

Measurement of Binary Diffusion Coefficients of Compounds at Infinite Dilution in Water as a Function of Temperature

Using Teledyne Isco Syringe Pumps



Syringe Pump Application Note AN26

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Introduction

Biomass substrates and other natural products comprise a number of biological solutes such as sugars, antioxidants, vitamins, proteins, and more. Such biological solutes are significant for application as alternative agents in food, pharmaceutical, and fuel industries. The extraction of such biological solutes from the natural sources using a “green” sustainable solvent such as water requires the knowledge of their thermodynamic and mass transfer properties as a function of temperature. However, such data is insufficient and in some cases, even non-existent in the literature. A flow apparatus developed using a **Teledyne Isco high precision syringe pump** at constant flow rate, a Rheodyne six-port injector, empty stainless steel tubing as a column, a constant temperature oven, and an absorbance detector, was used to measure the elution profiles of certain flavonoids and carbochemicals in water as a function of temperature, using the principle of chromatographic peak broadening. The solutes under study are extremely sensitive to such environmental factors as heat, light, and oxygen. The chromatographic peak broadening technique, or the Taylor-Aris dispersion technique, has been effectively used to determine the diffusion coefficients of the flavonoids in water until around 413 K, and of the carbochemicals in water until 473 K. The detection of the elution peaks for these compounds is based on their absorbance in UV and visible spectral wavelengths set in the absorbance detector.

Experimental Method

The experimental apparatus for measuring the diffusion coefficients of biological solutes in water as a function of temperature using the Taylor-Aris dispersion technique is shown in Figure 1 [1]. A stainless steel 316 tube (0.159 cm o.d. x 0.0228 cm i.d. x 3048 cm length; Waters Corporation, Milford, MA, USA) was placed in a constant temperature HP Model 5890 oven and coiled to form a coiled column with a diameter of 25 cm. Solute solutions (mole fraction of 10^{-4} to 10^{-5} in water) were made up with the solvent and injected into the apparatus using a Rheodyne Model 7725i injector (Upchurch Scientific, Oak Harbor, WA, USA) through a 20 μ L sample loop. A very precise delivery of solvent was achieved through use of a **Teledyne Isco 500D syringe pump** (Lincoln, NE, USA) at a constant flow rate of 0.1 mL/min. The solvent was initially preheated inside the oven to the set experimental temperature before a solute is injected into the column. The injector and a small amount of tubing held outside the constant temperature oven were completely insulated to prevent any thermal effects, i.e.

secondary dispersion. The solutes used in this study included phenolic compounds such as gallic acid monohydrate, protocatechuic acid, (+)-catechin hydrate, quercetin-3- β -D-glucoside, peonidin-3, O-glucoside and malvidin-3, 5-diglucoside, and carbochemicals such as furfural, 5-hydroxymethyl furfural (HMF), 2-furoic acid, vanillin, and syringaldehyde.

Water, degassed under vacuum to remove oxygen bubbles, was used as a solvent in the study. However, for determination of binary diffusion coefficients of the flavonoids in water, the solvent was spiked with 0.5% (v/v) with formic acid to reduce the tailing of the concentration profile of the solute caused by the adsorption of these highly hydroxylated compounds on the inner surface of the narrow bore column. The addition of the small quantity of formic acid also acted as a pH buffer (2.75-3.00 units) to maintain the anthocyanins in their flavylium cation form, which has greater thermal stability [2].

The solute solution effectively diffused in water as a function of the set experimental temperature, and exited the column to be detected using an absorbance detector (Dionex Corporation, Sunnyvale, CA, USA, Mode: AD-20). The output signal from the absorbance detector was converted to a digital signal using a Cole Parmer 18200-00 analog input module (Vernon Hills, IL, USA) and the chromatographic peak was recorded on a laptop module using Tracer DAQ software (Cole Parmer Instrument Company, Vernon Hills, IL, ver. 1.8.3). Different wavelengths were selected for different compounds based on their maximum absorptivity at such low concentrations in water. An adjustable back-pressure regulator (Upchurch Scientific, Oak Harbor, WA, USA, P/N#: P-880), rated to pressures between 2000 - 5000 psi, was attached to the exit of the absorbance detector to prevent flashing of water to steam at high temperatures (≥ 373 K). The temperatures at the injector and on the column were accurately measured using J-type thermocouples reading signal output via a Cole Parmer 18200-40 thermocouple module (Vernon Hills, IL, USA). Data indicated the variation in temperature of the oven and the column to be within a range of ± 0.5 K.

The system pressure and solvent flow rate was recorded and maintained using an **Isco SFX 200 controller module**. Studies have indicated that most processes using subcritical water as a solvent require pressures sufficient to maintain water in a liquid state above its boiling point. The pump pressure recorded on the Isco SFX 200 controller varied between 642 psi at 298 K to 598 psi at 473 K. It should be noted that the pump pressure refers to the total pressure recorded on the Isco pump to provide a constant solvent flow rate

through the preheat tubing, the coiled tubing, exit tubing, and UV-flow cell, and through the back-pressure regulator. The slight decrease in the pump pressure recorded at higher temperatures can be attributed to a decrease in the solvent viscosity and the adjustment of the back-pressure regulator to maintain the water in its

liquid state. The peak distribution obtained at the outlet of the Taylor diffusion apparatus is used to measure the diffusion coefficient of the solute in the solvent at a particular temperature.

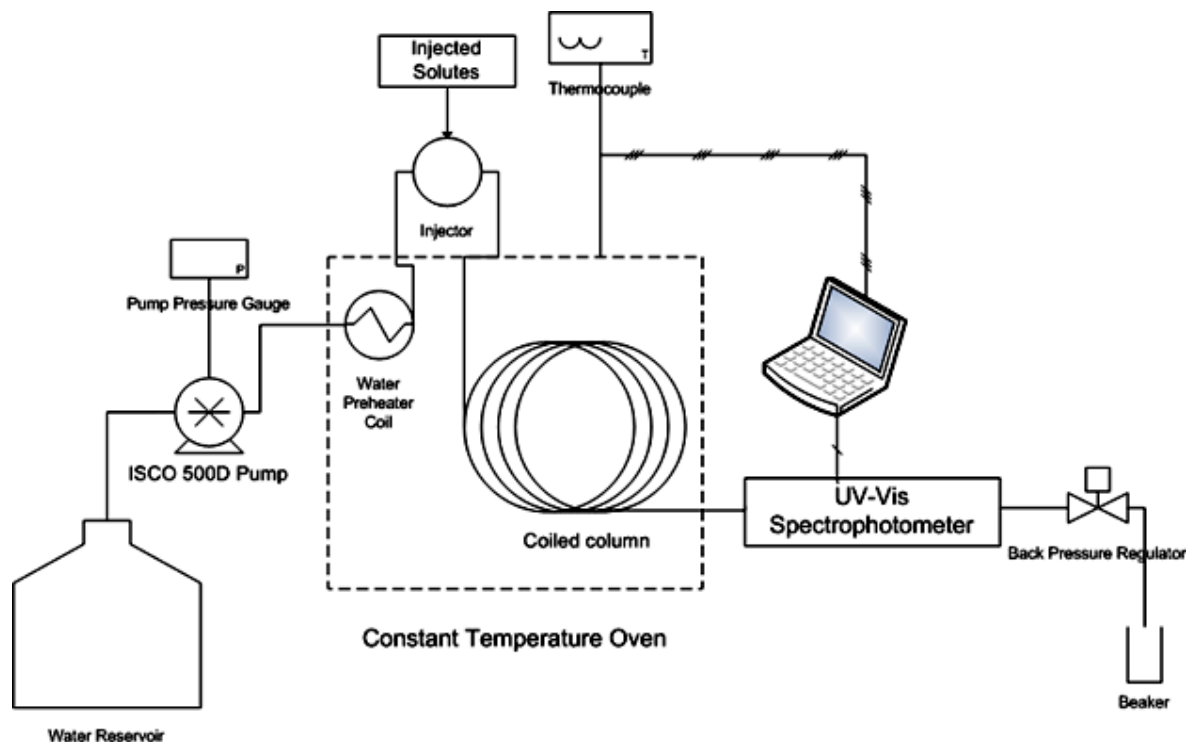


Figure 1: Apparatus for measuring the diffusion coefficients of the phenolic compounds using the Taylor – Aris dispersion method.

Theoretical Background

The dispersion of the solute pulse through the capillary tubing is influenced by molecular diffusion and, to a lesser extent, the axial bulk flow. The procedure to measure the diffusion coefficient of a solute using the chromatographic peak broadening method has been reported previously [3].

D_{eff} is the effective dispersion coefficient given by:

$$D_{eff} = D_{AB} + \frac{r_o^2 u_a^2}{48 D_{AB}} \quad (1)$$

For the concentration profile to be considered Gaussian, the value of $D_{eff} u_a L$ should be very small ($\ll 0.1$), where, r_o is the inner radius of the capillary column, L is the length of the column, D_{AB} is the diffusion coefficient of solute A in solvent B, and u_a is the solvent flow velocity through the column.

In the case of a coiled column, there is a probability of secondary flow effects in the column. In such a situation, the following condition must be satisfied for the above equation to be valid:

$$De \cdot Sc^{0.5} < 10 \quad (2)$$

where, De = Dean number and Sc = Schmidt number

$$De = \frac{\rho u_a d_{tube}}{\mu} \sqrt{\frac{d_{tube}}{d_{coil}}} \quad (3)$$

$$Sc = \frac{\mu}{\rho D_{AB}} \quad (4)$$

where, d_{tube} and d_{coil} are the inner diameters of the capillary column and the coil respectively, ρ is the density of the solvent, and μ is the dynamic viscosity of the solvent. However, some studies have reported that the secondary flow effects can be neglected as long as $De \cdot Sc^{0.5}$ is less than 18 [4].

The binary diffusion coefficient of the solutes in water was calculated from the following equation:

$$D_{AB} = \frac{u_a}{4} \left[H - \left(H^2 - \frac{r_o^2}{3} \right)^{0.5} \right] \quad (5)$$

The theoretical chromatographic plate height, HETP or H , was calculated from the peak width at half height of the Gaussian peak using the following equation:

$$H = \frac{LW_{0.5}^2}{5.545t_R^2} \quad (6)$$

where, $W_{0.5}$ is the peak width at half height of the Gaussian peak and t_R is the retention time of the solute in the column.

Results and Discussion

It was found that while the binary diffusion coefficients of the phenolic compounds in water [1] increased exponentially with temperature (Figure 2), that of the carbochemicals exhibited a linear trend as a function of temperature ($R^2 = 0.971 - 0.987$) as shown in Figure 3 [5]. Since the diffusion coefficients of the phenolic compounds in water were close to each other, the exponential trend in Figure 2 was shown by imposing a trend line ($R^2 = 0.922$) for the diffusion coefficient of malvidin-3, 5-diglucoside plotted as a function of temperature. The conditions for a Gaussian peak profile and negligible secondary flow effects, as discussed in the previous section, were maintained throughout the experiments, and the Reynolds number was found to vary between 5.0 and 50.0 over the selected temperature range.

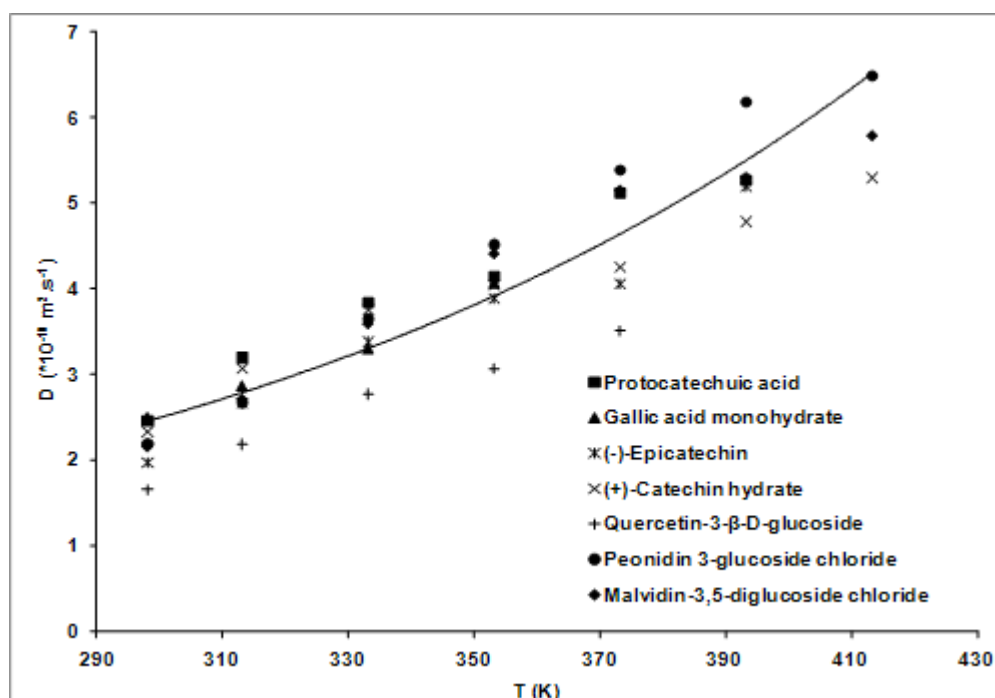


Figure 2: Variation of experimentally-measured diffusion coefficients of the phenolic compounds in water as a function of temperature.

The column used in the binary diffusion coefficient measurements was made by joining 10 stainless steel tubes of aforementioned inner and outer diameter and length of 304.8 cm with Swagelok zero-volume fittings. This was done due to the unavailability of a continuous stainless steel capillary tube of the required length and diameter specifications in the market for analytical measurements. In order to decrease the error in the

measurements, the effective internal radius of the column was calibrated by measuring the binary diffusion coefficients of known solutes such as benzene, toluene and phenol in water at 298 K, and compared with data available in literature. The effective internal radius of the column was calibrated at 0.015 cm and used in the diffusion coefficient calculations. This value of the internal column radius was slightly higher than the value quoted for the stainless steel tubing.

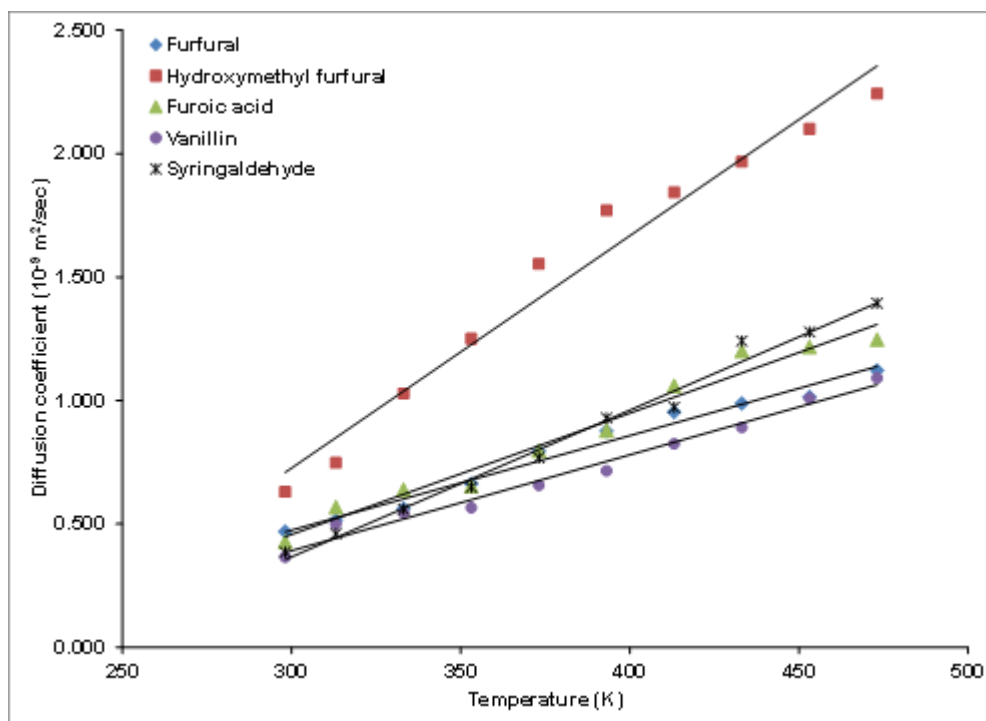


Figure 3: Variation of experimentally-measured diffusion coefficients of the carbochemicals in water as a function of temperature.

Using the effective internal radius of the column, the diffusion coefficient of phenol in water as a function of

temperature, measured using this technique, was compared with that available in the literature (Figure 4).

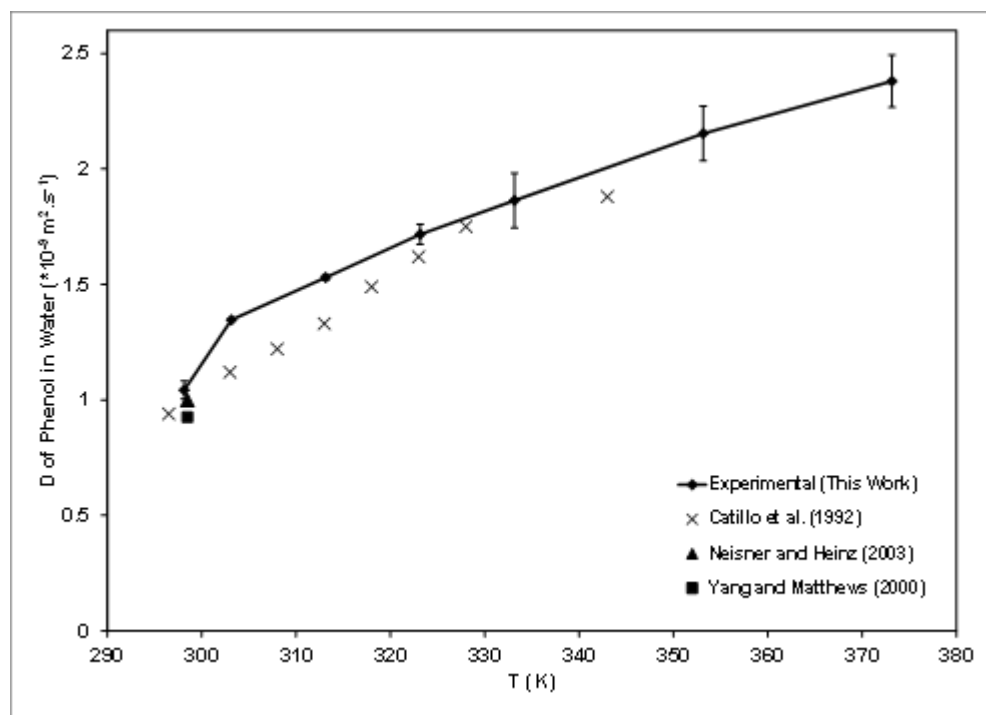


Figure 4: Comparison of the experimentally-measured diffusion coefficient of phenol in water as a function of temperature with that available in literature.

It can be seen here that the measured diffusion coefficient values of phenol showed close agreement with the diffusion coefficient values reported in the literature, lending evidence to the reliability of this measurement technique. The difference between the experimental and the literature values can be attributed to the slight tailing of the response peak due to strong solute polarity resulting in adsorption [6].

The experimentally-measured diffusion coefficients of the phenolic compounds and carbochemicals in water as a function of temperature can also be expressed in terms of solvent viscosity, and theoretical models based on thermodynamic factors can be used to make an accurate prediction of the diffusion coefficient

values of these compounds. Such calculations, and the procedure involved, have been reported in the literature [1]. It was found that the existing theoretical models based on the Stokes-Einstein equation have consistently failed to accurately predict the diffusion coefficients of these thermally sensitive compounds as a function of temperature. This is because the basic assumption of the Stokes-Einstein model is that the molecular compound is spherical in nature and its radius does not vary as a function of temperature. Although this assumption holds well for the selected biological solutes at close to ambient temperatures, it is not applicable as temperature increases above 333 K.

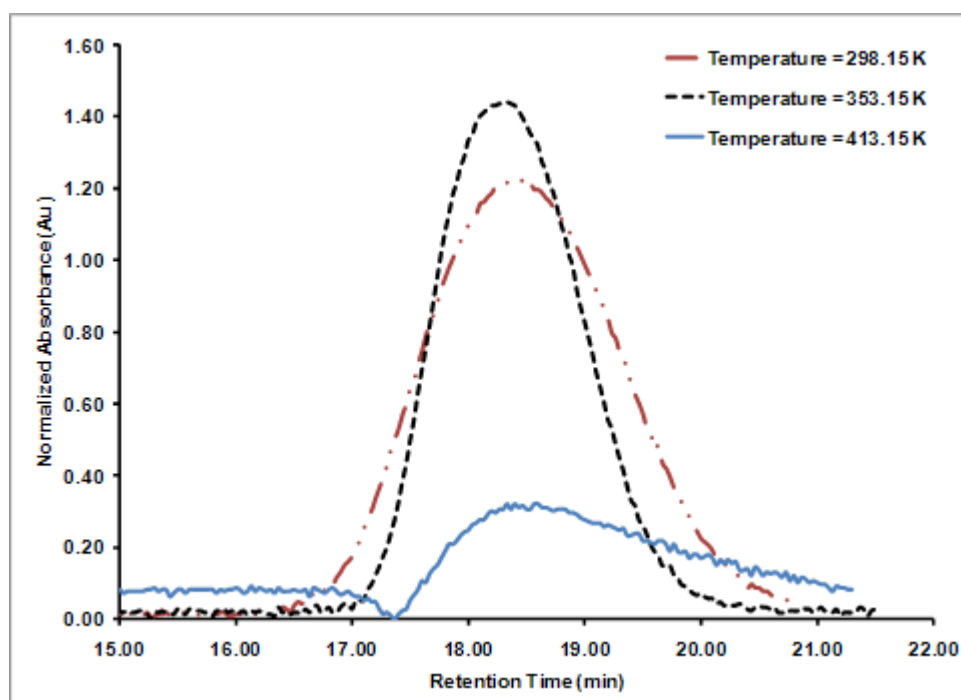


Figure 5: The peak profile response curves for protocatechuic acid measured at 280 nm at three different temperatures: 298.15 K, 353.15 K, and 413.5 K using the Taylor dispersion method

An example of thermal sensitivity of the compounds can be shown using the elution profile of protocatechuic acid at 298 K, 352 K, and 413 K as a function of its residence time along the length of the capillary column (Figure 5). Figure 5 shows that there is considerable peak broadening (peak height to width ratio) with an increase in temperature from 298 K to 352 K. However, at 413 K, there is a distortion in the response curve for protocatechuic acid in water. This peak distortion can be attributed both to adsorption effects due to solute polarity and thermal degradation caused by elevated temperature, low concentration of solute, and low solvent flow rate. Studies indicated the second reason to be more plausible, and also preventable by slightly increasing the solvent flow rate. However, it was found

that an increase in the solvent flow rate can result in an increase in the value of $De.Sc^{0.5}$ over the minimum value required to prevent secondary flow effects in the column. The study indicated that with the use of a **Teledyne Isco high precision syringe pump** at constant flow rate along with a narrow bore column, it was possible to experimentally determine the binary diffusion coefficients of various biological solutes in water as a function of temperature.

References

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