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EMERGENCE TEMPERATURES AS PHYSICAL CONSTANTS FOR MEASURING ANALYTE RETENTION IN PROGRAMMED TEMPERATURE GAS CHROMATOGRAPHY

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SUMMARY

Emergence temperatures are physical constants useful for describing analyte retention when programmed temperature gas chromatographic operating parameters are properly standardized. A combination of all the column operating conditions is standardized by the proposed method, therefore a wide variety of conditions (column length, program-rate, flow-rate, amount of stationary phase, etc.) may be used. Reproducibility is unaffected by the size, shape, or polarity of the analytes. In addition, tables of emergence temperatures provide an excellent means of identification of unknowns because reference compounds can be selected that emerge close to the compounds to be identified.

INTRODUCTION

The two most common ways of characterizing isothermal chromatographic data and identifying unknowns are by the use of relative retention times and by retention index systems, such as those introduced by Kováts[1,2].

Relative retention times offer the advantage of simplicity, for the data are taken relative to only one standard, which is frequently introduced into the sample to be analyzed. However, accuracy may suffer if the retention time of the compound of interest differs greatly from the reference compound. The retention index system introduced by Kováts overcomes this deficiency. It makes use of a series of closely related reference compounds so that the compound of interest is always bracketed with nearby reference compounds.

Both systems are temperature dependent. Consequently, their retention values may vary with a variation in column temperature. This is particularly true if the molecular dimensions of the sample and reference compounds differ[3,4]. When temperature programming is used, the problem is compounded since numerous parameters can affect the temperature range to which the solutes are subjected. Some of these are: (1) the temperature program-rate, (2) the carrier flow-rate, (3) the amount of liquid phase, and (4) the column length. A change in any one of these parameters may therefore limit the reproducibility of relative retention times and retention indices. To circumvent the problem of temperature dependency it has been necessary

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to strictly replicate all the column parameters[5,6], or to use a variety of reference compounds. For example, reference compounds have been chosen so they are similar in size and shape to the test compounds[7,8].

A second complication arises when element selective detectors are used (*e.g.* electron capture, electrolytic conductivity, flame photometric, etc.). Under these circumstances reference compounds must be chosen that contain the appropriate heteroatom in common with the test compounds[9].

As a consequence of these two effects it has been necessary to use a large number of reference compounds to meet the specific requirements of different analyses when programmed temperature gas chromatography (PTGC) is used. Thus much of the retention index data for PTGC has only limited application. A similar circumstance has been recognized as one of the drawbacks in the use of relative retention times[2,10,11].

A method of determining and reporting retention data for PTGC is proposed here that provides reproducible results even when any one or all of the four parameters listed above, which affect temperature, are changed. It will be shown that a single table of data can be developed for each stationary phase that will satisfy the problem of temperature dependency, and accomodate element selective detectors. This method makes use of the emergence temperatures of the analytes as an indication of their retention by the column, an approach that is consistant with the statement of Harris and Habgood that the emergence temperature is the most valuable retention parameter in PTGC[12].

The proposed method relies on arbitrarily assigning a specific emergence temperature to a suitable reference compound. Chromatographic conditions (column length, flow-rate, program-rate, etc.) are then adjusted so the reference compound emerges at the assigned temperature. The emergence temperatures of all other compounds, then, become physical constants dependent only on the nature of the stationary phase and the standardized (but flexible) operating conditions.

MATERIALS AND METHOD

Reagents

The compounds listed in Table I were injected from a solution containing 0.1 μg of each compound per μl, as were the pesticides eptam through delta-permethrin (Table IV). These two mixtures were used throughout this study.

Instrumentation

Column parameters are listed in Tables I and IV. Columns 1–5, and 8 were used in a Hewlett-Packard 5830 gas chromatograph which had been retrofitted with a Model 18835B capillary kit so that either a packed or capillary column could be used. The injection port heater was left off for the packed column experiments, to avoid a possible disproportionate influence that a high inlet temperature might have on the short columns. Although the injection port temperature increased each time the column oven temperature was increased, it was allowed to cool to within 10°C of the oven prior to each injection. Injections were made with a 4-in. needle so the solution could be deposited on a portion of the column within the oven. A flame ionization detector was used. Column 6 was used in a Tracor MT222 instrument

with an flame ionization detector. Injections were made with a standard 2-in. needle into the injection port area, and the temperature of the injection port was kept 40–50°C higher than the temperature of the oven during the determination.

Column 7 was used in a Tracor 565 instrument with a Hall electrolytic conductivity detector in the reductive mode. The injection port was unheated and the injection made with a 3-in. needle so as to deposit the solution on a portion of the column within the oven.

Oven temperatures were checked with a Supelco Model 175 chromel–constantan thermocouple which had been calibrated in a Thomas Hoover, capillary, melting point apparatus. This latter apparatus had been calibrated with the following melting point standards: vanillin, acetanilide, phenacetin, sulfanilamide, sulfapyridine, and caffeine.

Method

The chromatographic systems used were standardized by adjusting the carrier flow- and temperature program-rates of each so that the standardization compound, *n*-eicosane, emerged from the column just as it reached the preselected standardization temperature of 200 ± 1°C. During the standardization procedure, the initial temperature of the column was set below the threshold temperature of the standardization compound. The threshold temperature is that temperature at which a solute that is initially cold trapped at the head of the column first begins to move through the column[13]. The standardized conditions were then used for determining the emergence temperatures of other compounds.

Most element selective detectors respond poorly to hydrocarbons, but they respond well to chlorpyrifos because it contains a number of elements (oxygen, nitrogen, sulfur, phosphorous and chlorine) in addition to hydrogen and carbon. Thus, chlorpyrifos was selected as a secondary standardization compound, and was found to emerge at 194°C when methyl silicone (OV-1 or OV-101) columns were standardized so that eicosane emerged at 200°C. Column 7, which was connected to an electrolytic conductivity detector, was then standardized by adjusting the flow-rate and temperature program-rate so that chlorpyrifos emerged at 194 ± 1°C.

Emergence temperatures were calculated by the equation

$$T_{ex} = T_{es} + r(t_{Rx} - t_{Rs}) \tag{1}$$

where T_e is the emergence temperature, x refers to the test compound, s refers to the standardization compound, r is the temperature program rate, and t_R is the retention time. Even though the standardization compounds *n*-eicosane and chlorpyrifos may have emerged at ±1°C from the 200°C and 194°C standardization temperatures indicated in the method, exactly 200°C and 194°C respectively were used in the equation to obtain the data for this report.

RESULTS AND DISCUSSION

To test the proposed method rigorously, compounds differing in polarity, size, and shape were chosen for analysis. In fact one of the compounds, acenaphthylene, is known to have a large coefficient of variation relative to normal hydrocarbons as

the column temperature is varied[14]. As can be seen from the data in Table I, emergence temperatures are nearly constant when determined on a standardized system even though the following parameters are varied: column length, carrier flow-rate, column heating-rate, type of carrier gas and the amount of liquid phase.

TABLE I

EMERGENCE TEMPERATURES FROM A VARIETY OF CHROMATOGRAPHIC OPERATING CONDITIONS

Chromatographic column	1	2	3	4	5
Length (cm)	180	180	120	44	44
Internal diameter (mm)	4	4	4	4	4
Amount of liquid phase	5%	5%	5%	10%	3%
Carrier gas	He	N_2	N_2	N_2	N_2
Temperature program-rate (°C/min)	2.80	3.15	4.85	4.10	10.00
Flow-rate (ml/min)	49	56	51	34	25
t_R of C_{20} (min)	53	48	31	37	15
Compound	*Emergence temperatures (°C)*				
n-Decane	84	84	85	86	87
Acenaphthylene	137	137	138	138	138
n-Hexadecane	160	160	160	161	161
n-Eicosane (standard)	200	200	200	200	200
1-Octadecanol	208	207	206	206	207
n-Docosane	218	218	217	217	217
n-Tetracosane	234	234	233	233	233
n-Hexacosane	249	249	248	248	247
Testosterone cypionate	304	304	302	301	300

In addition to the parameter variations noted above, two additional parameters can be varied without affecting emergence temperatures, when the compounds of interest are initially cold trapped. These parameters are: (1) the starting temperature, and (2) the time interval that the starting temperature can be maintained prior to commencement of the temperature programming.

The independence of the emergence temperature from the starting temperature is evident in Fig. 1 in which Saxton's method 2[13] was used to determine threshold temperatures. For example, the emergence temperature of *n*-hexacosane (curve H in Fig. 1) remained constant although the starting temperature ranged from 30 to 190°C.

If it is impractical to lower the starting temperature below that of the threshold temperature of some of the compounds, the temperature at which they emerge will nevertheless be constant provided the starting temperature is accurately replicated. Emergence temperatures determined in such a manner, though, will not be true emergence temperatures as defined by the method. The starting temperature of *n*-decane of Table I, for example, was higher than its threshold temperature, nevertheless the temperature at which it emerged varied only slightly even when the column parameters were varied considerably. Despite the fact that the values tabulated for *n*-decane are not true emergence temperatures, they serve a useful purpose. For example, during pesticide screening analysis, considerable time saving could be realized by cooling the column to 50°C rather than trying to cold trap additional compounds by dropping to a much lower temperature.

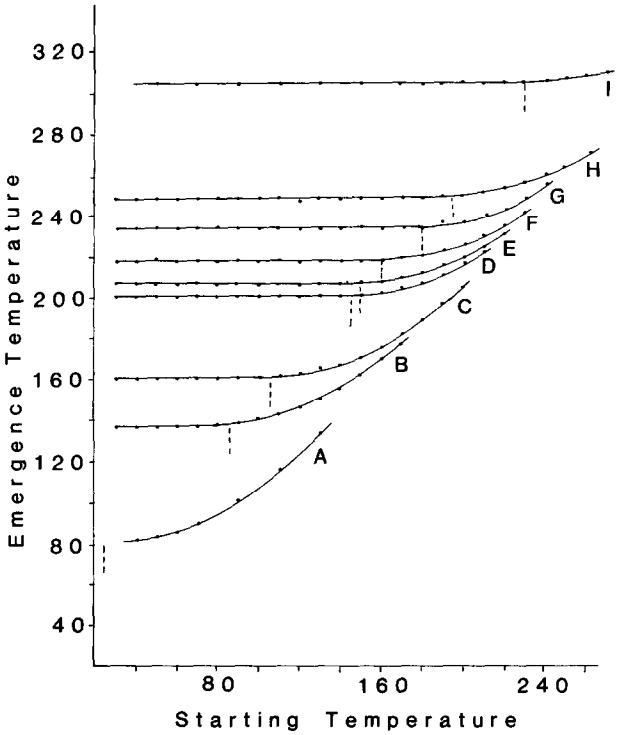

Fig. 1. Threshold temperatures (indicated by the dashed, vertical lines) for (A) *n*-decane, (B) acenaphthylene, (C) *n*-hexadecane, (D) eicosane, (E) 1-octadecanol, (F) *n*-docosane, (G) *n*-tetracosane, (H) *n*-hexacosane, and (I) testosterone cypionate. The figure demonstrates that the emergence temperature of a compound remains constant irrespective of the starting temperature as long as the starting temperature is lower than the threshold temperature. Column: 1.2 m × 4 mm containing 5% OV-101 on 80–100 mesh Chromosorb W HP, temperature programmed at 6.8°C/min. Gas chromatograph: Hewlett-Packard 5830 with a flame ionization detector.

The fact that emergence temperatures are unaffected by the time interval between the injection and the beginning of the temperature program for compounds that are cold trapped is evident in Table II. This can be a valuable asset for on-column and splitless injections in which the injection process may take considerable time. Note that *n*-decane was not initially cold trapped, and consequently its emergence temperature did vary with changes in the isothermal period.

Not only is it unnecessary to determine accurately the starting temperature as long as it is at or below the threshold temperature, it is also unnecessary to determine accurately the emergence temperature of the calibration compound. As can be seen by the examples in Table III, the temperature intervals determined by $r(t_{Rx} - t_{Rs})$ between eicosane and the test compounds that were initially cold trapped differed at most by only ± 1°C even though the calibration temperature ranged ± 5°C. Likewise the intervals just described varied by a maximum of ± 2°C even though the calibration temperatures ranged ± 10°C.

Although the method is quite forgiving of differences in oven temperature,

TABLE II

EMERGENCE TEMPERATURES FROM A CHROMATOGRAPHIC COLUMN IN WHICH THE ISOTHERMAL STARTING TEMPERATURE (50°C) WAS MAINTAINED FOR VARIOUS PERIODS OF TIME PRIOR TO STARTING THE TEMPERATURE PROGRAM

Compound	*Emergence temperature (°C)*		
	Isothermal period (min)		
	0	*1*	*5*
n-Decane	85	84	79
Acenaphthylene	138	138	138
n-Hexadecane	160	160	160
n-Eicosane (standard)	200	200	200
1-Octadecanol	206	206	206
n-Docosane	217	217	217
n-Tetracosane	233	233	233
n-Hexacosane	248	248	248
Testosterone cypionate	302	302	302

TABLE III

THE EFFECT THAT VARIATIONS IN THE EMERGENCE TEMPERATURE OF *n*-EICOSANE HAS ON THE TEMPERATURE INTERVAL BETWEEN THE EMERGENCE OF *n*-EICOSANE AND THE TEST COMPOUNDS

Compound	*Temperature interval (°C)*				
	T_e of n-eicosane (°C)				
	190	*195*	*200*	*205*	*210*
Acenaphthylene	62	62	62	62	62
n-Hexadecane	39	40	40	40	40
1-Octadecanol	6	6	6	6	6
n-Docosane	17	17	17	17	17
n-Tetracosane	33	33	33	33	33
n-Hexacosane	48	48	48	48	48
Testosterone cypionate	101	102	102	103	104

precision can be enhanced by use of relative retention time as a thermometer for standardizing column temperatures. For the chromatographic columns in Table I the ratio of the adjusted retention time of *n*-hexacosane relative to the adjusted retention time of *n*-eicosane was 8.14 when the oven was operated isothermally at 200°C. (The adjusted retention times were determined by subtracting the retention time of an unretained compound from the retention time of the compound of interest.) Thus the same standardization temperature can be established in other gas chromatographs by adjusting the temperature to obtain the same isothermal relative retention time for that pair of compounds. When element selective detectors are used, an oven temperature of 194°C can be established by setting the temperature so the ratio of the adjusted retention time of phosalone relative to the adjusted retention time of

chlorpyrifos is 5.73. (Phosalone has detection properties similar to those of chlorpyrifos because it contains the same variety of elements.)

If identical emergence temperatures are to be obtained from various gas chromatographic systems, then the temperature program-rate applied to eqns. 1 and 2 must be precisely determined. This can be achieved by using the invariance of the emergence temperatures to standardize the program-rates. For example, the temperature interval between *n*-hexadecane and *n*-hexacosane was 89°C for chromatographic columns 1 and 2 in Table I. Hence the temperature program-rates of other chromatographic systems were standardized by dividing 89°C by the time interval between *n*-hexadecane and *n*-hexacosane as determined on each additional system.

It is essential that this standardization procedure be used, to obtain identical emergence temperatures between systems, for two reasons: (1) the temperature program-rate indicated on the gas chromatograph may be in error, or it may not reflect the rate of temperature increase actually experienced by the column, and (2) the standardization procedure corrects for linear flow-rate changes.

The standardized temperature program-rate reflects the temperature interval per unit of time that is actually experienced by the column. Any measurement that relies on a thermocouple or similar device, is localized and may differ disproportionately at the two temperature extremes from that sensed by the column as a whole. Hence the rate of temperature increase of the column may not be reliably determined by timing the temperature rise indicated by such devices.

There is generally a positive correlation between carrier gas viscosity and temperature. Therefore resistance to flow increases with a rise in column temperature. The differential flow controllers used with packed columns are generally effective in maintaining a constant packed column flow, but since a constant head pressure is usually maintained for capillary columns, their flow-rate usually decreases as temperature programming proceeds. Consequently, when a capillary column is standardized there is a greater spread between emergence temperatures than occurs when the flow is constant. If the flow-rate change is nearly linear, use of the standardized temperature program-rate compensates for the flow-rate change, and emergence temperatures are obtained which are identical to constant flow-rate columns. If the flow-rate changes, then the standardized temperature program-rate is not the true temperature program-rate but instead represents a combination of the rate of temperature change per change in carrier flow-rate. The symbol r' is used here to designate the standardized temperature program-rate to distinguish it from the true temperature program-rate, r. Emergence temperatures listed in Table IV were calculated by substituting r' into eqn. 1. Both the true temperature program-rate r, determined by timing a temperature interval measured with the calibrated thermocouple, and the standardized value r' are listed in Table IV for comparison.

The data listed in Table IV show excellent precision even though nearly all column parameters except the nature of the liquid phase were varied. The flow through column 7 decreased from 35 to 33 ml/min as the column temperature increased, and the flow through the capillary column (column 8) decreased by 24% during the determination without adversely affecting the results.

When column 7, which was attached to the element specific, electrolytic conductivity detector, was standardized so that chlorpyrifos emerged at 194°C, emergence temperatures of the pesticides obtained from that system were in close agreement to those from the other chromatographic systems in Table IV.

TABLE IV

EMERGENCE TEMPERATURES CALCULATED BY APPLYING VALUES OF STANDARDIZED TEMPERATURE PROGRAM RATES, r', TO EQNS. 1 AND 2

Chromatographic column	1	2	3	4	5	6	7	8	
Inside diameter (mm)	4	4	4	4	4	4	2	0.25	
Length (cm)	180	180	120	44	44	180	180	3600	
Amount of liquid phase	5%	5%	5%	10%	3%	5%	5%	0.2 μm	
Carrier gas	He	N_2	N_2	N_2	N_2	He	He	H_2	
Flow-rate (ml/min)	49	56	51	34	25	64	35 → 33	41 → 31	
r	2.80	3.15	4.85	4.10	10.00	–	–	3.30	
r'	2.80	3.15	4.85	4.19	10.06	4.07	4.70	3.12	
t_R of eicosane (min)	53	48	31	37	15	39	–	46	
Compound	*Emergence temperatures (°C)*								*C.V.*
n-Decane	84	84	85	83	86	78	–	85	3.16
Acenaphthylene	137	137	138	137	138	135	–	136	0.78
n-Hexadecane	160	160	160	160	160	159	–	160	0.24
n-Eicosane (std.)	200	200	200	200	200	200		200	
1-Octadecanol	208	207	206	206	207	208	–	206	0.43
n-Docosane	218	218	217	218	217	217	–	218	0.25
n-Tetracosane	234	234	233	234	233	233	–	234	0.23
n-Hexacosane	249	249	248	249	248	248	–	249	0.22
Testosterone cypionate	304	304	302	303	301	302	–	307	0.65
Eptam	–	130	130	129	133	–	–	128	1.44
Lindane	–	169	170	169	170	–	170	170	0.30
Diazinon	–	177	177	177	178	–	–	178	0.31
Chlorpyrifos	–	194	194	194	194	–	194	194	0
Endosulfan II	–	214	213	214	213	–	214	214	0.24
pp-TDE	–	217	217	217	216	–	217	218	0.29
pp-DDT	–	224	224	224	223	–	224	225	0.28
pp-Methoxychlor	–	234	234	234	233	–	234	235	0.27
trans-Permethrin	–	252	251	251	250	–	252	252	0.32
delta-Permethrin	–	273	272	272	271	–	274	274	0.44

Thus, as shown, the proposed method enables a single table of data per stationary phase to have broad application. It is applicable to all types of compounds and detectors and to a wide variety of chromatographic conditions. This advantage will be realized, however, only if a convention is established in which a limited number of standardization conditions are used. We suggest that, whenever possible, chromatographic parameters be standardized for the determination of emergence temperatures such that *n*-eicosane emerges at 200°C and that r' be determined for such systems by dividing 89°C by the time interval between *n*-hexadecane and *n*-hexacosane. (When element selective detectors are used the same r' can be obtained by dividing 44.1°C by the time interval between chlorpyrifos and phosalone.)

Table V is an example of a functional presentation of emergence temperatures for characterizing peaks obtained using PTGC. Threshold temperatures are included in the table as an essential guide for setting the starting temperatures. The threshold temperatures are approximate, for they vary slightly in proportion to the column head pressure.

When a table of emergence temperatures, *e.g.* Table V, is used for the tentative

TABLE V

THRESHOLD (T_t) AND EMERGENCE TEMPERATURES (T_e) OF COMPOUNDS DETERMINED ON METHYL SILICONE (OV-1 AND OV-101) COLUMNS BY LINEAR PTGC

Compound	T_t ($\pm 5°C$) (°C)	T_e (°C)*
n-Decane	<35	84.1
Eptam	60	130.2
Acenapthylene	85	136.9
n-Hexadecane	105	159.6
Lindane	90	169.3
Diazinon	100	177.2
Chlorpyrifos	120	193.8
n-Eicosane (standard)	145	200.0
1-Octadecanol	150	207.2
Endosulfan II	140	213.7
pp-TDE	140	216.9
n-Docosane	160	217.6
pp-DDT	150	224.1
n-Tetracosane	180	233.7
pp-Methoxychlor	160	233.9
n-Hexacosane	195	248.6
trans-Permethrin	170	251.5
delta-Permethrin	190	273.1
Testosterone cypionate	230	304.2

* Data obtained from chromatographic column 2.

identification of unknowns, discrimination between compounds can be improved by selecting a reference compound that emerges close to the unknown and substituting the necessary parameters for it and the unknowns into eqns. 1 and 2. This procedure so enhances discrimination that it justifies tabulating emergence temperatures to the nearest 0.1 degree.

CONCLUSIONS

The proposed method relies on the use of emergence temperatures for measuring and tabulating the relative retention of various compounds when PTGC is used. Emergence temperatures are physical constants much like boiling points when the gas chromatographic systems are standardized as described (Fig. 1 and Tables I, II and IV). Hence under these conditions they are useful for establishing the identification of unknowns.

The standardization procedure allows flexibility in choosing parameters, such that the amount of resolution *versus* analysis time can be effectively adjusted to achieve the desired analytical results (Tables I and IV). For example, if the column length were increased for a given temperature program-rate, the temperature at which the analyte emerged would necessarily be higher. To compensate for this effect and to maintain the standardized system, the temperature program-rate can simply be reduced. Both changes, *i.e.* increasing the column length and decreasing the temperature program-rate, would tend to improve resolution and increase analysis time. Conversely, a short column would require a higher temperature program-rate to

attain the same standardized conditions. Since specific emergence temperatures would be reached in a short period of time for the latter system it would be useful for rapid screening analysis.

The proposed method allows the development of a single table of emergence temperatures per stationary phase. Such a table should have broad applicability as a means of characterizing complex mixtures of analytes, for it allows the use of a wide selection of chromatographic conditions with excellent precision.

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