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Gravitational Effects in a Passive Transmembrane Transport: The Flux Graviosmotic and Gravidiffusive Effects in Non-Electrolytes

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Abstract. In this paper the classification of the gravitational effects in a passive transmembrane transport is presented. Among these effects there are the flux and force gravitational effects (flux graviosmotic effect, osmotic pressure graviosmotic effect, flux gravidiffusive effect, osmotic pressure gravidiffusive effect, voltage gravielectric effect and current gravielectric effect). The volume flux graviosmotic and solute flux gravidiffusive effects model equations for a single-membrane system are elaborated. These models for binary and ternary non-electrolyte solutions have been verified using an experimental data volume and solute fluxes for osmotic-diffusion cell with horizontally mounted membrane. In the experimental set-up, water was placed on one side of the membrane. The opposite side of the membrane was exposed to binary or ternary solutions of densities greater than that of water (aqueous glucose or glucose-0.2 mole/l aqueous ethanol) and binary and ternary solutions of densities larger than that of water (aqueous ethanol or ethanol-0.05 mole/l aqueous glucose). These experimental results are interpreted in terms of the convective instability that increases the diffusive permeability coefficient of junction: boundary layer/membrane/boundary layer.

Key words: Concentration boundary layers, concentration polarization, Graviosmotic and gravidiffusive effects, Kedem-Katchalsky equations, membrane transport

1. Introduction

The gravitational field belongs to fundamental fields which influence the course of the evolutionary and adaptation processes of living world. Therefore, knowledge of the subject of gravitational influences on the working of living organisms operation is important [1]. The development of studies on how living organisms operate in conditions of microgravity during long-lasting space flights confirms this [2]. The changes of molecular organization, genetic, growth, of cell division and differentiation and morphology characteristics of single cell and whole organism were based on biological experiments carried out in outer space [2–12]. The understanding of reactions of organism on the state of weightlessness could cause improvement in medical care not only for people taking part in space flights but also for those living on the Earth. The influence of space flights on the human organism remind one of the processes accompanying ageing [2].

Many attempts have been put forward to explain the effects of gravity at the cellular level. The effects of gravity may be due to important changes in metabolism and/or molecular organization occurring within the cell that allow it to adapt to a new gravitational environment [2]. In essence, we have seen three theoretical ways of explaining the influence of gravitational force on the cell. Gravity could act (1) by means of molecules or organelles serving as gravity sensors (Pollard's model) [13], (2) by inducing an adaptation provoked through physicochemical changes in the cellular milieu (Schatz and co-workers model) [14, 15], and (3) in a holistic action of gravity (Mesland model) [16].

The lack of sedimentation and free convection in the state of weightlessness have an influence on nutrition, oxygen and by products gradients, because in the state of weightlessness these elements are only produced by diffusion. In this connection Schatz and co-workers [14, 15] infer that in conditions of microgravity in the cell-solution border could occur the membrane potential changes which cause changes of solution concentration leading to the formation of stationary boundary layers around the cells. Therefore, in the cells, in which the velocity of oxygen and nourishment consumption exceeds the rate of diffusion, cell metabolism could be distinctly disturbed. During high substrate consumption by the cells, like glucose, nearest to the cell surface the concentration gradient could also occur. The boundary layers, poor in glucose and oxygen, could be formed. Such boundary layers have to be taken into consideration during the study of growth bacterium or yeast cells in the bioreactor [17]. In order to obtain sufficient substrate mass transport from the surrounding fluid to the cell surface the intensive stirring of fluid is necessary. As a result of limited nourishment and oxygen considerable changes in cell metabolism are observed in yeast cells and mammal cells [2].

A directional action of gravity on single cells has been proposed by Mesland [16]. Based on the papers of Kondepudi and Prigogine [18], Mesland's model applies nonlinear nonequilibrium thermodynamics to living cells under changing conditions of gravity. Mesland suggests [16] that the lack of gravity alone could cause a cell experiencing weighlessness to behave differently from a cell experiencing normal conditions of gravity. This hypothesis implies that for each cell there must be a threshold gravitational force at which the system switches from gravitational to microgravitational behaviour or vice versa.

The transport processes of solute and solvent in single – or double-membrane osmotic-diffusion artificial cell lead to the formation on both sides of the membrane of concentration boundary layers (CBL). The CBL are regions in the external solutions, adjacent to the membrane interfaces where concentration gradients are maintained under steady-state conditions and act as pseudo-membranes in series with the physical membrane [19–22]. Consequently, they can produce substantial reductions in transmembrane flows [20–36]. The paper by Barry and Diamond [19] reviews the effects of boundary layers on membrane phenomena. The gravitational field exerts a relatively strong influence on thermodynamical systems and is one of factors modifying transmembrane transport [24–27]. Of especial interest is the

case of formation of CBL on both sides of horizontally mounted membranes. The gravitational field has a varied influence on the thickness of these layers and decreases value and change distributions of solution concentrations, which occur in them [24–37]. Ślęzak et al. [24] showed that the osmotic flux was significantly higher when the denser liquid was below the membrane in a horizontally mounted osmotic-diffusion cell. They explained this observation in terms of natural convection instability that reduced boundary layer thickness. These layers are thick and respectively high decreases of solution concentration occur on them. It is different in the case of layers on which the gravitational force has a destructive influence. These layers are relatively thin, and decreases of solution concentration are relatively small [28, 29]. Vigorous mechanical stirring of the external solutions minimizes the CBL thickness and flow reduction [21, 33]. The influence of the gravitational field on the transmembrane transport can be demonstrated by interferometric methods [28] and by measurements of the volume flux [24–26, 30, 33, 36], solute flux [25], membrane potential [27] and ionic electrical current [35]. Besides, the concentration profile, which is showed in Mach-Zehnder interferograms [28] can be calculated on the basis of adequate theoretical methods [28, 29, 31].

Gravitational effects on transmembrane volume and solute flows of the nonelectrolyte and electrolyte solutions in single-membrane system were reported in previous papers [24–37]. From these papers, the conclusion was drawn that the influence of the gravitational field on transmembrane transport manifests itself in Rayleigh-Taylor instability [24], rectyfying and amplifying properties of osmotic and solute fluxes [25, 26] and gravielectric effect [27]. In paper [36] the Kedem-Katchalsky-Zelman model equations (KKZ-model) of the volume and solute flows across an isotropic membrane were modified for cases in which the membrane separated two multicomponent, non-ionic and heterogeneous solutions at different concentrations. The Ω_{ik} factor, which determines the influence of concentration boundary layers on transmembrane transport, was introduced. For vertically [36] and horizontally [25] mounted membrane and for binary and ternary solutions the modified KKZ-model was verified. These findings were interpreted in terms of a convective gravitational instability that reduces boundary layer dimensions and increases the permeability coefficient of the complex system: boundary layer/membrane/boundary layer. A model equations of the transport across a horizontally mounted membrane was presented in [24, 25, 27, 30, 35–37]. In these models a concentration-gradient Rayleigh number is used.

2. A Classification of the Gravity Effects in a Passive Transmembrane Transport

The influence of the gravitational field on the transmembrane transport can be demonstrated by measurements in steady state of the thermodynamical fluxes or forces when the solutions were well stirred and when the solutions were unstirred. Let us denote the generalized flux and force measured in a conditions of well stirred

Table I. The definitions of the various types of gravitational effects in a passive membrane transport

Gravieffect	Signum	Definition
Flux graviosmotic effect	jGOE	jGOE = $J_v - J_v^i$
Osmotic pressure graviosmotic effect	πGOE	πGOE = $\pi - \Pi^i$
flux gravidiffusive effect	jGDE	jGDE = $J_s - J_s^i$
osmotic pressure gravidiffusive effect	πGDE	πGDE = $\pi - \Pi^i$
voltage gravielectric effect	vGEE	vGEE = $\Psi_M - \Psi_M^i$
current gravielectric effect	cgEE	cGEE = $\mathbf{I}_M - I_M^i$

solutions by J_k and X_k respectively. We denote the generalized flux and force in conditions of unstirred solution by J_k^i and X_k^i respectively. In this connection the definitions of the flux gravitational effect (*GJE*) and force gravitational effect (*GXE*) can be written as

$$GJE = J_k - J_k^i \tag{1}$$

$$GXE = X_k - X_k^i \tag{2}$$

where index *i* concern a suitable configuration of membrane system. Let us take into consideration the osmotic volume flux (J_v), the solute flux (J_s), the membrane potential (ψ_M), osmotic pressure (π) and the ionic electrical current (I_m) in conditions of well-stirred solutions and J_v^i, J_s^i, ψ_M^i, Π^i, I_M^i in conditions of mechanically unstirred solutions. In accordance with the definitions (1) and (2) we obtain: the flux graviosmotic effect (*jGOE*), osmotic pressure graviosmotic effect (πGOE), flux gravidiffusive effect (*jGDE*), osmotic pressure gravidiffusive effect (πGDE), voltage gravielectric effect (*vGEE*) and current gravielectric effect (*cGEE*). The definitions of these effects are listed in Table 1.

Till now, studying the influence of the gravitational field on transmembrane transport of non-electrolyte solutions, the modiyfied volume osmotic and/or solute fluxes were determined. In order to quantitatively study the influence of the gravitational field on passive osmotic and diffusive flows in single-membrane electrochemical cell, one needs determined suitable graviosmotic (*GOE*) and gravidiffusive (*GDE*) effects. With this in mind, in the present paper the volume flux graviosmotic effects (*jGOE*) and solute flux gravidiffusive effects (*jGDE*) in single-membrane osmotic-diffusive cell are described. On the basis of an irreversible thermodynamic method the *jGOE* and *jGDE* model equations for a single-membrane system was elaborated. In order to verify these models, the results of experimental studies and results of calculation for volume and solute fluxes in a single-membrane system containing binary and ternary nonelectrolyte solutions are presented. These experimental results are interpreted in terms of the convective instability that in-

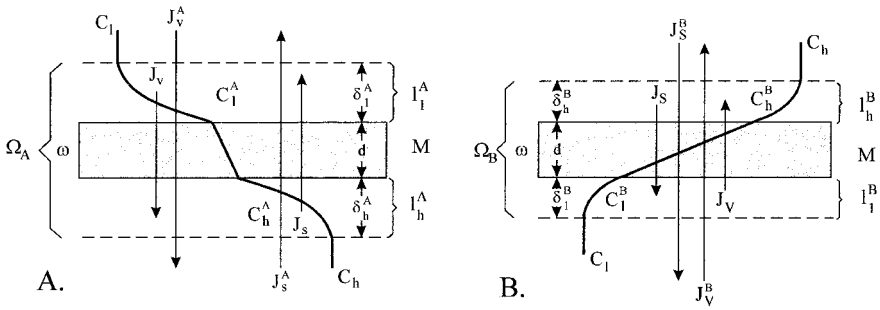

Figure 1. Two configuration (A and B) of the single-membrane system with the concentration profile. M, membrane; I_l^A, I_h^A, I_l^A and I_h^B, boundary layers in configurations A and B respectively; C_l, C_h, global solutions concentration; C_l^A, C_h^A, C_l^B and C_h^B local solutions concentrations in configurations A and B respectively; ω, Ω_A, and Ω_B, permeability coefficients of membrane and boundary layer-membrane-boundary layer complex (in configurations A and B) respectively; J_v, J_s, volume and solute fluxes across the membrane; J_v^A, J_v^B, J_s^A and J_s^B, volume and solute fluxes across the complex l/M/l in configurations A and B, respectively.

Table II. Values of the Nephrophane flat dialyze membrane transport parameters for glucose (subscript 1) and ethanol (subscript 2)

Coefficient	Value of parameter	
	Configuration A	Configuration B
$L_p \cdot 10^{12}$ [$m^3 \cdot N^{-1} s^{-1}$]	5.0±0.2	5.0±0.2
$\sigma_1 \cdot 10^2$	6.8±0.2	6.8±0.2
$\sigma_2 \cdot 10^2$	2.5±0.1	2.5±0.1
$\sigma_1^i \cdot 10^2$	0.24±0.01	3.4±0.1
$\sigma_2^i \cdot 10^2$	1.0±0.04	0.1±0.01
$\omega_{11} \cdot 10^{10}$ [$mole \cdot N^{-1} s^{-1}$]	8.0±0.3	8.0±0.3
$\omega_{22} \cdot 10^{10}$ [$mole \cdot N^{-1} s^{-1}$]	2.0±0.08	2.0±0.08
$\omega_{12} \cdot 10^{13}$ [$mole \cdot N^{-1} s^{-1}$]	8.1±3.5	8.1±3.5
$\omega_{21} \cdot 10^{12}$ [$mole \cdot N^{-1} s^{-1}$]	1.63±0.6	1.63±0.6
$\Omega_{11} \cdot 10^{10}$ [$mole \cdot N^{-1} s^{-1}$]	0.3±0.04	4.0±0.2
$\Omega_{22} \cdot 10^{10}$ [$mole \cdot N^{-1} s^{-1}$]	1.0±0.06	0.06±0.02
$\Omega_{12} \cdot 10^{13}$ [$mole \cdot N^{-1} s^{-1}$]	0.85±0.3	0.07±0.05
$\Omega_{21} \cdot 10^{12}$ [$mole \cdot N^{-1} s^{-1}$]	0.4±0.3	4.0±0.8

creases the diffusive permeability coefficient of junction: boundary layer/membrane/boundary layer.

3. Nature of the Graviosmotic and Gravidiffusive Effects

Let us consider two configurations (A and B) of the single-membrane system represented in Figure 1. We assume that this system contains the heterogeneous (not mechanically stirred) solutions of the same nonelectrolyte at concentrations C_l and C_k ($C_l < C_k$) separated by an isotropic and symmetrical membrane (M) of thickness d. The membrane is characterized by hydraulic permeability (L_p), reflection (σ) and diffusive permeability (ω) coefficients. The volume and solute fluxes across membrane are represented by J_v and J_s. In this steady state system, CBL of thickness δ_l^i and δ_h^i (indices i=A, B pertain to configurations A and B) are formed on both sides of the membrane. These layers can be treated as pseudo-membranes [24–27]. The reflection and diffusive permeability coefficients of the cBL/M/cBL complex are denoted by σ_i and Ω_i. The values of parameters L_p, σ, σ_i, ω and Ω_i for glucose solutions are listed in Table 2. The volume and solute fluxes across the complex CBL/M/CBL are represented by J_v^i and J_s^i. In both configurations the membrane has horizontally oriented planes and the respective concentration gradients are antiparallel and parallel to the gravitational force.

In configuration A, the thickness of boundary layers continually increases because of natural convection. In this instance the process forming of layers l_l^A and l_h^A is finished in a state of thermodynamical equilibrium. However we can assume that in a state when J_v = const. and J_s = const. the thickness of these layer are δ_l^A and δ_h^A. The value of thicknesses of the CBL formed on both sides of a horizontally mounted membrane can be determined using Nernst criterion on the basis of curves in distributions of concentrations [28]. This is well-founded by the inverted density gradient in solutions. In this connection in the stationary state of membrane transport, concentration of solutions at boundaries of membrane-solution increase from C_l to C_l^A ($C_l^A > C_l$) while concentration C_h to C_l^A ($C_h^A < C_h$).

In configuration B the process forming of layers l_l^B and l_h^B is finished in the moment of appearance of the process of natural convection and attains the stationary state by membrane system. Then the thickness of these layers (δ_l^B, δ_h^B) is constant, because the increases in thickness are limited by natural convection. In this connection the concentration of solution at boundaries of membrane-solution increases from C_l to C_l^B ($C_l^B > C_l$) whereas the concentration C_h decreases to C_h^B ($C_h^B < C_h$). Besides, $C_l^A > C_l^B$ and $C_h^A > C_h^B$. In the case of well-stirred solutions we obtain $C_l^i = C_l$, $C_h^i = C_h$, $J = J_v$, $J = J_s$, $\Pi_i = \Pi$, $\Omega_i = \omega$ and $\sigma_i = \sigma$.

For the case presented in Figure 1 we can write Van't Hoff's law as follows:

$$\Delta\Pi = RT(C_h - C_l) \tag{3}$$

$$\Delta\Pi^i = RT(C_h^i - C_l^i) \tag{4}$$

where RT is the product of the gas constant and absolute temperature, C_l and C_h the global solutions concentration, C_h^i and C_l^i the local solutions concentration, index i = A, B pertain to configurations A and B respectively. Besides, in the steady state

the following conditions are satisfied J_v = const., J_v^i = const., J_s = const. and J_s^i = const.

The definitions of the volume flux graviosmotic effect (j*GOE*) and solute flux gravidiffusive effect (*jGDE*) can be formulated as follows

$$(jGOE)_i = J_v - J_v^i \tag{5}$$

$$(jGDE)_i = J_s - J_s^i \tag{6}$$

where J_v, J_s are the volume and solute fluxes, respectively for mechanically well-stirred solutions; J_v^i, J_s^i (i = A, B) are the volume and solute fluxes for mechanically unstirred solutions.

For the mechanically well-stirred ternary nonelectrolyte solutions, the equations describing volume (J_v) and solute (J_s) fluxes, can be written in the following forms [32]

$$J_v = L_p\Big(\sum_{k=1}^{2}\sigma_k\Delta\Pi_k - \Delta P\Big) \tag{7}$$

$$J_s = J_v(1-\sigma_s)\tilde{C}_s + \sum_{k=1}^{2}\omega_{ks}\Delta\Pi_k \tag{8}$$

where L_p is the hydraulic conductivity of the membrane, ΔP is the hydrostatic pressure difference, σ_k and σ_s are the reflection coefficient of the membrane for the *k*-th and *s*-th substance respectively, $\Delta\Pi_k$ is the osmotic pressure difference for *k*-th substance, $\tilde{C}_s$ is an average solution concentration, ω_{ks} is the diffusive permeability coefficient of the *s*-th substance which passes across the membrane under the influence of a gradient of the *k*-th substance osmotic pressure difference $\omega_{ks} = \omega_{sk}\tilde{C}_s/\tilde{C}_k$ and s,k=1,2 s≠k.

Then, for the mechanically unstirred ternary nonelectrolyte solutions, the equations describing volume (J_v^i) and solute (J_s^i) fluxes, can be expressed as [25]

$$J_v^i = \zeta_v^i\Big((det\ K)^{-1}\sum_{k=1}^{2}\gamma_k^i\Delta\Pi_k\Delta P\Big) \tag{9}$$

$$J_s^i = J_v^i(1-\sigma_s^i)\tilde{C}_s^i + \sum_{k=1}^{2}\Omega_{ks}^i\Delta\Pi_k \tag{10}$$

Combining Equations (5)-(10) we obtain

$$(jGOE)_i = \sum_{k=1}^{2}(L_p\sigma_k(det\ K)^{-1}\zeta_v^i\gamma_k^i)\Delta\Pi_k + (\zeta_v^i - L_p)\Delta \tag{11}$$

$$(jGDE)_i = \sum_{s \neq k=1}^{2} (\omega_{ks} - \Omega_{ks}^i)\Delta\Pi_k + J_v(l - \sigma_s)\tilde{C}_s - J_v^i(l - \sigma_s^i)\tilde{C}_s^i \tag{12}$$

where [27]

$$0 \leq \frac{\Omega_{ks}^i}{\omega_{ks}} = \frac{1}{1 + 2RT\omega_{ks}\left[\frac{R_C^i V}{(D_{ks}^o)^2 g(\rho - \rho_o)_i}\right]^{1/3}} \leq 1 \tag{13}$$

$$\zeta_v^i = L_p\Big[1 + (det\ K)^{-1} L_p\Big(\sum_{k=1}^{2} \sigma_k det\ M_k^i\Big)\Big]^{-1}, det\ K = \omega_{11}\omega_{22} - \omega_{21}\omega_{12},$$

$$det\ M_1^i = \omega_{22}\big(\tilde{C}_1^i(1 - \sigma_1^i) - (1 - \sigma_1)\tilde{C}_1\big) - \omega_{21}\big(\tilde{C}_2^i(1 - \sigma_2^1) - (1 - \sigma_2)\tilde{C}_2\big),$$

$$det\ M_2^i = \omega_{11}\big(\tilde{C}_1^i(1 - \sigma_2^i) - (1 - \sigma_2)\tilde{C}_2\big) - \omega_{12}\big(\tilde{C}_1^i(1 - \sigma_1^1) - (1 - \sigma_1)\tilde{C}_1\big),$$

$$\gamma_1^i = \sigma_1 det\ K_{11}^i + \sigma_2 det\ K_{21}^i, \gamma_2^i = \sigma_1 det\ K_{21}^i + \sigma_2 det\ K_{22}^i,$$

$$det\ K_{12}^i = \omega_{22}\Omega_{21}^i - \omega_{21}\Omega_{22}^1,$$

$$det\ K_{21}^i = \omega_{11}\Omega_{12}^i - \Omega_{11}^i\omega_{12}, det\ K_{11}^i = \Omega_{11}^i\omega_{22} - \omega_{21}\Omega_{12}^i,$$

$$det\ K_{22}^i = \omega_{11}\Omega_{22}^i - \Omega_{21}^i\omega_{12},$$

R_C^i denotes the critical value of the concentration Rayleigh number, ν is the kinematic viscosity, ρ and ρ_o are the density of solution and solvent respectively, g is the gravitational acceleration and D_{ks}^o is the diffusion coefficient of s-th substance which passes in solution under the influence of a gradient of the k-th substance concentration.

With the above being taken into consideration, Equations (11)-(13) led to the model equations for the *jGOE* and *jGDE* being established. Below the experimental assessment of the validity of the model is described.

4. Experimental

4.1. MATERIALS AND METHODS

Studies on the $(jGOE)_i$ and $(jGDE)_i$ single-membrane osmotic-diffusion cell were carried out by means of the measuring apparatus whose detailed description has been given in previous papers [24, 25, 35]. Experiments were performed with flat sheet *Nephrophane* (cellophane) dialyzer membrane. The membrane was housed between two Plexiglas vessels, each with a volume of 200 ml, with 3.36 ± 0.2 cm^2 of available membrane area. Suitable values of the permeability parameters of this membrane are listed in Table 2, those of ρ and ν being collected in Tables 3 and

Table III. Values of the density (ρ) and kinematic viscosity (ν) of binary (water-glucose) and ternary (water-glucose-ethanol) solutions for various concentrations of glucose (C_1) and constant ethanol content (C_2)

C_1 [mole·l^{-1}]	$\rho \cdot 10^{-3}$ [kg·m^{-3}]		$\nu \cdot 10^6$[m^2·s^{-1}]	
	C_2 = 0 [mole·l^{-1}]	C_2 = 0.2 [mole·l^{-1}]	C_2 = 0 [mole·l^{-1}]	C_2 = 0.2 [mole·l^{-1}]
0.00	0.9980	0.9960	1.012	1.049
0.02	0.9992	0.9972	1.021	1.058
0.04	1.0004	0.9984	1.026	1.067
0.05	1.0010	0.9990	1.034	1.071
0.06	1.0016	0.9997	1.039	1.075
0.07	1.0022	1.0002	1.043	1.080
0.08	1.0028	1.0009	1.047	1.084
0.09	1.0034	1.0014	1.051	1.088
0.10	1.0040	1.0021	1.056	1.093

Table IV. Values of the density (ρ) and kinematic viscosity (ν) of binary (water-ethanol) and ternary (water-ethanol-glucose) solutions for various concentrations of ethanol (C_2) and constant glucose (C_1)

C_2 [mole·l^{-1}]	$\rho \cdot 10^{-3}$ [kg·m^{-3}]		$\nu \cdot 10^6$ [m^2·s^{-1}]	
	C_1 = 0 [mole·l^{-1}]	C_1 = 0.05 [mole·l^{-1}]	C_1 = 0 [mole·l^{-1}]	C_1 = 0.05 [mole·l^{-1}]
0.00	0.9980	1.0010	1.0120	1.0340
0.05	0.9975	1.0005	1.0212	1.0432
0.10	0.9970	1.0000	1.0305	1.0525
0.15	0.9965	0.9995	1.0397	1.0617
0.20	0.9960	0.9990	1.0490	1.0710
0.25	0.9955	0.9985	1.0583	1.0802
0.30	0.9950	0.9980	1.0676	1.0895

4. An additional support, consisting of a large mesh screen on each side of the membrane, was used to prevent buckling or tearing of thin dialysis membranes. The stirring speed in each chamber was maintained at approximately 500 rpm using independently controlled stirrer motors. One of vessels of the membrane system contained aqueous glucose and/or ethanol solution at varied concentration. This vessel was attached to a calibrated pipette, which allowed volume to be measured with accuracy $\pm$ 0.5 mm^3. The second vessel in all experiments contained pure water. This vessel was contacted to an external reservoir at the same height as the pipette. Each experiment was performed for two gravitational configurations of the membrane system: first, with water in the vessel above the membrane and the solu-

tion below (configuration A); second, with these positions reversed (configuration B). All experiments were performed at temperature T = (295 ± 0.1) K.

Volume flux, J_v, was determined from $J_v = (\Delta V)\cdot(S\cdot\Delta t)^{-1}$ where S is the membrane surface area and ΔV is the volume change occurring in time Δt. Evaluation of the global concentration exchanges in the solution was performed by standard isotope method. The measured value of the global concentration exchanges (dC) was used to calculate the solute flux (J_s) on the basis of following equation $J_s = (dC\cdot V_v)\cdot(S\cdot\Delta t)^{-1}$ where V_v is the volume of the measuring vessel. Measurements of J_v, J_v^i, J_s and J_s^i for both configurations were performed according to the following procedure. The first step involved the measurement of the volume or solute fluxes in the membrane system by means of mechanical stirring of the solution at 500 rpm. After achieving the initial steady state during which J_v or J_s was constant, stirring was stopped, and subsequently the evolution of volume or solute fluxes was measured up to the second steady state, while the J_v^i or J_s^i remained fixed. The same procedure was followed for each configuration. A plots of the time dependence for J_v and J_s in configurations A and B of the membrane system in the case of a 0.1 mole·l^{-1} aqueous glucose solutions and *Nephrophane* membrane were presented in a previous papers [25]. In Figures 1a and 1b, for example, a typical quantitative plots of time dependence for J_v and J_s in configurations A and B of membrane system are presented. Plots 1, common for both configurations, contains the results obtained under conditions of thorough mechanical stirring, and shows that J_v^o and J_s^o are independent of gravitational configuration of the single-membrane system. Plots 1A and 1B, obtained for configurations A and B respectively, demonstrate that the J_v and J_s values for both configurations differ ($J_v^A \neq J_v^B$ and $J_s^A \neq J_s^B$). The evolution of the J_v^o to the J_v^A or J_v^B and J_s^o to the J_s^A or J_s^B is a reflection of the process of formation of diffusion boundary layers on both sides of the membrane. Such behavior ceases on the attainment of a steady state. Thus, we can write, that $(jGOE)_A = J_v^o - J_v^A$, $(jGOE)_B = J_v^o - J_v^B$, $(jGDE)_A = J_s^o - J_s^A$ and $(jGDE)_B = J_s^o - J_s^B$. These expressions represent the definitions of flux graviosmotic effect ($jGOE$) and flux gravidiffusive effect ($jGDE$) in configuration A and B.

4.2. RESULTS

The experimental results obtained for the $jGOE$ are presented in Figures 3 and 4. The data illustrating the dependence $jGOE = f(\Delta C_1)$ are shown in Figure 3 for solutions of fixed ethanol concentration (C_2) and varying glucose concentration (C_1). For binary solutions progressive addition of glucose increases the density of solutions. For the solutions containing 0.2 mole·l^{-1} ethanol, progressive addition of glucose increases the density from below to above that of water. Figure 4 displays the data recorded at constant glucose concentration and varying ethanol levels. In contrast to the preceeding case, progressive addition of ethanol to 0.05 mole·l^{-1} lowers the density from above to that of water a value below.

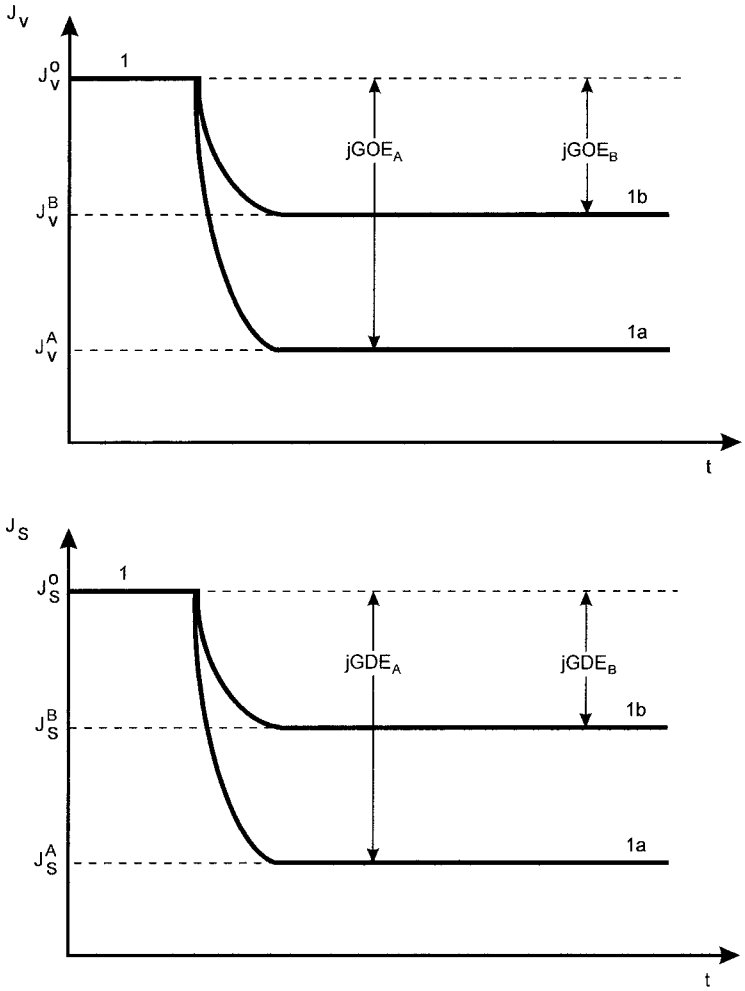

Figure 2. Evolution of the volume flux (J_v) from J_v^o to J_v^A or J_v^B and solute flux (J_s) from J_s^o to J_s^A or J_s^B in configuration A and B after turning off the mechanical stirrers.

Figures 5 and 6 display plots of the experimental *jGDE* for binary and ternary solutions. These results were obtained under conditions analogous to those for *jGOE* (see Figures 3 and 4). The results in Figure 5 were obtained using solutions of constant ethanol concentration (C_2 = 0.2 mole·l^{-1}) while varying the amount of glucose. The results in Figure 6 correspond to the reverse of this situation, i.e. fixed glucose (C_1 = 0.05 mole·l^{-1}) and varying ethanol concentration. Suitable values of *jGDE* in analogy with *jGOE*, can be interpreted in terms of a convective gravitational instability or stability of the boundary layers.

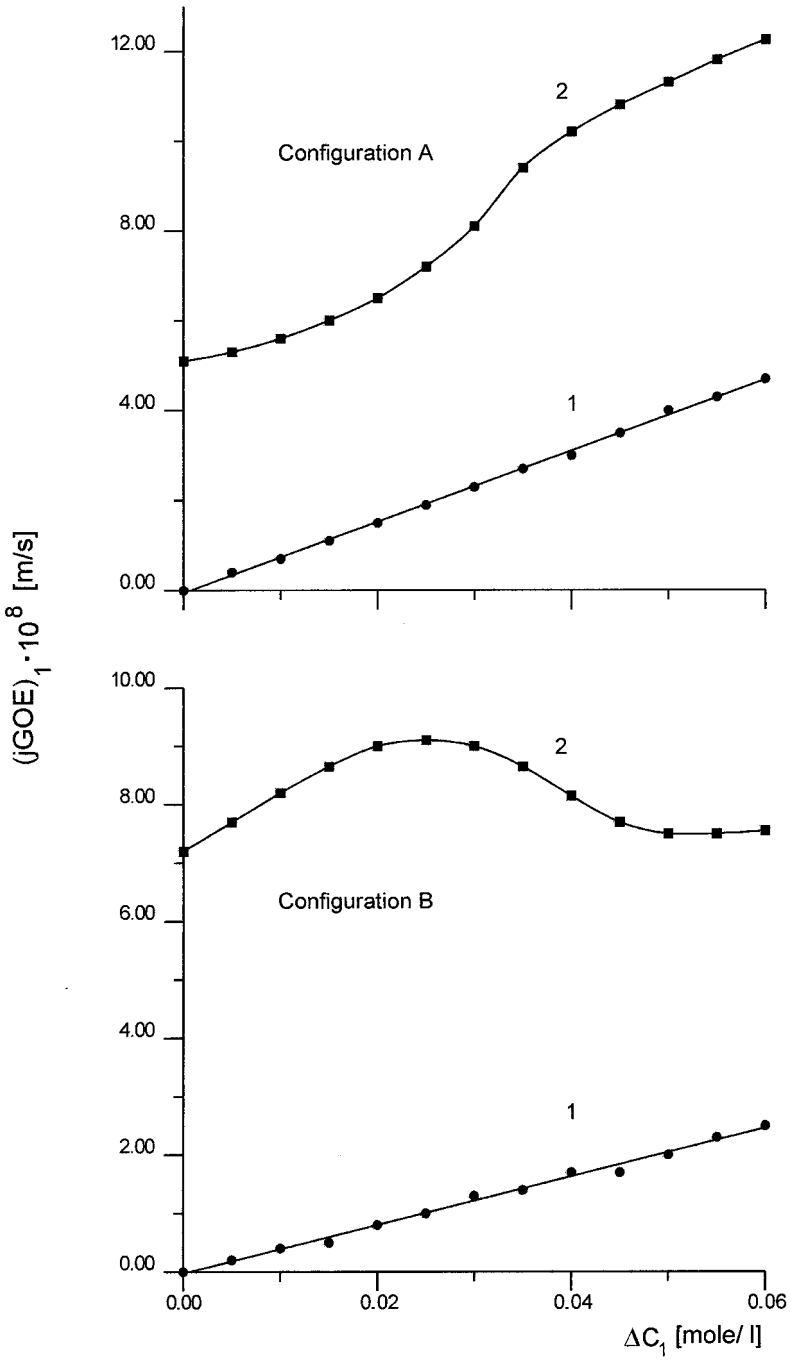

Figure 3. Experimental and calculated jGOE as a function of the glucose concentration difference (ΔC_1) in binary (plots 1) and in ternary (plots 2): (●) no ethanol; (□) ΔC_2 = 0.2 mole·l^{-1}. Solid lines illustrate the jGOE calculated on the basis of Equation (14) after taking into consideration $\Omega_{11}(\Delta C_1)$ and $\Omega_{22}(\Delta C_1)$.

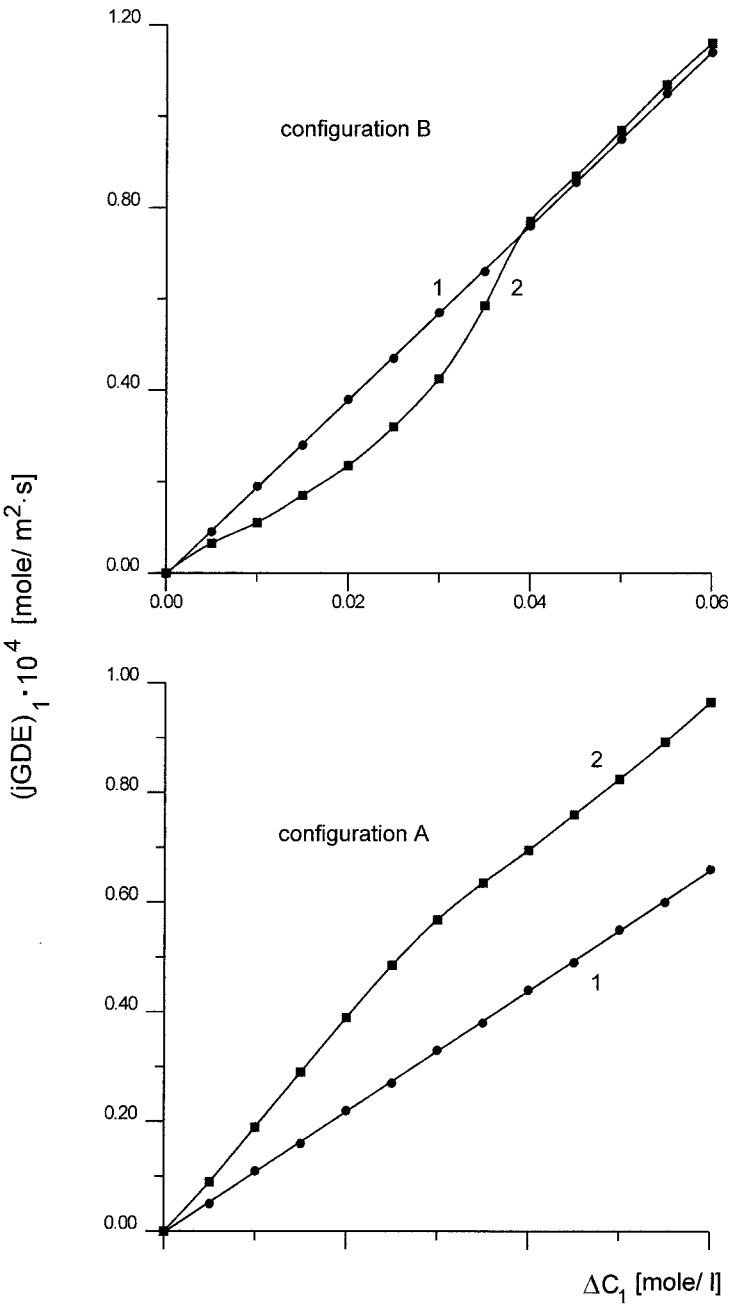

Figure 4. Experimental and calculated jGOE as a function of the ethanol concentration difference (ΔC_2) in binary (plots 1) and in ternary (plots 2) solutions: (●) no glucose; (□) ΔC_1 = 0.05 mole·l^{-1}. Solid lines illustrate the jGOE calculated on the basis of Equation (14) taking into consideration $\Omega_{22}(\Delta C_2)$ and $\Omega_{11}(\Delta C_2)$.

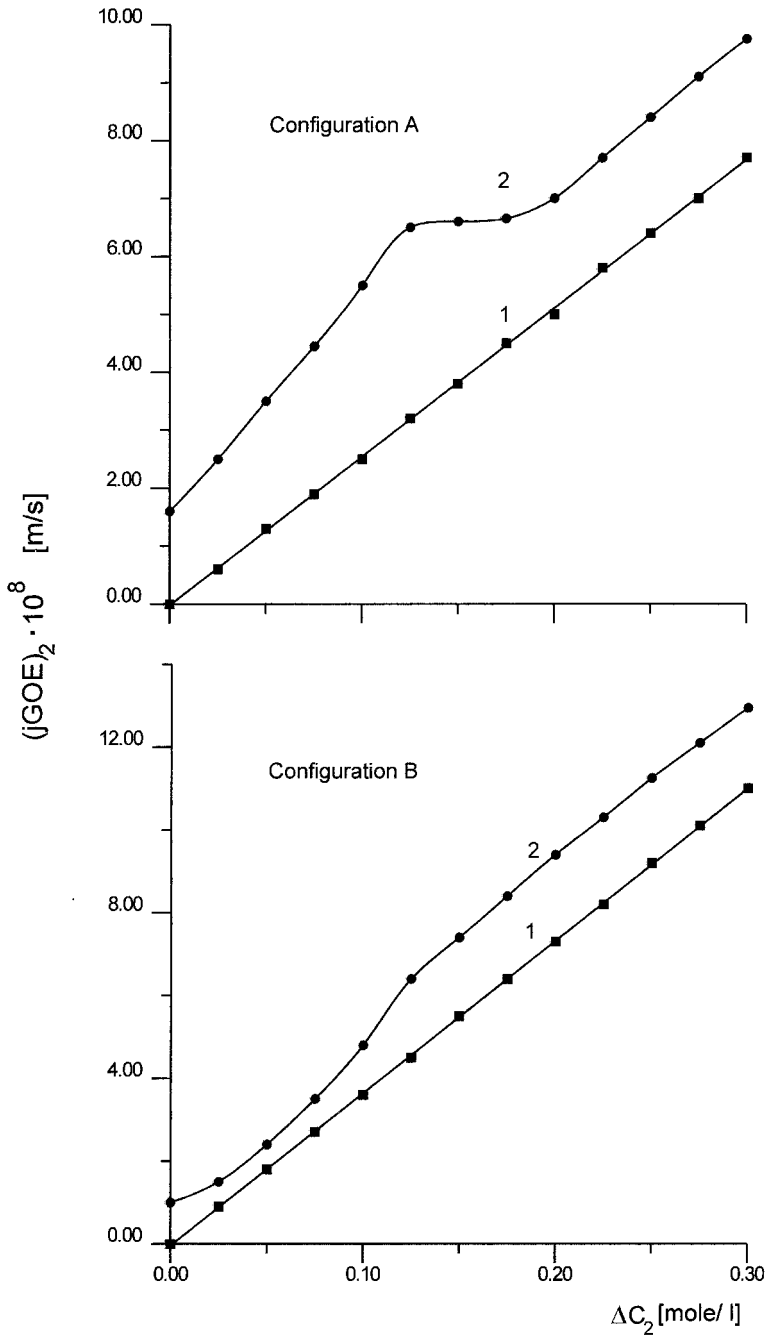

Figure 5. Experimental and calculated jGDE as a function of the glucose concentration difference (ΔC_1) in binary (plots 1) and ternary (plots 2) solutions: (●) no ethanol; (□) $\Delta C_2 = 0.2$ mole·l^{-1}. Solid lines illustrate the jGDE calculated on the basis of Equation (15) after taking into consideration $\Omega_{11}(\Delta C_1)$ and $\Omega_{22}(\Delta C_1)$.

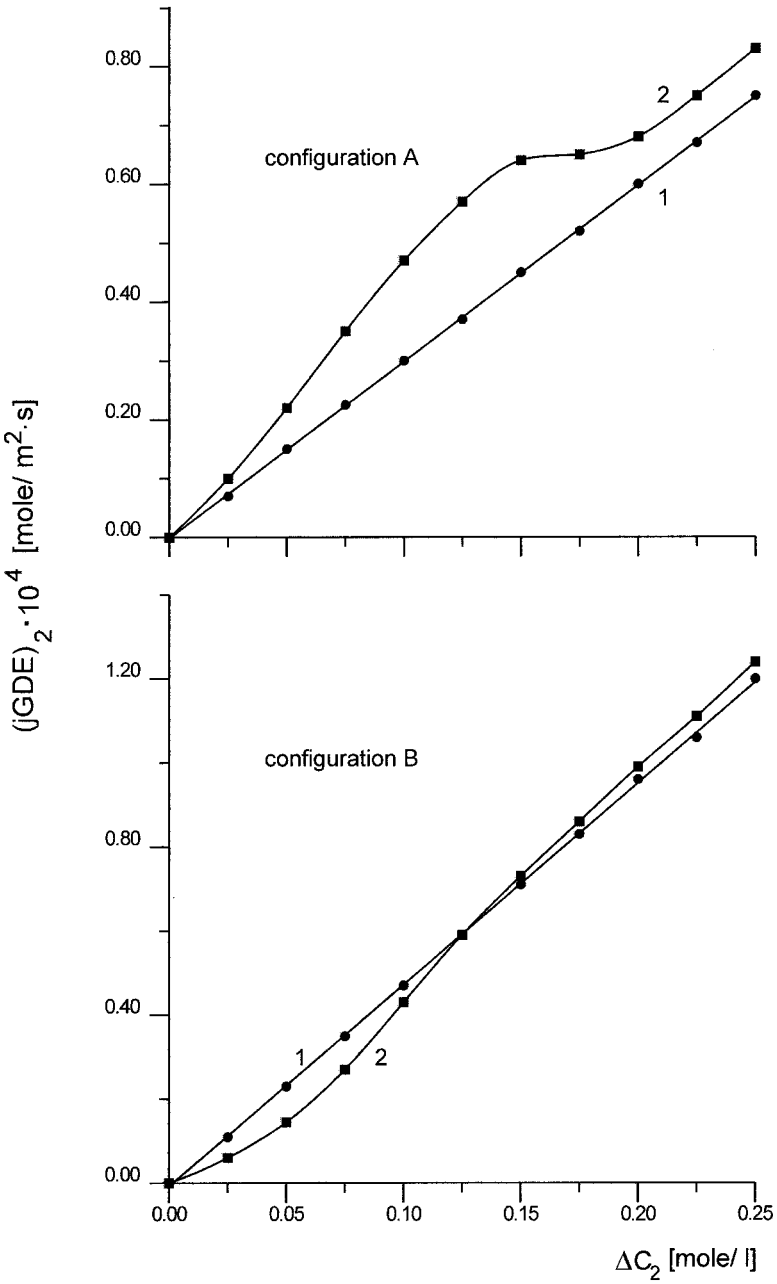

Figure 6. Experimental and calculated jGDE as a function of the ethanol concentration difference (ΔC_2) in binary (plots 1) and in ternary (plots 2) solutions: (●) no glucose; (□) ΔC_1=0.05 mole·l^{-1}. Solid lines illustrate the jGDE calculated on the basis of Equation (16) after taking into consideration $\Omega_{11}(\Delta C_2)$ and $\Omega_{22}(\Delta C_2)$.

5. Discussion

In order to establish the validity of the *jGOE* and *jGDE* model equations, the parameters L_p, σ_s, σ_s^i, ω_{ks} and Ω_{ks}^i for glucose and/or ethanol were evaluated experimentally. These parameters were defined in a previous paper [25]. It appears from equations (11) and (12) that, in order to characterize the permeability properties of a horizontally mounted membrane for ternary solutions, 19 coefficients are needed. Yet, the values of the fluxes J_v, J_v^i, J_s and J_s^i are determined by the diagonal coefficients. It appears from the results in Table 2 that the diagonal coefficients are greater than the non-diagonal coefficients by three orders of magnitude. Thus, this provides the basis of reduction of Equations (11)-(13) accepting $\omega_{12}=\omega_{21}=\Omega_{12}=\Omega_{21}\approx 0$. Regarding the above condition and the results presented in Figures 3–5, Equations (11) and (12) can be rewritten as

$$(jGOE)_i = \left[L_p\sigma_1 - \zeta_v^i\frac{\Omega_{11}^i(C)}{\omega_{11}}\right]\Delta\Pi_1 + \left[L_p\sigma_2 - \zeta_v^i\frac{\Omega_{22}^i(C)}{\omega_{22}}\right]\Delta\Pi_2 - (\zeta_v^i + L_p)\Delta P \quad (14)$$

$$(jGDE)_1^i = [\omega_{11} - \Omega_{11}^i(C)]\Delta\Pi_1 + J_v(1-\sigma_1)\tilde{C}_1 - J_v^i(1-\sigma_1^i)\tilde{C}_1^i \quad (15)$$

$$(jGDE)_2^i = [\omega_{22} - \Omega_{22}^i(C)]\Delta\Pi_2 + J_v(1-\sigma_2)\tilde{C}_1 - J_v^i(1-\sigma_2^i)\tilde{C}_2^i \quad (16)$$

where:

$$\zeta_v^i = L_p\{1-(\omega_{11}\omega_{22})^{-1}L_p\big[\sigma_1\omega_{22}\big(\tilde{C}_1^i(1-\sigma_1^i) - (1-\sigma_1)\tilde{C}_1\big) + \sigma_2\omega_{11}\big(\tilde{C}_2^i(1\sigma_2^i) - (1-\sigma_2)\tilde{C}_2^i\big)\big]\}^{-1}.$$

We have obtained equations including parameters (Ω) which refer to boundary layers. The equations can be used to analyse flux graviosmotic effect (*jGOE*) and flux gravidiffusive effect (*jGDE*) in any gravitational configuration of a single-membrane system.

Consider the results shown in Figures 3–6. It is clearly evident from this data that all dependencies $(jGOE)_i = f(\Delta C)$ and $(jGDE)_i = f(\Delta C)$ for ternary solutions are non-linear. In the case of binary solutions, one can use Equations 14–16 to describe the jGOE and jGDE with values L_p, σ, ω and Ω, being assumed, as listed in Table 2. However, for ternary solutions one also requires knowledge of the dependence of Ω_{ks} upon the concentration and configuration of the system in order to carry out the aforementioned analysis. That such an assumption is indeed valid is borne out by the data depicted in Figures 7 and 8. Curves A in a Figure 7 represent the glucose concentration dependence of the factor Ω_{11}/ω_{11} and Ω_{22}/ω_{22} (at constant ethanol) for configuration A. In configuration B the patterns of glucose concentration dependence of factors Ω_{11}/ω_{11} and Ω_{22}/ω_{22} are inverted as illustrated by curves B in Figures 7. Both Ω_{11}/ω_{11} and Ω_{22}/ω_{22} depend on ethanol concentration (see curves A and B in Figure 8). Consider the results shown in Figures 7 and 8. From these data we see that the curves A and B (in upper figure) are

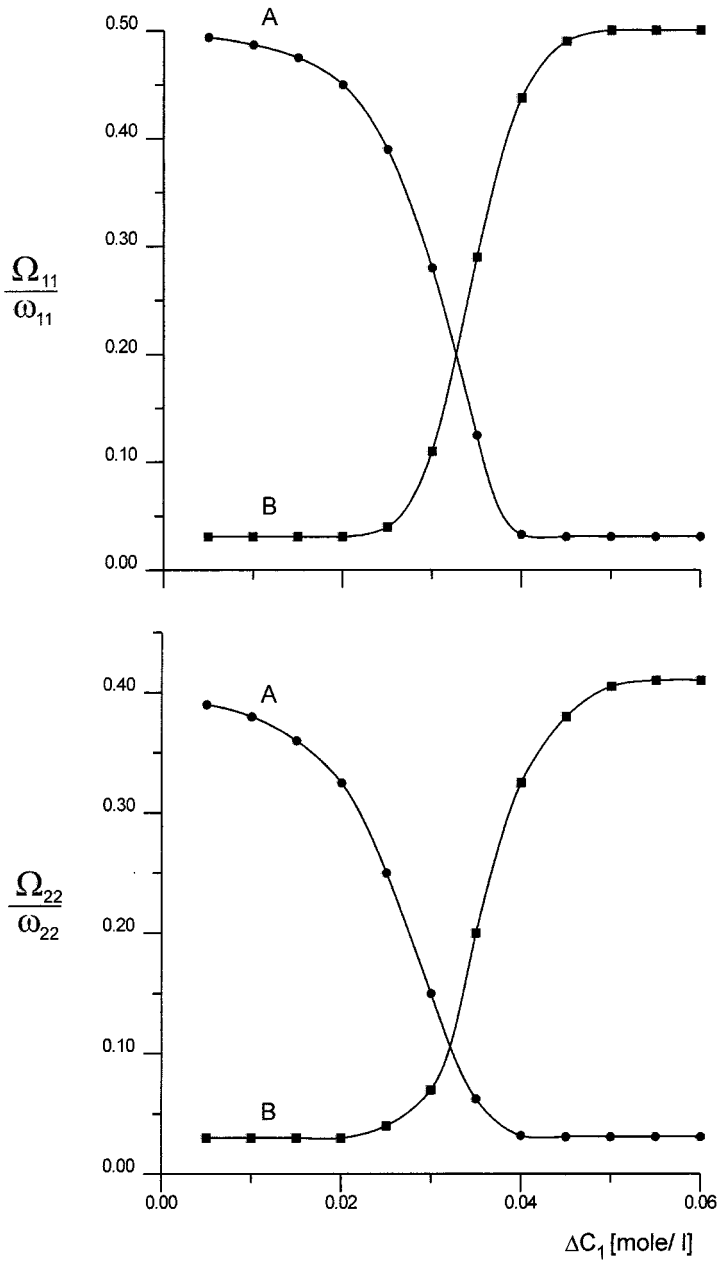

Figure 7. Concentration dependence of the factor Ω_{11}/ω_{11} and Ω_{22}/ω_{22} determined experimentally for glucose in 0.2 mole·l^{-1} aqueous ethanol solutions. Curves A correspond to configuration A; B – to configuration B.

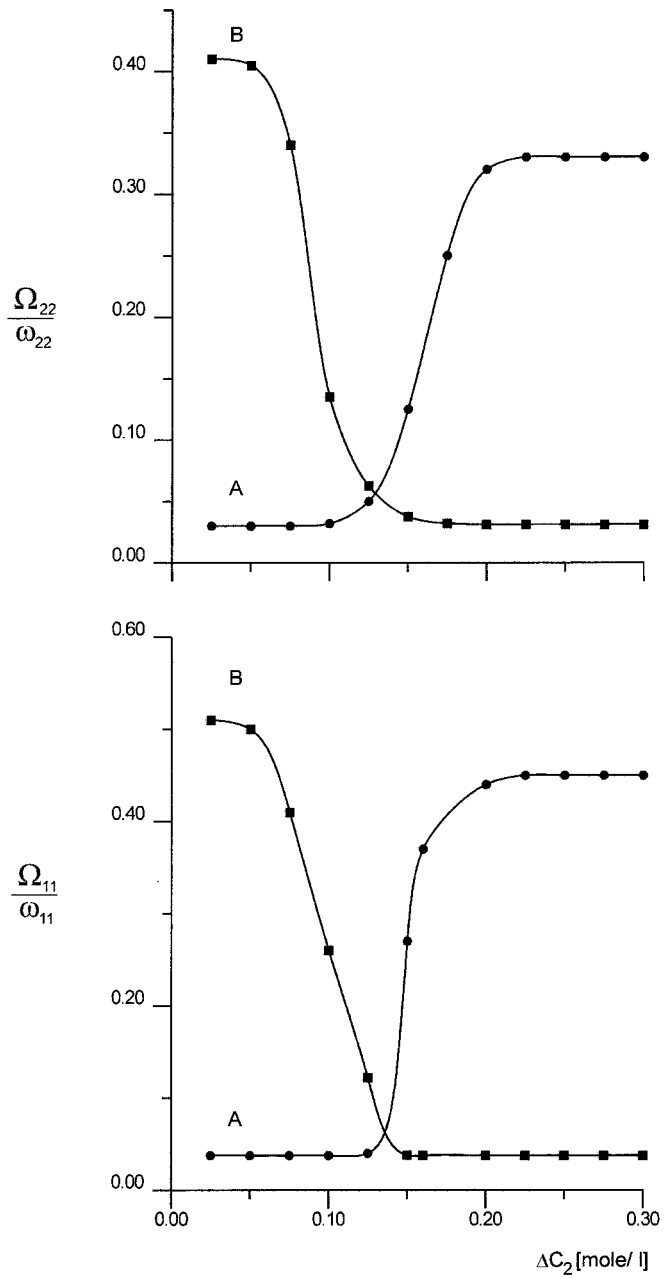

Figure 8. Concentration dependence of the factor Ω_{22}/ω_{22} and Ω_{11}/ω_{11} determined experimentally for ethanol in a 0.05 mole·l^{-1} aqueous glucose solution. Curves A and B correspond to configurations A and B, respectively.

crisscrossed in point with coordinates $(\Omega_{11}/\omega_{11})_{lim.} = 0.2$ and $(\Delta C_1)_{lim.} = 0.0325$ mole·l^{-1}, whereas curves A and B in the lower figure – in $(\Omega_{22}/\omega_{22})_{lim.} = 0.1$ and $(\Delta C_1)_{lim.} = 0.0325$ mole·$^{-1}$. From Figure 8 it results that the curves A and B (in upper figure) are crisscrossed at points with coordinates $(\Omega_{22}/\omega_{22})_{lim.} = (\Omega_{11}/\omega_{11})_{lim.} = 0.05$ and $(\Delta C_2)_{lim.} = 0.13$ mole·l^{-1}. The variation in the *jGOE* and j*GDE* were calculated, based on Equations (14)–(16), as functions of ΔC_1 or ΔC_2, with the experimental Ω_{11}/ω_{11} and Ω_{22}/ω_{22} concentration-dependent behavior in Figures 7 and 8 and parameter values listed in Table 2 being taken into account. The solid lines in Figures 3–6 correspond to the data obtained via calculation of the *jGOE* and *jGDE*. The results obtained experimentally and those from calculation are consistent with each other and, hence, Equations (14)–(16) yield a satisfactory fit to the curves from experiment, when the concentration dependence of Ω_{ks}/ω_{ks} is taken into consideration.

The experimental data presented in this paper indicate that gravitational force has an essential influence on volume osmotic flow and solute flow of electrolyte solutions. As was pointed out, the measure of this influence is flux graviosmotic effect (j*GOE*) and flux gravidiffusive effect (*jGDE*). The values of *jGOE* and *jGDE* depend on the sequence of solution position relative to membrane, located perpendicularly to the direction of gravitational force and on a composition and concentration of solutions separated by membrane. The situation in which the solutions are mechanically stirred, that is homogeneous in respect of concentration, illustrates a situation in which the gravitational field does not influence the passive membrane transport, because it does not create or eliminate local concentration gradients. This is the idealised case and was not found in living cells. As is known, in the case of lack of mechanical stirring boundary layers are created, on both sides of the membrane. Only in a situation when the sense of density gradient is opposite to gravitational force in the area near the membrane does free convection appear and causes weak solution stirring. Such a situation could occur in biological systems, present in earth gravitational field and in biological systems submitted to hypergravity. The state without convection responds to microgravity. This is the situation when the cell is provided with nutrients by diffusion. This means that in the close vicinity of cell membrane the concentration of nutrients is minimal. In the state without convection j*GOE* and *jGDE* obtain maximal values, increasing with increased solution concentration. Moreover in the state without convection $jGOE \approx J_v^o$ and $jGDE \approx J_s^o$. In the convective state $jGOE < J_v^o$ and $jGDE < J_s^o$. This means that gravitational force hampers passive membrane transport in the state without convection even almost totally.

In ref. [24–27, 30, 35, 37] the type of change in J_v^i and J_s^i was discussed in relation to the gravitational instability or stability of the boundary layers, i.e. low experimental values of J_v^i and $J^i - s$ indicate that the boundary layers are stable whereas high values correspond to their being unstable. Analogously, we interpret a high observed *jGOE* and *jGDE* as reflecting the instability of boundary layers in configurations A and B, whilst low values are indicative of their stability in this

configurations. Let us consider the equation (13). It appears from this equation that [37]

$$R_C^i = \frac{g(D_s)^2}{\omega_s RT)^3} \cdot \frac{(\rho_s^h - \rho_s^l)}{[(\upsilon_s^h \rho_s^h)^{1/4} + (\upsilon_s^l \rho_s^l)^{1/4}]^4} \cdot \frac{(1-\zeta_s^i)^4}{(\zeta_x^i)^3} \tag{17}$$

where $\zeta_x^i = \Omega_{ks}^i / \omega_{ks}$.

Choosing values $D_s = 0.69 \cdot 10^{-9}$ m^2·s^{-1}, g = 9.81 m·s^{-1}, ρ_s^h = 997.95 kg·m^{-3}, ρ_s^l = 996 kg·m^{-3}, $\nu_s^h = 1.063 \cdot 10^{-6}$ m^2·s^{-1}, $\nu_s^l = 1.049 \cdot 10^{-6}$ m^2·s^{-1}, T = 295 K, $\omega_s = 8 \cdot 10^{-10}$ mole·N^{-1}·s^{-1}, ζ_s^i = 0.19, equation (17) gives R_C^i = 150±10. This is not comparable with $*$ for the thermally driven instability.

The results of the study of gravitational force influence the passive membrane transport of electrolyte solutions presented in this paper, confirm rightness of Schatz and co-workers model [14, 15] and Mesland model [16].

6. Conclusions

1. The result of creating diffusion boundary layers determines the concentration profile [19, 21, 31]. This profile on the border between the membrane and the solution has a great effect on the solute and solvent fluxes across the membrane. Indeed, it determines the course of many physiological processes [1–16, 22, 23].
2. It has been demonstrated herein that the model equations for the j*GOE*, πGOE, *jGDE* and πGDE may be used in interpreting the data related on the volume flux and osmotic pressure graviosmotic effects and solute flux and osmotic pressure gravidiffusive effects in a single-membrane osmotic-diffusive cell. However, this is possible only when the dependence of Ω/ω on concentration is known. For binary solutions (Ω/ω) = const. The values of these parameters are governed by the hydrodynamic state of the CBL complex [27].

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