Mixture Pseudo-Chromatographic Separation by Matrix-Assisted Diffusion Ordered Spectroscopy

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Summary Pseudo-separation for phenylalanine (Phe), ibuprofen (Ibu), capsaicin (Cap) and norfloxacin (Nor) were studied by sodium dodecyl sulfate (SDS) or hydroxypropyl-β-cyclodextrin (HPβCD) assisted diffusion ordered spectroscopy (DOSY). Stokes-Einstein equation shows a linear relationship between the logarithms of apparent diffusion coefficients and logarithms of molecular weight (Mw). However, with matrix added, block factors became the important factors than molecular weight. Pseudo-separation was improved by SDS or HPβCD assist. Research showed different separation mechanism for SDS and HPβCD-assisted DOSY. The former used different lipo-hydro partition coefficient, and the latter used complexing mechanism.

Keywords: Matrix-assisted DOSY, NMR Chromatography, SDS, HPβCD.

Introduction

Nuclear magnetic resonance (NMR) is a widely used structure analysis tool in organic chemistry and pharmaceutical chemistry. However, for the absence of separation ability, NMR is limited for mixture analysis. A "hyphenated technique": LC-NMR has been led to resolve the deficiency of NMR in mixtures analysis. This powerful hyphenated technique necessitates dedicated hardware that is quite rare in organic chemistry or pharmaceutical chemistry laboratories and is also rather tedious to use as a routine method for various types of samples [1, 2].

In recently near, a new NMR technology that be named as diffusion ordered spectroscopy (DOSY) has been developed. Using DOSY method, mixture can be pseudo-chromatographic separated without actual physical separation [1-4]. Pulsed gradient spin echo (PGSE) sequence is introduced into DOSY experiment in which first dimension represents the regular chemical shift information and the second dimension separates species by diffusion coefficient which relate to hydrodynamic radius [5, 6]. Therefore, pseudo separation by DOSY is difficulty when the hydrodynamic radius, which relates to molecular weight, of mixtures are similar. Soluble compounds or silica gel have been used as the "stationary phase" [1, 4, 7-13] in DOSY separation of mixture to enhance the diffusion coefficient differences, just like normal-phase chromatography. This method taking example chromatography is named as matrix-assisted DOSY (MAD) or NMR chromatography [7, 8, 11]. For example, phenol (Mw=94.11) and methylbenzene (Mw=92.14) can be pseudo separated after the addition of hexamethylphosphoramide [12]. In another case, catechol, resorcinol, and hydroquinone, which are same in molecular weight, were distinguished by DOSY in the present of micelle [4]. Unfortunately, MAD appears to have attracted surprisingly little attention [4].

In this work, we studied sodium dodecyl sulfate (SDS) and hydroxypropyl-β-Cyclodextrin (HPβCD) assisted DOSY for phenylalanine (Phe), ibuprofen (Ibu), capsaicin (Cap) and norfloxacin (Nor) pseudo separation.

Results and discussion

Theory

Supposing all molecules are spherical, the relationship between diffusion coefficient (D) and hydrodynamic radius (R_h) of solute molecules can be described by Stokes-Einstein equation [9, 11].

$$D = \frac{kT}{6\pi\eta R_h} \quad (1)$$

k is Boltzmann's constant; T is absolute temperature; η is viscosity of dispersed medium. R_h and molecular weight (Mw) are related, so that the linear relationship is showed between log$D$ and logMw [9, 11]. Table-1 shows the relationship between diffusion coefficient and molecular weight of four molecules. Interestingly, diffusion coefficient of capsaicin obvious is smaller than that of norfloxacin, although molecular weight of capsaicin is smaller. It suggests that diffusion coefficient is not solely determined by M_W: some other factors, such as conformation, flexibility, ionization state, salvation, etc., all influence diffusion coefficient as well. Diffusion coefficient can be changed by changing factors using some assisted matrixes.

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Table-1: Relationship of diffusion coefficient and molecular weight.

	molecular weight	diffusion coefficient ($10^{-10}m^2s^{-1}$)
phenylalanine	**165.19**	**6.932**
ibuprofen	**206.28**	**6.229**
capsaicin	**288.40**	**4.816**
norfloxacin	**318.34**	**5.057**

SDS-Assisted DOSY

Because of the different of hydrodynamic radius, the four solutes are different in diffusion coefficients and can be separated in DOSY spectra. However, the different of diffusion coefficients is not very large when hydrodynamic radius of solute molecules is similar, especially for capsaicin and norfloxacin as show in Fig. 1a. To resolve the limitation of low separation, SDS in different concentration were added into solution with four molecules. Fig. 1b shows that separation is improved greatly in 80mM SDS condition.

Apparent diffusion coefficient is population weighted average value of free and bounded molecules [15]. It means that if more solute molecules are bound to micelles, the apparent diffusion coefficient changes more comparing with free molecules. In addition, mole fractions of free molecules are decided by lipo-hydro partition coefficient (log*P*). Fig. 2a shows diffusion coefficients of four molecules in different SDS concentrations. With SDS concentration increasing, diffusion coefficients of ibuprofen and capsaicin decrease rapidly, and the decreasing rate of capsaicin is faster than ibuprofen, meanwhile, diffusion coefficients of norfloxacin and phenylalanine are almost unchanged. Diffusion coefficient of ibuprofen is even smaller than norfloxacin when SDS concentration exceeds 40mM, although molecular weight of ibuprofen is smaller than norfloxacin. These phenomena show log*P* of capsaicin and ibuprofen are larger than norfloxacin and phenylalanine, in the meanwhile log*P* of capsaicin is larger than ibuprofen. After drawing the structures, Chemdraw software automatic calculate the log*P* of phenylalanine (-1.49), ibuprofen (3.75), capsaicin (4.44) and norfloxacin (1.96) respectively, consistent with the experimental results. It suggests that diffusion coefficient is not solely determined by M_W, log*P* is an important factor. The separation of molecules with different log*P* can be improved by adding SDS.

Equilibrium of free SDS and micelles exist in solution. Apparent diffusion coefficient of SDS (D_{app}) is equal to diffusion coefficient of free SDS under critical micelle concentration (CMC). However, ignoring viscosity influence, D_{app} is decided by equation 2 when concentration exceeds CMC [14, 16]:

$$D_{app}^{SDS} = D_{mic} + \frac{(D_{free} - D_{mic})CMC}{[SDS]} \quad (2)$$

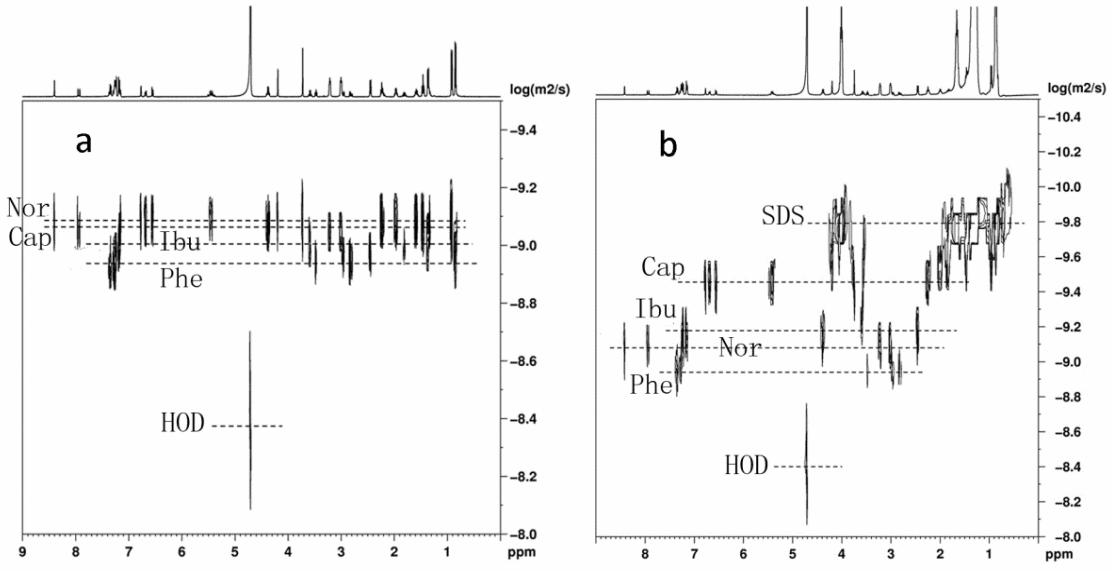

Fig. 1: DOSY spectra with (b) and without (a) 80mM SDS.

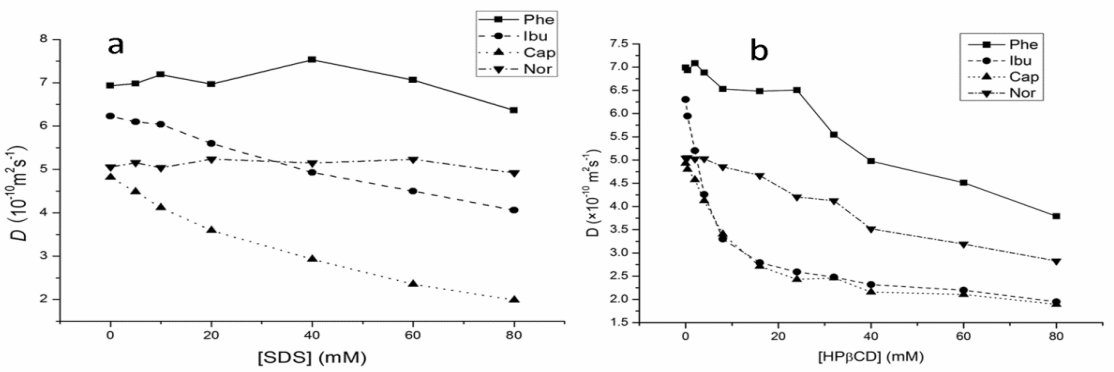

Fig. 2: Apparent diffusion coefficients in SDS-assisted (a) or HPβCD-assisted (b) DOSY at different concentrations.

D_{mic} is diffusion coefficient of SDS micelle, D_{free} is monomer SDS diffusion coefficient, [SDS] is SDS total concentration in solution. Equation 2 shows linearity relationship between SDS apparent diffusion coefficient and 1/[SDS] when SDS concentration is above CMC. At concentrations below the CMC, the apparent diffusion coefficient is equal to the diffusion coefficient of monomers. Fig. 3 shows the relationship bewteen SDS apparent diffusion coefficient and 1/[SDS] in mixture of four solutes. At higher concentration condition (upper 20 mM) well linearity emerges between D_{app} and 1/[SDS], and at lower concentration (around 10 mM) the linearity is not acceptable. Accroding to Trembleau and Rebek [17], the viscosity of SDS solutions rises significantly above 10–20 mM. This causes the plot of D versus 1/[SDS] to deviate from the linear form of equation 2 [16, 17]. The extrapolation of the line above the CMC to 1/[SDS] gives the value of D_{mic}. D_{free} is 2.1×10^{-10} m^2s^{-1} at 5mM. According to slope, the CMC value of SDS determined under these conditions from our data is 7.7mM.

HPβCD-Assisted DOSY

Fig. 2b shows diffusion coefficients of four molecules in different HPβCD concentrations. With HPβCD concentration increasing, diffusion coefficients of norfloxacin and phenylalanine decrease slower, and ibuprofen and capsaicin decrease more rapid. Meanwhile the decreasing rate of ibuprofen is faster than capsaicin at low HPβCD concentration (less than 4mM), after that diffusion coefficient trends of ibuprofen and capsaicin are almost the same. At high HPβCD concentration (higher than 20mM), diffusion coefficients changes of ibuprofen and capsaicin are tended to become mild. These phenomena are quite different from SDS-assisted experiments, suggesting the various mechanisms between SDS-assisted and HPβCD-assisted systems. HPβCD is frequently-used host molecule, and can form complex with guest molecule [18]. Apparent diffusion coefficient of host molecule is population weighted average value of free molecule and complex (D_{com}) [19, 20]:

$$D_{app}^{guest} = (1-X)\cdot D_{com} + X\cdot D_{free} \quad (3)$$

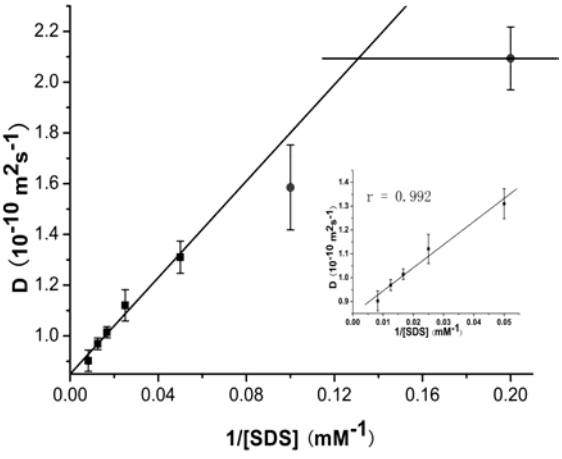

Fig. 3: Apparent diffusion coefficient (D_{app}), as a function of the reciprocal of SDS concentration (1/[SDS]). The insert shows linearity from 20 to 121.6mM. (statistics for the inset: N=5, Int=0.85±0.03× 10^{-10}, slope=9.7±1.5× 10^{-10}).

D_{com} is diffusion coefficient of complex; D_{free} is diffusion coefficient of free molecule; X is mole fraction of free guest molecule. (1-X) increases until approaching 100% with HPβCD (host) concentration increasing. So that apparent diffusion coefficient of guest (D_{app}^{guest}) approximately equal to D_{com}. Because the molecular weight of HPβCD is far greater than guest, molecular weight of complex is approximately equal to HPβCD, and diffusion coefficient of complex approaches to HPβCD. At lower HPβCD concentration, diffusion coefficients of ibuprofen and capsaicin decrease rapidly with HPβCD concentration increasing, and become mild until 20mM. It suggests ibuprofen or capsaicin form complexes with HPβCD, and both are almost included into hydrophobic cavity at high HPβCD concentration. At lower HPβCD concentration, diffusion coefficients of norfloxacin and phenylalanine are almost unchanged, suggesting that both of them do not form complexes. We think the viscosity of solution (η) or other block factors decrease the diffusion coefficients of norfloxacin and phenylalanine at high HPβCD concentration.

Fig. 4 shows the 2D ROESY spectrum of four molecules and HPβCD. Cross peaks appear between HPβCD and capsaicin or ibuprofen, but do not appear between HPβCD and norfloxacin or phenylalanine. It means capsaicin and ibuprofen are included into HPβCD hydrophobic cavity and norfloxacin and phenylalanine are outside, coinciding with DOSY experimental conclusions.

With the host (HPβCD) adding, complexation becomes a more important factor than Mw. The separation of molecules can be changed by adding HPβCD. The separation of molecules that be included or not be included into host, such as ibuprofen/phenylalanine or capsaicin/norfloxacin, was improved by adding HPβCD. However, the separation of both guests (capsaicin and ibuprofen) became poor because the Mw of both complexes are similar.

Experimental

Chemical and Instruments

Heavy water, NaOH and phenylalanine (Mw=165.19) were obtained from Aldrich (St. Louis). Hydroxypropyl-β-cyclodextrin (HPβCD, degree of substitution is 6.64) was purchased from Deli company (Xi'an, China). Ibuprofen (Mw=206.28), capsaicin (Mw=288.4) and norfloxacin (Mw=318.34) were purchased from Furen company (Zhengzhou, China). All the ^{1}H NMR, 2D DOSY and ROESY spectra were recorded by 400 MHz NMR spectrometer (Bruker AVANCE III spectrometer) which is equipped with a 5mm BBO probe with a z-axis gradient coil, maximum gradient strength was 50G/cm, and a Variable Temperature Unit (VTU).

NMR Spectroscopy

NaOD (0.1M) was prepared in D_2O, and all other solutes were prepared in this solution. Phenylalanine (4.0mM), ibuprofen (4.1mM), capsaicin (*3*.9mM) and norfloxacin (3.9mM) were prepared in HPβCD (from 0.4 to 80mM) or SDS (from 0.4 to 121.6mM) solution. NMR experiment temperature was all controlled at 303K.

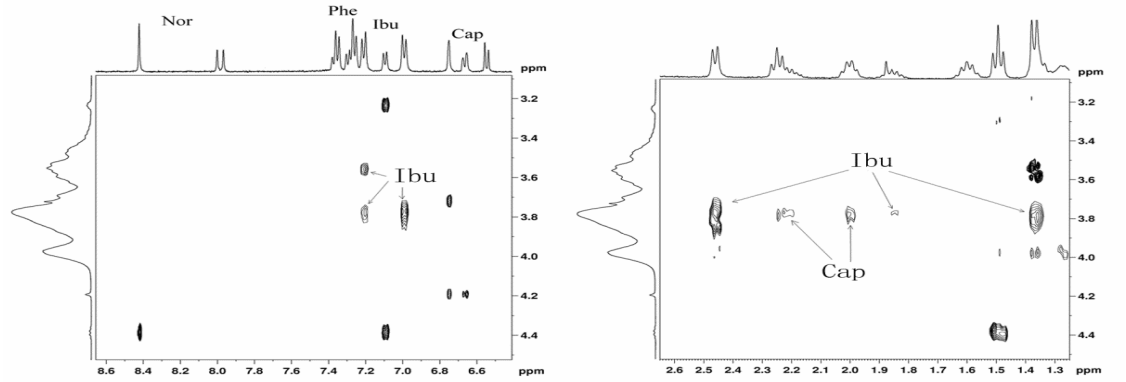

Fig. 4: Partial ROESY spectra. ROE signals are presented between capsaicin or ibuprofen and HPβCD.

Two-dimensional rotating-frame overhauser effect spectroscopy (2D ROESY) was acquired using Bruker standard parameters (pulse program roesyphsw) for the geometry of the inclusion complex. Each spectrum consisted of a matrix of 2K (F2) by 256 (F1). Size of FID covered a spectral width of 4000 Hz. The spectra were measured with a spin-lock mixing time (p15 pulse) of 200 ms, relaxation delay 2s. Gaussian apodization functions were applied in both dimensions. All DOSY experiments were performed using the bipolar pulse longitudinal eddy current delay (BPPLED) pulse sequence [4, 9, 12]. The duration of the magnetic field pulse gradients (δ) and the diffusion times (Δ) were optimized for each sample. The pulse gradients (g) were incremented from 2 to 95% of the maximum gradient strength in a linear ramp.

Conclusion

SDS or HPβCD using as assisted matrix can improve DOSY pseudo separation. Mechanism are different between SDS- and HPβCD-assisted DOSY. Lipo-hydro partition mechanism is used in SDS-assisted DOSY. All separation result can be improved at high SDS concentration. Complexing mechanism is used in HPβCD-assisted DOSY. This mechanism can great improve the separation between molecules inside and outside HPβCD cavity. However, if both molecules (ibuprofen and capsaicin) form complexes, they will not be separated at high HPβCD concentration. To adjust the HPβCD concentration carefully at low concentration can overcome this limitation. Experiments and theories show that apparent diffusion coefficients of guest molecules (ibuprofen and capsaicin) tend to the same values at high HPβCD concentration, and both are approximately equal to diffusion coefficient of free HPβCD.

References

1. J. S. Kavakka, I. Kilpeläinen and S. Heikkinen, *Organic Letters*, **11**, 1349 (2009).
2. J. S. Kavakka, V. Parviainen, K. Wähälä, I. Kilpeläinen and S. Heikkinen, *Magnetic Resonance in Chemistry*, **48**, 777 (2010).
3. D. A. Jayawickrama, C. K. Larive, E. F. McCord, D. C. Roe, *Magnetic Resonance in Chemistry*, **36**, 755 (1998).
4. R. Evans, S. Haiber, M. Nilsson and G. A. Morris, *Analytical Chemistry*, **81**, 4548 (2009).
5. D. Li, I. Keresztes, R. Hopson and P. G. Williard, *Accounts of Chemical Research*, **42**, 270 (2009).
6. K. F. Morris and C. S. Johnson, *Journal of the American Chemical Society*, **114**, 3139 (1992).
7. C. Pemberton, R. E. Hoffman, A. Aserin and N. Garti, *Langmuir*, **27**, 4497 (2011).
8. C. F. Tormena, R. Evans, S. Haiber, M. Nilsson and G. A. Morris, *Magnetic Resonance in Chemistry*, **48**, 550 (2010).
9. R. E. Hoffman, H. Arzuan, C. Pemberton, A. Aserin and N. Garti, *Journal of Magnetic Resonance*, **194**, 295 (2008).
10. S. Viel, F. Ziarelli and S. Caldarelli, *PNAS*, **9696**, 100 (2003).
11. J. S. Gounarides, A. Chen and M. J. Shapiro, *Journal of Chromatography* B, **725**, 79 (1999).
12. E. J. Cabrita and S. Berger, *Magnetic Resonance in Chemistry*, 39, S142 (2001).
13. C. Pemberton, R. Hoffman, A. Aserin and N. Garti, *Journal of Magnetic Resonance*, **262**, 208 (2011).
14. P. S. Denkova, L. V. Lokeren, I. Verbruggen and R. Willem, *The Journal of Physical Chemistry* B, **112**, 10935 (2008).
15. A. Guerrero-Martínez, G. González-Gaitano, M. H. Vinas and G. Tardajos, *The Journal of Physical Chemistry* B, **110**, 13819 (2006).
16. S. Lapenna, A. R. Bilia, G. A. Morris and M. Nilsson, *Journal of pharmaceutical Sciences*, **98**, 3666 (2009).
17. L. Trembleau and J. Jr Rebek, *Chemical Communications*, **1**, 58 (2004).
18. W. Liu, B. Zhao, Y. C. Li and H. M. Liu, *Magnetic Resonance in Chemistry*, **49**, 611 (2011).
19. K. S. Cameron and L. Fielding. *Magnetic Resonance in Chemistry*, **40**, S106 (2002).
20. P. S. Denkova, L. V. Lokeren and R. Willem, *The Journal of Physical Chemistry* B, **113**, 6703 (2009).