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ON THE MECHANISM OF EXTRACTION FROM SOLID BODIES OF CELLULAR STRUCTURE

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Abstract. Adequacy of mathematical model of the extraction process of solid bodies of cellular structure (plant material) was proved with taking into account its anatomical structure, namely the presence of cellular and intercellular environment. Experimental verification of the mechanism of extracting of intracellular substance during extraction process flow was made. An order of diffusion coefficients through cellular membrane – D_c and in intercellular environment D_m was confirmed.

Keywords: extraction, intracellular substance, intercellular environment, diffusion.

1. Introduction

While continuing the theme of extraction from solid bodies of cellular structure [1, 2] it is important to prove the adequacy of the developed model [1] not only on objects with target substance presented in cell volume [2] but also on those with target substance presented in intercellular environment.

As previously reported, a peculiarity of organogenic solids, in the most general form, is their cellular structure, which implies the presence of two environments: cellular and intercellular. Biologically-active substance (conventionally called the target substance) may be contained both in a cell as well as in the intercellular space. It is that substance that is the object of extraction.

2. Experimental

Theoretical solution of mathematical model, which takes into account anatomical organization of solid body of cellular structure, and actually plant material falls under such definition, is presented in the article [1]. In this work mathematical formulation of model of the extraction process from solid body of cellular structure is built in such a manner that diffusive resistance of cellular membrane, intercellular environment and size of the extracted particle of solid phase are taken into account and it looks like:

$$\begin{cases} \dfrac{dC_c}{dt} = -k_c(C_c - C) \\ \dfrac{dC}{dt} = k_c(C_c - C) - k_m(C - C_1) \\ Ve\,C_{co} = Ve\,C_c + V(1-e)C + WC_1 \\ t = 0; C = 0, C_c = C_{co}, C_1 = 0 \end{cases} \quad (1)$$

The system (1) is solved by an operating method relatively:

– C_c under condition: $t = 0, C = 0, C_c = C_{co}$; the solution is of the form:

$$C_c = C_{co} e^{-k_c t} \quad (2)$$

and answers the question how the concentration of intracellular (target) substance C_c changes with the lapse of time.

– C under condition: $t = 0, C = 0$; the solution is of the form:

$$C = C_{co} \frac{k_c}{(k_m - k_c)} [e^{-k_c t} - e^{-k_m t}] \quad (3)$$

and answers the question how the concentration of intracellular substance C changes in intercellular volume with the lapse of time.

– C_1 under condition that in the equilibrium state $C_{co} = C_c = C = C_{1p}$ the solution is of the form:

$$C_1 = C_{1p}\left(1 - \frac{1}{r+1}\exp[-(k_m - k_c)]t\right) \quad (4)$$

and answers the question how the concentration of intracellular substance C_1 changes in basic volume with the lapse of time.

Besides, the received result (4) allows defining first-passage time of the specified preassigned value of degree of extraction. If we make hypothetical assumption that the extraction proceeds from separate cells, whose

internal volume is limited by the cellular membrane, then Eq. (4) is rewritten as:

$$\left(1-\frac{C_1}{C_{1P}}\right)=\exp(-k_c t) \quad (5)$$

where: $k_c=\frac{D_c F_c}{d_c V_c}$ – coefficient of mass transfer through cell membranes.

After substitution of k_c value in (5), and taking into account the assumption made in [1, 2] that a cell has a form of sphere and the relation is:

$$\frac{F_c}{V_c}=\frac{6p\,d^2}{p\,d^3}=6/d \quad (6)$$

let's define first-passage time of the specified preassigned value of degree of extraction:

$$t=\frac{dd_c}{6D_c}\ln\left(1-\frac{C_1}{C_{1p}}\right) \quad (7)$$

Analyzing (4) it should be noted that the coefficient of mass transfer k in this equation is the quantity consisting of two values, namely, the coefficient of mass transfer through cell membranes k_c, and the coefficient of mass transfer in intercellular environment $k_м$.

Assuming that: $k=(k_m+k_c)$ at $t = t_p = \infty$ the value $A\cdot e^{-k\cdot t_\infty}$ is a small number that can be neglected, then: $C_1 = C_{1p}$; which correlates well with the data on kinetics of extraction at achievement of equilibrium.

Experimental study of the kinetics of extraction of biologically active substances from nettle leaf, coltsfoot leaf and peppermint leaf was conducted in an apparatus with a mixer at the temperature of 293 K. The raw material was milled using a laboratory herbal mill by a cutting method to the sizes: $1\cdot10^{-3}$; $2\cdot10^{-3}$; $3\cdot10^{-3}$; $4\cdot10^{-3}$ and $5\cdot10^{-3}$ m. Solid phase particle size was defined by sieve test. Solid body : liquid phase ratio was 1:30. The container was covered with a cap and a stirrer was switched on. The samples were collected over determined periods of time with such calculation that the amount of the selected extract did not influence the concentration of extractive substances in an extract. Content of extractive substances in an extract was determined according to [5]. The active substances of leaves of nettle are chlorophylls, tannins while those of coltsfoot are polysaccharides. For these two types of plants target substances are located in cellular environment. The principal substances of peppermint leaves are essential oils, which are located in intercellular environment (see below Figs. 3 and 4) [3].

By inserting experimental data of extraction kinetics of grinded leaves of nettle, coltsfoot and peppermint of different sizes in Eq. (4) in logarithmical coordinates, we can calculate the values, $\ln\left(1-\frac{C_1}{C_{1p}}\right)$, using which we build a series of kinetic curves (Fig. 1), on the basis of which we can find the values of parameter k as tangent of slope of the straight part of the curve and preexponential multiplier; A as a distance, which is cut by the direct prolongation of straight part of each of the obtained curves on the ordinate axis. Parameter A is called wash-out coefficient, because it characterizes the quantity of open or ruined cells in grinding process for extracted material.

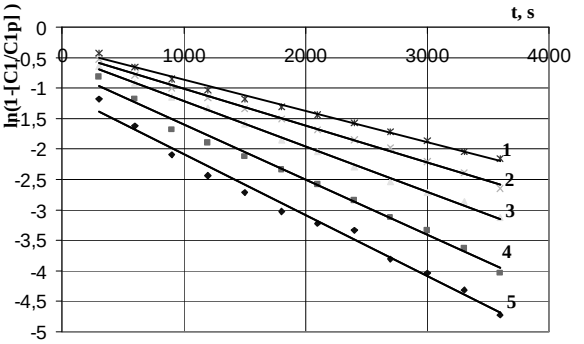

Fig. 1. Logarithmic dependence of the kinetics of extraction leaves of coltsfoot grinded to sizes: $1\cdot10^{-3}$ (1); $2\cdot10^{-3}$ (2); $3\cdot10^{-3}$ (3); $4\cdot10^{-3}$ (4) and $5\cdot10^{-3}$ m (5)

3. Results and Discussion

As one can see from Fig. 1, particle size of plant material does not greatly influence the equilibrium achievement time of the extraction process. The equilibrium achievement time increases with the increase of size of the extracted particle. It can be explained by the increase of the diffusion way of target substances from the internal environment of raw material to the border of division of phases. Analyzing the data of Fig. 1 one can expressly define two periods of extracting. In the first (I) period up to 300 s, dissolution and rapid washing of target substances from the destroyed cages take place while in the second period (II) of extracting slow diffusion of target substances from integral cells occurs. The relative amount of target substances, extracted in the first period of extracting, characterizes the amount of the destroyed cells and determines a numerical value of the coefficient of washing, as this is a segment which is cut off by direct branch on ordinate axis [4], *i.e.* value of *A*.

More detailed analysis of obtained mass transfer coefficients k depending on the grinding degree of plantain leaves; d allows to claim that this dependence is linear in nature (Fig. 2) and is described by an analytical equation:

$$k = 11.89\cdot10^{-4} - 0.142d \quad (8)$$

and parameter A is determined by this dependence (Fig. 2):

$$A = 88.0d + 0.302 \quad (9)$$

overall kinetic equation of extraction of leaves of coltsfoot:

$$C_1 = 2.26(1- [88.0d + 0.302] \cdot \cdot \exp(-[11.89 \cdot 10^{-4} - 0.142d]\, t) \quad (10)$$

By an analogical method the experimental results of kinetics of extracting of leaves of nettle and peppermint were interpreted. It was determined that dependence $k = f(d)$, for the leaves is described by linear function within the range of $1 \cdot 10^{-3}$ to $6 \cdot 10^{-3}$ m. Summary kinetic equation of extractions of nettle leaves (11) and peppermint leaves (12) are:

$$C_1 = 0.37(1 - [44.0d + 0.440] \cdot \cdot \exp(-[\, 12.17 \cdot 10^{-4} - 0.187d]t) \quad (11)$$

$$C_1 = 1.7 \cdot 10^{-4}(1 - 0.87 \cdot \cdot \exp\,(-[0.355d - 27.35 \cdot 10^{-4}])t) \quad (12)$$

The obtained experimental data of kinetics of extracting are explained by the anatomic structure of leaf (Fig. 3). In the dried leaf, which was subject to extracting, after penetration of the extractant through stoma and side surface, which appeared as a result of grinding, partial renewal of anatomic integrity takes place in the internal volume of a leaf, i.e. during a contact with an extractant an intercellular and cellular environment are formed and all internal space is renewed.

Intracellular substance, which interdiffused through a cell membrane, continues to diffuse through intercellular space to the surface of a leaf. Then partly through the stomata of leaves target components pass to the extractant. Another way of diffusions (and obviously quite a powerful one) is represented by side surface or surface of grinding, as its increase (reduction of the size of particle of hard phase) is accompanied by a proportional increase of the mass transfer coefficient k. After the substitution of mean value of diameter of herbal cell in dependence $k = f(d)$, one can obtain value of the mass transfer coefficient through cell membrane k_c, by which, using formula (5), one can find an order of the coefficient of diffusion through the cellular membrane D_c.

The thickness of vegetable cellular membrane is $\delta_c = 2$ μm, and its diameter is $d = 50$ μm [3]. The order of the diffusion coefficient through a cellular membrane according to the calculations we made in works [4, 5] is 10^{-14} [m^2/s]. Under such circumstances first-passage time of the specified preassigned value of degree of extraction from (7) was 1150.5 s.

The obtained result correlates well with experimental kinetics of the extraction of nettle leaf, coltsfoot leaf and peppermint leaf reduced to the identical size of $3 \cdot 10^{-3}$ m. As was marked before, target substance (essential oil) of peppermint leaf is contained in glandular trichomes, which are located in intercellular environment, not in internal cell volume. Therefore the extraction time must decrease by value of time of diffusion of average molecules through a cellular membrane Indeed, comparing the results of extraction kinetics of nettle leaf and coltsfoot leaf with extraction kinetics of peppermint leaf, reduced to the identical size, one can see that first-passage time of the specified preassigned value of degree of extraction for peppermint leaf is achieved at the average faster by the determined value t (see Table).

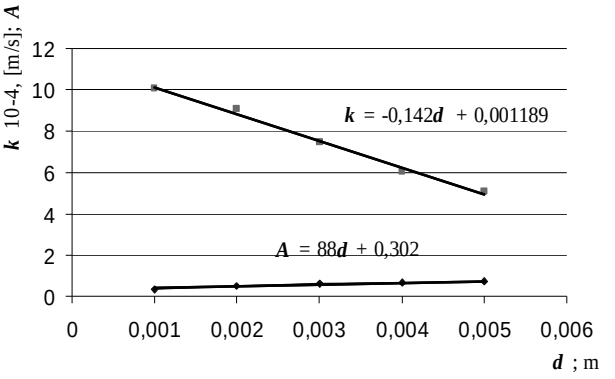

Fig. 2. Dependence of mass transfer coefficients k, and coefficient A on size d in the process of extracting of target substances from the leaves of coltsfoot

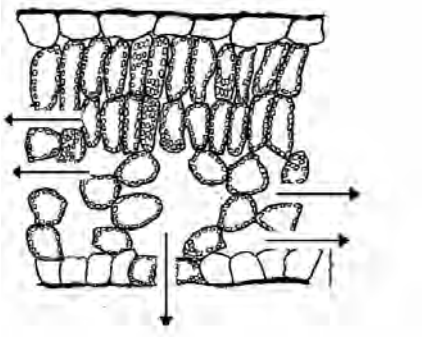

Fig. 3. Schematic cross-cut of typical herbal leaf according to the Ref. [3]
→ direction of diffusion of target substance to the phases division border

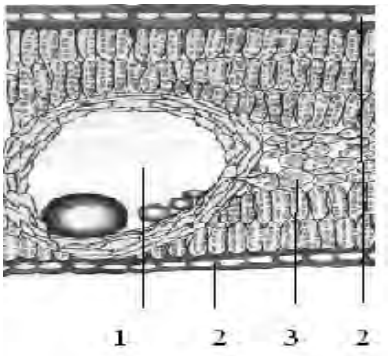

Fig. 4. Localization of essential oils in peppermint leaf: receptacle of essential oil (1); epidermis (2) and vacuoles (3) according to Ref. [3].

Table

Kinetics of leaf extraction

t; s	500	900	1200	1500	1800	2100	2400	2700	3000	3300
Nettle; C_1, kg/m^3	5.04	7.76	9.13	10.1	10.9	11.5	12.0	12.3	12.6	12.8
Coltsfoot; C_1, kg/m^3	1.06	1.54	1.66	1.80	1.90	1.97	2.03	2.08	2.11	2.13
Peppermint; C_1, kg/m^3	0.82	1.38	1.49	1.58	1.64	1.68	1.70	1.70	1.70	1.70

4. Conclusions

The obtained result demonstrates correctness of the order of diffusion coefficient D_c through a cellular membrane 10^{-14} m^2/s, which attests adequacy of the developed mathematical model (1) to the processes of transfer of target substance from cellular environment through intercellular environment to the basic volume of extractant.

5. Abbreviation

C – concentration in intercellular volume; C_c – concentration in cell volume; C_{co} – initial concentration in cell volume; C_1 – concentration of extractant; D_c – diffusion coefficient through a cellular membrane; D_m – diffusion coefficient in intercellular volume; F_c – area of surface of the cell; d – diameter of the cell; $R_{екв}$ – equivalent diameter of cell; t – time; t_p – time development equilibrium; δ_c – thickness of cellular membrane; V_c – volume of the cell; W – volume of extractant; $V\varepsilon$ – volume occupied by cells; e – porosity of raw material.

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ПРО МЕХАНІЗМ ЕКСТРАГУВАННЯ ІЗ ТВЕРДИХ ТІЛ КЛІТИННОЇ БУДОВИ

Анотація. *Доведено адекватність математичної моделі процесу екстрагування твердих тіл клітинної будови (рослинна сировина), враховуючи її анатомічну будову, а саме наявність клітинного та міжклітинного середовища. Експериментально підтверджено механізм екстрагування внутріш-ньоклітинної речовини в процесі перебігу екстракційного процесу. Підтверджено порядок коефіцієнту дифузії через клітинну обоонку – D_c та в міжклітинному середовищі D_m.*

Ключові слова: *екстракція, клітинна речовина, міжклітинний простір, дифузія.*