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Kinetics of Bioactive Compounds Extraction from Plant Material Using Boiling Solvent

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Abstract—A model of extracting the natural products through boiling ground plant material in a solvent using a bain-marie is proposed. The model trait is that it has only one adaptation parameter, i.e., the diffusion coefficient of the solute in the plant material, which depends on the solvent and plant material properties. The model is applied to study the kinetics of extraction, and the minimal solvent volume, which is used for extraction, is theoretically determined. A comparison of the model with the experimental data of the extraction of hydrophobic diterpene acids from sage leaves and hydrophilic flavonoids from common knotgrass herb is in good agreement with the experiment. Experiments performed with finely ground plant material (particle diameter is less than 1 mm) show that, for most extraction conditions, more than 90% of the target compounds are extracted by the time the solvent starts to boil.

Keywords: extraction kinetics, mathematical simulation, flavonoid, diterpene
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INTRODUCTION

Currently, the vast majority of herbs applied in medical research have been subjected to standardization based on the content of various groups of biologically active substances, e.g., flavonoids, saponins, or alkaloids. Determinations are made using a variety of methods, including photometric, chromatographic (GC and HPLC), electrochemical, etc.; however, in all cases, the extraction procedure of target compounds from plant material foregoes the quantitative measurements. The extraction of biologically active substances from plant material is the most laborious and time-consuming part of the standartization procedure. This is because the full and complete extraction of the target compounds is achieved by the prolonged and repeated extraction from the plant material. Reasons of this approach raise doubts, since it has been observed that most of the target compounds are extracted to the solution during first minutes of a single extraction.

This observation leads to an idea of modification of the accepted methods of quantitative determination of target compounds in order to reduce significantly the extraction time. To investigate this question, a mathematical model of the process has been proposed, which is described in detail in the first part of the article. This model takes into account various approaches proposed in the literature [1–5] and simplifies some of them. At the same time, the formulated model seems to be general enough to describe all of the observed effects and contains only one adaptation parameter depending on the interaction of target substances and raw material internal structure. Based on the proposed model, the extraction kinetics of target substances has been studied in case of single and multiple extractions with new portions of solvent.

In the second part of the article, the results of model validation on extraction experiments with knotweed grass and sage leaves are discussed. In conclusion, based on these results, the main parameters which give a possibility to reduce the time of complete extraction are highlighted.

MATHEMATICAL MODEL OF EXTRACTION PROCESS

The extraction process using a bain-marie may be divided into two stages. The first one is a preparatory step, and its duration t_0 may vary greatly depending on the properties of the solvent used. At this stage, ground plant material of mass m_0 and density ρ_0 is placed in a flask with a solvent that permeates the raw material at room temperature. Then, the flask is covered by a reflux condenser and set on a hot bain-marie, where the solvent is brought to a boil and its solubilizing power increases [1–3]. The impregnation of raw material with solvent and a gradual rise in temperature up to the boiling point leads to the gradual release of

the target substances from cells of the raw material to internal transport channels.

They are formed from pores (cell walls, the intercellular space), which originally exist in the dried plants. The volume fraction of these channels is designated as e. The impregnation of the sample with solvent increases the size of ground particles, leads to the pores opening, and the formation of transport channels. The extractable substances start to diffuse via these channels to the particles surface at the first stage of extraction. The coefficient of volume expansion caused by the impregnation of material is denoted by α.

A detailed mathematical description of the processes that occur in the raw material at the preparatory stage is complicated for the following reasons. During the heat of the solvent (usually $t_0 > 3$ min), the structure of the raw material at the cellular level undergoes major alterations (at sufficiently high temperatures, the proteins coagulate and cell membranes are destroyed), and the chemical properties of the solvent (solvent power and the diffusion rate of the extractives increase) vary significantly. Therefore, in the simulation of the first extraction step, we propose to consider only its final moment, the moment when the solvent starts to boil. From this moment we start counting extraction time t.

When $t = 0$, the second (main) step of extraction starts. Its mathematical description is given in this paper. For this, the density C_0 of the extract in solution at $t = 0$, as well as the average mass θ_{max} of extractable substances per unit volume of transport channels and available for extraction at the second stage are defined. These values are initial for the corresponding parameters which are used to express solute mass balance at the main stage of extraction.

Hereinafter, V is the volume of the solvent used for extraction and V_s is the amount of free solvent that is not absorbed in the raw material as shown below

$$V_s = V - V_0 E, \quad E = e\alpha.$$

Here, V_0 is the total volume of dry ground particles, defined as the ratio of the sample mass to the raw material density ρ_0, and $V_0 E$ is the volume of transport channels of impregnated material, containing absorbed solvent. Since boiling condition leads to major convective (mixing) flows in the solvent, the model is confined to a homogeneous approximation of the solvent and the current solute density $C(t)$ is equal to its average density, i.e., to the ratio of the current mass m of the solute in the flask to the solvent volume V_s.

Denoting the initial mass concentration of target compounds in the raw product per unit mass of dry plant as θ_0 and considering that at the boiling point the solvent is capable to infinitely dissolve extractable matter, we write the mass balance equation for target compounds at moments $t = 0$ and $t \to +\infty$ as follows:

$$\theta_{max} V_0 E + C_0 V_s = \theta_0 m_0 = C_{max} V.$$

Hence, we obtain the following expressions for θ_{max} and the maximum solute density C_{max}:

$$\theta_{max} = \frac{1}{E}\left(\theta_0 \rho_0 - C_0 \frac{V_s}{V_0}\right), \quad C_{max} = \frac{\theta_0 m_0}{V}.$$

It is assumed that ground particles are isotropic and isometric. The radius of the dry particles is denoted by a', and a is a corresponding radius of the particles impregnated with solvent:

$$a = a'\left(1 + \alpha e - e\right)^{1/3}.$$

In general, the sample of ground raw material consists of particles of different sizes. The particle size distribution is described by the function of the volume distribution $F(a)$ with density $f(a)$. By definition, $dF = fda$ is the volume fraction of particles with sizes from a to $a + da$.

A spherical coordinate system is introduced inside each particle, and $0 \le r \le a$ is the distance from its center. Then, assuming that the target compounds diffuse inside ground particles according to the Fick's law with the diffusion coefficient D and the process is independent of the presence of other substances, the following mass balance equation in the channels of raw material particles [6] could be written:

$$\frac{\partial \theta}{\partial t} = \frac{D}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial \theta}{\partial r}\right), \tag{1}$$

where $\theta(r, t, a)$ is the current solute density in the channels of raw material. At the initial moment,

$$\theta(r, 0, a) = \theta_{max}. \tag{2}$$

We assume that the rate of mass transfer from the surface of the particles due to the boiling of the solvent is large enough comparing to the internal diffusion rates. Then, the boundary conditions in each particle are:

$$\left.\frac{\partial \theta}{\partial r}\right|_{r=0} = 0, \quad \theta|_{r=a} = C(t). \tag{3}$$

Eventually, in our assignments, the solute mass balance equation in the free solute volume V_s for a polydisperse batch is written as follows:

$$\frac{dC}{dt} = -D\frac{\gamma}{1-\gamma}\int_0^{+\infty} \frac{3}{a}\left.\frac{\partial \theta}{\partial r}\right|_{r=a} f(a)da, \quad \gamma = \frac{V_0 E}{V}, \tag{4}$$

where dimensionless complex γ is equal to the pore volume in the impregnated raw material to the volume of solvent used.

Starting at the beginning of the main phase of the extraction, the density of the extract is C_0 and the initial condition for Eq. (4) takes the following form:

$$C|_{t=0} = C_0. \tag{5}$$

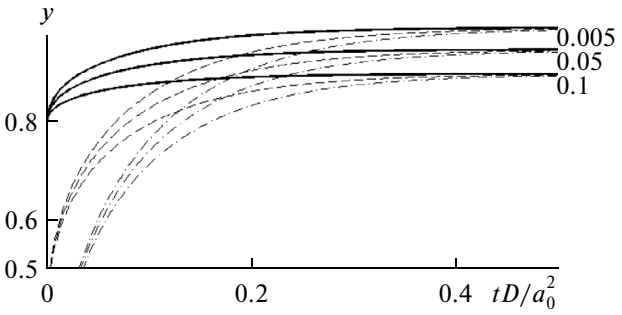

Fig. 1. Normalized OECs for different values of y_0 and γ. Solid lines correspond to $y_0 = 0.8$, dashed lines correspond to $y_0 = 0.4$, dash–dot lines correspond to $y_0 = 0$. Numbers under the curves are the corresponding values of γ. Three curves with different values y_0 correspond to each value γ and vice versa.

In the discrete case when, e.g., n homogeneous groups of particles with mass fractions $f_1, f_2, ..., f_n$ and respective characteristic sizes $a_1 < a_2 < ... < a_n$ are isolated as a result of the sieve analysis of raw material, Eq. (4) takes the following form:

$$\frac{dC}{dt} = -3D\frac{\gamma}{1-\gamma}\sum_{k=1}^{n}\frac{f_k}{a_k}\left.\frac{\partial\theta}{\partial r}\right|_{r=a_k}. \qquad (6)$$

The model (1)–(6) is similar to the basic equations of the one from [1], since the same laws of mass transfer in the raw material are considered at the model formulation. Two important differences in these models should be noted. Firstly, the proposed model takes into account the polydispersity of a sample. The necessity of such approximation for a similar extraction process is shown in [7, 8]. However, the main difference lies in the reduction of the number of adaptive parameters to one (instead of four, introduced in [1], and the two found in [2]). The decrease in the number of adaptive parameters is acheived due to the consideration of the first extraction stage in its final moment. In this case, it is sufficient to know only two values, e.g., C_0 and θ_{max}, that are determined experimentally. Density C_0 generally depends on the particle size, the type and volume of the solvent, the type of raw material, and the process temperature. However, at the same time, this parameter has a clear physical meaning.

Thus, the formulated model contains the given parameters ρ_0, e, α, which characterize the investigating material. Values θ_0 and C_0 are determined during extraction, and the diffusion coefficient D, depending on the type of solvent, process temperature and the type of plant material, is an adaptive model parameter.

Basic information about the kinetics of target substances extraction is given by the overall extraction curve $Y(t)$ (**OEC**). It is a function of time and equal to the ratio of the extracted solute to the sample mass m_0. It is further used to configure the parameters of the model. In our notations,

$$Y(t) = C(t)V_s/m_0.$$

Kinetics of Single Extraction

The consideration of the dimensionless variables

$$\mathrm{Fo} = \frac{tD}{a_0^2},\quad y = \frac{Y}{\theta_0},\quad y_0 = \frac{Y_0}{\theta_0},\quad c = \frac{C}{C_{max}} \qquad (7)$$

for a monodisperse sample with a particle size a_0 has shown that the kinetics of extraction of target compounds is determined by two dimensionless complexes, i.e., γ and y_0. Value y_0 means the solute mass fraction extracted from the raw material to the moment $t = 0$. The typical shape of the normalized overall extraction curve y at different values of these complexes is given in Fig. 1.

A series of computational experiments showed that, when $\gamma \le 0.01$, the normalized OECs differ slightly (less than by 1%) at any fixed value y_0. Hence, we come to the universal condition

$$V_0E \le \frac{V}{100}, \qquad (8)$$

which means that when the volume of solvent used is 100 times greater than the volume of the pores in the impregnated raw material, a further increase of volume V at constant y_0 does not affect the shape of OEC. Thus, when condition (8) is satisfied, only the dimensionless complex y_0 affects the total extraction time that is proportional to the ratio a_0^2/D. Therefore, reduction of the full extraction time can be achieved by fine material grinding and the selection of a suitable solvent that provides the highest diffusion D, as well as by increasing the value of the variable y_0.

It shoud be emphasized that, if the values V_0, E, V do not satisfy criterion (8), additional systematic errors would appear in the measurement results which lead to the underestimation of the full solute content (Fig. 1, curves for $\gamma > 0.005$).

EXPERIMENTAL METHODS AND MODEL ADAPTATION

Description of the Experiment

Sage leaves (*Salvia officinalis* L., fam. *Lamiaceae*) in filter bags manufactured by JSC St-Medifarm purchased at a retail pharmacy were used to study the extractability of diterpene acids. According to the requirements of the pharmacopeia article the particle size (diameter) of the raw material in the filter bags should not exceed 2 mm for sage leaves. Sieve analysis was applied to study the fractional composition of the raw material in the filter bags, sieve mesh sizes are 0.25, 0.385, 0.5, 1, and 2 mm. The content of diterpene acids was determined by personally developed

methodology, based on the spectrophotometric method. For this purpose, about 2 g (accurately weighed) of sage leaves (ground and sieved through a sieve with 1 mm-mesh size) were placed in a 200-mL flask and extracted with 60 mL of petroleum ether (40–70) at moderate boiling on a bain-marie. When studying the mass transfer rates dynamics of diterpene acids, extracted solute mass determination was performed immediately after the solvent began to boil and after 5, 10, 20, 30, and 60 min of solvent boiling in a flask. For this, the flask was removed from the water bath and cooled to room temperature, after which the solution was filtered through filter paper into a 50-mL volumetric flask. The solution in a volumetric flask was diluted to the fixed volume with petroleum ether, stirred, and the aliquot was measured photometrically at 285 nm using the specific absorption rate of carnosolic acid equal to 40.92.

Knotweed grass (*Polygonum aviculare* L., fam. *Polygonaceae*) harvested in May–June 2013 in Tatarstan was used to study the extractability of flavonoids. The content of flavonoids was determined by the State Pharmacopoeia method using spectrophotometric method after the color reaction of flavonoids with aluminum chloride [10]. In each experiment, 1 g (accurately weighed) of ground knotweed grass sieved through a 1-mm-mesh sieve was extracted with water solution of ethyl alcohol (which concentration varied in the range of 40–95%) boiling on a water bath. The total flavonoid content in the raw material was determined as a result of three consequent 30 min extractions using 40, 30, and 30 mL, respectively. In order to study flavonoids extraction their mass in the solution was measured when the solvent started to boil and after 5, 10, 15, and 30 min of solvent boiling in the flask.

Each experiment was repeated at least three times, and the optical density was measured on a LAMBDA 25 spectrophotometer (Perkin Elmer, United States).

Model Adaptation

Mathematical model adaptation requires to match the theoretical dependence $Y(t)$ with experimental OECs obtained for concrete values of the solvent volume V, the sample mass m_0, and other parameters of the extraction process. To adapt the model it is also necessary to set the characteristics of raw material, e.g. E, V_0, θ_0, y_0, D, as well as sample particle size and histogram of the distribution function. Further, the proposed method of parameters adjustment is discussed in detail.

Since $E = e\alpha$, the estimation for e and α are needed. For the porosity of raw material one can assume that $e \le 0.1$, as the volume fraction of cell walls and intercellular space in the wet raw material is about 0.01 of its volume. Similarly, we assume that $\alpha \le 2$; i.e., the pore volume increases by no more than twofold due to impregnation. Then, the changes in particle size can be neglected and $E \le 0.2$. Using this estimate, we can strengthen the criterion (8), excluding E, as follows:

$$\frac{V_0}{V} \le 0.05. \qquad (9)$$

Then, it is necessary to determine the volume V_0 of sample particles and to check the criteria (8) and (9). For this purpose, in addition to the volume of the solvent, it is necessary to know the sample mass m_0 and raw material density ρ_0, which is proposed to determine using the formula $\rho_0 = \rho_b/(1 - e_b)$, where ρ_b is bulk density of the material, and $e_b = 0.55$ is a sample porosity at the ρ_b determination (density values are given in Table 1). Finally, the total volume of ground particles is, by definition, equal to the ratio of the sample mass and density, $V_0 = m_0/\rho_0$. It was found that for each series of experiments criterion (9) and, consequently, criterion (8) are satisfied.

Table 1. Characteristics of raw material and solvent

Raw material	Sage	Knotweed
Sample mass, m_0, g	2	1
Raw material density ρ_0, g/cm^3	0.845	0.645
Raw material particles volume V_0, mL	2.37	1.55
Bulk density of raw material ρ_b, g/cm^3	0.38	0.29
Porosity of dry material e	0.1	0.1
Coefficient of volume expansion α	2	2
Solvent volume V, mL	60	100

Parameter values y_0 and θ_0 are determined from experimental OEC. The first parameter is equal to the initial value of the curve, at $t = 0$, and the second one is found based on its maximum value in the case of fine grinding when $t \to +\infty$. Since criterion (8) is satisfied in the described experiments, it can be assumed that $V_s = V$. Therefore, the total content of the extracted substances is approximately equal to the maximum value of OEC, $Y_{\max} = \theta_0$.

The last group of parameters determines the fractional composition of sample. For the knotweed grass, every sample is considered in a monodisperse approximation and the radius a_0 is taken equal to 0.25 mm. The effects associated with sample polydispersity are demonstrated on the basis of sage leaves extraction. As

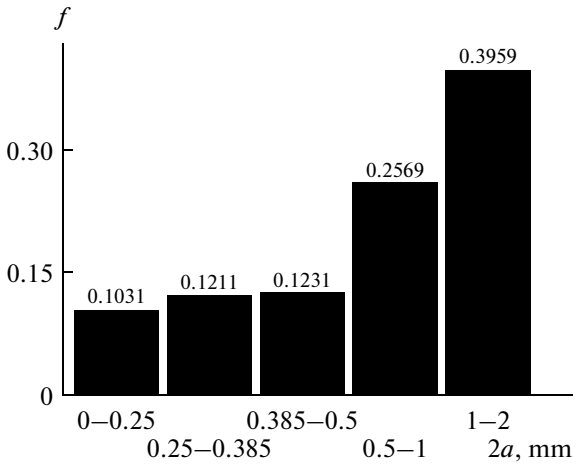

Fig. 2. Histogram of the distribution function of particles of ground sage leaves from filter bags.

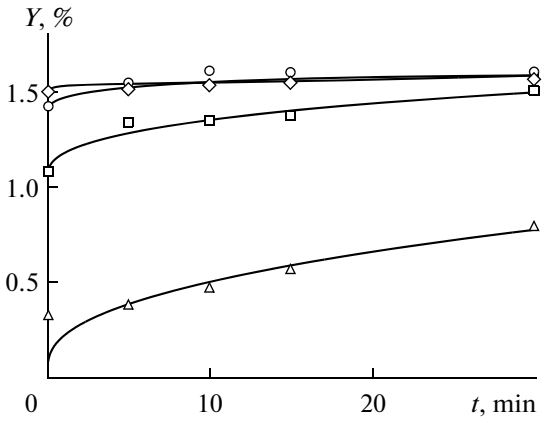

Fig. 3. Extraction of flavonoids from knotweed grass. Adaptation of the model (solid line) and experimental results (markers) at four different concentrations of the alcohol in the solvent (triangles represent 96%; circles represent 70%; rhombs represent 60%; squares represent 40%).

a result of sieve analysis, the mass fractions in the sample of particles with various sizes are 0.1031, 0.1211, 0.1231, 0.2569, and 0.3959 (histogram in Fig. 2). The average particle size of each fraction is assumed to be $a_1 = 0.0625$ mm, $a_2 = 0.16$ mm, $a_3 = 0.22$ mm, $a_4 = 0.375$ mm, and $a_5 = 0.85$ mm, respectively. For the fine grinding of sage leaves, the average particle size is set to be $a_0 = 0.12$ mm.

Thus, the only adaptation parameter is the effective diffusion coefficient D which depends on the type of solvent (a series of experiments on the extraction of knotweed grass) and independent of the particle size (a series of extraction experiments with sage leaves). Figures 3 and 4 show the results of the model adaptation for the extraction of target compounds from a sample of ground material (sage and knotweed, respectively). Due to the selection of the adaptation parameter D, it was possible to achieve satisfactory agreement between the theory and experimental data, and its values (for sage, $D = 2.5 \times 10^{-12}$ m^2/c; for knotweed, see Table 2) are within the expected range [9].

RESULTS AND DISCUSSION

Knotweed Grass

For extraction curves of knotweed grass corresponding to 60 and 70% water solution of ethyl alcohol (rhombs and circles in Fig. 3, respectively), it should be noted that at the moment when the solvent hits the boiling point, more than 95% of the target substances have been isolated from the raw material and the curves differ slightly. When compared with other OECs (96 and 40% alcohol), it can be concluded that the concentration range of 60–70% is the optimum for extraction of flavonoids from knotweed grass and it can also be optimal for flavonoids extraction from other herbs, e.g., calendula flowers or birch leaves. The small difference in the full content determined after triple extraction (Table 2) may be due to several factors, including different characteristics of raw material (grass was collected during the vegetation phase and, in some instances, could be more developed), the error of measuring the solution concentration, or the possible degradation of target substances due to the high temperature of the process. Therefore, in the simulation of the results for knotweed grass, for each OEC, it is assumed that $\theta_0 = 0.0158$.

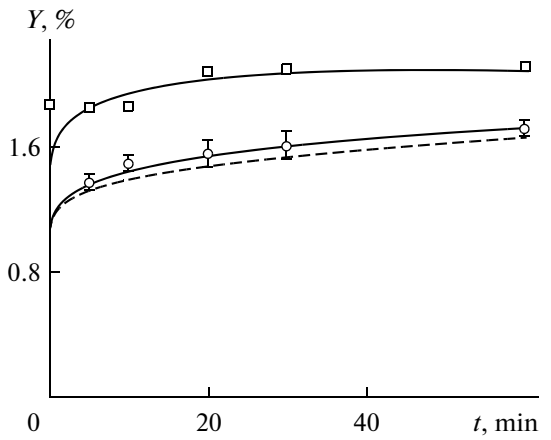

Fig. 4. Extraction of diterpenes from ground sage leaves. Adaptation of the model (lines) and experimental results (markers) for different degrees of grinding of raw material (squares represent fine grinding, and circles represent raw material from the filter bags). Dashed line corresponds to the model adaptation in monodisperse approximation of raw material sample from the filter bag, value a_0 is equal to the average particle size in the sample.

Table 2. Model parameters for knotweed grass

Alcohol concentration	40%	60%	70%	96%
Diffusion D, 10^{-12} m^2/s	4.5	6.4	11.2	1.05
Parameter y_0, %	1.07	1.49	1.42	0.035
Total content according to the procedure of triple extraction, %	1.6874	1.61135	1.55572	1.5478

The accepted pharmacopeia determination method of the total flavonoid content in the knotweed grass implies triple extraction. As it follows from the analysis of the experimental results, the model adaptation results, numerical calculations, and criteria (8) and (9) a commensurable fraction of target compounds are extracted during the first extraction using the pharmacopoeia method (solvent volume is 40 mL), under the same experimental conditions as in this work. A minor amount of the target substances (less than 5%) should be extracted during the other two extraction procedures (when 30 mL of solvent were used). Therefore, if 5% accuracy is acceptable when determining the total content, triple extraction can be superfluous.

For a more detailed research of this question, it is planned to study the extraction dynamics of flavonoids with different volumes of solvent, as well as to measure the mass of extractive substances isolated during the second and the third extraction procedures with the same sample. More accurate estimates of E might be required.

Sage Leaves

As in the vast majority of experimental data on the plant extraction, fine grinding (squares in Fig. 4) significantly increases the total extraction yield. Moreover, before the boiling point of the solvent (petroleum ether) is attained, a substantial part of the extract is isolated from raw material and the extraction is completed in 20–30 min of boiling. The second series of experiments (circles in Fig. 4) corresponds to the coarse grinding of polydisperse sample (raw material from filter bags). These experiments were carried out for two different packs of the same lot. Circles in Fig. 4 are the average results for the two packs, and the ends of vertical segments mark the results for each pack. For the polydisperse approximation of the sample (solid line) the model adaptation is in good agreement with the average result (circles), and the calculated curve (dashed line) is significantly lower in the case of monodisperse approximation for $a_0 = 0.48$ mm (mean particle radius) than the experimental data.

Thus, the results of the observations may vary, even within the same batch of packed material, as well as depend significantly on the fractional composition of the ground particles, for which it is insufficient to consider the monodisperse approximation. Therefore, at the preparation of the experiment, it is recommended not only to preliminary mix all raw material prepared for experiments, but also to conduct a sieve analysis in order to take into account the effects associated with sample polydispersity.

CONCLUSIONS

The proposed model of extraction of target substances from plant material allows the simulation of the extraction kinetics for different types of raw materials and solvents. The adequacy of the model has been demonstrated based on the extraction experimental data with knotweed grass and sage leaves. As it follows from the model the extraction rates can be increased by adjusting three independent parameters, e.g., a_0, D, and y_0. In the case of the fixed solvent, the last parameter depends on the ratio of the raw material mass to the solvent volume, and its value corresponds to a more than a 90% yield of the desired compounds under the considered conditions in most experiments with fine grinding.

The process analysis at the stage of model formulation and its further investigation revealed two important points. Firstly, it is necessary to consider the finiteness of time needed to acheive the desired temperature of the system and, therefore, to point out accurately which time moment (e.g., time of immersion of raw material in the solvent, the moment of bringing the solvent to a predetermined temperature, and others) is selected as the initial moment of extraction. Secondly, a criterion is found which satisfaction allows to experimentator to select an economically optimal ratio of raw material mass to the solvent volume, as well as to reduce the measurement error associated with the retention of the solvent by raw material after extraction.

The results obtained from model validation allowed to determine the optimal extraction mode of diterpene acids from the leaves of *Salvia officinalis* when developing a method for quantifying it and offering the option to quickly determine flavonoids content in knotweed grass, which leads to a significant reduction

in extraction time. In particular, for the approximate determination of the total content, it is suggested that multiple extractions should be replaced by a single one. A detailed description of these methods is beyond of the scope of this article.

In each set of experiments, considerable information about the extraction of target compounds is received from OEC. For example, it allows to confidently talk not only about the completeness of extraction, but also about the possible degradation of the extract components at sufficiently high extraction temperatures [11]. Therefore, the study of the target compounds extraction kinetics in the case of single and multiple extraction is crucial for the development of various techniques of quantitative determination.

NOTATION

a—radius of particle impregnated with the solvent, m;

a_0—particle radius at the monodisperse approximation of the sample, m;

a'—radius of dry sample particles, m;

C—current density of the extract in the solution in the flask, kg/m^3;

C_0—extract density in the solution in the flask at $t = 0$, kg/m^3;

C_{max}—extract density in the solution in the flask at $t \to +\infty$, kg/m^3;

c—current density of the extract in the solution in the flask normalized on the maximum density;

D—diffusion coefficient of target compounds in the raw material, m^2/s;

E—ratio of the volume of the transport channels in the impregnated raw material to the volume of the dry material;

e—porosity of raw material before immersion in the solvent;

F—volume distribution function with respect to particle size;

f—density of F, m^{-1};

m_0—sample mass, kg;

n—number of fractions in the polydisperse sample;

r—radial coordinate in particles, m;

t—time, s;

t_0—duration of the first, preparatory, stage, s;

V—volume of solvent used, m^3;

V_s—volume of solvent washing the particles during extraction, m^3;

Y—the overall extraction curve (OEC);

Y_0— Y value at $t = 0$;

y —overall extraction curve normalized on the total reserves of extractives;

y_0—value of y at $t = 0$;

α—coefficient of volume expansion of the pores in the raw material;

γ—ratio of pore volume in the wet raw material to the volume V of the solvent;

θ —current density of extractives in the channels of raw material, kg/m^3;

θ_0—total extractives content in the raw material, kg/kg;

θ_{max}—average density of extractives in the raw materials in the channels at t = 0, kg/m^3;

ρ_0—raw material density, kg/m^3;

Fo—Fourier criterion (homochronicity criterion).

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