

CHROMSYMP. 759

SOLVENT PROPERTIES AND THEIR EFFECTS ON GRADIENT ELUTION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

I. BULK AND MOLECULAR PROPERTIES OF WATER AND ACETONITRILE

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SUMMARY

The most common baseline anomalies observed in gradient elution high-performance liquid chromatography can be explained by the non-ideal behaviour of the solvents used. The bulk and molecular properties of mixed solvents, as they affect the performance of the liquid chromatograph, are explored. The concept of mole flow-rate, useful in heat balance and partition mechanism studies, is introduced and put to use in a preliminary heat balance study which could be used to size heat exchange equipment useful in thermostating a liquid chromatograph. The inter-dependence of these properties is shown to produce cumulative effects in the pumping system, the column and the detector of the liquid chromatograph. The properties of water-acetonitrile mixtures are used for illustration.

INTRODUCTION

Gradient elution high-performance liquid chromatography (HPLC) finds use in virtually every area of application in HPLC except size exclusion chromatography. With the increasing popularity of this technique, and the increasing use of extreme or complex gradients, have come reports of baseline anomalies. A baseline anomaly is a repeatable phenomenon whose occurrence is not related to the presence or absence of sample in the chromatographic system. It is, rather, related to the choice of chromatographic conditions including (1) the choice of mobile phase components; (2) the choice of detectors and detector operating conditions; (3) the effects of the

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bulk and molecular properties of mixed solvents on gradient elution HPLC; and (4) the dramatic effects that mixing has upon the physical properties of liquids.

Berry, in an article published in 1982¹, reported a phenomenon which he termed the "Mid-gradient hump". This phenomenon was described as a baseline disturbance produced by the conditions of the separation. It was attributed to UV absorbance mismatch, although the responsibility for the mismatch was never assigned definitely to either of the solvents composing the system. The presence of "aqueous eluent-derived ghost peaks" was noted, as was the presence of other ghost peaks. Berry quotes earlier work by Rabel², who attributes certain "ghost" peaks to contamination of the acetonitrile. Experiments performed in our laboratories indicate that both attributions are correct. Our work shows, clearly, that the impurities in the aqueous mobile phase elute as an early group of peaks, whereas the peaks attributable to acetonitrile contamination elute later, usually during the regeneration (re-equilibration) period of the gradient (*vide infra*). In a later work, Berry and Dolan³ allude to "amine-like impurities" present in acetonitrile, although the nature of these impurities is not further specified.

Bakalyar and co-workers, in earlier work⁴⁻⁸, reported the influence of dissolved gases on gradient HPLC. Much of the detrimental effect of dissolved air in the mobile phase is attributed to the dissolved oxygen as it was by Snyder⁹. The work of Bakalyar *et al.* focuses on two aspects of the general problem of dissolved gas in the mobile phase:

(1) The quantitative aspect, *i.e.* how to obtain quantitative results from digital integrators if the contribution of dissolved gases to the total absorbance at any point in one chromatogram is different from that in a second chromatogram to which the first is to be compared; and

(2) How best to rid the chromatograph of the dissolved gases?

Bakalyar also discussed the non-linearity of the solubility of several gases in various mobile phase mixtures as a function of composition. The same work compared the efficiency of various methods of de-gassing. The result of these considerations was a patent on an apparatus and method for removing dissolved gases from components of the mobile phase⁷. Bakalyar's studies were subsequently confirmed and extended by Hobbs¹⁰ working independently and by experiments undertaken in our own laboratories and described below.

The role of temperature in the anomalous performance of short-wavelength gradient HPLC is often neglected. Campbell *et al.*¹¹ demonstrated that this oversight can prove detrimental to the quality of the chromatogram, from spectral considerations alone, without considering that temperature changes can produce retention order inversion¹² in isocratic HPLC.

The non-ideality of the bulk properties of mixed solvents manifests itself in such forms as pressure variations with composition at constant pump output and the appearance of humps or troughs in the reported chromatogram. These problems become even more pronounced as the use of short-wavelength UV absorbance detection and wide gradients gain popularity.

Our research has noted the influence of composition dependence of viscosity, composition dependence of excess enthalpy, composition and wavelength dependence of refractive index, changes in pH with composition, composition and pressure dependence of excess volumes of mixing, and effects of solvent purity upon the gradient

HPLC baseline. The work presented here examines the water–acetonitrile gradient in the light of physical-chemical knowledge of the influence of these properties. The differences between several brands of “HPLC-grade” acetonitrile are demonstrated, and “clean-up” experiments are described.

GENERAL OBSERVATIONS

The mole flow-rate concept

Mole flow-rate is defined as the number of moles of a solvent (pure or mixed) which pass through the column per unit time. Its units are either moles/min or gram-formulae/min. Although it was originally an *ad hoc* term, it appears to have some use in describing the events of gradient elution HPLC in terms of the Hildebrand solubility parameter¹³. It is, therefore, potentially useful in relating the occurrences of reversed-phase HPLC to other solubility phenomena.

The concept finds further use in quantitative comparisons of physical-chemical properties with findings from chromatographic experience. For example, the heat flux through the chromatograph's mixer can be calculated from the excess enthalpy of the mixture and the mole flow-rate. Mole flow-rate is calculated in the following way:

$$F_m = F_i \rho_f / (x_a m_a + x_b m_b)$$

where F_i is the inlet total volumetric flow-rate (the sum of the flow-rates of each pump) at temperatures T and pressure P , ρ_f is the mixture density at temperature T and pressure P , x_a is the mole fraction of solvent a , m_a is its molecular weight and F_m is the mole flow-rate of the mixture in moles/min. Formula density must be read from a table or chart (*vide infra*), and the mole fraction of each solvent can be calculated. We further assume isothermal mixing. If this is not the case, a temperature correction based on the mixture's isobaric heat capacity (C_p) and excess enthalpy of mixing (*vide infra*) can be introduced.

Fig. 1 shows mole flow-rates as functions of temperature and composition. The original importance of mole flow-rates was to explain the pressure fluctuation observed in the ion source of a mass spectrometer during gradient elution direct liquid introduction/liquid chromatography–mass spectrometry¹⁴. The same sorts of considerations apply to calculations of background absorbance in LC–UV according to the Beer–Lambert Law. Absorbance is a molecular phenomenon, so any change in the number of moles of an absorbing species in the light path must result in a proportional change in the measured optical density. A change in the number of moles present in the light path might arise from compositional changes in the mobile phase, changes in the cell pressure, or thermally induced isocratic density changes. It is, therefore, important to have achieved an isotherm before the mobile phase enters the detector cell, for two reasons: firstly, so that the viscosity of the mobile phase will not undergo unforeseen changes, and thereby induce density changes in the flow cell, and secondly, so that the density of the mobile phase will not undergo direct changes due to the wall temperature of the cell. The latter has been observed by the authors to induce such severe Schlieren that the flow cell was “eclipsed” by refractive index disturbances. The temperature–absorbance effect was previously noted and reported¹¹.

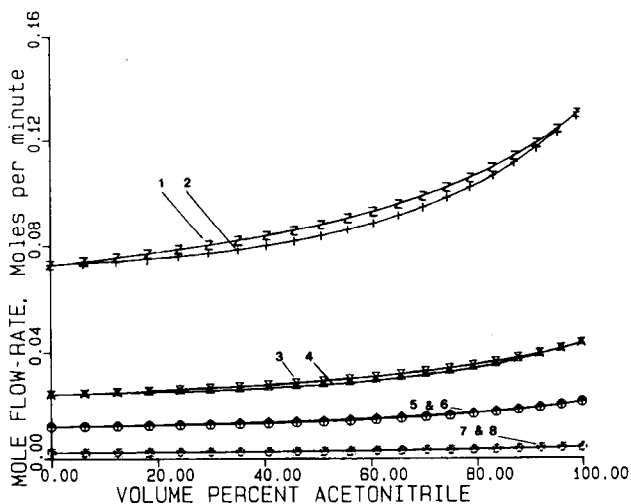


Fig. 1. Mole flow-rate as a function of temperature and composition. Experimental points use density data of Moreau and Douheret¹⁵. Theoretical points use linear prediction based on mole fraction composition and densities of pure solvents. Curve 1: flow-rate = 3.0 ml/min. Experimental density data at 25°C. Curve 2: flow-rate = 3.0 ml/min. Linearly-predicted density data at 25°C. Curve 3: flow-rate = 1.0 ml/min. Experimental density data at 25°C. Curve 4: flow-rate = 1.0 ml/min. Linearly-predicted density data at 25°C. Curve 5: flow-rate = 0.5 ml/min. Experimental density data at 25°C. Curve 6: flow-rate = 0.5 ml/min. Linearly-predicted density data at 25°C. Curve 7: flow-rate = 0.1 ml/min. Experimental density data at 25°C. Curve 8: flow-rate = 0.1 ml/min. Linearly-predicted density data at 25°C.

Pressure variations at constant flow

The pressure inside the liquid chromatograph changes, even though each pump maintains a constant output. This phenomenon has been attributed to pump failure or column deterioration. Our investigations show that such an explanation is rarely correct. Moreau and Douheret¹⁵ report the viscosities of various compositions of water and acetonitrile at several temperatures. The viscosities reported deviated markedly from the usual linear predictions, as Fig. 2 shows. These deviations, termed "excess viscosity", appear identical to the pressure profile observed in a 0–100% acetonitrile in water gradient. Quantitatively, there are some differences between the isothermal viscosity curves shown in Fig. 2 and the pressure profile (Fig. 3), because there is usually no attempt made to thermostat the mobile phase at the mixing node. Indeed, most attempts at temperature control begin at the column inlet, rather than before it. This can lead to serious problems.

The viscosity of water–acetonitrile mixtures is bi-modal as Fig. 2 shows. Viscosity reaches a maximum at 10% (v/v) acetonitrile and decreases non-linearly to a minimum at approximately 70% (v/v) acetonitrile. The viscosity then increases to another smaller maximum at about 90% (v/v) acetonitrile and then decreases to the viscosity of pure acetonitrile. Viscosity in the water–acetonitrile system becomes more nearly a linear function of composition at higher temperatures, as Fig. 2 shows. It may, therefore, be preferable, from the point of view of the third term of the Van Deemter equation, to operate at a temperature above ambient where diffusion is facilitated and where the diffusion constants in the chromatograph are more nearly

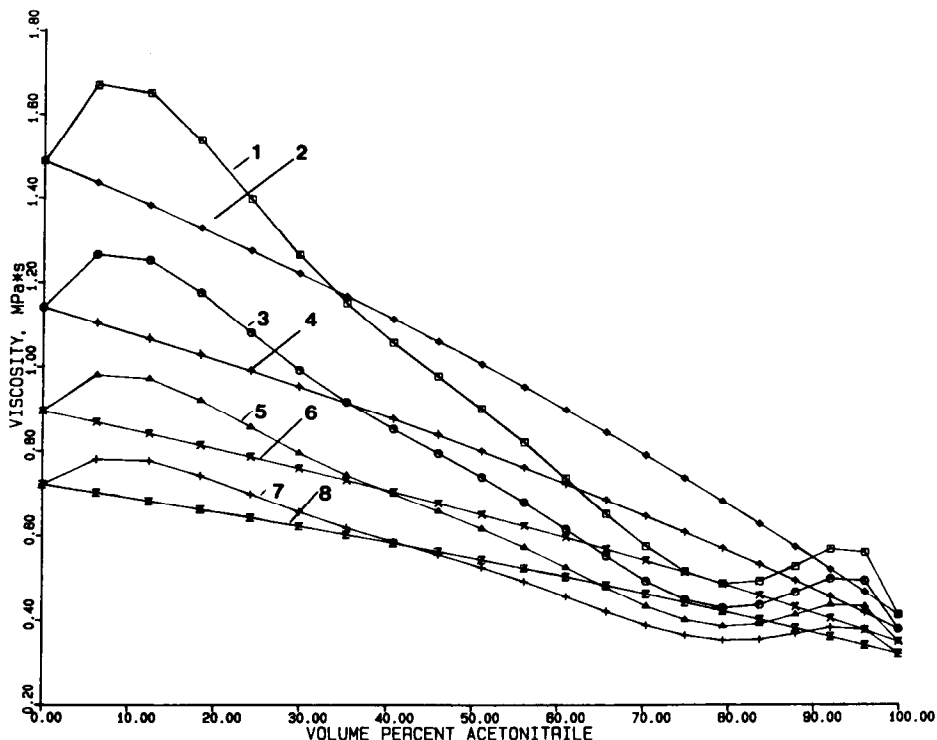


Fig. 2. Viscosity of water-acetonitrile mixtures as functions of temperature and composition. Experimental data courtesy of Moreau and Douheret¹⁵, interpolated by McCown; theoretical data are linear predictions based on mole fraction composition. Curve 1: experimental density data at 5°C. Curve 2: linearly-predicted density data at 5°C. Curve 3: experimental density data at 15°C. Curve 4: linearly-predicted density data at 15°C. Curve 5: experimental density data at 25°C. Curve 6: linearly-predicted density data at 25°C. Curve 7: experimental density data at 35°C. Curve 8: linearly-predicted density data at 35°C.

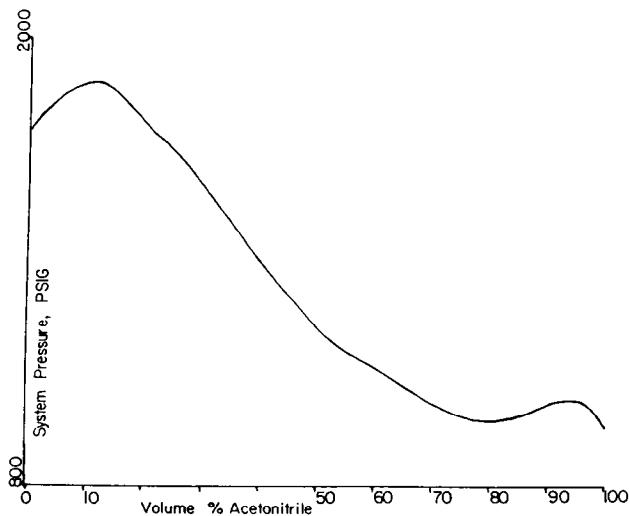


Fig. 3. Pump pressure profile in gradient liquid chromatography. Pumps: Beckman Model 100A. Controller: Beckman Model 421. Mixer: Beckman 2.8 ml internal volume, dynamic. Column: Beckman Ultrasphere ODS, 250 × 4.6 mm. Recorder: Beckman/Kipp. Mobile phase: A = water, B = acetonitrile. Gradient: 0 to 100%B in 20 min. Flow-rate: 1.0 ml/min.

constant. Diffusion constants typically exhibit an inverse relationship to viscosity¹⁶; consequently, temperature control at slightly elevated temperatures will have the effects of regularising and narrowing the shape of chromatographic peaks. Practical considerations dictate that the thermostating temperature should be 10–15°C above ambient for control, and the entire chemical system of the chromatograph should be thermostatted.

Influence of composition on pressure and temperature changes

The formation of mixtures of water and acetonitrile involves the liberation of some heat at some temperatures and compositions. A very considerable amount of heat is consumed in the formation of most compositions of water and acetonitrile. These temperature changes arise from the “excess enthalpy of mixing” of water and acetonitrile. Morcom and Smith¹⁷ present mixing enthalpies for water and acetonitrile at three temperatures and ambient pressure. Reddy¹⁸ extends the temperature range of the available data, and, in passing, makes the point that mixing enthalpy is a function of composition and temperature only at low to moderate pressures. Goetze and Schneider¹⁹ present data which extend the pressure range beyond that usually encountered in HPLC.

Mixing water and acetonitrile is, by chromatographic standards, an energetic operation. It is necessary, for example, to supply about 1100 J/g formula weight of mixture to keep the mixture at 25°C when making an acetonitrile–water (70:30) mixture from components at 25°C.

The sign of the system’s excess enthalpy is not even consistently positive¹⁷: there is a small exotherm in the 0–6 mole% range at temperatures below 35°C (Fig. 4). This exotherm is limited to the 0–4 mole% range at 25°C and disappears altogether at 35°C. These data can be used to calculate the heat flux in the mixer of the chromatograph by the product of molar excess enthalpy on mole flow-rate:

$$Q(T,P,X) = F_m(X) \cdot H_m(T,P,X)$$

where $Q(T,P,X)$ is the heat flux in J/min at constant temperature, constant pressure and constant composition, $F_m(X)$ is the mole flow-rate at constant composition and $H_m(T,P,X)$ is molar excess enthalpy at pressure P , temperature T and composition X . The results of typical calculations can be seen in Table I and Fig. 5. Positive values mean that heat must be supplied (in the indicated quantity) in order to keep the mixture at the temperature of the starting components. Negative values mean that heat must be removed to keep the mixture’s temperature from rising. The numbers shown here are for atmospheric pressure mixing, with the components at 25°C. The numbers will be different for other starting temperatures¹⁷ or for mixing at other pressures¹⁹.

If the heat flux is divided by the heat capacity of the mixture produced, it is possible to calculate the expected temperature change in °C/min (Fig. 6). The temperature of the mobile phase will decrease (in this case), and continue to drop until the endotherm is balanced or offset by heat input from the environment.

The mixer in the liquid chromatograph is, therefore, in a continuous state of thermal non-equilibrium. The mixture, forming continuously, liberates a certain amount of heat to the surrounding chamber or tubing. This heat must be removed

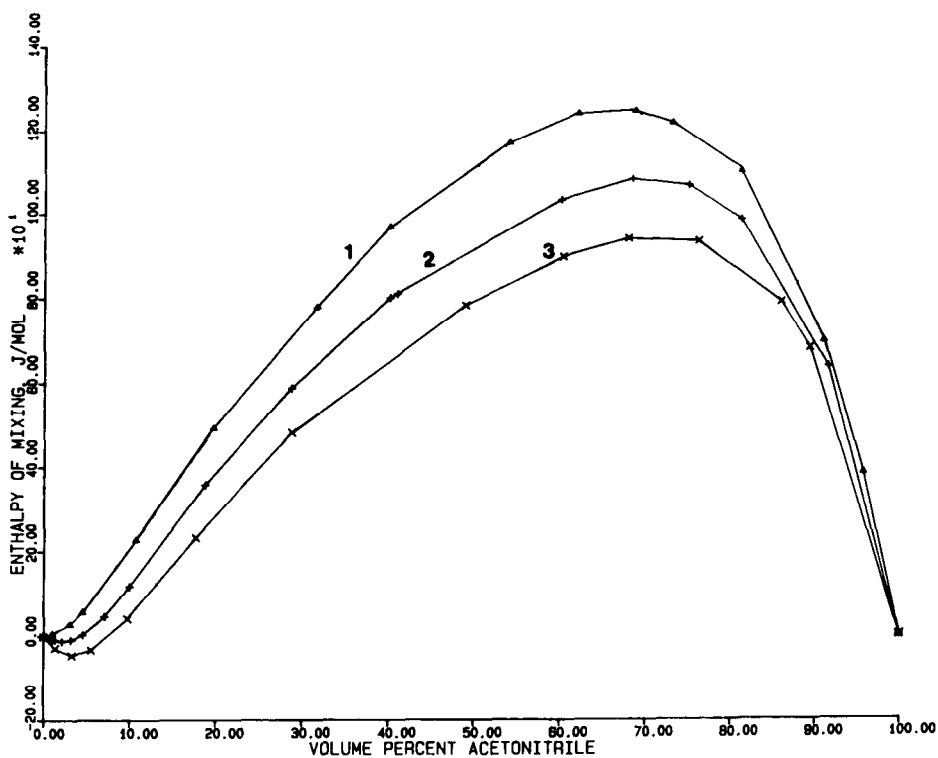


Fig. 4. Excess enthalpy of water-acetonitrile mixtures. Experimental data courtesy of Morcom and Smith¹⁷, interpolated by McCown. Curve 1: excess enthalpy, 35°C. Curve 2: excess enthalpy, 25°C. Curve 3: excess enthalpy, 15°C.

to keep it from disturbing the pseudo-equilibrium of the column. It is preferable to liberate and remove the heat of mixing at one point in the chromatograph, so complete mixing must occur in the smallest possible volume at the summing node (the place where the pumps are hydraulically joined).

Failure to establish isothermal conditions at the mixer can lead to the appearance of anomalous peaks at the system's exchange volume. The width of such peaks is usually about equal to the mixer's clearing volume (approximately three times its nominal volume). These peaks are usually due to the elution of a segmented band of mobile phase whose temperature is markedly different from that of the surrounding liquid. The refractive index of this solvent band is partially responsible for the apparent absorbance effect. The balance of the observed peak is due to a true absorbance effect attributable to the thermally induced change in density in the band. This effect was observed by the authors in their laboratories.

The retention time of the "ghost" peak could be changed by changing the flow-rate. Its area remained unchanged by this change, but its width changed inversely with flow-rate. The peak area was proportional to the integral of heat flux calculated for the flow-rate and rate of change of composition. The peak could be made to disappear by placing a 35°C heater between the mixer and the column.

TABLE I
HEAT FLUX DUE TO MIXING IN THE WATER-ACETONITRILE SYSTEM

Acetonitrile (%, v/v)	Acetonitrile (mole fraction)	Formula density (25.15°C)	Molar excess enthalpy (25.15°C)	Heat flux (J/min)											
				Volumetric flow-rate (ml/min)											
				0.10	0.25	0.50	0.7	1.00	1.25	1.50					
0	0	0.9971	0	0	0	0	0	0	0	0	0	0	0	0	0
2.3	0.0081	0.9939	-6.90	-0.04	-0.09	-0.19	-0.2	-0.38	-0.47	-0.57					
2.9	0.0104	0.9930	-8.10	-0.04	-0.11	-0.22	-0.3	-0.44	-0.55	-0.66					
4.8	0.0168	0.9904	-11.30	-0.06	-0.15	-0.30	-0.4	-0.61	-0.76	-0.91					
5.0	0.0177	0.9901	-11.01	-0.06	-0.15	-0.30	-0.4	-0.59	-0.74	-0.89					
11.6	0.0250	0.9872	-8.50	-0.05	-0.11	-0.23	-0.3	-0.45	-0.56	-0.68					
14.5	0.0360	0.9828	4.10	0.02	0.05	0.11	0.1	0.21	0.27	0.32					
10.0	0.0366	0.9826	5.29	0.03	0.07	0.14	0.2	0.28	0.34	0.41					
14.9	0.0566	0.9753	47.30	0.24	0.60	1.19	1.7	2.39	2.99	3.58					
15.0	0.0569	0.9752	48.05	0.24	0.61	1.21	1.8	2.43	3.03	3.64					
20.0	0.0787	0.9868	113.53	0.57	1.41	2.83	4.2	5.65	7.07	8.48					
20.3	0.0803	0.9848	118.40	0.59	1.47	2.94	4.4	5.87	7.34	8.81					
25.0	0.1022	0.9578	189.74	0.89	2.23	4.46	6.7	8.93	11.16	13.39					
30.0	0.1277	0.9482	272.65	1.23	3.09	6.17	9.2	12.34	15.43	18.52					
34.7	0.1538	0.9386	357.60	1.56	3.89	7.79	11.6	15.58	19.47	23.37					

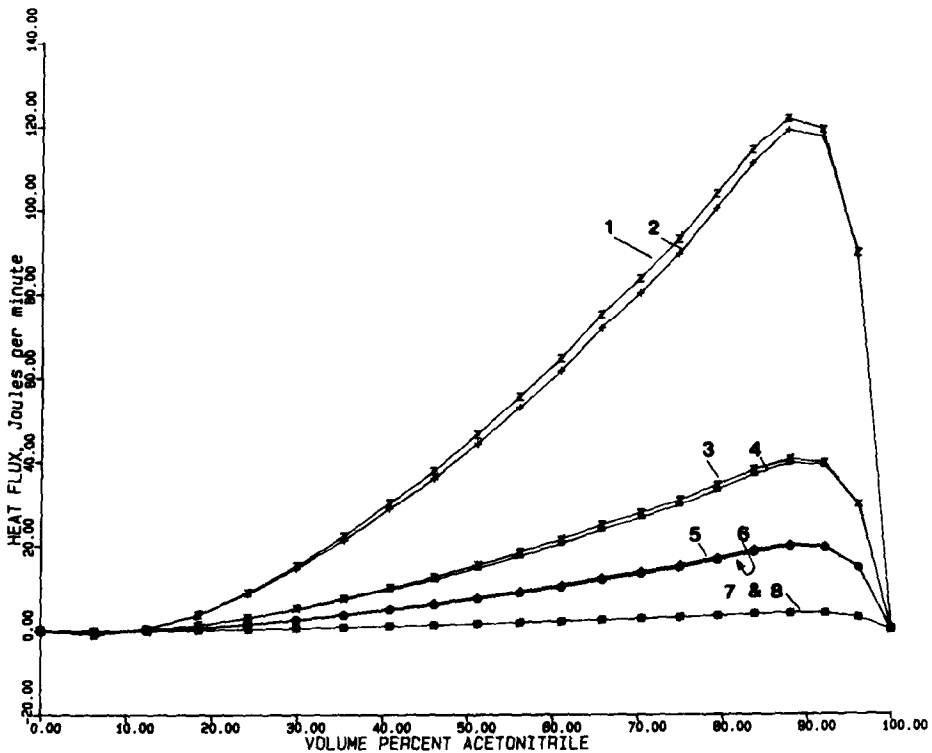


Fig. 5. Heat flux at 25°C due to mixing of water and acetonitrile at various flow-rates and compositions. Curve 1: flow-rate = 3.0 ml/min. Experimental density data. Curve 2: flow-rate = 3.0 ml/min. Linearly-predicted density data. Curve 3: flow-rate = 1.0 ml/min. Experimental density data. Curve 4: flow-rate = 1.0 ml/min. Linearly-predicted density data. Curve 5: flow-rate = 0.5 ml/min. Experimental density data. Curve 6: flow-rate = 0.5 ml/min. Linearly-predicted density data. Curve 7: flow-rate = 0.1 ml/min. Experimental density data. Curve 8: flow-rate = 0.1 ml/min. Linearly-predicted density data.

Excess heat capacity

The heat capacity of the water–acetonitrile system deviates markedly from expectation. Partial molar heat capacities for water and acetonitrile in mixtures at 25°C have been reported by Benson *et al.*²⁰. Isentropic compressibilities were reported in the same work. Partial molar heat capacities were also reported by De Visser *et al.*²¹.

The true mixture heat capacity (expected + excess, Table II) can be used to predict the temperature of the mixture in the familiar way. The results can then be used to predict the capacity of heat exchanger required to maintain isotherm at a given temperature when mixtures are formed.

A related property, thermal conductivity of water–acetonitrile mixtures was estimated by the method of Li (see ref. 22):

$$TC_{\text{mix}} = \sum_i \sum_j f_i \cdot f_j \cdot TC_{ij}$$

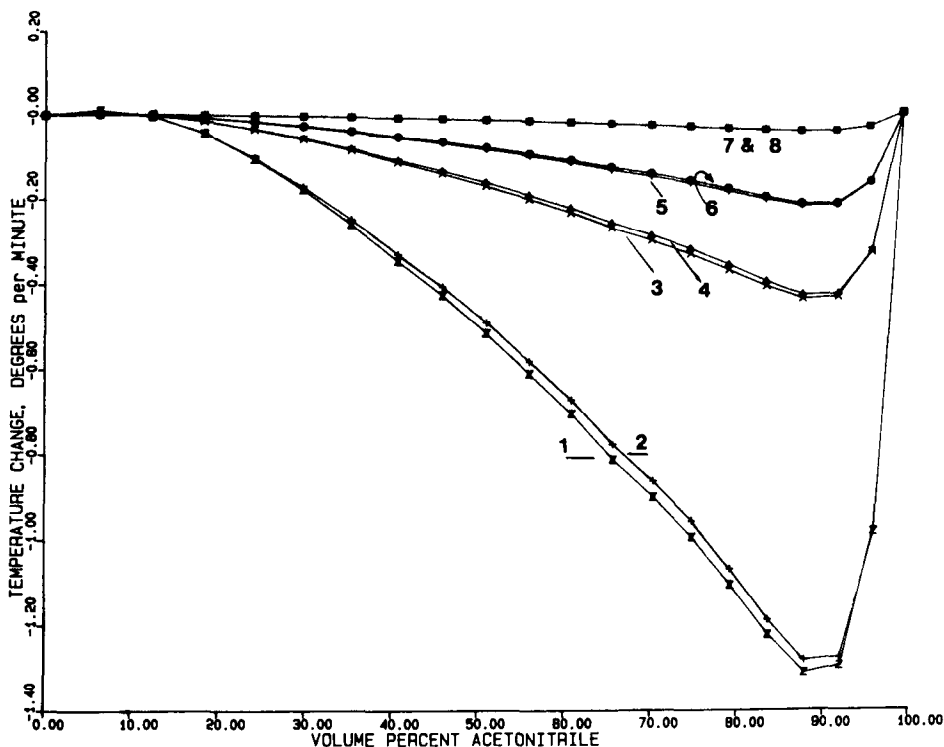


Fig. 6. Rate of temperature change due to mixing enthalpy at various flow-rates and compositions. Curve 1: flow-rate = 3.0 ml/min. Experimental density data at 25°C. Curve 2: flow-rate = 3.0 ml/min. Linearly-predicted density data at 25°C. Curve 3: flow-rate = 1.0 ml/min. Experimental density data at 25°C. Curve 4: flow-rate = 1.0 ml/min. Linearly-predicted density data at 25°C. Curve 5: flow-rate = 0.5 ml/min. Experimental density data at 25°C. Curve 6: flow-rate = 0.5 ml/min. Linearly-predicted density data at 25°C. Curve 7: flow-rate = 0.1 ml/min. Experimental density data at 25°C. Curve 8: flow-rate = 0.1 ml/min. Linearly-predicted density data at 25°C.

where f_i is the volume fraction of i in the mixture, f_j is the volume fraction of j in the mixture, and TC_{ij} is defined:

$$TC_{ij} = 1/2(1/TC_i + 1/TC_j)$$

where TC_i is the thermal conductivity of i and TC_j is the thermal conductivity of j . The method is stated to be accurate to about 10%. Results of typical calculations can be seen in Table III.

CHANGES IN MEASURED FLOW AT CONSTANT TOTAL PUMP OUTPUT

It has been observed that even pumps which are well-calibrated and deliver perfectly linearly over the anticipated flow-rates of the gradient do not deliver the volume of a mixture expected from the sum of their outputs. The excess can be predicted from the excess volume of the mixture. A useful tabulation of excess

TABLE II
MIXTURE HEAT CAPACITY (J/mole)

<i>Acetonitrile</i> (%, v/v)	<i>Water</i> (mole fraction)	<i>Acetonitrile</i> (mole fraction)	<i>Excess capacity,</i> <i>mixture</i>	<i>Total capacity,</i> <i>mixture</i>
0	1.0000	0	0	75.29
5.0	0.9606	0.0394	2.94	78.85
10.0	0.9202	0.0798	4.24	80.79
15.0	0.8791	0.1209	4.83	82.02
20.0	0.8370	0.1630	5.28	83.15
25.0	0.7938	0.2062	5.82	84.37
30.0	0.7497	0.2503	6.41	85.65
35.0	0.7045	0.2955	7.02	86.97
40.0	0.6581	0.3419	7.51	88.19
45.0	0.6107	0.3893	8.03	89.46
50.0	0.5620	0.4380	8.22	90.42
55.0	0.5123	0.4877	8.30	91.28
60.0	0.4612	0.5388	8.18	91.97
65.0	0.4080	0.5920	8.00	92.63
70.0	0.3546	0.6454	7.64	93.11
75.0	0.2996	0.7004	7.04	93.38
80.0	0.2429	0.7571	6.11	93.34
85.0	0.1846	0.8154	4.69	92.84
90.0	0.1248	0.8752	2.94	92.03
95.0	0.0633	0.9367	1.17	91.23
100.0	0	1.0000	0	91.06

TABLE III
MIXTURE THERMAL CONDUCTIVITY AT 25°C (J/mm · s · K)

<i>Acetonitrile</i> (%, v/v)	<i>Water</i> (mole fraction)	<i>Acetonitrile</i> (mole fraction)	<i>Thermal conductivity</i> <i>at 25°C</i>
0	1.0000	0	3.00
5.0	0.9606	0.0394	3.03
10.0	0.9202	0.0798	3.06
15.0	0.8791	0.1209	3.10
20.0	0.8370	0.1630	3.13
25.0	0.7938	0.2062	3.17
30.0	0.7497	0.2503	3.20
35.0	0.7045	0.2955	3.24
40.0	0.6581	0.3419	3.28
45.0	0.6107	0.3893	3.32
50.0	0.5620	0.4380	3.35
55.0	0.5123	0.4877	3.39
60.0	0.4612	0.5388	3.43
75.0	0.2996	0.7004	3.55
80.0	0.2429	0.7571	3.59
85.0	0.1846	0.8154	3.63
90.0	0.1248	0.8752	3.67
95.0	0.0633	0.9367	3.71
100.0	0	1.0000	3.75

density (hence excess volume) of water–acetonitrile mixtures is given by Moreau and Douheret¹⁵. Details of the effect of pressure upon excess properties are given in the work by Goetze and Schneider¹⁹.

Implicit in the pressure dependence of excess volume is a pressure distortion of an already-distorted linear velocity profile. To begin with, the volumes pumped by each pump in a multi-pump gradient system are not additive. The pressure gradient from column inlet to outlet is often assumed to be linear from the inlet pressure to atmosphere pressure, ± 50 p.s.i. More often, flow-rates are roughly proportional to $\Delta P^{3/2}$, where $\Delta P = P_i - P_0$, P_i is the inlet (head) pressure and P_0 is the outlet pressure.

Consequently, the volumetric flow-rate must be correct at the summing node or mixer, or at the column inlet, or at the column outlet. In the case of water–acetonitrile systems, if the mixer input flow-rate is correct, the mixer output flow-rate will be low due to the excess volume of mixing. A 1–2% error would be typical, but the extent of the error depends on the composition and pressure. The volume change caused by temperature changes further exacerbates the situation. Depending on the composition and endotherm, the net error can be 3–5% in a water–acetonitrile system. Then, because the excess volume of water–acetonitrile systems varies inversely with pressure, the column outlet flow-rate will be greater than the inlet flow-rate. If the walls of the column are inelastic, the change in volume must result in a change in linear velocity. Because excess volume is not a linear function of composition, we can expect the linear velocity of the system to reach a maximum governed by the volumetric flow-rate, the system pressure, the solvents' temperatures, and the mobile phase composition.

COLLIGATIVE PROPERTIES

The net effects of mixing water and acetonitrile on the refractive index of the mixture are predictable from the foregoing; however, we note that Moreau and Douheret²³ tabulate values of the refractive index of water–acetonitrile mixtures as a function of composition at several temperatures, together with dielectric constants and dipole moments. The non-linearity of dielectric constants in water–acetonitrile mixtures helps explain the difficulty of using gradient elution with electrochemical detection. The purity of the acetonitrile explains why some chromatographers find it impossible to “zero” their electrochemical detectors.

Fig. 7 shows that the refractive indices (589.3 nm) of the water–acetonitrile system are influenced by changes in temperature. The Eyckman equation provides a means of calculating the refractive index of a particular mixture at reasonable temperatures from information more readily available, *viz.* temperature and density:

$$dn/dT = c \cdot [(\eta + 0.4)/(2\eta + c\rho)] (d\rho/dT)$$

where n is refractive index, T is temperature, ρ is density, $(d\rho/dT)$ is the temperature dependence of density, and

$$c = (\eta^2 - 1)/(\eta + 0.4) \cdot \rho$$

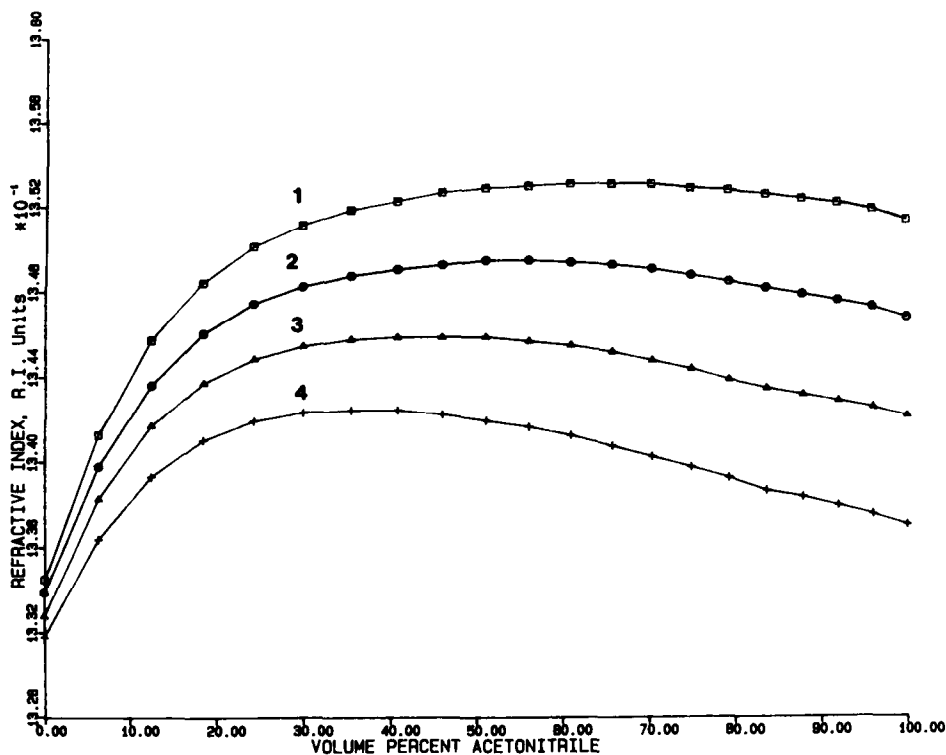


Fig. 7. Refractive indices of water-acetonitrile mixtures. Data courtesy of Moreau and Douheret²³, re-interpolated by McCown. Curve 1: 5°C. Curve 2: 15°C. Curve 3: 25°C. Curve 4: 35°C.

Refractive indices are also dependent upon the wavelengths at which they are observed, as the following equation demonstrates:

$$\eta_{\lambda} = A + B/\lambda^2 + C/\lambda^4$$

where λ is wavelength and A, B and C are constants evaluated by solving the equation simultaneously at three wavelengths. The only stipulation is that the absorbance of the solvent must be small at the three wavelengths chosen.

The literature values²⁴ of the refractive index of acetonitrile at several appropriate wavelengths are given in Table IV. No similar values for water-acetonitrile

TABLE IV

LITERATURE VALUES OF REFRACTIVE INDEX AT FOUR WAVELENGTHS

Wavelength (nm)	Refractive index*
656.3	1.34427
589.3	1.34596
486.15	1.35004
434.06	1.35333

* Measured at 16°C²⁴.

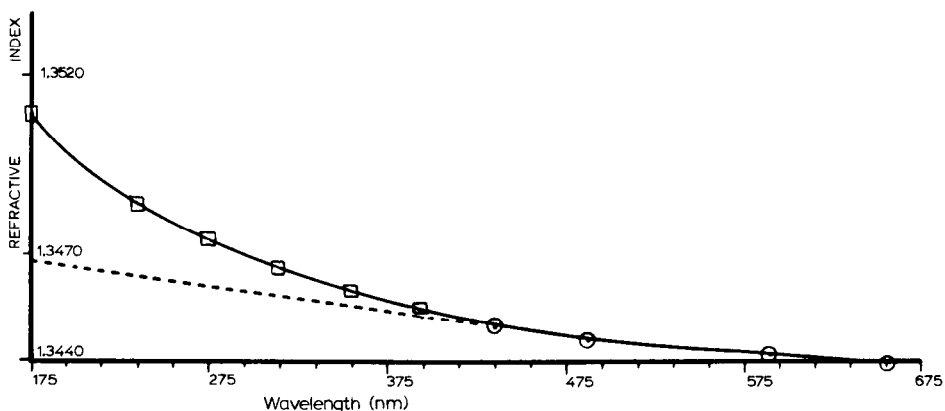


Fig. 8. Refractive index of acetonitrile as a function of wavelength. This figure was obtained by the extrapolation of the fourth order polynomial (see text) fitted to the four points given in Table IV. ○: Measured value; □: 2nd-order least-squares extrapolation.

mixtures have been found in the literature from 1967 to the present. It is clear, however, from an inspection of the equation, that the refractive index of the mixture must be inversely proportional to the wavelength at which it is measured. The importance of this is that refractive changes which at 254 nm might be unimportant will assume considerable importance at shorter wavelengths. The refractive changes become, however, indistinguishable from absorptive changes at extremely short wavelengths. Fig. 8 shows the change in the refractive index of acetonitrile as a function of wavelength.

THE INFLUENCE OF THERMODYNAMIC CHANGES ON ABSORBANCE

The net effect of mixing water and acetonitrile on the spectral properties of the mixture is complex. From the foregoing, it can be seen that the non-linear composition–density curve of water–acetonitrile mixtures will affect the spectra. Since in HPLC these mixtures are not allowed to reach equilibrium, but rather pumped down the column to the detector, the number of molecules of the absorbing (in this case, the acetonitrile) species present in the detector cell is influenced by the mixture's temperature and pressure, as well as its density. The fact that all of these conditions are changing at the same time makes attribution of an effect to a single cause difficult. The isothermal composition–density curves, the isocratic temperature–density relationships deducible from them, and the isentropic compressibility of the mixture combine to indicate that under ordinary HPLC conditions, there will be more acetonitrile in the detector cell than linear interpolation would predict. Furthermore, the excess of acetonitrile is not linear with composition. The absorbance of water being small by comparison to the absorbance of the mixture at most wavelengths, it is only surprising that no more serious baseline anomalies have been reported. We conclude that as one uses shorter wavelengths for detection, the physical occurrences one may not observe at longer wavelengths will make themselves manifest.

The addition of the column into these considerations indicates that the solvent will probably not have attained an isotherm by the time it reaches the detector, and

it will have the same temperature as the detector only by coincidence unless the entire separating and detecting portion of the chromatograph is thermostatted.

GAS SOLUBILITY

Solubilities of gases in water have been reported with greater or less reliability by various authors. The best review of the available data, together with an evaluation of their reliability, is that of Battino and Clever²⁵. The solubility of gases in liquid is of direct concern to the liquid chromatographer for at least three reasons, *viz.*:

- (1) cavitation in the liquid head of the pump during refill;
- (2) enucleation on or in the detector cell, with its attendant effect; and
- (3) changes in background absorbance due to changing gas concentrations in the mobile phase.

Yet other reasons for concern include the possible decomposition of certain labile samples and the alteration of spectra, especially due to the presence of oxygen. The authors were unable to find references, in the open literature, to the solubility of gases in water-acetonitrile mixtures. That notwithstanding, Bakalyar concluded:

- (1) solubility of molecular gases is greater than that of atomic gases;
- (2) solubility is inversely proportional to temperature; and
- (3) solubility is greater in the organic phase than in the water.

We expect the water-acetonitrile system to prove no exception.

The chromatographer is variously enjoined to de-gas or not to de-gas, depending on the question he asks. Modern reciprocating pumps usually have little trouble filling with air-saturated solvents, but some have difficulty priming themselves or clearing bubbles from the liquid head. A more persuasive reason to de-gas is that air-saturated mobile phases tend to form bubbles in the detector cell and sometimes in the column itself.

The solubility of air gases (especially N₂ and O₂) in mixtures is not a linear function of composition in the two bulk components; in fact, it appears to be sharply lower in the mixture than in the pure organic phase. As a result, the mixture made with air-saturated components will very likely be super-saturated at the pressure of the column. It follows, therefore, that as soon as the pressure drops enough, bubbles will form, usually in the flow cell.

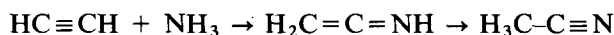
The presence of dissolved gases in the solvent leads to increased background in most detectors. This background is most noticeable (in an absorbance detector) at short wavelengths and high sensitivity, where it leads to higher limits of detection. It is implied in the literature of some manufacturers, and in their technical advice, that de-gassing is a "once-a-day" operation, and that no significant deterioration of solvent transparency can occur before the passage of about twenty-four hours. This advice is purely notional. It is intuitively clear that the process of re-dissolution begins, in earnest, as soon as the de-gassing process ends. Consequently, vacuum de-gassing and boiling the mobile phase are processes of little practical use, as they must be stopped before separations can be made. Ultrasound is presently too costly for routine application here, apart from which it does not appear to be as effective as sparging. The merits and shortcomings of various de-gassing procedures were discussed by Snyder⁹. The consensus is that de-gassing is worthwhile for several reasons, but only if it is carried out in a way which does not affect the intended

composition of the mobile phase and if it is continued throughout the analytical day in order to avoid the return of the problem. The authors prefer sparging with helium at 20–50 ml/min.

THE MANUFACTURE AND PURIFICATION OF ACETONITRILE

The origin of many of the impurities to be found in commercially available acetonitrile is in the method of its manufacture. The first commercial production of acetonitrile took place in 1952. Considerable effort has been expended in the purification of the solvent, but the consensus appears to be that one must suit the purification to the end use.

There are three processes for the preparation of acetonitrile reported in the literature. The first was reported in a German patent²⁶ and involves the catalysed reaction of ammonia with acetylene. March²⁷ says that the mechanism involves the intermediate formation of the enamine which can tautomerise to the imine. This mechanism could explain the presence of late-eluting compounds if the ethyleneimine produced as an intermediate self-coupled. Such an event would produce compounds which would be highly retained in reversed-phase systems. This process was used until the development of the SOHIO process (for the manufacture of acrylonitrile, *vide infra*). The mechanism given by March for the reaction of ammonia with acetylene is shown here, both for interest and because the mechanism of the SOHIO acrylonitrile process would appear to be similar:



This method is no longer used in the United States because of the availability of acetonitrile as a side product from the cheaper SOHIO process.

The method by which all of the HPLC-grade acetonitrile in the United States is produced, is the SOHIO process. Acetonitrile is produced as a by-product of the manufacture of acrylonitrile by the catalysed reaction of ammonia with propylene. The acetonitrile appears to be produced from the ethylene present in the propylene, and by cracking. Although the Monsanto Corporation produces acetonitrile, only E. I. DuPont and SOHIO Chemicals (formerly Vistron) supply the chromatographic demand²⁸.

DISCUSSION

The non-ideal properties of binary mixtures can exhibit considerable influence on the performance of a liquid chromatograph. Some of the properties of water–acetonitrile mixtures are reviewed here. Chief among the properties of water–acetonitrile mixtures which affect the chromatograph is the excess enthalpy of mixing, which, in the case of water–acetonitrile mixtures, is not even monomodal at low concentrations of acetonitrile. Typical evolved energies here are orders of magnitude greater than typical differences in standard free energy changes of elution, which means that the formation of a mobile phase mixture evolves enough energy to alter a separation. Because mixing enthalpy is both composition and temperature dependent, a separation which is partly produced by an enthalpic temperature change will

be affected by a change in the temperature of the solvent reservoirs, quite apart from the distortion of the analyte's band shape which results from thermal instability in the column, as Part II²⁹ will show.

Enthalpic temperature changes also affect the mean linear velocity of the mobile phase, but not in a linear way. Density of polar mixtures is not a linear function of composition, nor of temperature, so the effect of composition upon flow-rate is complex. Because the net effect of composition upon flow-rate includes a double dependence upon temperature (for excess enthalpy of mixing and for density), the flow-rate is expected to be bimodal around the expected value.

The better to deal with the effects of non-ideal properties of mixtures, we introduce the mole flow-rate concept. Its use in determining the heat flux and temperature change resulting from the formation of mixtures has been shown.

ACKNOWLEDGEMENTS

The works of Morcom and Smith, Moreau and Douheret, and Goetze and Schneider to which we refer in the bibliography have been the *sine qua non* of our work. We are grateful for their interest in our work and their willingness to allow us to use their data.

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