisorption proceeds with non-zero activation energy (dotted line in Fig. 6), then losses will be both concentration-dependent and time-dependent.

Test procedures

Evidently, the most obvious way to measure time-dependent effects is to change the retention time of the solute, keeping other variables, such as injected amount and temperature, constant[7,8]. Temperature programming is to be avoided, since the decomposition rates are then difficult or impossible to calculate. On-column injections are difficult to use, since the solvent-trapping effect is dependent on the carrier gas pressure and the column temperature. A split injector with high split flow or a moving-needle injector is preferred.

Analogously, concentration-dependent effects are measured by varying the amount of solute injected. The total amount of solvent injected should be the same, as well as the splitting ratio, if split injection is used. Attention should be paid to potential problems connected with the handling of solutions of widely different dilutions.

Useful information can also be gained by looking at the temperature dependence of the two types of losses. It can be used for qualitative tests, where adsorption is distinguished from catalysis[9,10], or for quantitative tests, where the apparent activation energy is measured[5].

Utility of activity tests

What practical use is there of a column test comprising several injections, to justify the time spent on testing? First, it is possible to compare different columns on the basis of quantitative data on column activity. Secondly, test data can be extrapolated to "real-life" conditions, when the column is put to use. It is, of course, essential that the tests be carried out with the analytes of interest.

TABLE II

RETENTION AND DECOMPOSITION RATES OF THREE DIFFERENT TEST SUBSTANCES ON SIX COLUMNS WITH DIFFERENT STATIONARY PHASES

Columns: 25 m × 0.3-mm Pyrex, HCl-leached and silylated with hexamethyldisilazane and diphenyltetramethyldisilazane[11]. Static coating with 0.2% solutions.

Phase	*bis(trifluoroacetyl)metoprolol, 200°C*		
	k'	*k · 10³ (sec⁻¹)*	*$t_{1/2}$ (min)*
CP™-Sil-5	3.4	0.27	43
SE-33	2.9	0.46	25
SE-33 CL	2.8	1.1	10.3
SE-54	3.6	0.23	49
SE-54 CL	3.5	1.4	8.1
OV-1701	4.1	6.5	1.8
	Isosorbide 5-mononitrate, 140°C		
CP™-Sil-5	2.2	<0.1	>110
SE-33	1.5	2.0	5.8
SE-33 CL	1.6	0.40	30
SE-54	2.8	3.3	3.5
SE-54 CL	2.8	3.1	3.7
OV-1701	8.5	0.14	83
	Propoxyphene, 200°C		
CP™-Sil-5	5.6	0.19	62
SE-33	5.0	<0.05	>200
SE-33 CL	5.0	<0.05	>200
SE-54	6.0	0.09	130
SE-54 CL	6.0	0.12	98
OV-1701	5.2	0.83	14

Applications

In a previous paper[5] different types of columns, all coated with methylsiloxane phases, were tested. The catalytic activity toward bis(trifluoroacetyl)metoprolol and bis(heptafluorobutyryl)metoprolol varied over one-and-a-half orders of magnitude. Results from duplicate columns differed by less than a factor of 1.5.

Differences in catalytic activity can also be seen when the same type of column is coated with different stationary phases, some of which were crosslinked. The results are shown in Table II. The most inert columns for bis(trifluoroacetyl)metoprolol were coated with CP™-Sil-5 and SE-54 without dicumylperoxide treatment. An OV-1701 column was twenty times more active. The activity against nitrate esters was somewhat different. The three most inert columns were those with CP™-Sil-5, OV-1701 and immobilized SE-33. In the case of OV-1701 it must also be considered that the four-fold higher retention means higher retention times or higher elution temperatures and that it leads to increased decomposition of the nitrate esters.

In some cases, losses of an analyte show both concentration and time dependence, and this was the case for isosorbide 5-mononitrate[12]. This may either be due to chemisorption with non-zero activation energy, or to separate adsorptive and catalytic effects. If chemisorption were responsible for the observed losses, then it should be possible to measure higher decomposition rates for smaller injected amounts, according to the model given above. For isosorbide 5-mononitrate, we were unable to observe such a concentration dependence of the decomposition rate. Instead, we could locate adsorption effects in the injector. When these were minimized and a proper column installed, virtually no concentration-dependent loss could be seen when 0.5–10 ng isosorbide 5-mononitrate were injected with a splitting ratio of 50:1 (10–200 pg on-column). With low- or zero-split flows, used for biological samples, adsorption in the injector could not be totally eliminated.

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