Bulgarian Chemical Communications, Volume 50, Issue 1, (pp. 63 – 68) 2018

Effect of controlled volume variation on the osmotic rate in aqueous solutions

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Received August 8, 2016; Accepted January 10, 2017

The evolution of the osmotic pressure in aqueous solutions was studied experimentally as a function of time in two different regimes: of constant and variable solution volume. Quantitative dependence of the solvent osmotic rate on the relative solution volume variation was established as well. Glucose, a biologically active substance, was chosen as a reference solute for the complex tests. A custom made osmotic cell was used. A novel operative experimental approach, employing controlled limited variation of the solution volume was developed and applied for the purpose. First of all, the obtained kinetic dependencies reveal strong divergence in the rates of the process at the two experimental regimes. The rise of pressure is much faster at constant solution volume, while the solvent influx is many times greater in the regime of variable volume. Moreover, the rate of the osmotic process is being modified by varying the solution volume. We consider the effects established here by means of an artificial semipermeable membrane to be of relevance for the processes taking place in the real living cells and tissues.

Keywords: membrane permeability, semipermeable membrane, osmotic kinetics

INTRODUCTION

Osmosis, i.e. the passage of fluid (usually water) through a semipermeable membrane, has been known for almost two centuries. Although always being on the agenda, for rather long time it seems not to have drawn largely the attention of the researchers. Yet, it needs not be surprising that in the latest years, with the discovery of the role of aquaporins as selective pores in water transport, the interest to this phenomenon has undergone genuine revival. As K. Alleva *et al.* have formulated the issue in their excellent review paper (Aquaporins: Another piece in the osmotic puzzle) [1]: "The elucidation of osmotic phenomena will help to understand central issues such as the identification of the causes of previously identified syndromes ….. and could also aid in finding adequate therapies for various pathologies, the comprehension of water management by plants, and the development of efficient methods for water purification. Therefore, unveiling the osmotic process is important both at the biological and technological level".

The driving force of the osmotic process is the concentration difference between two solutions separated by a semipermeable membrane. It creates pressure difference across the membrane (*osmotic pressure)*. Solvent transport takes place from the more diluted solution to that of higher concentration, until equilibrium is reached. J. H. van't Hoff was the first [2] to propose a theory and a formula, named the *van't Hoff law*, for the (equilibrium) osmotic pressure, Π, resulting from the transfer of solvent through the membrane:

$$\Pi = cRT \,, \qquad (1)$$

where $\boldsymbol{c}$ (mol/m^3) is the molar concentration of the dissolved substance, R (8.314 J/mol K) is the universal gas constant, T (K) is the absolute temperature. This equation is still in use, along with a number of more complex formulae for Π that have been produced since as well [3-8]. Although we are aware of the supposedly more precise formulae, we have found the original van't Hoff law to be entirely sufficient for the tasks considered here, as discussed further.

Equilibrium studies of osmosis, whether theoretical or experimental, predominate in the literature, but the interest to the kinetic aspects of the process has persisted through the years [9-12]. Osmotic equilibrium is considered to be well understood from thermodynamic viewpoint and does not pose serious ambiguities. In contrast, the dynamic aspects of the process frequently exhibit new and even surprising effects, which are difficult to explain within the frames of the traditional kinetic models.

The aim of our present investigation was to examine in detail the specific features of the osmotic process in an aqueous solution under dynamic conditions as a function of time, while applying two different experimental regimes: of

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constant and variable solution volume. In the case of confined volume, Krustev, Kolikov *et al.* [13] have introduced the term 'confined osmosis', defined as "…osmosis at which practically constant solution volume is maintained by external mechanical influence, resulting in an increase of the hydrostatic pressure in this volume".

The specific tasks of the present investigation required a novel approach and modification of the classical experimental setup. In the classical membrane osmometry Π is directly determined by the hydrostatic pressure value established in an "open mode" – through the rise of the liquid level in the solution compartment. Of course, such an approach is only suitable at moderate elevation – of the order of decimeters – which, accordingly, means small concentration differences: up to a few tens of millimoles per liter (see Eq. 1). An alternative mode, without such limitations, is conducting the process in a closed constant volume [4, 5, 8] and determining Π by means of an appropriate pressure sensor. Here we put forward an operative hybrid method, which combines the advantages of the two above: it comprises controlled variation of the solution volume, which permits measuring much higher pressure levels, when subjected to the "open mode".

For the complex tests we have chosen glucose: a low molecular mass compound that is a popular biological agent, already used elsewhere as a reference in osmotic studies [8].

MATERIALS AND METHODS

Glucose Braun G-5 (5% glucose of high purity; B. Braun Melsungen AG, Germany) was employed in all experiments. Polyamide composite semipermeable Koch RO (reverse osmosis) membranes were used within the prescribed ranges (pH = 4-11; temperature < 50°C). All solutions were prepared with Elga Labwater (model PURELAB Option-Q7) deionized water. The membrane osmometer employed in our experiments was specially designed and built for the purpose [14]. It consists of two cylindrical plastic shells: for solvent and solution, respectively. A semipermeable membrane of 5.0 cm diameter was sealed between the shells and was supported against deformations by two additional perforated Plexiglas disks on either side. The operative area of the membrane (the integral surface area of orifices) was ca. 5 cm^2, Fig. 1. Such a kind of a membrane osmometer, with solution compartment of constant volume, has been employed already in our preceding study of equilibrium osmotic pressure [15].

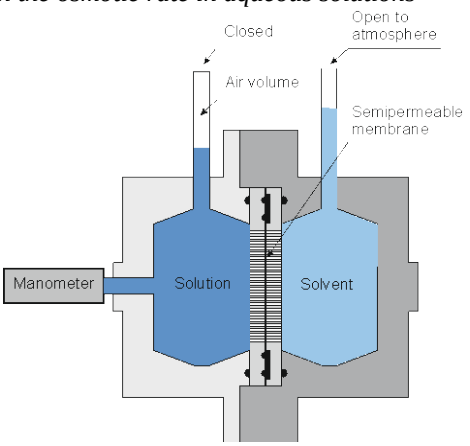

Fig. 1. Schematic of the membrane osmometer (osmotic cell).

However, the specific tests of the present investigation, primarily, the comparison between osmotic rates at variable and constant solution volume, required further refinement. We applied here our novel "hybrid" modification of the cell with limited variation of the solution volume. Thin graduated 1.3 m long transparent plastic tubing of 2 mm radius was attached to the solution chamber to provide control over the liquid and air amount, and measure the solution level rise at variable volume. Thus, with initial capacity of the solution compartment of 60 cm^3, the attached tube provided variable additional volume of ca. 16 cm^3, that is, a possibility of volume change by up to some 25 %. We consider this sufficient for our present purpose. Of course, we could have supplied even larger span of volume variation, but such a step would have brought further complication, due to the substantial dilution of the studied solution upon time.

A unique and promising feature of this novel modification is its potential to control the rate of pressure rise. As pointed out by a reviewer of our work: „This is relevant not only for an understanding of biological systems but may have interesting technical applications, as potentially damaging abrupt pressure changes can be avoided".

Of course, as required by the gas laws, in the case of (limited) volume variation the 'solvent influx *vs.* pressure' dependence is not linear: the liquid flux per unit pressure steadily decreases upon building osmotic pressure, as illustrated by Fig. 2. Yet, for the purpose of our comparison here such non-linearity does not create any problems. The ultimate solution concentrations were derived by means of the amount of solvent passed through the membrane. The corresponding osmotic pressure was registered by a 16-bar electronic pressure sensor (reading ±0.01 bar). All experiments were conducted at a temperature of 22^{O}C.

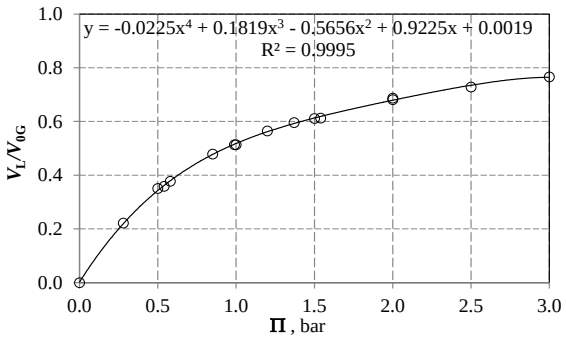

Fig. 2. Solvent influx V_L, relative to the vacant initial (gas) volume V_{0G}, as a function of the osmotic pressure Π in the regime of limited variation of the solution volume.

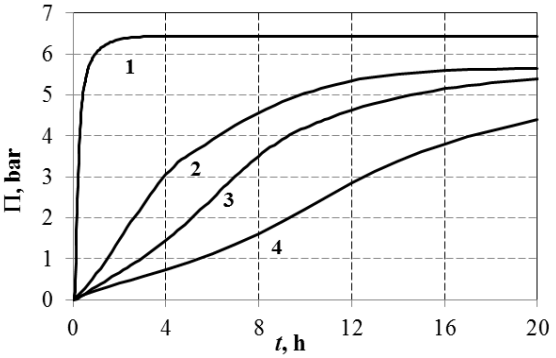

Fig. 3. Osmotic pressure Π vs. time *t* dependence for the two experimental regimes: (1) Constant volume); (2) Variable volume (+ 4 cm^3); (3) Variable volume (+ 8 cm^3); (4) Variable volume (+ 16 cm^3).

RESULTS AND DISCUSSION

The solute concentration as chosen for the comparison of the osmotic rates for processes at constant and variable solution volume was 5% = 0.278 mol/L glucose. We shall remind here that, at such a level of solute concentration, we have found that the use of the original van't Hoff law as a reference for the equilibrium osmotic pressure values proved entirely sufficient for the tasks considered here. The results of the extensive study of Grattoni *et al.* [8] have clearly shown, that the divergencies between the equations describing the equilibrium osmotic pressure in the references cited above [2-8] become significant at solute concentrations above ca. 0.5 mol/L, corresponding (at room temperature) to maximal (equilibrium) osmotic pressure values of the order of 12 bar. At our chosen concentration of solute c = 0.278 mol/L, we have operated in the range of moderate levels of osmotic pressure values below 7 bar. In this range, the deviations between all the above cited equations are well within the limits of the experimental scatter, as established in ref. [8] for a number of nonionic, low-molecular solutes, including glucose.

The obtained experimental results are presented in Figs. 3-6 and Table 1.

The juxtaposition of the obtained kinetic dependences, as presented in Fig. 3, demonstrates the drastic differences in the rates of osmotic pressure rise for the two regimes.

With variable cell volume, the osmotic pressure rise occurs at much slower rate. However surprising at first sight, this finding can be regarded as a quite natural result. The amount of solvent, which has to pass into the solution compartment of the cell, in order to lift the osmotic pressure, differs dramatically in the two regimes.

For example, employing the value for the coefficient of compressibility of pure water of 4.6×10^{-5} bar^{-1}, one estimates that for a closed cell of solution volume of 60 cm^3 the amount of solvent needed to raise the pressure by one atmosphere is 2.76×10^{-3} cm^3 (= 1.53×10^{-4} moles of H_2O). Concurrently, in our case of limited solution volume variation, even by the addition of as little as 4 cm^3 to the initial 60 cm^3, the amount of solvent necessary to lift the pressure up to a level of Π = 1.0 bar will be *ca*. 2.08 cm^3 (= 0.115 moles of water; conf. Fig. 2). The latter amount is some 750 times larger than that at constant volume and, of course, will definitely require longer time for transport. For the sake of comparison we can also employ the classical case of unlimited solution volume variation. For an osmotic cell connected to an open tube of radius as small as 2 mm (= 0.2 cm), the amount of solvent necessary to lift the solution level by 10.2 m (in order to impose hydrostatic pressure of 1 atmosphere) would be $4\pi\times10^{-2}$ (cm^2) $\times$ 1.02×10^3 (cm) = 128 cm^3 (= 7.1 moles of H_2O)!

The '$d\Pi/dt$ *vs.* t' dependences, as derived from the data of Fig. 3 and presented in Fig. 4 (a,b), exhibit marked differences in the kinetic behaviour of the studied systems at the two regimes:

Firstly, the rate of pressure increase reaches many times greater values at the regime of constant volume and the temporal dependence passes through a sharp maximum.

Secondly, one can observe distinct differences in the $d\Pi/dt$ pattern at different volume expansion. At the lowest level of 4 cm^3, a well-defined maximum in the temporal dependence is still present. However, as the additional volume increases, the maximum becomes shallower, and at the largest level of volume variation (of 16 cm^3) it turns into a wave-shaped dependence, exhibiting first a shallow minimum, followed by a shallow maximum.

Thirdly, the maximal values of the rate of osmotic pressure rise, $\mathrm{d}\Pi / \mathrm{d}t$, steeply decrease with the volume expansion: from ca. 25 bar/h at constant volume (Fig. 4a) down to 0.32 bar/h at ΔV = 16 cm^3 (Fig. 4b).

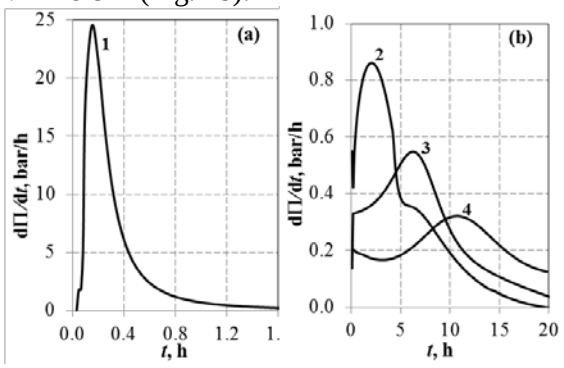

Fig. 4. Rates of osmotic pressure increase, $\mathrm{d}\Pi / \mathrm{d}t$ *vs. t* for the two experimental regimes: (a) Constant volume regime (1); (b) Variable additional volume regime: (2) 4 cm^3; (3) 8 cm^3; (4) 16 cm^3.

The integral temporal dependences for the amount of solvent transfer, 'Δn_L *vs. t*', presented in Fig. 5 (a,b) depict yet another remarkable finding. While the osmotic pressure rise is always faster at constant volume, the flow through the membrane is much faster in the regime of variable volume. As it must be noted, the scales for Δn_L in the two sections of Fig. 5 differ by three orders of magnitude! Thus, the solvent influx rates at variable regime turn to be practically by two orders of magnitude larger practically in all studied cases.

The above conclusion is reinforced by the differential temporal dependences, '$\mathrm{d}n_L / \mathrm{d}t$ *vs. t*', presented in Fig. 6. At constant volume the osmotic process appears to start at a slower rate and sharply accelerate with time to pass through an expressed maximum, beyond which the rate of solvent transfer rapidly declines. The picture is rather different in the regime of varied solution volume. Almost from the very start of the process the solvent transfer rates uniformly diminish with time at all such cases of different level of volume expansion.

All these results are outlined in Table 1, which presents the osmotic characteristics, as estimated in SI-units for the two different regimes: maximal total solvent influx values (Δn_L) at the final t = 20 h, maximal rates of osmotic pressure rise at constant volume $(\mathrm{d}\Pi / \mathrm{d}t)_{con}$, at variable volume $(\mathrm{d}\Pi / \mathrm{d}t)_{var}$ and their ratios for the different volume expansions; the corresponding values of the solvent transfer rates $(\mathrm{d}n_L / \mathrm{d}t)_{con}$ at constant volume, at variable volume $(\mathrm{d}n_L / \mathrm{d}t)_{var}$ and their ratios for the different volume expansions $\frac{(\mathrm{d}n_L / \mathrm{d}t)_{var}}{(\mathrm{d}n_L / \mathrm{d}t)_{con}}$; the times corresponding to the maximal pressure increase rates (τ_p) and maximal solvent influx rates (τ_n), and the ratios of instant to equilibrium pressure values at the solvent influx maxima ($\Pi_{(\tau_n)} / \Pi_{eq}$). The solvents influx rates were computed using an estimated value for the active membrane area of 4.65 cm^2.

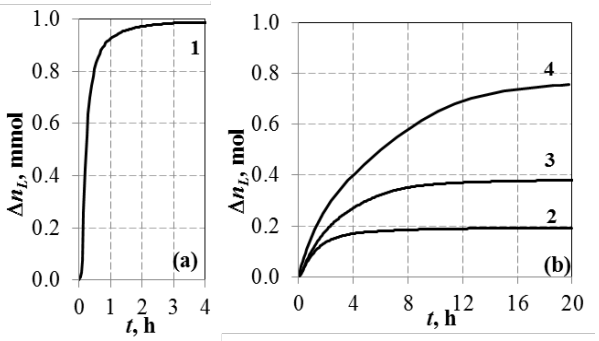

Fig. 5. Total solvent influx Δn_L as a function of elapsed time *t* dependences for the studied variations of solute volume: (a) Constant volume regime (1): Δn_L *is shown in millimoles*; (b) Variable additional volume regime: (2) 4 cm^3; (3) 8 cm^3; (4) 16 cm^3 (Δn_L *is shown in moles*).

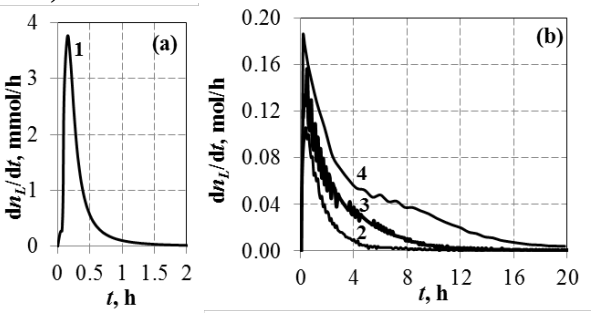

Fig. 6. Solvent rates of transfer differential dependences $\mathrm{d}n_L / \mathrm{d}t$ as a function of lapsed time *t* for the two regimes. (a) constant volume regime (1); (b) Variable additional volume regime: (2) 4 cm^3; (3) 8 cm^3; (4) 16 cm^3.

Among the obtained results some are quite surprising and far from easy to interpret at once. For instance, we would have rather expected fairly steady pressure and liquid transfer rates, especially in the initial stages, away from equilibrium. Nevertheless, the initial increase may be attributed to a delayed response of the semipermeable membrane to the early impact of solvent, to which it needs time to adjust.

Table 1. Comparison of the kinetic characteristics of the osmotic process in aqueous glucose solutions at the two different regimes (subscripts 'var' and 'con' indicate *variable* and *constant* solution volume. Active area of the semipermeable membrane S_M = 4.65 cm^3

	Constant volume	Varied volume		
	V_{0G} = 0 cm^3	V_{0G} = 4 cm^3	V_{0G} = 8 cm^3	V_{0G} = 16 cm^3
Δn_L [mol] (t=20h)	1.53×10^{-4}	0.19	0.38	0.75
$dp/dt\ (maximal)$ [mbar/s]	6.80	0.239	0.153	0.089
$\frac{(dp/dt)_{con}}{(dp/dt)_{var}}$	-----	28.5	44.5	76.5
$dn/dt\ (maximal)$ [mmol/m^{2}s]	1.97	65.7	95.6	113.5
$\frac{(dn/dt)_{var}}{(dn/dt)_{con}}$	-----	33.3	48.5	57.6
τ_p [s]	0.54×10^3	6.48×10^3	21.24×10^3	38.88×10^3
τ_n [s]	0.54×10^3	2.52×10^3	2.16×10^3	0.72×10^3
$p(\tau_n)/p_{eq}$ [%]	26.7	6.37	2.79	1.09

In any case, the onset of the decline beyond the maxima appears to occur too early to be interpreted in terms of the decreasing difference between equilibrium and instant osmotic pressure values (the driving force of the osmotic process toward equilibrium). The pressure value at the maximum is still sufficiently far from the respective upper limit of Π.

Besides those already observed in Figs. 3-6, there are more tendencies to note in Table 1 for the determined characteristics upon changing the experimental conditions. Such are e.g. the reverse trends in the time-span of reaching the maximal pressure ascent rate, τ_p, and the maximal solvent influx rate, τ_n. Concurrently, the pressure level at which the maximal influx rates, $\Pi_{(\tau_n)}$, are reached noticeably declines when the additional solution volume is enlarged and are definitely lower than those reached at the respective maximal pressure ascent rates, $\Pi_{(\tau_p)}$.

We can summarize in brief the present findings as follows:

- The novel approach of limited variation of solution volume applied here has proved efficient and productive for the osmotic experiments.
- The obtained 'pressure *vs.* time' dependences attest that the rise of pressure is much faster at constant solution volume.
- Inversely, the solvent influx through the semipermeable membrane toward the solution is many times greater in the regime of variable volume.
- The values of flow rate at constant solution volume pass through expressed and well defined maxima, while at variable volume they exhibit a steady decline with time, starting practically from the onset of the process. The latter effect may be principally attributed to the applied technique of limited variation of solution volume. Concurrently, the dilution of the operative solution in the progress of the process can only account for a small fraction of the decline.

CONCLUSIONS

The set off here study of aqueous solutions under different osmotic regimes employs a new experimental approach of limited solution volume variation. The results obtained demonstrate the applicability and the advantages of the new method when comparing the osmotic behaviour at different regimes. Most remarkably, the kinetic rate values for the two regimes are very different. Qualitatively speaking, the fact that the pressure increase at constant solution volume occurs at much faster rate is a natural result, considering the amount of solvent transferred into the solution compartment. In fact, the picture in terms of solvent flow rates is exactly the reverse: transfer of liquid is much faster in the case of variable volume.

Summing up, we consider the effects established here for the osmotic process by means of an artificial semipermeable membrane to be of

relevance for processes taking place in nature and technology. For instance, our present results are in accord with the recognized now vision about the feasible mechanism of self-maintained cell homeostasis. The living cells rapidly achieve osmotic equilibrium in confined volumes upon changes in the environment mostly by means of protein channels in the lipid membranes, despite osmosis being considered a slow process in general.

The data generated in the present investigation have allowed our deriving definite qualitative and semi-quantitative conclusions about the distinctions in the kinetics of the osmotic process under the different regimes (of constant and variable solution volume). In stricter quantitative terms, the interpretation of the obtained differences is much more complex and would demand additional considerations. This, however, is beyond the scope of the present initial investigation and is meant to be a subject of further studies of ours.

***Acknowledgment**: This study is financially supported by Project № 13/2015 by the Sofia University "St. Kliment Ohridski".*

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ВЛИЯНИЕ НА КОНТРОЛИРАНАТА ПРОМЯНА НА ОБЕМА ВЪРХУ СКОРОСТТА НА ОСМОЗАТА ВЪВ ВОДНИ РАЗТВОРИ

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Постъпила на 8 август, 2016 г.; Приета на 10 януари, 2017 г.

(Резюме)

Изменението на осмотичното налягане във водни разтвори е изследвано експериментално като функция от времето при два различни режима: на постоянен и променлив обем на разтвора. Установена е количествена зависимост на скоростта на осмозата в разтворителя от относителната промяна на обема на разтвора. Глюкозата, като биологично активно вещество, е избрана като референтен разтворен компонент в комплексните изследвания. За целта е изработена специална осмотична клетка. Разработен е нов експериментален подход, използващ контролирана ограничена промяна на обема на разтвора. Получените кинетични зависимости показват съществени различия в скоростта на процеса при двата експериментални режима. Повишаването на налягането е много по-бързо при постоянен обем на разтвора, докато притокът на разтворител е много по-голям в режим на променлив обем. Освен това, скоростта на осмозата се променя при промяна на обема на разтвора. Ние считаме, че зависимостите установени в настоящата статия с помощта на изкуствена полупропусклива мембрана са от значение и за процесите, протичащи в реалните живи клетки и тъкани.