

On ultrasonic spectroscopy and fractal analysis in the study on progressive aggregation of humic substances in diluted solutions

MILOSLAV PEKAŘ

Faculty of Chemistry, Institute of Physical and Applied Chemistry & Materials Research Centre
Brno University of Technology
Purkyňova 112, 612 00 Brno
CZECH REPUBLIC
pekar@fch.vutbr.cz <http://www.fch.vutbr.cz>

Abstract: - Recently published paper on application of ultrasound spectroscopy with high resolution and fractal analysis to the study of solution behavior of humic substances is commented. It is shown that the concentration increment of ultrasound velocity used to analyze ultrasonic data is mathematical construction which is very sensitive to experimental data uncertainties and should be accompanied by analysis of the very ultrasound velocities which are really measured. Fractal analysis does not take into account the compressibility dependence of ultrasound velocity properly.

Key-Words: aggregation, critical micellar concentration, fractals, humic substances, surfactants, ultrasound velocity

1 Introduction

Recently, a paper on application of ultrasound spectroscopy with high resolution and fractal analysis in the study of solution behavior of humic substances has been published in WSEAS Transactions on Environment and Development [1]. Especially the former method represents a new technique in humic research which can significantly contribute to ongoing discussion on the structural character of humic acids and their aggregation properties. However, the paper is not free of shortcomings which can mislead readers. This note should point out the main discrepancies.

Humic substances are formed during decomposition of organic matter mainly of vegetable origin and represent unique and the most important component of natural organic matter. Due to their beneficial effects on the physical, chemical and biological properties of soils humic substances can be included among the factors which are indispensable for the existence of life on the Earth [2, 3]. Humic substances are found in soils, sediments, waters, caustobiolites (particularly peats and lignites). In natural environments they play really versatile roles – they regulate many chemical and biochemical processes, transport plant nutrients or pollutants, retain water, control pH etc. [4]. As a result of the large number of different organic compounds in living organisms their degradation and recombination processes lead to nearly infinite number of molecules. Humic substances are

operationally classified into three groups on the basis of their solubility in aqueous solutions of different pH. Humic acids are insoluble in acidic solutions and are isolated as the precipitate which is formed when the pH is decreased below 1-2. Fulvic acids are soluble regardless the pH and are isolated from the supernatant obtained after the removal of humic acid precipitate. The last group – humins – refer to that part of humic substances which is insoluble at any pH and is received as a solid residue after alkaline extraction of humic and fulvic acids. However, the distinction of three different types of humic substances does not indicate the existence of three distinct structures of organic molecules. In fact, all the three types are complex mixtures formed by many molecular entities the structure of which still remains unresolved [2, 3]. Even the discussion on the principal molecular structure of humic substances has not been closed. Early concepts were based on the development in macromolecular and polymer science. According to these concepts humic substances are macromolecules forming random coils that can elongate in basic or low-ionic-strength solutions [2]. Later alternative concepts of humic substances appeared which disputed their macromolecular nature and brought experimental support for “low molecular” theory of humic substances. These newer concepts regard humic substances as aggregates of low molecular weight components. Two basic models of the structure and formation of

humic aggregates have been introduced. Micellar model was inspired by micelles – aggregates formed by classical surfactants – in which intermolecular interactions produce interior hydrophobic domains separated from aqueous surroundings by exterior hydrophilic layers. This model originated especially from experiments with fluorescent polarity probes dissolved in humic solutions [5-7]. Wershaw [8] also suggested formation of more intricate surfactant-like humic structures, like bilayers or membrane-like coatings, on the surfaces of soil minerals which accompany humic substances in their natural environments. The second aggregate view on humic substances was presented by Piccolo and his collaborators [9, 10] – that of the supramolecular association. In this model many relatively small and chemically diverse organic molecules form clusters linked by non-covalent forces, especially by hydrogen bonds, hydrophobic and π - π interactions. Hydrophobic forces are easily disrupted by simple organic molecules penetrating large hydrophobically bonded aggregates which are thus separated into smaller associations held together by more strong hydrogen bonds [9-11]. Fulvic acids, in contrast to humic acids, possess lower hydrophobicity and higher negative charge which do not support formation of large associates through hydrophobic or hydrogen bonding interactions. Consequently, the apparent average molecular size of fulvic acids demonstrated minimal change under various solution conditions [10].

Ultrasound velocimetry is a universal method for characterization of colloids including their aggregating behavior [11, 12]. Particularly the high resolution ultrasound spectroscopy [13-15] provides unprecedented sensitivity and ability to distinguish ultrasonic behavior of structurally similar compounds. In a typical ultrasonic experiment ultrasound wave of preselected frequency passes through sample and changes in its characteristics due to interactions with the sample are analyzed. Two characteristics are measured – ultrasound velocity and attenuation. The former is simply the speed with which the wave travels through the sample, the latter refers to decreasing amplitude of the wave when passing through the sample. Only the velocity is a subject of this note.

Ultrasound wave moves by periodic compressions and decompressions of media. Hence, it probes intermolecular interactions within the sample which in the case of solution include not only the interactions between solute molecules but also between solute and solvent (solvation or, in aqueous solutions, hydration). Spreading of ultrasound wave is determined by elasticity (compressibility) and

density of media. Compressibility effects are usually dominating. The relationship between the ultrasound velocity v and the compressibility (β) and density (ρ) of media is given by following equation [12]

$$v = 1/\sqrt{(\rho\beta)} \quad (1)$$

sometimes called the Laplace equation.

Ultrasound velocimetry was applied also in studies of surfactants aggregation and formation of micelles, see e.g. ref. [16-18]. Ultrasound velocity measured in samples of surfactant solutions is usually compared with the velocity measured in pure solvent (water) under the same conditions. Especially in studies performed using the high resolution ultrasound spectroscopy this comparison is based on the concentration increment of ultrasound velocity [12, 13, 18] which is calculated from measured data as follows:

$$I = (U - U_0)/(U_0 m \rho_0) \quad (2)$$

where I is the concentration increment, U is the ultrasound velocity of the sample measured in the sample cell of HRUS equipment, U_0 is the ultrasound velocity measured in the pure solvent cell at the same conditions, m is the sample mass concentration and ρ_0 is the density of pure solvent (water) at the conditions of ultrasonic measurements. Concentration increment is proportional to the slope of the dependence of differential ultrasound velocity (the difference between the velocity in sample and in the solvent) on sample concentration. Consequently, it is very sensitive to measurement uncertainty when calculated directly from data points, especially at low concentrations. It is therefore more appropriate to calculate it from mathematical model fitting the experimental data.

2 Problem Formulation

Commented paper [1] presents experimental results on measurement of ultrasound velocity in aqueous solutions of various samples of humic substances. Interpretation of results is based on comparison with results obtained in similar measurements with traditional surfactants. Analysis is based on the concentration increment of ultrasound velocity which can give strange results even in the case of well-defined surfactant. The problem consists in mathematics of defining equation of the increment and is closely related with apparently negligible scattering of experimental data points which is natural consequence of limited precision of any

measurement. Potential problems can be easily revealed by checking the raw, really measured data, i.e. the ultrasound velocities.

Fractal analysis used in ref. [1] for additional evaluation of experimental data gives equations

humic concentration (cf. Figure 3 in the paper [1]), it was concluded that humic substances aggregate already at (very) low concentrations. However, this straightforward interpretation is unjustified for at

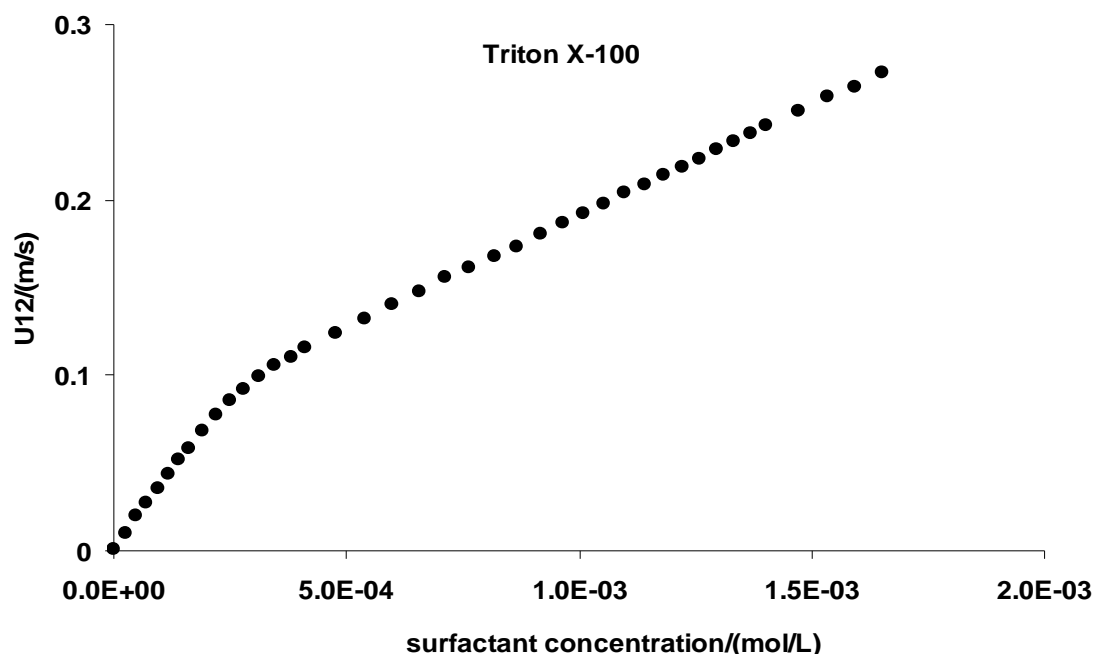


Fig. 1. Example of concentration dependence of ultrasonic velocity difference for Triton X-100 surfactant; change of slope occurs at the CMC

which are not fully consistent with established theory of ultrasound propagation. Sources of discrepancies are pointed out.

3 Discussion

The first part of the commented paper [1] reports on ultrasound velocimetry of humic solutions. Analysis of results is based on the concentration increment of ultrasonic velocity and on comparison of its concentration dependence measured for humic substances with that measured for common surfactants. Figure 2 in the paper [1] shows that the increment of a common surfactant is concentration independent below the surfactant critical micellar concentration (CMC) and decreasing with increasing surfactant concentration above CMC. Because the increment measured for humic substances was only decreasing with increasing

least following four reasons.

Reason 1. The paper presents no theory supporting that the decreasing increment is a result of the aggregation. Probably the only one published theory on ultrasonics of surfactant aggregation [17] does not lead to this result and is ignored in the paper.

Reason 2. In fact, the decreasing concentration increment is not a direct result of aggregation but a mathematical result of the concentration dependence of the ultrasound velocity. Fig. 1 of this note shows example of this dependence for Triton X-100 mentioned in the paper [1] which has been measured also in the same laboratory (as well as the other data in this note). Ultrasound velocity is given as a difference between the velocity in the surfactant solution and in the pure solvent (water) denoted U_{12} . Below the CMC, velocity is linearly increasing with concentration which is quite common behavior

of many various low-molecular as well as macromolecular substances. Above the CMC, the increase is still essentially linear but the slope is much lower.

Dependence of the concentration increment on the concentration at a given temperature is

calculated from measured data as is the case of the concentration increment. An example is on Fig. 2.

Fig. 2 clearly shows that humic substance (sodium salt of leonardite preparation 1S104H) does not show the (abrupt) change of slope upon micellization (aggregation), as is clearly seen for

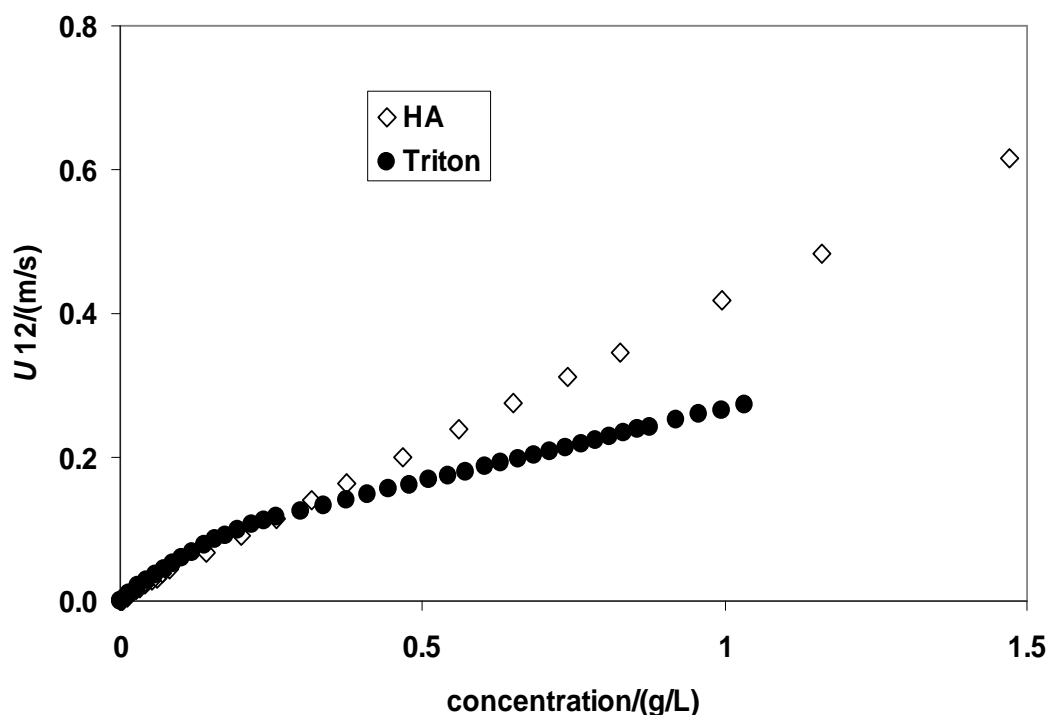


Fig. 2. Comparison of ultrasound velocity difference as a function of concentration for surfactant Triton X-100 and sodium salt of humic acid (HA) 1S104H

determined by the fraction U_{12}/c , where c is the concentration, because the other quantities in the original expression for the increment are constant at constant temperature. As illustrated in Fig. 1, below the CMC, $U_{12} = k_1 c$, where k_1 is the constant slope; consequently, $U_{12}/c = k_1$ and the concentration increment is independent on concentration. Above the CMC, $U_{12} = k_2 c + q$ and $U_{12}/c = k_2 + q/c$ and the concentration increment is decreasing function of concentration because of the term q/c .

Reason 3. If the argumentation in the paper is based on analogy only, why not to compare also the ultrasound velocities, which are really measured quantities, and not to compare only quantities

surfactant, and its dependency still resembles that of the surfactant in the pre-micellar region. Similar dependencies were measured also for the other humic substances discussed in the paper [1]. Consequently, humic aggregation is not proved by this analogy, which compares original, really measured data.

Reason 4. If the concentration dependence of U_{12} for humic substances does not correspond with that of traditional surfactants in the micellar (aggregation) region why then is the humic concentration increment of ultrasonic velocity still decreasing with increasing concentration, as illustrated in the commented paper? In principle, it is a result of measurement uncertainty especially at

very low concentrations, close to instrument sensitivity (resolution). In other words, it is a result

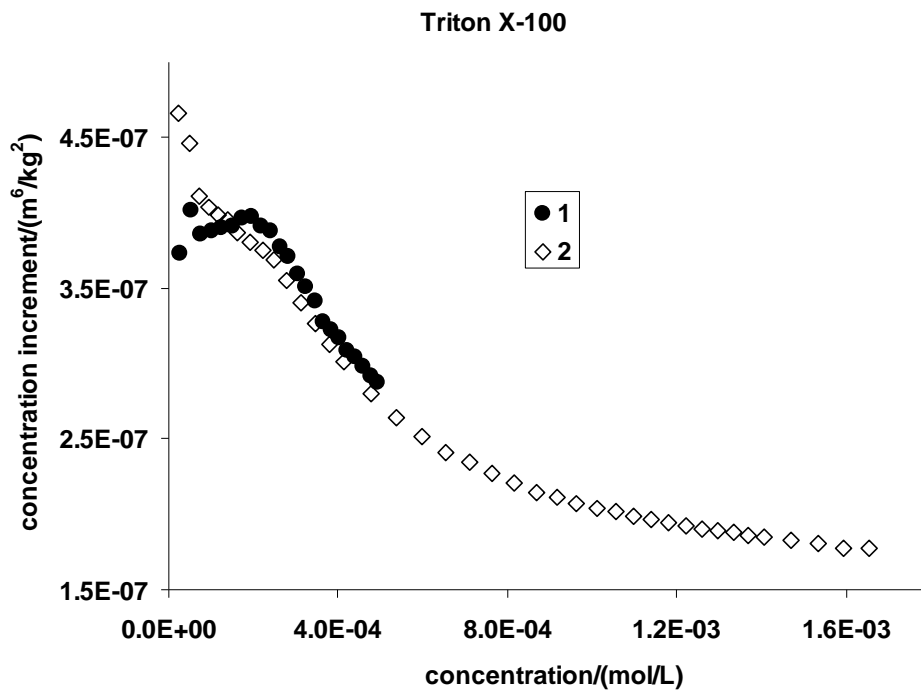


Fig. 3. Concentration increment of ultrasound velocity as a function of concentration for two replicated measurements with Triton X/100

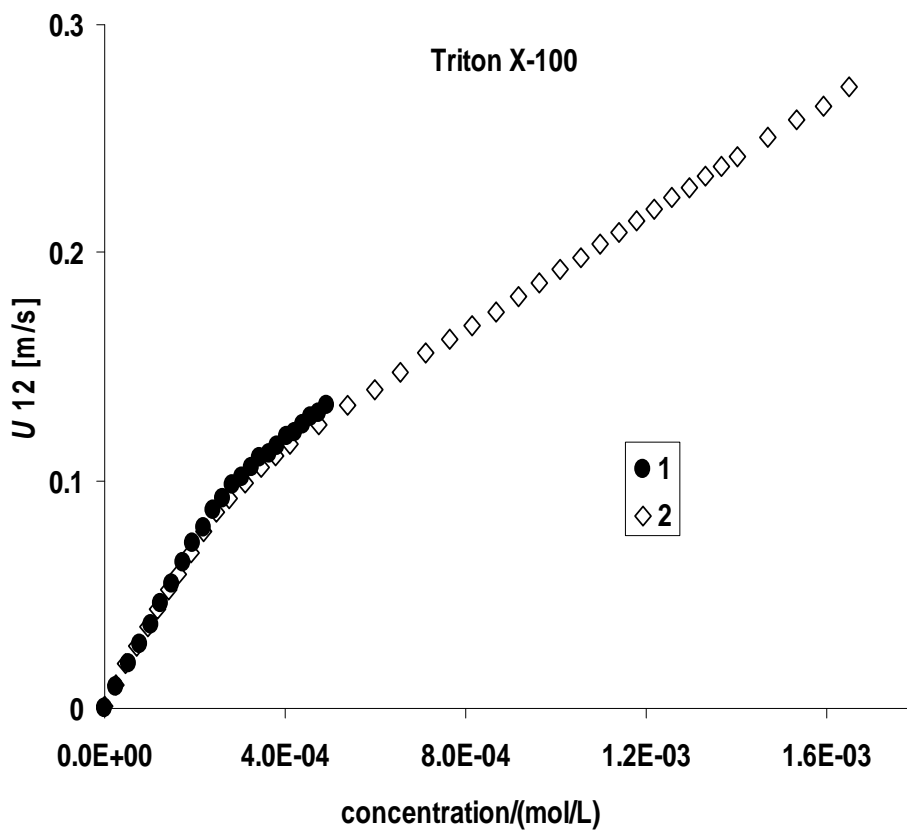


Fig. 4. Ultrasound velocity difference as a function of concentration for the same replicates as in Fig. 3

of ordinary experimental errors. Actually, the function $U_{12} = k_1c$ is not measured exactly but with some error ε : $U_{12} = k_1c + \varepsilon$. Recalculating to the increment gives: $U_{12}/c = k_1 + \varepsilon/c$ and the error ε/c

The fact that the increment is much more sensitive to measurement uncertainties than U_{12} , particularly at low concentrations, is illustrated on Figs. 5-7.

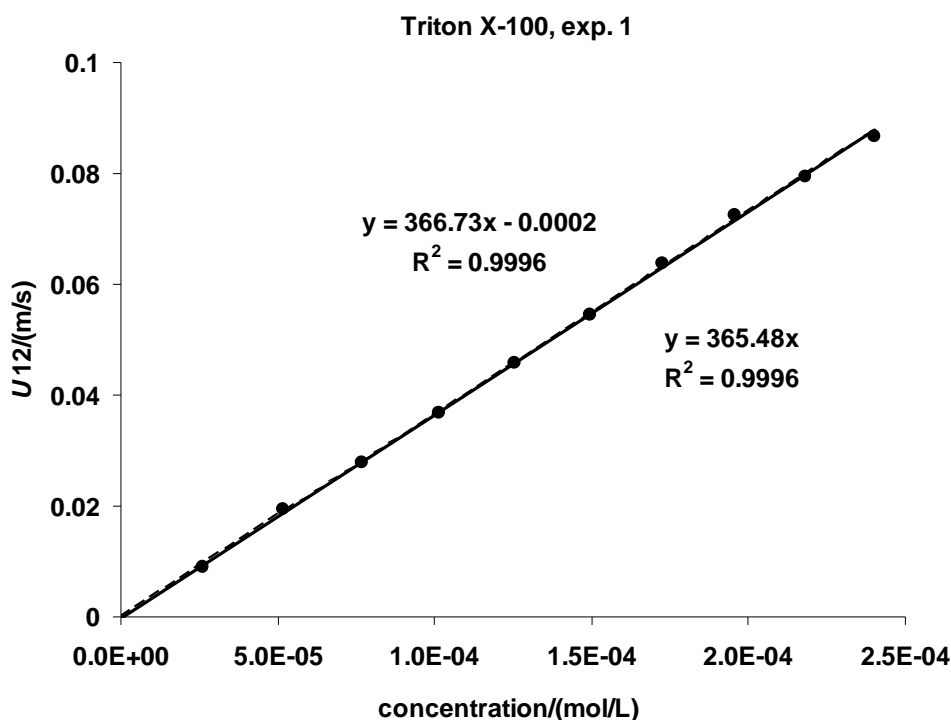


Fig. 5. Ultrasound velocity difference as a function of concentration in the pre-micellar region for measurement Nr. 1 from Fig. 3 or 4

term, which is a decreasing function of concentration, becomes significant especially at low concentrations. All this is illustrated on following figures.

Fig. 3 shows results of two replicated measurements for the surfactant, expressed as the concentration increment. Measurement Nr. 1 gave more or less constant increment in the pre-micellar region and decreasing increment after the CMC. On contrary, the second measurement produced only decreasing increment which should, according to the approach presented in the commented paper, indicate micellization, which is erroneous conclusion; Fig. 3 shows further that increment reproducibility is very bad. However, the concentration dependence of the ultrasound velocity difference (U_{12}) is much better reproducible and the change of slope, indicating the CMC, is well evident in both cases at practically the same surfactant concentration, cf. Fig. 4.

Figs. 5 and 6 show that data can be well fitted by two different straight lines. The first line, which is of the type $U_{12} = kc$, corresponds to what should be expected because when $c \rightarrow 0$, the liquid sample approaches the pure solvent for which $U_{12} = 0$ by definition. The second line, which is of the type $U_{12} = kc + \varepsilon$ mentioned above, has in the case of experiment Nr. 1 more than ten times lower the (absolute) value of the y -intercept (ε) than for experiment Nr. 2. Thus, in the former, the error term has much smaller effect on the calculation of the concentration increment. This is confirmed by Fig. 7 showing the concentration increments calculated from the fitting lines $U_{12} = kc + \varepsilon$ – increment of Nr. 1 is almost constant, whereas that of Nr. 2 is decreasing. Of course, fitting lines of the type $U_{12} = kc$ always give a constant value of this increment.

As an example of similar dependence in the case of humic substances, Fig. 8 illustrates U_{12} measured as a function of concentration for the

Pahoee peat humic acid. It is clear that both types of fitting line describe the data with comparable accuracy and cannot be distinguished.

compressibility (β). Usually, the effect of compressibility significantly outweighs the effect of density. However, there is no compressibility in

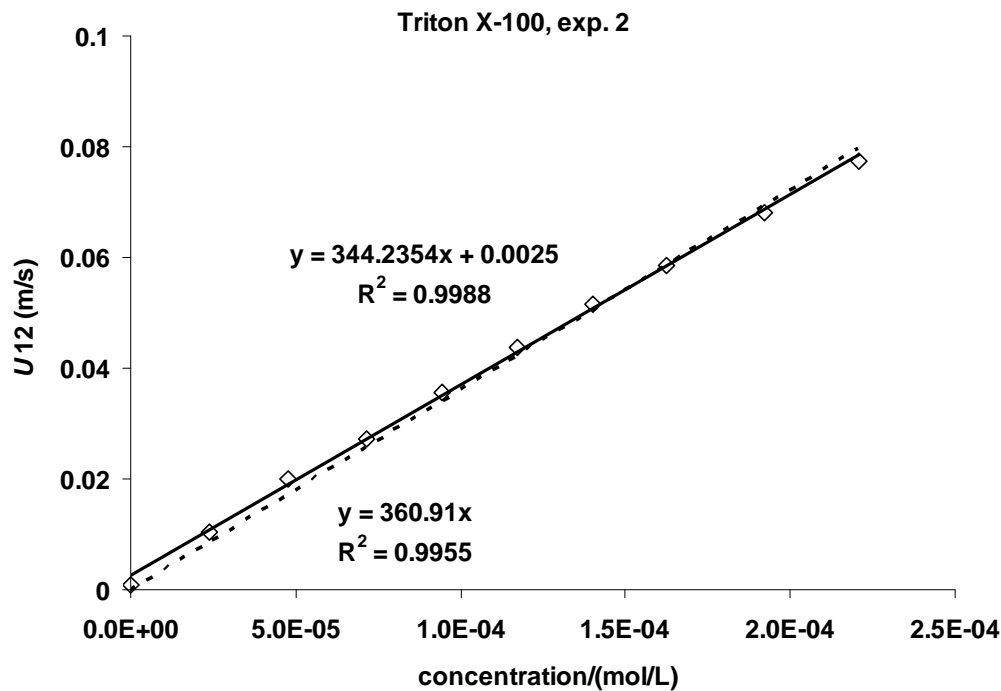


Fig. 6. Ultrasound velocity difference as a function of concentration in the pre-micellar region for measurement Nr. 2 from Fig. 3 or 4

The second part is devoted to the fractal analysis of the concentration increment. Besides the fact that this analysis is applied to problematic data (concentration increment) there are additional problems with the very fractal analysis; the most important are summarized in what follows.

Problem 1. Equation (6) in the paper [1], which is reproduced here as Equation (3)

$$v = \sqrt{\frac{k_v K}{D(D-1)} \left(\frac{\rho}{k_\rho K} \right)^{\frac{D-1}{2(D-3)}}} \quad (3)$$

(v is the sound velocity, k_v is the proportionality constant in fractal expression for potential [1], K is the fractal measure, D is the fractal dimension, ρ is the density, k_ρ is the proportionality constant in fractal expression for density), was derived to describe the dependence of ultrasound velocity on density. There is a well-known equation, sometimes called the Laplace equation, see (1), which is used for many decades to demonstrate that the ultrasound velocity depends on density *and*

equation (3). It might be hidden in the other parameters or quantities appearing in this equation but simple dimensional analysis shows inconsistent units.

Problem 2. Equation (3) cannot be used for systems with $D = 3$, i.e., for “smooth” (regular, “normal”, “non/fractal”) three-dimensional particles or homogeneous media (pure solvent), because in this case the exponent (and the ultrasound velocity) $\rightarrow \infty$.

Problem 3. Equation (7) in the commented paper [1], which is reproduced here as Equation (4)

$$U - U_0 = \sqrt{\frac{k_v K}{D(D-1)} \left(\frac{\rho - \rho_0}{k_\rho K} \right)^{\frac{D-1}{2(D-3)}}} \quad (4)$$

should describe the difference between the ultrasound velocity measured in the humic solution (U) with the density ρ and the velocity measured in the solvent (water; U_0) of the density ρ_0 . This equation was derived by a very strange direct

substitution of velocity and density differences into equation (3). It is absolutely not clear why the difference was not expressed as a difference between two terms given by equation (3) as expected:

$$U - U_0 = \sqrt{\frac{k_v K}{D(D-1)}} \left(\frac{\rho}{k_\rho K} \right)^{\frac{D-1}{2(D-3)}} - \sqrt{\frac{k_v K}{D(D-1)}} \left(\frac{\rho_0}{k_\rho K} \right)^{\frac{D-1}{2(D-3)}} \quad (5)$$

Original equation (1) is claimed to express the dependence of the velocity of sound on the density of media but $U - U_0$ is not the velocity in media of

decreasing function of humics concentration analyzed in the commented paper [1] is mathematical artifact resulting from improper data treatment and all subsequent discussions are doubtful.

- Equations used to apply the fractal analysis were derived in a very strange or even incorrect ways. Unless these discrepancies are resolved, the fractal analysis is useless.

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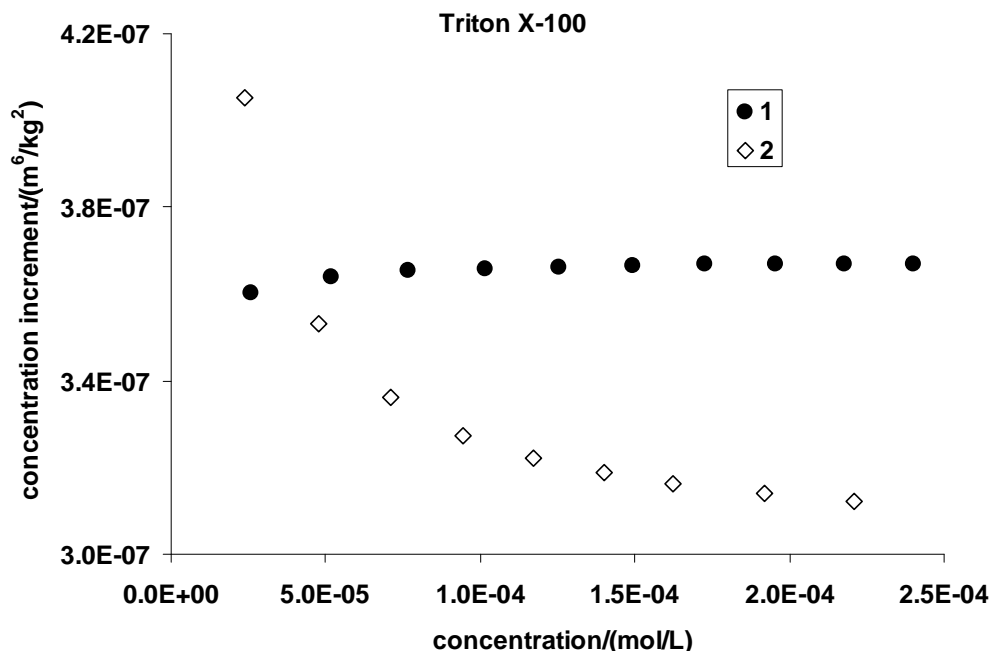


Fig. 7. Concentration increment of ultrasound velocity calculated from fitting lines $U_{12} = kc + \varepsilon$ shown in Fig. 5 and 6

density $\rho - \rho_0$ as should then follow from Equation (2). $U - U_0$ is just the difference between two velocities independently measured in media with density ρ and ρ_0 .

4 Conclusion

To summarize:

- Very probably, the concentration increment of ultrasonic velocity as a

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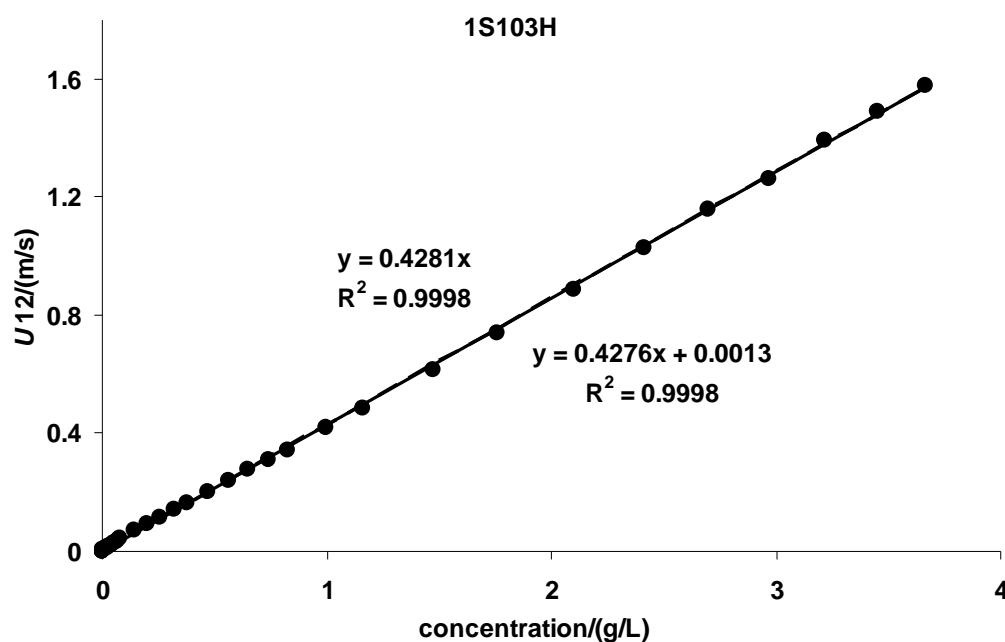


Fig. 8. Ultrasound velocity difference as a function of concentration measured for the sodium salt of the Pahokee Peat humic acid 1S103H

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