Reversed Headspace Analysis for Characterization, Identification, and Analysis of Solid and Liquid Matrices: Part I.

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Abstract

This paper offers a methodology of an experimentally simple reversed headspace (RHS) analysis for measuring of matrix effects and their use for identification and characterization of condensed matrices such as pharmaceuticals, polymers, chromatographic packing, etc. applicable for both quality control monitoring and research and development investigation. In RHS methods, the matrix is spiked and equilibrated with a mixture of volatile chemicals containing various functional groups (molecular sensor array or MSA mixture). Headspace chromatograms of the same spikes of a sample and an empty vial are compared. Examination of basic headspace theory shows that matrix specific constants (M), rather than partition coefficients (K), can be calculated from the headspace chromatograms and $M = (K - 1) \times \beta$, where β is a degree of matrix volume change during equilibration. Matrix specific constants can be plotted against any property of chemicals (polarity, dielectric constant, solubility parameter, vapor pressure, etc.) or just against a set of consecutive numbers, each representing a chemical in MSA. This plot is, in a sense, a molecular affinity spectrum (MAS) specific for a given matrix at a given temperature and is independent of an instrument. Changes in MAS that correspond to chemicals with a particular functional group give an insight to the type of differences between matrices and may quantitatively define them.

Introduction

This part of the paper presents the foundations of reversed headspace (RHS) methodology for measurements of condensed matrix properties, examines problems with basic assumptions of headspace theory, and shows experimental difficulties of partition coefficient measurements. Instead of partition coefficients, it introduces concepts of "matrix specific constants" that can be easily determined from headspace chromatograms and "molecular affinity spectrum" (MAS) that can be constructed from these constants and used for monitoring, investigation, and identification of condensed matrices and changes in them.

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General

Headspace analysis is a proven analytical tool that is mostly used for the determination of volatile impurities in condensed (liquid and solid) matrices. Many articles and books (1–3) have been written on the subject, and various designs of automatic headspace samplers are available from analytical instrument manufacturers. The general headspace equation that most of the authors used for the description of headspace analysis is given below:

$$Cg = Co/(K + Vg/Vc) \qquad \text{Eq. 1}$$

where Cg is an equilibrium concentration in gaseous phase and Co is an original concentration of the analyte in the matrix in terms of weight or moles per volume. Vg and Vc are volumes of gaseous and condensed phases in a sealed headspace vial. K is a partition coefficient defined as a ratio of equilibrium concentration in the condensed phase (Cc) to equilibrium concentration in the gaseous phase (Cg), K = Cc/Cg.

Generally speaking, K is a constant only when the analyte is present in trace quantities, and the changes in its concentration do not appreciably affect the composition and structure of the matrix.

The major difficulty of headspace analysis is the so-called "matrix effect". These matrix effects are results of unexpected variations in the matrix that affect K values and calibrations.

Several techniques were developed to combat these matrix effects (2,4–6). Some of them require several analytical runs for generation of a single concentration value or require significant sacrifices in sensitivity (4).

RHS analysis makes use of these matrix effects, which are the problem of regular headspace analysis, and presents a methodology for their quantitative measurements.

Approach to matrix characterization

Changes in K are the results of changes in the matrix itself, and a specific analyte can be used as a molecular sensor to detect the changes in the matrix.

According to the equation 1, if Cg and Co are known, than partition coefficient K can be calculated as:

$$K = (Co/Cg) - (Vg/Vc) \quad \text{Eq. 2}$$

Equations 1 and 2 describe the equilibrium conditions arising from evolution of volatile chemicals from samples into gaseous phase. In the spiking experiments, the value of Co can be defined as the amount of spike (m) divided by the volume of the sample (Vc) for the chemicals that were not present in the sample matrix to begin with.

In other words, if a headspace vial containing a known volume of matrix (Vc) is spiked with a known amount of volatile chemical (Co = m/Vc) and the headspace concentration (Cg) is measured after equilibration, the K value can be calculated. If the sample is spiked with the mixture of chemicals, then all their partition coefficients can be determined in a single analytical run for all the peaks resolved on the chromatogram.

This mixture of volatile chemicals with various functional groups and properties can be used as a molecular sensor array (MSA) for monitoring many chemical and physical changes in the matrix that may be of interest to quality control or investigation of matrix properties.

For example, if there are extra unreacted acidic chemicals in polymer matrix, a sharp reduction in headspace concentration of volatile amines will be observed and vice versa.

Uncertainties in partition coefficient determination

There are some experimental and theoretical problems with using equation 2 for the determination of partition coefficients. First, there is a difficulty in measurements of (Vg/Vc) volume ratios at the temperature of equilibration that can be quite different than this ratio at sample preparation (ambient) conditions. Second, it is the validity of assumptions made in deriving equation 1. Indeed, equation 1 was derived assuming that the volume of condensed phase doesn't change appreciably with temperature and the volume of sample taken for analysis at ambient conditions (Vc^0) is equal to the volume of condensed phase after equilibration (Vc).

Strictly speaking, these two quantities are not exactly the same because of partial evaporation of the matrix and its thermal expansion on heating. This difference is expressed in equation 3.

$$Vc = Vc^0 - Ve + \Delta Vt, \quad \text{Eq. 3}$$

where, Ve – is the loss of volume due to evaporation, and ΔVt is an increase in volume caused by thermal expansion. These two corrections are opposing each other and are presumed to be small. The effect of Ve is, indeed, small for solids at lower temperatures and larger sample volumes [Vc > 5–20% of total volume of the vial (Vo)]. However, when small sample volumes at higher temperatures are used, like in full evaporation technique (FET) methods (4), the evaporative loses of volatile sample matrices or matrix solutions can be very significant resulting in several folds changes in Vc^0/Vc ratio. On the other hand, the effect of ΔVt is smaller when Vc is smaller.

The equation that takes into account these changes of volume was derived and is given below:

$$Cg = Co/(Vg/Vc^0 + K \times (Vc/Vc^0)) \quad \text{Eq. 4}$$

Equation 4 is reduced to equation 1 when there is no change of matrix volume on heating and $Vc = Vc^0$. The changes in the volume of condensed phase result in the gaseous phase volume changes as well, as to Vg = Vo – Vc, where Vo is an internal volume of the headspace vial.

To eliminate Vg as a variable, equation 4 can be re-written into the following:

$$Cg = Co/(Vo/Vc^0 + (K - 1) \times (Vc/Vc^0)) \quad \text{Eq. 5}$$

In some cases, the value of Vc can be calculated when saturated vapor pressure of the matrix and its thermal expansion coefficient are known, such as in the case of water-based matrices. Knowledge of Vc value permits the use of headspace technique (Cg measurements) to determine true partition coefficients. However, for many real world samples, these values are not readily available.

All headspace techniques for determination of partition coefficients that presume the equality of condensed matrix volume before and after equilibration do not generate the true values of partition coefficients as compared with values derived from independent analyses of equilibrated gaseous and condensed phase separately. Subsequently, all thermodynamic functions, like activity coefficients, free energies, enthalpies of evaporation, etc., calculated from these K-values, have an inherent error.

Matrix specific constant

However, in spite all these drawbacks, a matrix specific constant (M) that uniquely characterizes the analyte–condensed phase system can be measured and used, instead of partition coefficient, for characterization and comparison of matrices. In addition to the partition coefficient, this constant includes changes in matrix volume attributable to its volatility and its thermal expansion characteristics as shown in equation 6.

$$M = (K - 1) \times (Vc/Vc^0) = (Co/Cg) - (Vo/Vc^0) \quad \text{Eq. 6}$$

The set of these matrix constants constitute a MAS that is unique for every matrix. Strictly speaking, M is also a function of sample volume (see equation 6). However, in cases of non-volatile matrices or larger vial loadings (> 20%), the effects of evaporative losses for most common matrices are much smaller than volume gains caused by thermal expansion.

In other words, at Ve << Vc, $(Vc/Vc^0) = \beta$, where β is a degree of volumetric thermal expansion on heating from ambient temperature to the temperature of equilibration. It is a function of the difference between these temperatures multiplied by volumetric thermal expansion coefficient that is also matrix specific. The value of M-constant can be expressed by the following equation:

$$M = (K - 1) \times \beta \quad \text{Eq. 6a}$$

Subsequently, the general headspace equation 5 can be

re-written using the M constant as equation 7:

$$Cg = Co/(M + Vo/Vc^0) \quad \text{Eq. 7}$$

Cg is the only quantity in equation 7 that has to be measured at equilibration conditions for the determination of M-constants. Indeed, $Co = m/Vc^0$, where "m" is the amount of spike and the volumes (Vo and Vc^0) are easily measurable quantities.

It follows from equation 6a that at $K < 1$ (dissolved gases), M can be negative. However, Cg as given by equation 7 cannot be negative even at very small Vo/Vc^0 volume ratios and K-values approaching zero. Indeed, Vo/Vc^0 cannot be less than β; otherwise, the volume of equilibrated (thermally expanded) condensed phase (Vc) will exceed the total volume of the vial (Vo). It follows from equations 6a and 7 that even at minimum possible volume ratios = β, Cg is positive and its theoretical maximum can be expressed by equation 7a.

$$Cg \leq Co/(K \times \beta) \quad \text{Eq. 7a}$$

Practice of M-constant determination via peak areas

It is easy to show that M-constant can be calculated without any quantitative calibrations of the instrument. Knowledge of peak areas, initial matrix volume, and total volume of the headspace vial are the only parameters needed for generation of consistent spectra (MAS).

The expression of M-constant through peak areas and volumes is derived from equation 7 and is given in equation 8:

$$M = (Ao/As - 1) \times Vo/Vc^0 \quad \text{Eq. 8}$$

where Ao and As are the areas generated by headspace analysis of an empty headspace vial and vial containing a sample spiked with an identical amount of MSA. In the practice of the technique, Ao is determined in the following fashion. A linear plot $Ao = f(m)$ is generated with empty vials using various masses of MSA (m) and Ao for every chemical sensor is found from this plot as a point corresponding to the actual amount of MSA added to the sample matrix under investigation. The amount of sample spike should be less than the linear region of "Ao" versus "m" plot for all chemical sensors. The linear region of the plot is the region of total evaporation of MSA mixture in the headspace vial at given temperature.

Equation 8 shows that the ratio of areas is a characteristic of the matrix if the procedural parameters, like total vial volume (Vo) and the amount of sample (Vc^0), are held constant.

The user of the methodology can tailor the mixture composition (MSA) to optimize for the sensitivity to a specific matrix property of interest (see Results and Discussion section). This work will show that the value of M-constant is quite sensitive and useful for characterization and identification of many important matrices that include polymers, pharmaceuticals, construction and household materials, etc.

These spectra also can be generated by plotting M-constant against any property of MSA chemicals (polarity, dielectric constant, solubility parameter, acidity, molecular weight, vapor pressure, critical constants, etc.) or just against a set of consecutive numbers, each designating a chemical in MSA. In addition, it also can be plotted against specific property of matrix such as porosity, surface area, particle size, percent of any ingredient, electroconductivity, viscosity, density, moisture content, percent of solids, etc., and spectra can be used for measurements of these properties. The data also can be treated with any pattern recognition software to deduce many important parameters such as fat content in foods and grains.

Experimental

Instrumentation

An Agilent 6890 GC equipped with a 5973 MSD (Agilent Technologies, Palo Alto, CA) was interfaced with an ASIST-150 dual needle autosampler (ASIST Inc., Cleveland, OH). The autosampler was run in a single needle mode, using time control injection technique. Headspace vials with poly(tetrafluoroethylene) lined septa and internal volumes of 24.5 mL were used in all experiments.

Headspace and chromatographic conditions were varied depending on the matrix and MSA mixture used (see figure captions).

MSA mixture preparation

The work presented here involved two MSA mixtures. Approximately 0.5 mL of pure chemicals were weighed on analytical balances and added into a vial. The density of the mixture was determined by weighing of 1-mL aliquot of the mixture.

Composition of mixture I (MSA-11)

The mixture included toluene, ethyl acetate, methylene chloride, acetic acid, acetonitrile, heptane, *n*-butanol, pyridine, picoline, dimethylformamide (DMF), and decane.

Composition of mixture II (MSA-19)

The mixture included Freon 7100, heptane, perfluoropentane, ethyl acetate, methyl ethyl ketone (MEK), methylene chloride, isopropanol, acetonitrile, chloroform, toluene, 1,4-dioxane, *n*-butanol, dodecane (*n*-C12), pyridine, picoline, DMF, acetic acid, propanoic acid, and dimethyl sulfoxide. (A typical headspace gas chromatography–mass spectrometry chromatogram of the MSA-19 mixture is presented in Figure 1).

While designing a composition of MSA, it is advisable to have at least two sensors with the same functional group but having different volatilities to maximize the sensitivity of MSA to various matrix changes. As long as the objective is the determination of M-constants and generation of MAS, it is not necessary to know a precise quantitative composition of MSA mixture. However, the same MSA should be used for spiking of empty vial (calibration curve) and the sample.

Estimation of spike amount

There are two reasons to keep the spiking amount as small

as possible. First, as was mentioned earlier, it should be completely vaporizable in the headspace available to avoid formation of a separate phase. Second, the effect of spiking on the matrix composition should be minimal to maintain a very low concentration of every chemical in the matrix, providing for a near ideal solution of the chemicals in the matrix (Henry law region). Calculations of the maximum amount of a particular chemical (sensor) that can be evaporated in the headspace vial were described in the literature (4) using equation 9.

$$Po > P = nRT/Vg \qquad \text{Eq. 9}$$

where Po is a saturated vapor pressure of a chemical, P is the pressure that would be developed by "*n*" moles of this chemical in the headspace volume Vg (liters) at absolute temperature T (K). R is the universal gas constant, which equals 62.4 if pressures are expressed in mm Hg.

The maximum amount Wt (mg) of a chemical that can be evaporated in the headspace volume Vg (milliliters) is calculated using equation 10.

$$Wt = Po \times M_w \times Vg/RT \qquad \text{Eq. 10}$$

where M_w is molecular weight of a chemical. Po can be found in many reference books or can be calculated for every chemical at different temperatures using the Antoine equation (7).

For example, in the case of acetonitrile and empty headspace vial of 23.4 mL, Po = 758 mm Hg at 80°C. Placing these values in the equation 10 yields: $Wt = 758 \times 41 \times 23.4/(62.4 \times 353) = 33$ mg

Similar calculations for acetic acid and pyridine yield approximately 13 and 20 mg at 80°C and 25 and 37 mg at 100°C, respectively.

The amount of every chemical in the spike should be several times less than the maximum vaporizable amount to avoid any possibility of condensation on the cold spot of analytical system and to overcome the effects of possible non-ideality in gaseous phase (fugacity). Ideally, the amount of spike should be as small as experimentally feasible and sufficient to provide measurable concentration in the gaseous phase. Our experience showed that it is not necessary to spike more than a few microliters of MSA per vial.

Spiking procedure

It is not advisable to make spikes directly onto the surface of solid matrices, primarily because high local concentration of organics may modify local area and re-equilibration may take an unreasonably long time. To avoid direct contact of liquid spike with the matrix, the experiment is conducted as shown in Figure 2.

A small, unsealed glass vial was placed on top of the sample inside of the headspace vial, and the spike was made into the internal volume of the small vial. Regular 1.7-mL liquid autosampler vials were used for this purpose. This way, the matrix contacted sensors uniformly via the vapor phase only. The volume of the glass of the vial (~ 1 mL in these experiments) inserted into a headspace vial must be taken into account during data reduction correcting Vo value.

The amount of matrix and amount of spike can be varied; however, it is recommended, especially for solid samples, to keep their ratio as small as experimentally feasible (mg/g). Typical chromatogram of MSA-19 mixture is shown in Figure 1, and repeatability of spiking procedure is demonstrated in Table I for MSA-11 and in Table II for MSA-19. Percent relative standard deviation (RSD) values of Table I indicate that the spectral differences in MAS in the excess of 5% may be significant.

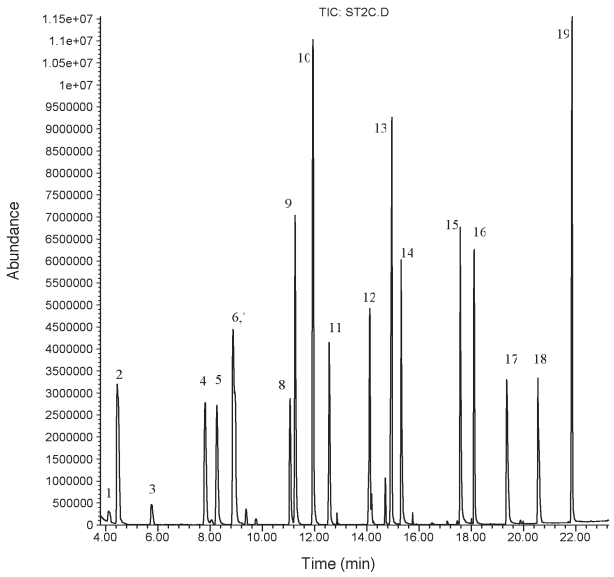

Figure 1. Typical chromatogram of MSA-19 mixture. Sample: 2 µL of the mixture. Headspace and conditions: temperature, 150°C; pressurization time, 2 min; injection time, 3 s; column, HP-Innowax (60 m × 0.25 mm × 0.25 µm); column flow, 1 mL/min; split flow, 20 mL/min; detector temperature, 270°C; and injector temperature, 200°C. Oven program: 40°C for 3 min, rate 4°C/min up to 60°C, and 10°C/min up to 260°C (hold for 20 min). Total scan mode: 29–350 *m/z*. Peaks 6 and 7 were not completely chromatographically separated; however, the quantitation was conducted using the *m/z* 84 specific for methylene chloride and *m/z* 45 for isopropanol.

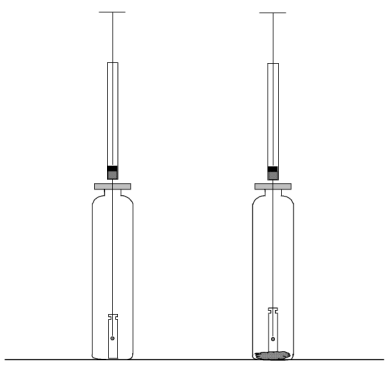

Figure 2. Illustration of spiking procedure. Small glass vials were placed inside of headspace vials. One of the headspace vials contains sample. Both headspace vials are spiked with the same amount of MSA mixture, closed, and equilibrated.

Results and Discussion

Data reduction

The raw data generated with 11 components MSA (mixture I) for an empty vial (Ao) and several polymer compositions (As) are presented in Table III.

In this experiment, the gaseous volume of an empty headspace vial (Vo) was 23.5 mL (taking into account the volume of glass insert vial). The weight of the polymers has been chosen to maintain 0.5 mL volume of it for all the samples.

All vials were spiked with 5 µL (4.5 mg) of MSA 11 mixture, as shown in Figure 2. The values of M constants were calculated using equation 8 and are compiled in Table II.

MAS presentation and manipulation

There are several ways to present MAS. Figure 3 shows a spectrum of polymer sample P-003 in column and smooth line formats. The values of M constant varied greatly from one sensor chemical to another, and it was often more convenient to express the spectra in terms of relative area reduction (Figure 4). As follows from equation 9, relative peak area reduction also could be used for comparison of matrices if volumes of sample and headspace vial were held constant. This way of presentation permits keeping the data from chemicals with widely different M constants on the same scale (from 0 to 1 for common samples); however, it required an equivalency of sample volumes in the experiments that may be difficult to achieve with some matrices.

Normalization of the spectra to MAS of a reference matrix may be useful for quality control (QC) purposes. Figure 5 shows normalized spectra of several styrene copolymers using 50% butadiene copolymer with butylated hydroxyanisole (BHA) antioxidant (P 003) as a reference. The values of standard deviations of spectra among all sensors or selected ones can be used as an objective QC measure of matrix identity to a reference material. Calculations showed that the formulation

Table I. Repeatability of Spiking Technique

MSA solution		Raw peak areas for replicates				
Chemical	Peak no.	R1	R2	R3	Mean	RSD%
n-Heptane	1	1338910	1341378	1328410	1336233	0.52
Ethyl acetate	2	951277	912812	932570	932219	2.06
CH_2Cl_2	3	643520	642660	647883	644688	0.43
n-Decane	4	2181495	2171688	2196192	2183125	0.56
Acetonitrile	5	881192	879807	884048	881682	0.25
Toluene	6	2362777	2343180	2380487	2362148	0.79
Butanol	7	1374128	1364722	1383585	1374145	0.69
Pyridine	8	1974987	1960197	1995487	1976890	0.90
Picoline	9	1535893	1523445	1553753	1537697	0.99
DMF	10	1029217	1023367	1039647	1030743	0.80
Acetic acid	11	933810	926660	949810	936760	1.27

Table II. M Constants for Different Polymers Calculated from Table III

MSA mixture		M constants for different polymers						
Chemical	Peak no.	P-003	P-176	P-115	P-148	P-152	P-159	P-057
n-C7	1	122	115	109	124	67	41	66
Ethyl acetate	2	63	69	53	72	68	77	50
CH_2Cl_2	3	52	48	39	54	42	66	22
n-C10	4	1000	1003	999	1047	706	307	540
Acetonitrile	5	31	29	17	31	37	97	28
Toluene	6	352	331	269	315	285	289	180
n-Butanol	7	136	127	84	117	118	237	130
Pyridine	8	469	447	216	314	512	489	306
Picoline	9	1339	1329	541	833	1949	1261	951
DMF	10	505	484	202	306	723	1284	469
Acetic acid	11	204	191	65	109	187	918	211

Table III. Raw Data for Empty Headspace Vial (HS vial) and Seven Polymers with MSA-11 Spiking Solution

	MSA 11 preparation			Peak areas							
Chemical	Peak no.	Wt (g)	Wt (%)	HS vial	P-003	P-176	P-115	P-148	P-152	P-159	P-057
n-C7	1	0.7405	4.26	1336233	371415	387459	403017	366792	549257	712149	556993
Ethyl acetate	2	1.2951	7.44	932219	399527	376816	437868	367600	380893	351928	449629
CH_2Cl_2	3	2.4918	14.32	644688	307089	317814	353184	300450	339109	268870	437863
n-C10	4	1.2876	7.40	2183125	98004	97722	98108	93750	136238	289709	174943
Acetonitrile	5	1.1944	6.86	881682	532843	547441	646781	533590	491019	287702	549664
Toluene	6	1.2856	7.39	2362148	278044	293844	351843	306334	334760	329964	490029
n-Butanol	7	1.2597	7.24	1374145	352632	371306	492633	393194	391941	227258	365667
Pyridine	8	1.6081	9.24	1976890	180152	188200	353698	257670	166350	173203	263385
Picoline	9	1.2787	7.35	1537697	52150	52525	122940	82170	36217	55262	72421
DMF	10	2.3303	13.39	1030743	87759	91247	194179	137204	62942	36411	93850
Acetic acid	11	2.6307	15.12	936760	175722	185025	391388	283066	188340	45622	170520

P176 (different lot # of P003 formulation) had 5% standard deviation, though formulations with isoprene, different butadiene content, and different antioxidant packages, have standard deviations 21%, 22%, 23%, and 29% for compositions P 057, P 148, P 115, and P 152, respectively.

Changing an order of chemicals on the *X*-axes in Figures 3–5 will change the appearance of the spectra. Once chosen, this order should be held constant for spectra comparison purposes.

Sometimes it is of interest to evaluate M constant values against some property of selected sensors. Figure 6 is the M constants of three polymers plotted against boiling points of chemical sensors reflecting their volatility, and Figure 7 is the same polymers characterized against refractive index that reflects chemical polarizability. This way of presentation sets up a specific order of chemicals according to an increase in a specific property value.

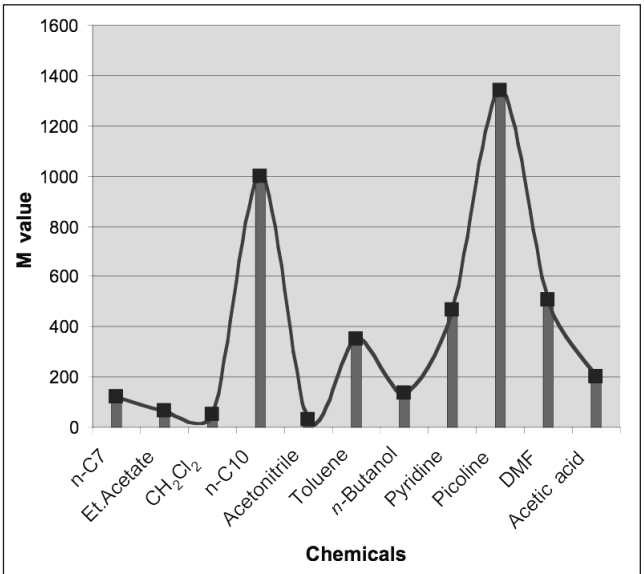

Figure 3. MAS for polymer P-003 using MSA-11 mixture. The discrete points corresponding to values of M-constants for specific chemical sensors are connected with a smoothed line for a better visualization of spectra and, especially, spectral differences. Sample (0.5 g) spiked with 5 µL of the mixture. Headspace conditions: temperature, 125°C; pressurization time, 2 min; and injection time, 3 s. GC–MS conditions were the same as described in Figure 1.

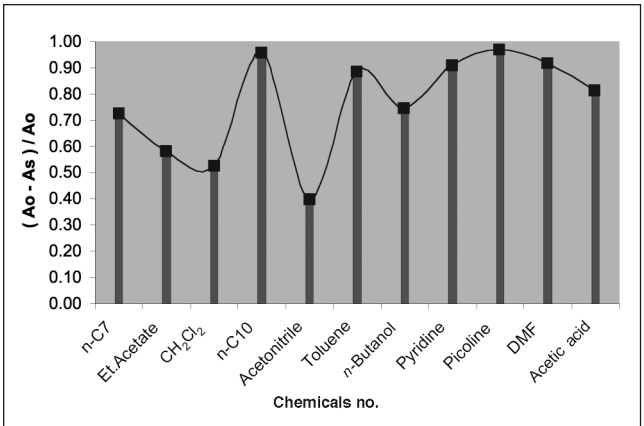

Figure 4. Relative area reduction for polymer P-003 spiked with MSA 11. This figure is generated from the same experimental data as Figure 3 and plotted in terms of "relative peak area reduction".

Selection of sensors and requirements to MSA solution

To avoid additional analyses and to use the simplest data reduction procedure, the matrix to be characterized should not contain MSA constituent or chromatographically interfering components in quantities comparable with its quantity in MSA spike. Otherwise, an analysis of un-spiked matrix should be performed and, somewhat, more complicated equations should be used to deduce the values of M-constants.

Table IV was generated with MSA-19 mixture on empty vials and four aspirin samples heated to 150°C. Table IV shows data

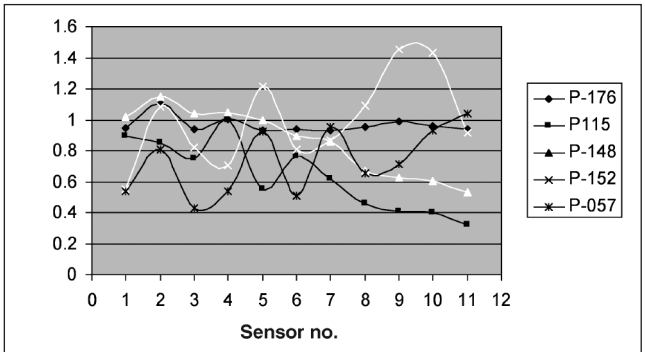

Figure 5. MAS of various styrene copolymers normalized to reference matrix (P-003). This plot is generated from the data in Table III by dividing M constants for every polymer by the value of corresponding M value of a standard polymer (P-003).

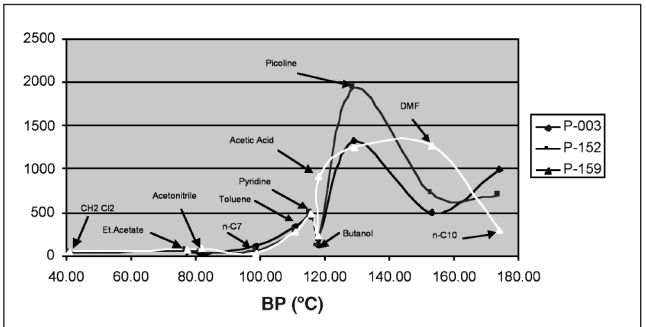

Figure 6. Matrix constants for polymers P-003, P-152, and P-159 plotted versus boiling point of MSA chemicals.

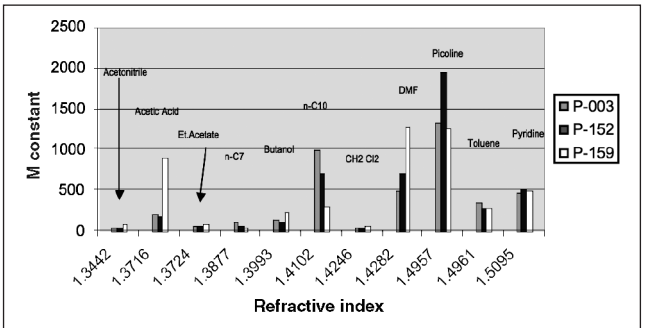

Figure 7. Matrix constants for polymers P-003, P-152, and P-159 plotted versus refractive index of MSA chemicals.

for the standards, percent RSD for the method, relative peak area reduction for samples, and sensor selection choices for MAS generation. Aspirin was chosen because it is available from different suppliers that claimed an identical amount of active ingredient (325 mg/tablet), and it represented matrices that may decompose at the equilibration temperature. Acetic acid was omitted because it was found in headspace of all samples to begin with (it is believed to be a product of decomposition), and acetonitrile was present in samples P and W. The other omitted sensors showed near complete absorption by the matrices or chemically reacted with matrices and offer very little information for comparison purposes. Figure 8 is MAS of aspirins from different suppliers using 11 selected sensors out of 19 chemicals solution (MSA-19) added.

The spectra indicated that sample P is quite different from the rest of the samples, and samples B and D were quite similar. Sample W was also similar to B and D with the exception of its lower affinity to dodecane. It turned out that after normalization of all spectra to the spectrum of sample "B", the average standard deviations among all selected sensors from unity were 6%, 32%, and 43% for samples "D", "W", and "P", respectively. However, when the dodecane sensor was omitted, the values of percent RSD changed to 5%, 9%, and 29%, respectively. Apparently, some nonpolar constituent was not present (or formed) in sample "W", whose behavior, otherwise, was similar to "B" and "D", and the sample "P" was decomposed to a quite different matrix.

It is not the intent of this paper to interpret the differences detected. They can be very benign, like variability of moisture, or sample matrices may be changing differently under equilibration conditions. Nevertheless, the spectra indicate that they are different. It is up to the user of this methodology with a deeper knowledge about the matrix and its properties to interpret the nature of the differences (composition, technology of preparation, or storage conditions).

It may be useful to introduce standard matrices for unification of spectra generated in different laboratories with different instrumentation and headspace sampling techniques. Glycerol and silicone oil were used as low volatility matrices of highly different polarities whose volumes in headspace vials can be easily controlled. The data generated with MSA-19 for glycerol and silicone oil are shown in Figure 9.

The figure shows that sensors like dodecane, dimethyl sulfoxide, and DMF were the most sensitive to the changes in matrix polarity, and sensors like MEK and 1,4-dioxane were not at 125°C. Depending upon the property under a study, different reference matrices can be selected and some property index scale may be created (e.g., glycerol–silicone oil polarity index at 125°C). (G/S – 125) can be defined in the following fashion. The difference between M constants of silicone oil and glycerol for every sensor can be assigned a value of 100 units, where M indices of glycerol have G/S – 125 = 100 and all indices of silicone oil have D/S – 125 = 0. The discussion on G/S and other standardization indices is reserved for the second part of the paper. However, it may be appropriate to mention here that M values determined on standard matrices should not

Table IV. Sensor Selection for MAS of Aspirin Samples*

Sensors	Three standards						
Chemical	Mean Ao	RSD (%)	B	W	P	D	Sensor selector
Freon 7100	5228281	0.9	0.208892	0.200432	0.490765	0.176568	OK
Heptane	15966719	1.9	0.247094	0.240451	0.507071	0.217092	OK
Freon HPF	2262641	3.0	0.239995	0.246807	0.519309	0.219854	OK
Ethyl acetate	1.08E+08	2.5	0.501366	0.455521	0.617393	0.466497	OK
MEK	35181504	2.6	0.478982	0.484383	0.62012	0.469504	OK
CH_2Cl_2	76639425	2.7	0.297043	0.29487	0.516192	0.271055	OK
Iso-propanol	1.03E+08	2.8	1	0.966703	1	1	Low info.
Acetonitrile	74436652	2.5	0.459843	–0.74664	–0.60317	0.448933	Present
Chloroform	1.36E+08	2.3	0.326509	0.338993	0.533783	0.304617	OK
Toluene	2.12E+08	2.1	0.396001	0.42085	0.574154	0.378593	OK
1,4-Dioxane	58683625	2.9	0.655917	0.671281	0.748867	0.662063	OK
n-Butanol	47635560	30.	1	0.996632	0.998046	0.998468	Low info.
Dodecane	87230546	2.6	0.863405	0.448359	0.628599	0.863128	OK
Pyridine	1.02E+08	2.1	0.998902	0.992836	0.988597	0.98937	Low info.
Picoline	98436896	2.8	1	1	1	1	Low info.
DMF	83889157	5.9	0.99699	0.995534	0.969828	0.996768	Low info.
Acetic acid	34404207	5.2	–76.4754	–68.538	–60.8381	–71.234	Present
Propanic acid	33758966	1.1	0.858031	0.865752	0.989042	0.881004	OK
DMSO†	1.05E+08	0.9	1	1	1	1	Low info.

* Relative area reduction was calculated as (Ao – As)/Ao. The sensors marked "OK" were selected for plotting MAS in Figure 8. Sensors marked "Low info." were absorbed by the matrix to near completion and are low on information about sample differences. Sensors marked "Present" were present or were generated in the matrix during equilibration. All selected sensors had RSD% values 3% or better.

† Dimethyl sulfoxide.

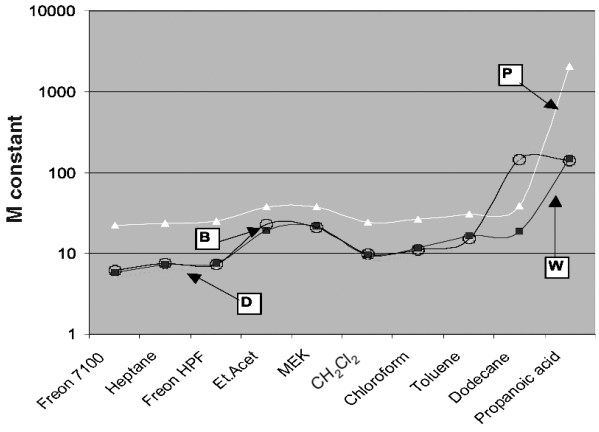

Figure 8. M constants (log scale) of aspirin for selected sensors. Sample (0.5 g) spiked 2 µL of the mixture. Headspace conditions: temperature, 150°C; pressurization time, 2 min; and injection time, 3 s. GC–MS conditions were the same as described in Figure 1. The plot is generated from Table IV data. The sensors marked "Low info." and "present" were omitted.

vary from laboratory to laboratory or from instrument to instrument. In order to ensure that a headspace method can be transferred from one instrument to another, the settings on the second instrument should be adjusted in such a way that the standard MASs on both instruments would match.

A competitive absorption or adsorption among various MSA chemicals may have significant effect on MAS appearance. This factor in itself may also be a contributor to the sensitivity of MAS to even small changes in matrices. The composition of MSA may be adjusted to suppress or to enhance this phenomenon.

Effect of sample size and experimental errors

The inversion of equation 7 shows that there was a linear relationship between reciprocal concentration in gaseous phase (Cg or As) over the matrix and the matrix volume taken placed in the headspace vial.

$$1/Cg = M/Co + (1/Vc^0) \times (Vo/Co) \qquad \text{Eq. 11}$$

Taking into account that $Co = m/Vc^0$ (m is the amount of a chemical in the spike), equation 11 is transformed into equation 12.

$$1/Cg = M \times Vc^0/m + Vo/m \qquad \text{Eq. 12}$$

The effect of sample volume in terms of peak areas can be expressed by equation 12a.

$$Ao/As = M \times (Vc^0/Vo) + 1 \qquad \text{Eq. 12a}$$

Equation 12a shows how the precision of M determination and, subsequently, an ability of MAS to distinguish between similar materials, is related to the precision of peak area measurements. This relationship is demonstrated in Figure 10.

The figure shows that errors of M determination were minimized when peak area over the sample (As) was much smaller than Ao. It follows that larger M values can be determined with a better precision. Ao/As ratio can be controlled by adjusting sample size (Vc^0) and by the composition of MSA.

Often, it is more convenient and accurate to control sample size by weight (Wt) rather than by volume, especially for solid samples. Equation 12a can be re-written as equation 12b in terms of sample weight and the density of the matrix (d) at the temperature of sample preparation (ambient).

$$Ao/As = (M/d) \times (Wt/Vo) + 1 \qquad \text{Eq. 12b}$$

Density was also a matrix property and, therefore, could be included in the definition of matrix constants that were used for comparison and identification of matrices. This weight-based matrix constant ($M' = M/d$) can be determined via the same experiment and calculated via equation 13:

$$M' = Vo \times (Ao/As - 1)/Wt \qquad \text{Eq. 13}$$

Matrix parameters that influence the specificity of this matrix constant M' are combined in the equation 14.

$$M' = (K - 1) \times \beta/d \qquad \text{Eq. 14}$$

In other words, matrix constant reflects chemical affinity at equilibration temperature, density at ambient conditions, and matrix volume changes on heating from ambient to the equilibration temperature. Matrix constants and MAS will be sensitive to the changes in any of these parameters. The discussion of physical chemical meaning of matrix constant and its temperature dependence are outside the scope of this paper. However, it may be of interest to note here three special cases:

(*a*) at $M' = -\beta/d$ ($M = -\beta$, $K = 0$), the matrix is impermeable to the sensor molecules and acts as an inert filler occupying a part of a vial interior; (*b*) at $M' = \infty$ ($M = \infty$, $K = \infty$), the matrix totally absorbs or chemically modifies the sensor molecules and there are no molecules of the sensor in the vapor phase after equilibration; and (*c*) at $M' = 0$ ($M = 0$, $K = 1$), the sensor chemical makes no distinction between the matrix and gaseous phase at the equilibration temperature.

These three cases are the consequences of equations 6a and

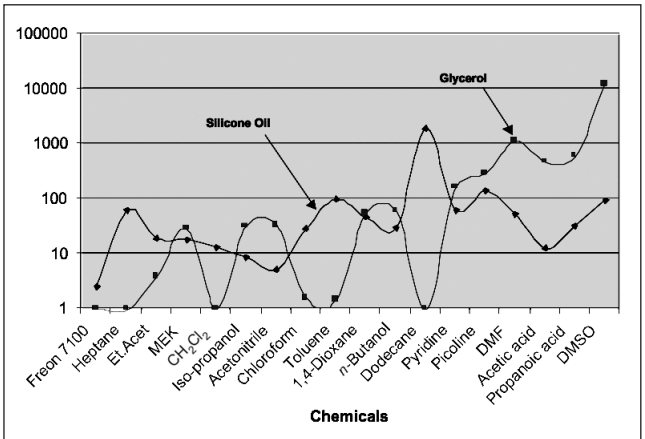

Figure 9. Molecular affinity spectra of glycerol and silicone oil. Log scale was chosen for a better visualization of spectral differences for chemicals with widely different M constants. Freon PFP was omitted from the MSA-19 set because of chromatographic interferences.

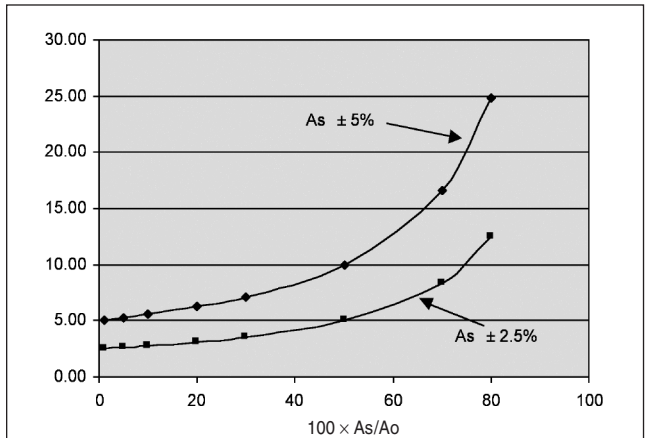

Figure 10. Error in M constant versus percent As of Ao. Errors in M constant determination as a function of peak area ratio for 5% and 2.5% uncertainties in peak area measurements.

14 that define M and M' as the measures of chemical affinity of the matrix to a particular chemical and basic matrix properties.

Conclusion

RHS methodology is, in a sense, a form of image recognition techniques that generates a picture of matrix affinities to, or interactions with, various molecules rather than the picture of interactions with electromagnetic radiation as it is the case in a variety of spectroscopic techniques. Condensed matrices can be identified, studied, and characterized by headspace analysis of their spikes with a mixture of volatile chemicals called MSA.

The paper suggests that a regular headspace instrument in combination with a gas chromatograph or any other analyzer can be used for identification, quality control, and studies of condensed matrices. The sensitivity of contemporary instruments provides for the detection of very small quantities of volatile chemicals dissolved in an equilibrated sample matrix and having a minimal effect on the original matrix structure.

The paper introduces a definition of matrix specific constant (M constants) that can be determined from a simple headspace experiment. It presents theoretical foundations of the method, modifies a basic headspace equation taking into account thermal properties of matrices, and establishes relationship between M constant and true partition coefficient.

It is shown that a set of M constants (all determined in one experiment) forms an MAS that is specific for a given matrix at a given temperature. A step-by-step procedure of MAS generation (data reduction) from raw peak areas to the formation of spectra is demonstrated with several examples (polymers and pharmaceuticals). Suggestions for sensor selection, quantity of spikes, quantity of sample, and compositions of MSA are made.

The use of standard experimentally convenient matrices is suggested to eliminate effects of instrument-to-instrument variations on the spectra (MAS).

In addition to identification and quality control purposes, this RHS approach to analysis can be useful for measurements of specific properties of matrices such as degree of crystallinity of polymers, salinity of waters and biofluids, determination of specific additives to chemical and pharmaceutical formulations, measurements of surface areas, etc. Some of these applications will be demonstrated and discussed in Part II of this RHS study.

These initial studies of the methodology indicate that MAS are repeatable and quite sensitive to variations in matrix composition and can serve as informative investigation, quality control, and analytical tools.

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