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EVALUATION OF CAPILLARY COLUMNS FOR PERFLUOROACYLATED OPIATES*

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SUMMARY

Perfluoroacylated opiates are sensitive to decomposition on capillary columns during gas chromatography. Capillary columns prepared according to different methods of surface pre-treatment and deactivation were compared. Decomposition rate constants for perfluoroacylated morphine and codeine were determined in order to compare the catalytic activities of the columns. The decomposition rate constants at 220°C and the energies of activation varied between $(0.092–0.70) \cdot 10^{-3}$ sec^{-1} and 33.8–52.1 kcal/mole, respectively. The most inert columns were obtained after etching with hydrogen chloride–hydrogen fluoride and methylpolysiloxane deactivation.

INTRODUCTION

It was shown in previous work[1] that perfluoroacylated morphine and codeine are sensitive to decomposition on capillary columns. Loss of the analyte increased with longer residence times of the analyte and with higher temperatures of the column. A qualitative test was used to compare some columns deactivated by aminol polymerization or Carbowax deactivation. There has been a rapid development in capillary column technology during the last decade. Great efforts have been made to produce columns that are inert towards the substances to be chromatographed. Deactivation of the column surface with aminol polymers or Carbowax has been replaced by deactivation with siloxanes or by silanization. The introduction of fused-silica column materials and the possibility of cross-linking the stationsry phase have further improved the inertness and thermal stability of modern capillary columns. Glass columns are usually pre-treated with acid to remove alkaline material from the glass surface. This can be achieved by leaching with hydrochloric acid[2] or by etching with hydrogen chloride[3]. The acid-treated glass surface contains a large number of silanol groups, which can be deactivated at high temperature by reaction with disilazanes[2], cyclic siloxanes[4] or polysiloxanes[3]. Seven columns, prepared according to the methods mentioned above, have been evaluated in this study.

Capillary columns are often tested with a polarity test mixture containing

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hindered acids and bases, to determine acid–base properties, and also alkanes for the determination of coating efficiency. These tests are usually performed at low temperature where adsorption effects are pronounced. A more demanding test is required to differentiate between adsorption and degradation effects, which usually take place at high column temperature (*e.g.*, in biomedical applications). De Nijs *et al.*[5] measured the first-order rate constant for the degradation of endrin in order to compare the activities of different columns. Ahnoff *et al.*[6] measured the catalytic activity against perfluroacylated amino alcohols by determining the decomposition rate constants. In this work, adsorption effects were studied by injection of 10–400 pg of perfluoroacylated morphine together with aldrin as inert internal standard and with electron-capture detection. Decomposition rate constants were determined by variations of flow-rate or by chromatography of codeine homologues, in line with the technique described by Ahnoff *et al.*[6].

EXPERIMENTAL

Sample preparation

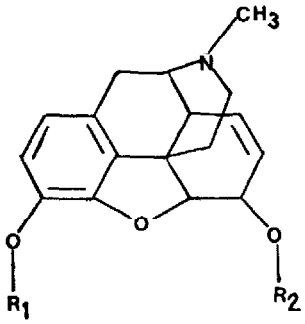
Morphine, ethylmorphine and codeine were obtained from the World Health Organization Centre for Chemical Reference Substances (Solna, Sweden). Pentafluoropropionic anhydride (PFPA) was purchased from Massanalys (Stockholm, Sweden). Tetrapentylammoniumhydroxide (QOH) was prepared from tetrapentylammonium iodide and silver oxide. Propyl- and butylmorphine were prepared by extractive alkylation as follows: 50 mg of morphine, 3 ml of 0.14 *M* QOH and 5 ml of 5% alkyl iodide in dichloromethane were shaken for 30 min. The organic phase was evaporated and the residue was dissolved in 3 ml of diethyl ether and filtered. The ether was washed with 1 ml of 0.1 *M* sodium hydroxide solution, dried over sodium sulphate and evaporated. The residues contained 90% alkylmorphine by spectrophotometric analysis. The alkylmorphines were dissolved in dichloromethane and diluted, together with codeine, ethylmorphine and docosane, to concentrations in the range 17–30 μg/ml. A 1-ml volume of this solution was evaporated in a test-tube, followed by reaction with 0.1 ml of PFPA at 55°C for 30 min. The PFPA was evaporated and the residue was dissolved in 1 ml of toluene. Solutions containing PFP-acylated morphine and codeine, together with docosane, were prepared in the same manner. Volumes of 1 μl of these solutions were injected into the gas chromatograph for determination of decomposition rate constants. The chemical structures of the compounds are shown in Fig. 1.

Capillary columns

All columns were made of soft glass. A summary of the different procedures used for surface pre-treatment and deactivation is presented in Table I. Methylsilicones were dissolved in pentane and OV-1701 in dichloromethane, prior to static coating. The stationary phase of the SE-54 column was immobilized by vulcanization with dicumyl peroxide. All columns were conditioned at 250°C overnight prior to testing.

Gas chromatography

A Hewlett-Packard gas chromatograph and integrator, equipped with a con-



	R_1	R_2
PFP-Morphine	$CF_3CF_2C(=O)-$	$CF_3CF_2C(=O)-$
PFP-Alkylmorphines	$CH_3(CH_2)_n-$	$CF_3CF_2C(=O)-$

Fig. 1. Structures of compounds.

TABLE I

COLUMN PREPARATION

Surface pre-treatment methods: 1, HCl–HF dynamic etching according to Schomburg *et al.*[3]; 2, HCl leaching according to Grob *et al.*[2]. Deactivation methods: 1, methylpolysiloxane deactivation[3]; 2, silanization[2]; 3, siloxane deactivation with cyclic siloxanes[4].

Column No.	*Surface pre-treatment*	*Deactivation method*	*Deactivation agent and conditions*	*Stationary phase*
1	Etching	1	OV-101, 1 h, 450°C	OV-1
2	Etching	1	OV-101, 3 h, 450°C	OV-1
3, 4	Leaching	3	Octamethylcyclotetrasiloxane, 16 h, 400°C	SE-30
5	Leaching	3	Hexaphenylcyclotrisiloxane, 16 h, 400°C	OV-1701
6	Leaching	2	HMDS + DPTMDS, 16 h, 400°C	SE-30
7	Leaching	3	Octamethylcyclotetrasiloxane	SE-54*
8	Chrompack (Middelburg, The Netherlands), AR-glas			Cp-Sil5
9	Orion (Helsinki, Finland), fused silica			SE-30

* Immobilized with dicumyl peroxide.

stant-pressure regulator and a falling-needle injector (LKB, Bromma, Sweden), was used. The column was connected to the detector with the aid of a glass-lined tee for make-up gas. Flame-ionization detection was used for the determination of decomposition rate constants and electron-capture detection for adsorption tests.

Adsorption test

Adsorption effects were studied by injection of 10–400 pg of perfluoroacetylated morphine, together with aldrin as internal standard. A plot of the relative peak heights *versus* amounts injected was used to interpret the results.

Determination of decomposition rate constants from variation of flow-rate

Decomposition rate constants for perfluoroacylated morphine and codeine

were determined by injection of the substances, together with docosane as internal standard, at 4–6 different flow-rates of the carrier gas in the range of 8–60 cm/sec. Decomposition rate constants were estimated by linear regression of the rate equation:

$$\ln A/A_{C_{22}} = \ln A^0/A_{C_{22}} - kt'_R \quad (1)$$

where A = area of analyte peak, A^0 = area of analyte peak without decomposition, $A_{C_{22}}$ = area of inert internal standard docosane peak, t'_R = adjusted retention time = retention time(t_R) – retention time of unretained compound (t_0), and k = decomposition rate constant.

Determination of decomposition rate constants from chromatography of homologues

Decomposition rate constants for codeine were determined by separation of four codeine homologues (methyl- to *n*-butylmorphine, a homologous series with 0–3 methylene groups). The homologues have different elution times (t_R). One pair of data is obtained for each homologue ($A_n/A_{C_{22}}$, t'_R; n = number of methylene groups in the homologue). Area quotients without decomposition ($A_n^0/A_{C_{22}}$) were obtained from the rate equation, determined by the flow-rate method. One rate constant was obtained from each chromatogram by linear regression of the rate equation:

$$\ln (A_n/A_{C_{22}}: A_n^0/A_{C_{22}}) = -kt'_R \quad (2)$$

Determination of activation energy

The Arrhenius equation was used:

$$\ln k = \ln F - E_a/RT \quad (3)$$

where E_a = activation energy, T = absolute temperature, R = 1.98 cal/mole and F = frequency factor.

Temperature dependence of the distribution coefficient

The temperature dependence of the distribution coefficient for perfluoroacylated codeine between the stationary phase and the gas phase was determined from retention time data with aid of the following equations:

$$K = (t_R - t_0)/t_0 V_g/V_l \quad (4)$$

$$d\ln K/d(1/T) = \Delta H_e/R \quad (5)$$

where K = distribution coefficient between liquid and gas phases, V_g/V_l = phase volume ratio of gas and liquid phases and ΔH_e = heat of evaporation.

RESULTS AND DISCUSSION

It is assumed that there is a limited number of active sites for adsorption or catalytic decomposition on the column surface. The stationary phase may also contain impurities or functional groups that cause decomposition. If adsorption effects are present, the fraction of analyte adsorbed will decrease with increasing amount

injected, owing to saturation of active sites. Catalytic sites are more difficult to saturate if decomposition products are reversibly adsorbed.

In catalytic decomposition, the rate will be proportional to the amount of analyte adsorbed on the active sites, if the decomposition reaction is the rate-limiting step. The decomposition rate constant at a given temperature can be used as a measure proportional to the number of active sites, and the activation energy can be used to compare active sites in different columns.

In this work, adsorption effects were studied by injection of 10–400 pg of perfluoroacylated morphine. The response was proportional to the amount injected, indicating that adsorption losses were of minor importance at injection of 10 pg (Fig. 2). At constant temperature, losses increased with longer residence times and appeared to follow first-order kinetics (Fig. 3).

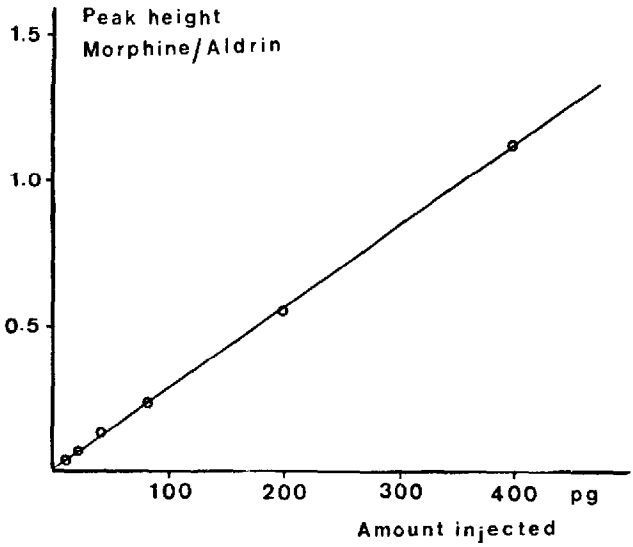


Fig. 2. Relative peak height of perfluoroacylated morphine/aldrin *versus* amount injected. Column temperature, 195°C; column No. 6.

Decomposition rate constants were determined by variation of the flow-rate (Table II) or by chromatography of codeine homologues (Table III). Chemical structures of the compounds are shown in Fig. 1. Provided that the decomposition rates and activation energies are equal for the homologues, they can be used for the determination of decomposition rates. The similarity in reaction was checked by determination of rate constants for butylmorphine and codeine by the flow-rate method and by comparing the results obtained by both methods. The quotients were close to unity: ($k_{\text{homologues}}/k_{\text{flow-rate}} = 0.99$ and $k_{\text{codeine}}/k_{\text{butylmorphine}} = 1.0$, where k = decomposition rate constant).

The differences in retention times between the homologues allow the same information to be obtained in one injection (eqn. 2) compared to with several runs by the flow-rate method (eqn. 1).

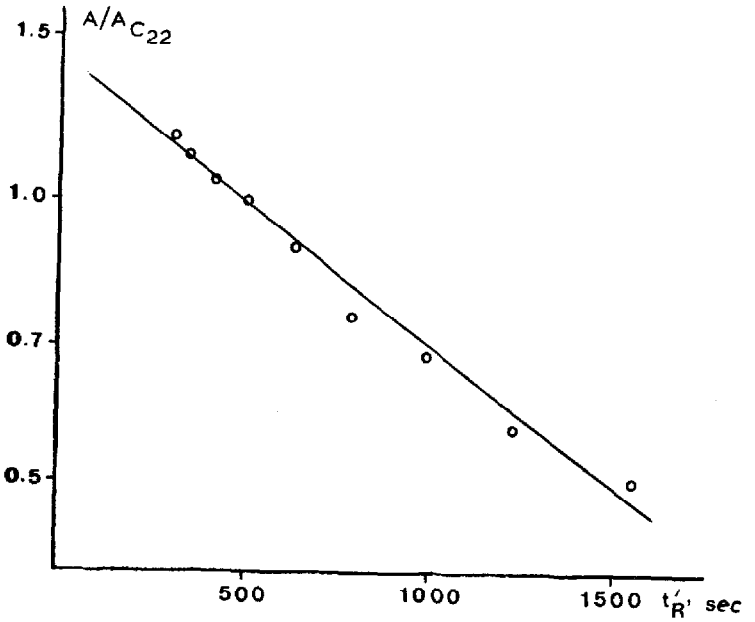


Fig. 3. Relative peak area of perfluoroacylated codeine/docosane *versus* time. Column No. 5.

TABLE II

DECOMPOSITION RATE CONSTANTS FROM VARIATIONS OF FLOW-RATE ($k \times 10^3$, sec^{-1})

Derivative	*Temperature* (°C)	*Column*					
		8 *Cp-Sil5*	*3* *SE-30*	*4* *SE-30*	*6* *SE-30*	*5* *OV-1701*	*9* *SE-30*
PFP-codeine	195				0.072		
	200			0.081			
	220	0.35	0.46	0.26		0.70	0.32
	225				0.43		
	240		1.26	121	1.28*		1.03
	260				4.90		
PFP-morphine	195				0.11		
	200			0.21			
	220	0.27	0.40	0.27		0.51	0.27
	225				0.34		
	240		1.20	1.05	0.89		1.01
	260				4.39		

* Rate constant for butylmorphine determined to be 1.23 at the same conditions.

Codeine was found to be more sensitive to decomposition than the bis-PFP derivative of morphine ($k_{codeine}/k_{morphine} = 1.2$).

The decomposition products were less retained than the parent compounds and appeared as a raised baseline in front of the parent compounds (Fig. 4). The long-lasting rise in the baseline indicates that decomposition proceeds along the entire

TABLE III

DETERMINATION OF DECOMPOSITION RATE CONSTANTS FROM CHROMATOGRAPHY OF HOMOLOGUES ($k \times 10^3$, sec^{-1})

Temperature (°C)	*Column*					
	1 OV-1	*2 OV-1*	*4 SE-30*	*6 SE-30*	*7 SE-54*	*9 SE-30*
220	0.135	0.092	0.279	0.297	0.147	0.216
225	0.177	0.130	0.359	0.394	0.201	0.308
230	0.505	0.275	0.495	0.565	0.375	0.528
235	0.630	0.530	0.719	0.780	0.435	0.711
240	0.887	0.804	1.11	1.16	0.726	1.04
245	1.41	1.24	1.56	1.37	1.40	1.50
250		1.68	2.19	2.20	2.02	2.22
Activation energy ± S.E. (kcal/mole)	49 ± 5	52 ± 4	36 ± 1	34 ± 1	41 ± 2	39 ± 1

column and not just at the inlet or outlet. Decomposition products were not observed when the column temperature was decreased from 240 to 220°C (Fig. 4). The decomposition involved is probably the elimination of pentafluoropropionic acid from the alcoholic ester group (R_2 in Fig. 1). Elimination of trifluoroacetic acid (TFA) from the bis-TFA derivative of α-hydroxymetoprolol during gas chromatography has

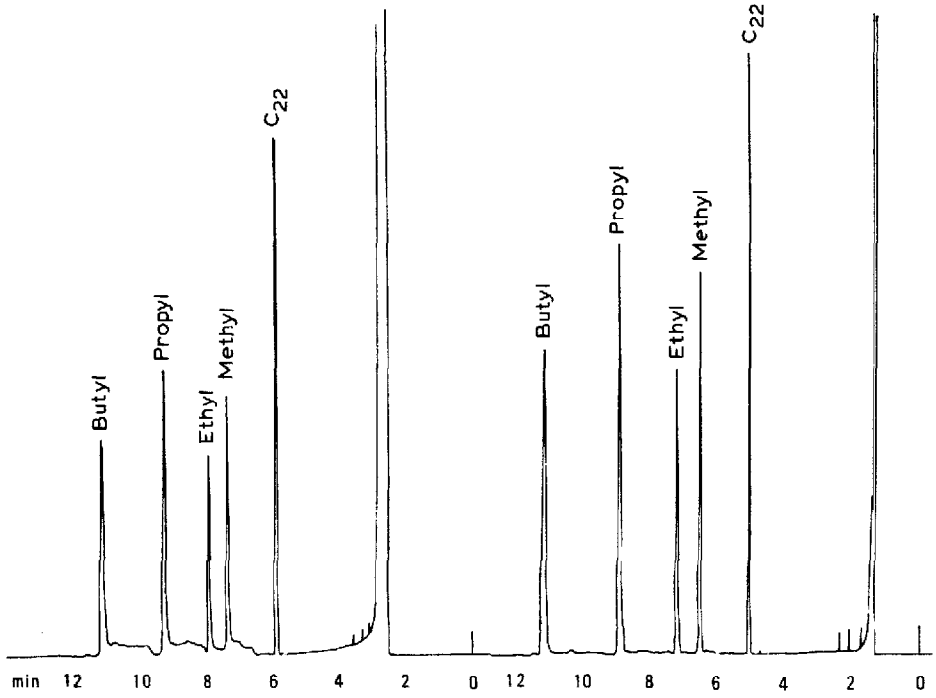


Fig. 4. Separation of codeine homologues. Column No. 6. Column temperature: 240°C (left); 220°C (right).

been reported by Ervik *et al.*[7], the structure of the decomposition product being elucidated by gas chromatography–mass spectrometry.

The differences in rate constants between different brands of methylsilicones (Cp-Sil5, SE-30, OV-1) can be obtained from Tables II and III. The most inert columns were obtained with OV-1, but this probably depends on a better surface deactivation after HCl–HF etching and methylpolysiloxane deactivation, which was used for OV-1 columns. The highest energy of activation was obtained after HCl–HF etching. This may depend on a change in the silica lattice structure on the glass surface after HF treatment, resulting in a more inert surface. The most active column was obtained with OV-1701 (Table II). This probably depends on less efficient deactivation with hexaphenylcyclotrisiloxane compared with octamethylcyclotetrasiloxane, as well as the content of cyano substituents in the stationary phase. OV-1701 contains phenyl and cyano substituents, which in itself is detrimental for certain TFA derivatives[8].

How should decomposition be kept as low as possible for a given column? A decrease in column temperature will decrease the decomposition rate ($\ln k = \ln F - E_a/RT$), but will have the reverse effect on retention time ($\ln K = \Delta H_e/RT$; K = distribution coefficient between stationary and gas phases). However, a decrease in column temperature at constant flow-rate will reduce decomposition if $E_a > \Delta H_e$. ΔH_e was determined from retention time data to be 17.1 $\pm$ 0.2 kcal/mole for perfluoroacylated codeine with OV-1 as the stationary phase. This value of ΔH_e is far below all values of E_a determined in this work (Table III), and consequently low column temperatures and high carrier gas flow-rates should be used to reduce decomposition.

Less than 5% decomposition was obtained on most of the prepared columns at column temperatures below 210°C ($K = 0.085 \cdot 10^{-3}$ sec^{-1} and $t'_R = 600$ sec). The use of inert columns will improve the speed of analysis as higher column temperatures can be used. Inert columns will also decrease the detection limit, as a larger fraction of analyte injected will reach the detector, and the use of higher column temperatures will increase the peak height and thus decrease the detection limit. Inert columns will also improve the accuracy and precision of analysis.

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