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Carbohydrate Research 341 (2006) 1890–1895

Carbohydrate
RESEARCH

Concentration measurements of sucrose and sugar surfactants solutions by using the ^{1}H NMR ERETIC method

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Received 9 March 2006; received in revised form 13 April 2006; accepted 18 April 2006
Available online 15 May 2006

Abstract—The ERETIC method has been used to determine precise concentrations of aqueous solutions of sucrose and sugar surfactants, namely octyl glucoside and fatty acid sucrose esters by ^{1}H NMR spectroscopy. The effects of NMR tuning, acquisition parameters, and spectrum processing on the measurement have been assessed in these particular cases. The linearity upholds over the whole concentration range, with both sucrose and octyl glucoside, whatever the physicochemical phenomena occurring, either an increasing viscosity or the micellization of the surfactant. For sucrose solutions, an accuracy of 2% is measured for concentrations between 0.1 and 200 mmol/L, which is consistent with literature data.

Keywords: Calibration; Carbohydrate; Surfactant; Sucrose ester; Alkyl glucoside; Micelle

1. Introduction

Sucrose esters (Fig. 1c) are non-ionic surfactants issued from renewable resources, which are used notably as emulsifiers in food and cosmetics. During our work on the characterization of the physicochemical properties of pure sucrose monoesters, we used PFGSE-NMR for determination of the self-diffusion coefficients of these compounds in water.[1] The self-diffusion depends on phenomena occurring in solution when the concentration increases. For example, for concentrations greater than the critical micellar concentration (CMC), amphiphilic sucrose esters aggregate in micelles and the diffusion coefficient measured by PFGSE-NMR undergoes a rapid decrease. To determine precisely the CMC value and other parameters such as the micelle hydrodynamic radius, it is of the utmost importance to have an accurate measure of the concentration. Sucrose esters being quite hygroscopic compounds, the concentration determined by weighting small amounts of freeze-dried compounds when preparing the solutions can be slightly inaccurate. Having in hand an additional approach to measure the solution concentration should be of great interest, particularly if the method could directly measure the concentration in the NMR sample, whatever the sample preparation procedure is.

The ERETIC method (electronic reference to access in vivo concentrations) was developed by Akoka

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doi:10.1016/j.carres.2006.04.034

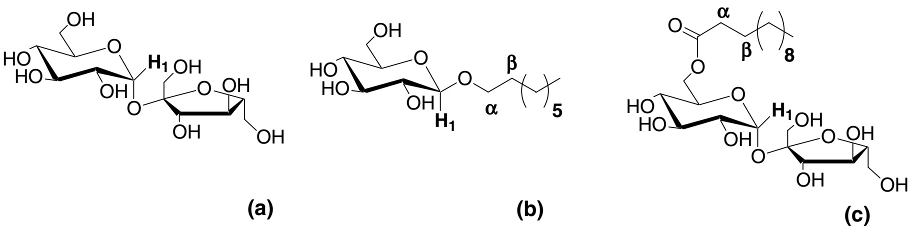

Figure 1. (a) Sucrose, (b) octyl β-D-glucopyranoside, (c) sucrose monolaurate (6-*O*-regioisomer represented). In bold, the protons used for quantification.

et al.[2–7] It has been also used recently for quantitative measurements in solid state NMR.[8] This NMR technique provides an external reference signal that is generated electronically during the acquisition and can be placed anywhere in the NMR spectrum. After calibration, the concentration of a given molecule in the NMR sample can be determined by comparing the intensity of one of its signal to the intensity of the reference signal.

Prior to applying this method to the sucrose esters samples, we checked its accuracy by reviewing and testing the parameters that could possibly affect the quality of the measurement on D_2O-solutions of sucrose (Fig. 1a) and octyl β-D-glucopyranoside (further referred to as 'octyl glucoside') (Fig. 1b). The effects of both NMR acquisition parameters were evaluated (^1H and ^{13}C probes tuning and matching, shim, calibration of the 90° pulse, number of acquisition scans ns and relaxation delay d_1—and spectrum processing:—phase correction, baseline correction, and adjustment of the integral slope and bias). In the case of octyl glucoside, we also looked for possible drifts of the calibration of the external signal whether the anomeric proton of the sugar part or the protons of the fatty chain are chosen as a reference (Fig. 1). To ascertain that the measurement is not affected by physico-chemical changes occurring in solution when the concentration of the sample increases, two calibration curves have been built in the range 0.1–200 mmol/L: the first one from sucrose solutions, for which the viscosity and interactions via hydrogen bonding increase with the concentration; the second one from octylglucoside solutions, which undergo association phenomena (micellization) when the concentration increases.

2. Results and discussion

The effect of the parameters on the measurement during tuning and processing, has been assessed by using sucrose solutions. Concentration measured by ERETIC is denoted C or C_E.

(a) When testing the effect of a parameter drift, deviations have been calculated with respect to the data obtained with the optimal tuning and processing, by using the formula:

$$d = \left|\frac{C - C_{\text{opt}}}{C_{\text{opt}}}\right| \times 100$$

(b) The stability of the measurements has been assessed by performing several experiments with exactly the same parameters and processing methods. The resulting dispersion of the results have been calculated as the relative standard deviation (coefficient of variation).

(c) When accuracy has been assessed, the following formula has been used:

$$d = \left|\frac{C - C_{\text{exp}}}{C_{\text{exp}}}\right| \times 100$$

(d) When difference of C_E depending on the signal used for quantification (H-1, $(CH_2)_5$ or CH_3) has been determined, the formula used has been specified in the column entries Table 2.

2.1. NMR spectrum acquisition and implementation of the ERETIC method for phase correction

NMR spectra have been acquired on a BRUKER DRX500 spectrometer with a gradient triple channel $^1H/^{13}C/^{15}N$ probe. The ERETIC method has been modified to provide a very simple procedure with no change in the spectrometer wiring and no phase pre-calibration. The first point was fulfilled by injecting the reference signal on the ^{13}C channel of the probe, without changing any connection. For the second point, in the original procedure, the reference signal was generated by a second RF channel, phase synchronized, and set at the same frequency as the first one. Phase modulation of the shape pulse allowed the proper frequency positioning of the reference signal, but it was necessary to align perfectly its phase with that of the spectrum prior to acquisition. Moreover, we observed that the phase of the spectrum varies slightly with the number of scans, which complicates the procedure for low level signals, which need to be averaged. To simplify the experiment, the reference signal and the FID were acquired with the same parameters in two separate experiments, phased separately and added at the end for easy quick visual inspection and possibly for quantification. However,

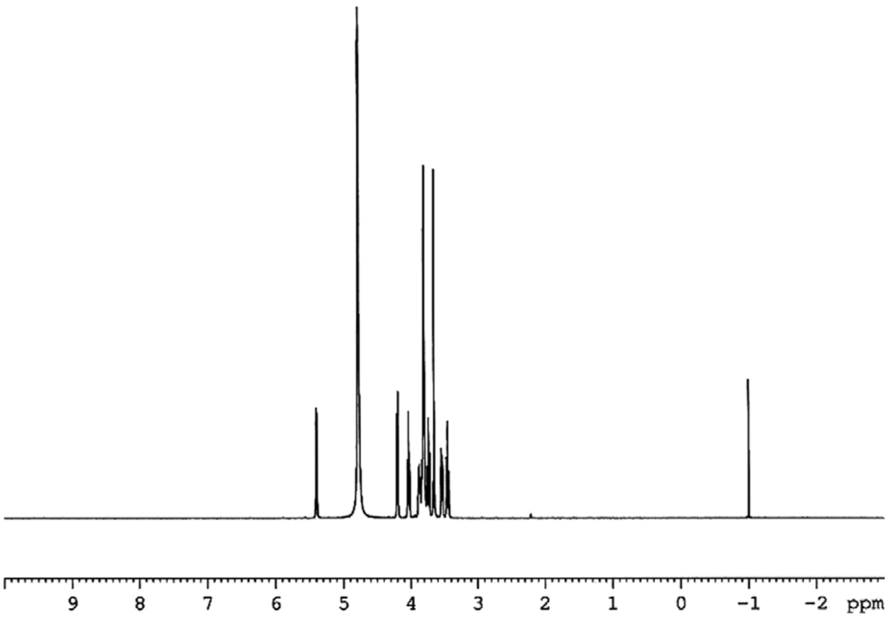

Figure 2. NMR spectrum of a sucrose solution in D_2O obtained during an ERETIC experiment. The reference signal is placed at −1 ppm.

this additional procedure deteriorates the signal-to-noise ratio, because the resulting noise standard deviation increases with a factor of $\sqrt{2}$, whereas NMR signals keep their own amplitudes. Consequently, one may use this procedure only for high S/N spectra. When it is necessary to accumulate the spectrum for low concentrated solutions, it is mandatory to integrate the two spectra independently in absolute mode to preserve the signal S/N of the accumulated spectrum. By this way, splitting in two the standard ERETIC experiment, phase correction of the composite spectrum is no longer a problem, which saves both setup and processing times. The ERETIC signal generated with the shape tool consists in an exponential decaying signal centered at −1 ppm (Fig. 2). The acquisition time was fixed once, alleviating to recalculate the shape pulse for each new acquisition. The sample was not spinning.

2.2. NMR spectrum processing and stability of the measurement

After a line broadening set at 1 Hz, Fourier transform and phasing, further processing consisted in global baseline correction and local baseline correction around the reference peaks. The integral value of the ERETIC signal, and thus, the resulting measured concentration are highly sensitive to the quality of integration. In every case, when the integrals appeared correct from a routine spectrum processing point of view, small additional corrections quickly led to non-negligible variations in quantification. In order to get the smallest error possible, well-defined integration areas were chosen around H-1 and the ERETIC signal. Slope and bias corrections of the integrals were carried out with the best accuracy by obtaining the longest and most horizontal lines on both sides of the integral signals. The stability of the quantification obtained by such processing has been checked on nine sucrose solutions, ranging from 0.1 to 200 mmol/L (Table 1). Two to five experiments (acquisition and processing), for which the probe tuning is optimal (see below), have been performed for each tube. A mean value of the concentration has been thus determined, and the deviation from this mean value has been calculated for each experiment. The resulting dispersion of the results has been calculated as a relative standard deviation (Table 1). This latter lies within 2%. The systematic error made during the signal integration, all other parameters being optimized, is thus 1–2%. These

Table 1. Stability of the measurement: mean concentrations are calculated on a series of experiments at same ns, d_1, with optimal tuning and processing, for each concentration

Sucrose solutions, C_{exp} (mmol/L)	ns, d_1 (s)	Mean ERETIC concentration, $\overline{C}_{\mathrm{E}}$ (mmol/L)	$\left\lvert\frac{\overline{C}_{\mathrm{E}}-C_{\mathrm{exp}}}{\overline{C}_{\mathrm{E}}}\right\rvert$ (%)	Relative standard deviation (%)
203.62	1, 1	202.66	0.47	0.35
102.69	1, 1	104.40	1.66	0.42
49.93	1, 1	50.20	0.53	1.74
10.33	1, 1	10.75	4.1	1.28
5.01	32, 10	4.87	2.8	0.89
	32, 15	4.82	3.7	0.68
2.17	32, 10	2.25	3.6	0.84
	32, 15	2.24	3.3	0.21
1.01	32, 10	0.99	1.8	1.62
0.104	64, 10	0.102	1.5	0.75
	128, 10	0.101	3.3	1.32

results are consistent with those described by the authors of the method.[3]

The maximal deviations, compared to the mean concentration measured, for each set (at same concentration, ns and d_1), range within 1% for 75% of them, within 2% for 22% of them, and 3% have shown a drift of about 4%. The drifts tend to be higher for the least concentrated samples (2–4% for 1 mmol/L and less), illustrating that the accuracy of integration depends on the signal to noise ratio.

2.3. Probe tuning

Fine tuning of the ^{1}H probe appeared to be the second origin of coarse drifts in the quality of quantification. Tuning and matching screws have been tuned with the help of the wobbulation curve and diodes signals, which can be applied either on a 4 or a 1 MHz scale. The quantification shows a linear correlation with probe tuning with significant variation even for a small drift (Fig. 3).

The result is quite sensitive to tuning and the 1-MHz mode of the wobbulation curve should be systematically used in order to tune with the best accuracy. For the ^{13}C probe tuning and matching, a deviation occurs only for strong mistuning.

2.4. Acquisition parameters (P1 and shim)

The effect of the pulse length has been checked by performing the experiment with values of 5.7, 6.0, 6.3, and 6.6 μs around a 90° pulse of 6.3 μs. The deviation remains within 0.2% for the three longest pulses, and is higher (2%) for the shortest one, which is consistent with the small variation of a sine function around 90°. Excluding a mistuning of the pulse length more than 5%, the pulse length is then not a crucial parameter. In the same way, substantial deviation from the optimal shim by varying Z_1 or Z_2, without visual major line distortion of peaks, does not affect the result.

2.5. Number of scans and relaxation delay

For the least concentrated samples, the number of acquisition scans had to be increased in view to obtain a precise spectrum integration. The relaxation delay then has to be taken into account. First, T_1 were assessed on a sample of intermediate concentration of sucrose monolaurate (4.4 mmol/L), and gave $0.9 < T_1 < 1.6$ s for sucrose signals (1.2 s for H-1); $0.5 < T_1 < 0.6$ s for CH_2 signals, and $T_1 = 0.9$ s for CH_3 signal. Thus, taking $d_1 = 10$ s ensured at least five times the maximal T_1 for complete relaxation to occur, in the case of accumulated spectra. Further, the effect of delay was assessed on a less concentrated sample (about 1 mmol/L). Delay has been given the values: 1, 5, 10, 15, 20, 25, 30, and 60 s, for a 0.944 mmol/L sucrose solution acquired with 32 scans, while the ERETIC signal remained acquired with $d_1 = 1$ s and ns = 1 and scaled according to the number of scans used for the spectrum. The standard delays (10 or 15 s) gave good results, while drifts larger that 2% are observed for too short ($d_1 = 1$ s) or too long delays ($d_1 = 60$ s). This last point, quite surprising, is probably due to the long term unstability.

For the series of sucrose solutions from 0.1 to 200 mmol/L, delays of 10 or 15 s have been tested, with either 32, 64, or 128 scans, when 1-scan experiments were no longer suitable. It appeared that 32 scans was the minimum for sucrose solutions at 5, 2, and 1 mmol/L, and 64 or 128 scans were needed for the solution at 0.1 mmol/L to obtain accurate results. For the

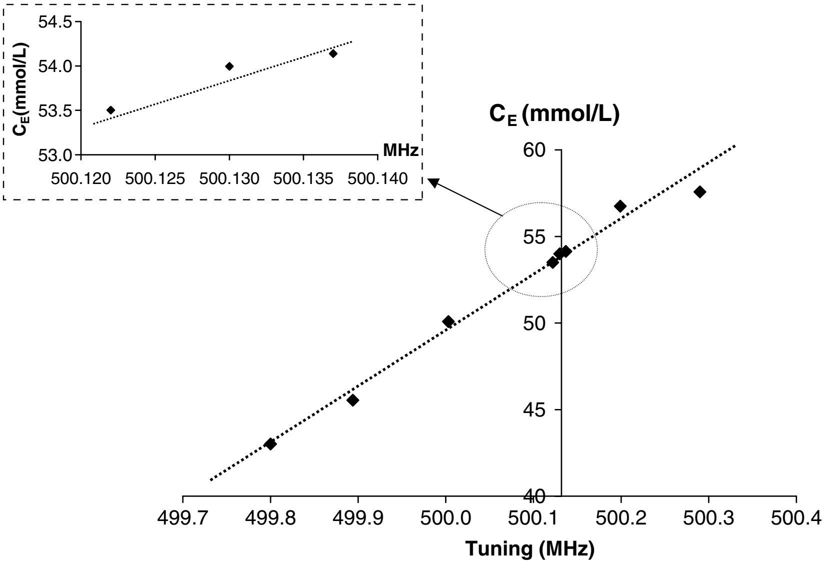

Figure 3. Effect of tuning on the measured concentration of a sucrose solution ($C_{exp} = 51.4$ mmol/L).

sucrose solutions at 5 or 2 mmol/L acquired with 32 scans, differences between $d_1 = 10$ s or $d_1 = 15$ s range within 1%. A delay of 10 s was chosen for further experiments (Table 1).

2.6. Octyl glucoside solutions

In the case of octyl glucoside, either the H-1 signal of glucose or one of the chain signals ($CH_{2\alpha}$, $CH_{2\beta}$, $(CH_2)_5$, or CH_3) can be used for comparison with the reference signal for quantification (Fig. 1). Since the signal of $(CH_2)_5$ exhibits the best signal to noise ratio at all concentrations, it was useful to know whether taking it as a reference will lead to a difference in quantification. Table 2 shows the deviation in the measured concentration depending on which signal is used as a reference. Quite high differences were observed between integrations of the $(CH_2)_5$ signals compared to the H-1 signal, ranging within 3% for solutions up to 5 mmol/L, and higher for the less concentrated ones. Differences between the $(CH_2)_5$ and the CH_3 signal remains in the range of 1% for solutions up to 5 mmol/L and 2% for the less concentrated ones.

The effects of delay (10, 15, and 20 s) and scan number (32 or 64) on these differences of integration from CH_2 or H-1 have been assessed for solutions at 10.11 and 4.84 mmol/L. In the whole, the delay does not affect strongly the results, since the differences still lie within 3%, $d_1 = 10$ s remaining the best value.

2.7. Calibration curves

The ERETIC signal has been calibrated from eight sucrose solutions (Table 1), showing an excellent linearity even at the lowest and the highest concentrations. Once fixed the integral of H-1 proton to one, the integral I_{ERETIC} of the ERETIC signal gave the concentration by using the formula: $C = k/I_{\mathrm{ERETIC}}$. The correlation coefficient $k = 0.0362$ has been obtained, with a fitting coefficient $R^2 = 0.9999$. As for the octyl glucoside solutions (ten solutions, Table 2), calibration with the H-1 signal gave the same correlation coefficient $k = 0.0362$, with $R^2 = 0.9998$ whereas calibration with the $(CH_2)_5$ signal gave a correlation coefficient $k = 0.0370$, with $R^2 = 0.9998$. No deviation to linearity was observed at higher concentrations, showing that neither viscosity increase, nor association phenomena disturbed the measurement in our case.

Table 2. Deviations between concentrations measured by using the H-1 signal, the $(CH_2)_5$ signal or CH_3 signal, for octyl glucoside solutions

Octyl glucoside solutions, C_{exp} (mmol/L)	ns, d_1 (s)	$\left\lvert\frac{C_{\mathrm{E}}\mathrm{CH_2} - C_{\mathrm{E}}(\mathrm{H\text{-}1})}{C_{\mathrm{E}}(\mathrm{H\text{-}1})}\right\rvert$ (%)	$\left\lvert\frac{C_{\mathrm{E}}(\mathrm{CH_2}) - C_{\mathrm{E}}(\mathrm{CH_3})}{C_{\mathrm{E}}(\mathrm{CH_3})}\right\rvert$ (%)
198.89	1, 1	1.61	0.52
99.70	1, 1	2.13	0.12
50.00	1, 1	3.18	0.17
29.36	1, 1	1.86	0.64
22.42	1, 1	0.36	0.65
15.29	1, 1	1.67	0.14
10.11	1, 1	1.32	0.02
4.84	32, 10	2.65	0.04
1.92	32, 10	4.59	1.73
0.104	128, 20	12.7	1.94

2.8. Application to sucrose esters

For sucrose esters quantification, used for the self-diffusion coefficient measurements described in Ref. 1, the calibration coefficient obtained by calibrating on the $(CH_2)_5$ signal of octyl glucoside was preferred, first because in this kind of molecules, the $(CH_2)_n$ signal remains the most accurate at low concentrations, and also because when dealing with mixtures of isomers of sucrose esters, there is more than one signal for the anomeric proton, making its integration difficult. For experiments on sucrose monolaurate solutions in D_2O, ranging from 75 to 0.4 mmol/L, the deviation to the experimental values (obtained from weighting), spreaded from 1.5% to 9.5% (mean = 5%) by using ERETIC, and from 2 to 12% (mean = 6%) by using HPLC quantification. For sucrose solutions, this deviation lies within 0.5 to 4% (see Table 1). Thus, the ERETIC method giving a precision within 2%, as shown for octyl glucoside and sucrose, when applied to sucrose esters in the optimized way, allowed a good decrease of the imprecision obtained by the usual weighting of the samples.

3. Conclusion

The measurement of concentrations by ERETIC for sucrose and octyl glucoside solutions gave excellent correlations in the whole range and no deviation to linearity at higher concentrations was observed. These results showed that, in the concentration range studied (0.1–200 mmol/L), and for the molecules studied, the physicochemical changes occurring in solution, such as an increase in viscosity, micellization, and changes in molecular interactions do not affect the linearity of the method. However, in the case of octyl glucoside, a slightly different correlation coefficient with the synthetic signal is obtained if the calibration is made on the basis of the chain signals instead of the anomeric proton (H-1), probably due to differences in relaxation for these two sets of protons. In our case, the method was implemented by acquiring the reference signal and the spectrum of interest in two different experiments, thus allowing a perfect phase correction of both parts before addition. In this method, the two crucial parameters for a correct quantification are a sharp ^{1}H probe tuning and a perfect signal integration.

4. Experimental

4.1. Sample preparation

Powdered sucrose (Béghin-Say, MW = 342.3) was dried for 30 h in an oven at 70 °C under diminished pressure and allowed to stand at room temperature in a desiccator with activated sieves (4 Å) for 48 h. Octyl β-D-glucopyranoside (Aldrich, 98%, MW = 298.4) has been dried over activated molecular sieves (4 Å) for 48 h. Sucrose monolaurates have been prepared according to procedures described in earlier papers, and were freeze-dried samples.[1] The most concentrated solns (200 and 100 mmol/L) were prepared by weighting sucrose or octyl glucoside and dissolving it in either 2 or 5 mL D_2O in a volumetric flask. The solns have been weighted in order to calculate their exact massic concentration C_w, used for allowing a better accuracy of the subsequent dilutions. Less concentrated solns have been prepared by weighting a definite quantity '*m*' of a more concentrated soln and diluting it to 2 mL ($= V_{tot.}$) in D_2O. The molar concentration is thus calculated as $C_{exp} = [m \times C_w/342.3]/V_{tot.}$, for sucrose and $C_{exp} = [m \times C_w/298.4]/V_{tot.}$ for octyl glucoside solns. Experimental concentrations of the solns prepared are reported in the tables, along with concentration measured by ERETIC. NMR tubes have been sealed after bubbling of argon, in order to avoid evaporation and biological degradation of the solns.

4.2. Determination of the concentration by ERETIC

The value of 1 is given to the integral H-1 and the resulting value of the reference signal is reported. For octyl glucoside, when the chain signals (CH_3, $(CH_2)_5$, $CH_{2\beta}$ chain) have been used for quantification, their respective integration values 3, 10, 2, have been given to each one. When more than 1 scan is used for the spectra (32, 64, or 128), while the reference signal remains acquired with 1 scan, the relevant multiplying factor is affected to the reference signal for calculation.

Acknowledgements

This work was achieved in the former research facility UMR 143 CNRS-Béghin-Say, c/o Béghin-Say, C.E.I., 66 boulevard Niels Bohr, B.P. 2132, F-69603 Villeurbanne, France. Financial support from Béghin-Say and the CNRS and a grant from ANRT to V.M. are greatly acknowledged.

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