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# Octanol–water partition coefficient of glucose, sucrose, and trehalose

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**Abstract**—The octanol–water partition coefficients ($P$) of glucose, sucrose, and trehalose were measured at temperatures between 5 and 20 °C using an enzymatic method. The measured $\log P$ is compared with calculated and experimental data previously reported. In the case of trehalose, the measured $\log P$ differs considerably from the theoretically estimated values and agrees with the expected value for a disaccharide. Some methods of assessing the partition coefficients are also analyzed and it is concluded that the atom/fragment contribution method overestimates the hydrophilicity of disaccharides and, probably in a larger extension, that of trisaccharides. The knowledge of $P$ for these sugars is valuable both for basic or applied purposes, including food and biomolecules stabilization.
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## 1. Introduction

The partition coefficient of solutes between octanol and water, $P$, is extensively used in environmental and biomedical sciences as a descriptor of the lipophilicity–hydrophilicity properties of different compounds.[1] Also, several quantitative structure–activity relationships (QSAR) use $P$ for predicting such compound properties as permeability through membranes,[2] binding at receptor sites,[3] bacterial spore germination, inhibitory activity,[4] and others.

The octanol–water system is a reference for modeling the partition between water and a biophase. The reason is simple: octanol has a polar head and a long non-polar tail, thus resembling the features of phospholipids and proteins found in many biological systems.

The aim of this work is to measure $\log P$ for trehalose, because the theoretically estimated value for this disaccharide seems to overestimate its hydrophilicity as compared with sucrose. Both saccharides are widely used in the stabilization of foods and biomolecules,[5–7] and thus the knowledge of $P$ should provide useful information on the sugar distribution in macroscopic samples, and the degree of interaction with the molecules to be cryoprotected. Even when the trehalose-protection effect is related to specific polar interaction of –OH sugar groups with the polar head of the phospholipidic bilayers, $\log P$ gives us an indication of the preference of the sugar to interact with water instead of the phospholipid head.

Table 1 shows the $P$ values reported for a monosaccharide (glucose), disaccharides (sucrose and trehalose), and a trisaccharide (raffinose) by several authors. While the experimental values for glucose are in good agreement, those for sucrose spread over one order of magnitude. For trehalose and raffinose there are no experimental values, but they have been estimated using the atom/fragment contribution method.[21]

The differences observed among the octanol–water partition coefficients of these sugars from several references are noteworthy. The estimated $\log P$ value for raffinose,[20] calculated using the group contribution relationship,[22] indicates that raffinose is less lipophilic than sucrose. There is an unexpectedly large difference in the lipophilicity of trehalose and sucrose, taking into

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**Table 1.** Reported octanol–water partition coefficients ($P$) of several carbohydrates

| Sugar | $P$ | $\log P$ | $T$, °C | Method (Ref.) |
|---|---|---|---|---|
| Glucose | $7.9\times10^{-4}$ | −3.10 | 25 | Radiochemical[8] |
| | $9.5\times10^{-4}$ | −3.02 | [a] | Liquid chromatography[9] |
| | $1\times10^{-3}$ | −3.00 | [a] | Radiochemical[10] |
| | $1.58\times10^{-2}$ | −1.8? | [a] | Not reported[11] |
| | $5.7\times10^{-4}$ | −3.24 | [a] | Experimental (?) cited in lit.[12] |
| Sucrose | $3.9\times10^{-4}$ | −3.41 | [a] | Radiochemical[13] |
| | $4.6\times10^{-4}$ | −3.34 | 37 | Liquid chromatography[14] |
| | $2\times10^{-4}$ | −3.7 | Ambient | Radiochemical[15] |
| | $5.7\times10^{-4}$ | −3.24 | [a] | Radiochemical[16] |
| | $9.7\times10^{-4}$ | −3.01 | [a] | Not reported[11] |
| | $1.9\times10^{-3}$ | −2.70 | [a] | Radiochemical[17] |
| | $2.1\times10^{-4}$ | −3.67 | [a] | Radiochemical[18] |
| | $2.0\times10^{-4}$ | −3.7 | Ambient | Experimental (?) cited in lit.[12] |
| | $7.9\times10^{-5}$ | −4.1 | [a] | Estimated[19,b] |
| Trehalose | $3.31\times10^{-6}$ | −5.48 | [a] | Estimated[12,c] |
| Raffinose | $5.49\times10^{-5}$ | −4.26 | [a] | Estimated[20,d] |
| | $1.7\times10^{-7}$ | −6.76 | [a] | Estimated[1,c] |

[a] Not reported in the original paper.
[b] Computational method.
[c] Atom/fragment contribution method.
[d] Group contribution relationship.

account the similar group contribution to $\log P$ expected for both disaccharides. Also remarkable is the disagreement between the $\log P$ values estimated for raffinose using the atom/fragment contribution method[12] and the group contribution relationship.[22]

These facts prompted us to measure the octanol–water partition of trehalose, sucrose, and glucose at 5 and 20 °C, using the same experimental procedure. We also recalculated the $\log P$ values for several sugars to test whether the observed differences in the estimated $\log P$ values were artifacts due to errors in the calculations or a failure of the theoretical method. The aim of performing the experimental measurements at two temperatures is to assess the effect of temperature on the partition coefficient, since these sugars are usually employed in biomolecule stabilization below room temperature.

### 1.1. Theory

If two liquid phases containing arbitrary concentrations of the same solute are in contact, the solute crosses the interface and is distributed between the liquids until the partition equilibrium is reached. The thermodynamic condition of this partition equilibrium is the equality of the chemical potential ($\mu$) of the solute in both phases, water and octanol. In our case

$$\mu_i^{\mathrm{w}}(T, c_i^{\mathrm{w}}) = \mu_i^{\mathrm{oct}}(T, c_i^{\mathrm{oct}}) \tag{1}$$

where $T$ is the temperature and $c_i^{\mathrm{w}}$ and $c_i^{\mathrm{oct}}$ are the solute concentrations in water and octanol, respectively.

The expression for the chemical potential of the solute in each liquid phase, assuming that the solution behaves as ideal (activity coefficients equal 1 in both phases), is given by

$$\mu_i = \mu_i^{\mathrm{o}} + RT \ln c_i \tag{2}$$

where $\mu_i^{\mathrm{o}}$ is the standard chemical potential. Thus, combining Eqs. 1 and 2 we obtain

$$\frac{\mu_i^{\mathrm{w}} - \mu_i^{\mathrm{oct}}}{2.303RT} = \log\left(\frac{c_i^{\mathrm{oct}}}{c_i^{\mathrm{w}}}\right) = \log P \tag{3}$$

where the partition coefficient of the solute between octanol and water is defined as

$$P = \frac{c_i^{\mathrm{oct}}}{c_i^{\mathrm{w}}} \tag{4}$$

Therefore, $\log P$ is a measure of the difference of chemical potential of the solute between water and octanol in the standard state (ideal 1 mol dm$^{-3}$ solution).

Leo et al.[22] showed that the chemical potential of the solute can be arbitrarily divided into lipophilic and hydrophilic contributions, that is,

$$\mu_i = \sum \mu_i^{\mathrm{lip}} + \sum \mu_i^{\mathrm{hyd}} \tag{5}$$

The partition equilibrium condition of the solute molecule can be applied to each group, yielding the general relationship

$$\log P = \sum \log P_i^{\mathrm{lip}} + \sum \log P_i^{\mathrm{hyd}} \tag{6}$$

where $P_i$ is the contribution of the $i$ group to the partition coefficient of the solute.

The additivity of a wide variety of groups was proposed by Leo et al.[22] defining a substituent constant $\pi$

$$\pi_X = \log P_X - \log P_H \tag{7}$$

where $P_X$ is the partition coefficient of the $X$-substituted molecule and $P_H$ the partition coefficient of the unsubstituted molecule.

Using the additivity concept, the $\log P$ of the trisaccharide raffinose can be estimated using the relationship

$$\log P_{\text{raffinose}} = \log P_{\text{galactose}} - \log P_{\text{sucrose}} - \log P_{\text{glucose}} \quad (8)$$

yielding the value $\log P = -4.26$ reported in Table 1.[20]

The atom/fragment contribution method[21] proposed to determine octanol–water partition coefficient of organic compounds is based on the assignment of atom/fragment contribution values and correction factor occurring in the molecule

$$\log P = A + \sum_i n_i B_i + \sum_j C_j \quad (9)$$

where $A$ is a constant, $n_i$ the number of fragment $i$ in the molecule, $B_i$ is the fragment contribution to $\log P$ and $C_j$ is the $j$th correction term. The values of $A$, $B_i$, and $C_j$ were obtained by multiple linear regression on more than 1000 compounds.

## 2. Experimental

### 2.1. Materials and sample preparation

D-Glucose (Merck, Darmstadt, Germany), sucrose (Riedel-de Haën, Germany), and trehalose dihydrate (Hayashibara, Okayama, Japan) were used without any further purification. 1-Octanol, anhydrous 99%, was obtained from Merck, Darmstadt, Germany, p.a. grade. Milli-Q water (>10 MΩ cm) was used for all experimental work. The enzymes invertase (β-fructofuranosidase from *Saccharomyces cerevisiae*) and trehalase (α,α-trehalose glucosidase from porcine kidney) were from SOLVAY, Bioproducts Div., BsAs, Argentina and from Sigma Chemical Co., St. Louis, MO, respectively.

Equal volumes (2 mL) of the octanol and the aqueous sugar solution (concentration 1 M in all cases) were placed in 15 mL vials. The two phases systems were mixed by inversion (10 times) and were incubated at 5 and 20 °C. Two octanol samples (approximately 1.5 mL) were collected and weighted as a function of time of storage, and the amount of sugar in the organic phase was determined as described below.

### 2.2. Sugar determination

The concentration of sucrose or trehalose in the organic phase was determined after enzymatic hydrolysis to its component monosaccharides (glucose and fructose for sucrose and glucose for trehalose) using an enzymatic method[23,24] based on the oxidation of glucose by glucose oxidase to gluconic acid and hydrogen peroxide (glucose concentrations around 0.1% w/w was found optimal for this purpose). The latter compound, in the presence of peroxidase, causes the oxidative coupling of phenol with 4-aminophenazone, forming a red-colored chromogen whose absorbance was measured spectrophotometrically at 505 nm. As octanol interfered with the enzymatic method employed for glucose determination, the organic solvent was first evaporated-off placing the samples in a vacuum glass chamber at 70 °C for 3 h.

**2.2.1. Trehalose hydrolysis.** After evaporation of octanol the residue was resuspended in 0.25 M $Na_2CO_3$. The reaction mixture consisted of 10 μL of the dissolution, 5 μL of AcOH (1 M), 5 μL of NaOAc (300 mM, pH 5.5), and 10 μL of the enzyme trehalase (49 enzymatic units (EU)/mL of buffer, where one unit is the amount of enzyme required to produce 1 μmol of glucose per 120 min at pH 5.7 at 37 °C). The solution was mixed and incubated for 120 min at 37 °C. After incubation, 10 μL of 0.33 M $Na_2CO_3$ was added to inactivate the enzyme. Glucose was then determined as previously described. The amount of trehalose was calculated as

$$\text{trehalose (g)} = \text{glucose (g)} \cdot (342/180) \cdot (1/2)$$

A standard curve was obtained with five known concentrations of trehalose in the range of 0.025–0.25% (w/w) ($R^2 = 0.992$), the standard deviation calculated from eight measurements of the same concentration was 0.005.

**2.2.2. Sucrose hydrolysis.** After evaporation of octanol the residue was resuspended in 100 μL citrate buffer (0.1 M, pH 5). Enzyme invertase (10 μL) was added (320 EU/mL of citrate buffer, where one unit is the amount of enzyme required to produce 1 μmol of glucose per hour at pH 5 at 37 °C) and the vials were incubated for 60 min at 37 °C. After incubation, 15 μL of 0.33 M $Na_2CO_3$ was used to inactivate the enzyme. Glucose was then determined as previously described and the amount of sucrose calculated as

$$\text{sucrose (g)} = \text{glucose (g)} \cdot (342/180)$$

A standard curve was obtained with five known concentrations of sucrose in the range of 0.025–0.25% (w/w) ($R^2 = 0.998$); the standard deviation calculated from eight measurements of the same concentration was 0.006.

## 3. Results and discussion

The octanol–water partition coefficient of glucose, sucrose, and trehalose was determined from the ratio of the molar concentration of the compound in octanol

to that in the water phase after equilibration (7 days) at the selected temperature. Due to the small concentration of the saccharides in the octanol phase, their concentration in the aqueous phase was taken as the initial concentration, prior to equilibration with the octanol phase.

The averaged values of 10 determinations of $P$ are reported in Table 2 for each sugar.

It is observed that $\log P$ for the three saccharides studied in this work seem to be independent of temperature within the experimental uncertainty. Therefore, the comparison with literature values where temperature has not been indicated appears worthwhile.

The values obtained in this work for glucose (summarized in Table 2) are in relatively good agreement with those reported by Bas et al.[9] and Lennernas et al.[10] (see Table 1). For sucrose, our results are in very good agreement with those reported by Glynn and Yazdanian[14] and close to the values obtained by Cornford[16] and by Johnson et al.[13] The new value seems to corroborate that the data by Martin and Edington[17] and Levin[18] deviates from the average value reported in the literature for sucrose.

Using Eq. 9, it is possible to estimate $\log P$, as quoted in Table 1 for several sugars. The values reported in Ref. 12 could not be reproduced in this work using the values of $B_i$ and $C_j$ reported by Meylan and Howard.[21] For instance, we obtained $\log P$ values of $-3.14$, $-6.23$, $-5.77$, and $-9.87$ for glucose, sucrose, trehalose, and raffinose, respectively. In the case of raffinose, the difference with the estimated value reported[12] is particularly large.

The estimated value reported[12] for the monosaccharides mannose and galactose ($\log P = -2.43$) as compared with that of glucose ($\log P = -3.24$) is also unexpected because these monosaccharides only differ in the equatorial or axial orientation of the OH groups, which is not taken into account in the atom/fragment contribution method.

The experimental $\log P$ measured for sucrose differs significantly from the values estimated by Genty et al.[19] using a computational method[25] and the calculated in this work ($\log P = -6.23$). Other theoretical methods, such as the UNIFAC models[26,27] were not successful in predicting the octanol–water partition coefficient of sugars.[28]

**Table 2.** Measured octanol–water partition coefficients ($P$) of glucose, sucrose, and trehalose

| Sugar | $T$, °C | $P$ | $\log P$ |
|---|---|---|---|
| Glucose | 5 | $(1.4 \pm 0.1) \times 10^{-3}$ | $-2.84 \pm 0.03$ |
| | 20 | $(1.5 \pm 0.1) \times 10^{-3}$ | $-2.82 \pm 0.04$ |
| Sucrose | 5 | $(4.9 \pm 0.4) \times 10^{-4}$ | $-3.31 \pm 0.04$ |
| | 20 | $(5.0 \pm 0.4) \times 10^{-4}$ | $-3.30 \pm 0.03$ |
| Trehalose | 5 | $(1.75 \pm 0.3) \times 10^{-4}$ | $-3.76 \pm 0.06$ |
| | 20 | $(1.71 \pm 0.2) \times 10^{-4}$ | $-3.77 \pm 0.05$ |

It should be noted that recommended $\log P$ values[12] are higher than the experimental ones reported in the present work for glucose, sucrose, and trehalose. For trehalose the value quoted in the SRC Database[12] ($\log P = -5.48$) was estimated using the atom/fragment contribution method[21] and differs significantly from the $\log P$ measured here ($\log P = -3.76$). This value looks reasonable as compared to those experimental $\log P$ reported for sucrose (Table 1), and it would indicate that the lipophilicity of trehalose is lower than that of sucrose but not to the extent predicted by theory. Although one could expect that the $\log P$ criteria have low comparative–quantitative predictive power related to stabilization by sugars of cells in the dry state, Crowe et al.[29] concluded that under ideal conditions of drying and storage, the efficiency of trehalose and other sugars is similar, a result that suggests that, even under these conditions, similar hydrophilicity would lead to similar protective efficiency.

The use of fragment methods to estimate $\log P$ could lead to conflicting values[22,25] due to different ways for decomposing the molecule in fragments. From the values of $\log P$ reported in Table 1 and those recalculated in this work, we conclude that the atom/fragment contribution method overestimates the hydrophilicity of the sugars, and the differences with the experimental results increase with from mono- to tri-saccharides.

## 4. Conclusions

The octanol–water partition coefficient was measured at 5 and 20 °C using an enzymatic method. The $\log P$ measured for glucose is in quite good agreement with that reported in the literature, while for sucrose our measurements agree with the set of experimental values around $\log P = -3.3$.

The $\log P$ obtained for trehalose is the first experimental value reported for this important disaccharide widely used in cryoprotection. As expected, its lipophilicity is not very different from that of sucrose, confirming that the estimated $\log P$ value[12] using the atom/fragment contribution method is not reliable.

In general, the estimated $\log P$ values of saccharides reported by different authors, using the same theoretical method or different ones, are in disagreement with both, present and published experimental values.

The group-contribution method overestimates the hydrophilicity of sugars, and the differences with the experimental results are larger in the case of di- and probably tri-saccharides (an experimental determination of $\log P$ for raffinose would help to confirm this statement). The application of these methods for the estimation of the lipo/hydrophilicity of sugars should be discouraged until a new set of experimental data on di-, tri-, and oligosaccharides can be incorporated in

the regression analysis to improve the contribution of the fragments/groups relevant for these compounds and the corresponding correction terms.

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