A New Approach To Description Of Equilibrium State In The Extraction System Of Vegetal Raw Material-Extractant

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Abstract—**Aim of this study is the theoretical justification and experimental confirmation of new approach to description of equilibrium state in extraction system vegetal raw material-extractant. In framework of this study the milled raw material was used containing the particles sized 0,1-0,5 mm: roots and rhyzomes of Glycyrrhiza glabra or uralensis, leaves of Uva-urzi, leaves of Eucalyptus viminalis, flowers of Helichrysum arenarium, flowers of Calendula officinalis and etc. The process of equilibrium establishment was studied at temperature levels such as 4, 20, 40 and 60±1°C, wherein the method of simple maceration was implemented for 24 hours of decoction. Interphase distribution of BAS was studied using the proportion of raw material weight / extractant volume as 1:5, 1:10, 1:20, 1:40. The watery ethanol solution of 70 or 80±1 % vol. was chosen as an extractant for the purpose of this study. Assay test of BAS in raw material was carried out with the help of RP HPLC instrumentation method. Experimental points for all the studied BAS from various types of vegetal raw materials are well-approximated by regression lines built up in theoretically predicted coordinates, which is confirmed by the high value of determination coefficient R²≥0.99. The new approach has been proposed to description of equilibrium state in extraction system vegetal raw material-extractant, which is based on the promotion of hypothesis, according to which the mechanism of interphase molecular distribution of BAS in extraction system is explained and described by classic Boltzmann distribution for discrete values of molecular energy (or Fermi-Dirac quantum distribution). The developed and experimentally confirmed mathematical model was based on the promoted hypothesis, and it successfully described the relation of the main parameters of extraction system, which allows to develop the rational technology of tincture or extracts manufacturing.**

Keywords—extraction, vegetal raw material, biologically active substances, equilibrium, Boltzmann distribution for discrete values of molecular energy, Fermi-Dirac quantum distribution.

I. INTRODUCTION

At the moment the worldwide situation is that biologically active substances (BAS) of natural origin (obtained from microorganisms, fungi, plants and etc.) are continuing to play their important role in development of new drug products on their bases. According to the US scientists' assessment, out of 1211 molecules of FDA approved BAS for development of new drug products, in between 1981 up to 2014 there are approximately 36% of substances have synthetic origin, approximately 14% are of semi-synthetic and 34% are natural, including the vegetal ones [1-4]. It's important to note that among anticancer and antimicrobial substances, the amount of natural BAS significantly overshoots 60% [5, 6].

According to the data from national authors from Russian Federation, the percentage of vegetal drug products equals around 20-25% of the overall number of registered drug products [7, 8]. The most often vegetal drug products find their use in treatment of gastrointestinal, respiratory, nervous and urogenital system diseases [7-10]. Moreover,

BAS of vegetal raw materials attract the attention of scientist due to their valuable anticancer and antimicrobial properties that can be helpful to solve the issue of antimicrobial resistance to well-known medical antibiotic drug products, especially in the field of stomatology [11-15].

The information provided above witnesses of significant potential of BAS of natural origin, in particular vegetal raw materials, especially those ones having antimicrobial properties for development the new drug products on their base for local use in stomatology, dermatology and otolaryngology.

As the rule, the content of targeted BAS in vegetal raw material is low, and technological approaches of their extraction and purification is multi-stage, and they are also material and energy demanding [16, 17]. It's important to note that extraction process of BAS from vegetal raw material is a leading one in manufacturing technology of any drug products of natural raw material [18, 19]. Hence, the lot size, quality and costs of drug products, also rate of exhaustion and usage of vegetal raw material are depended on the approach of manufacturing process organization [20]. However, although many studies have been performed in this field, the extractant choice justification and selection of optimal extraction conditions for various types of vegetal raw materials are still done via experiment, but they aren't backed by any theory so far [21-23].

This is why those studies are relevant, that are aimed at determination of sense of facts and processes that take place to be in extraction system and will allow to develop the mathematical model of BAS extraction from vegetal raw materials in order to optimize this manufacturing stage in terms of material and energy resources.

Aim of this study is the theoretical justification and experimental confirmation of new approach to description of equilibrium state in extraction system vegetal raw material-extractant.

II. EXPERIMENTAL

Vegetal raw material

In framework of this study the milled raw material was used containing the particles sized 0,1-0,5 mm: roots and rhyzomes of Glycyrrhiza glabra or uralensis, leaves of Uva-urzi, leaves of Eucalyptus viminalis, flowers of Helichrysum arenarium, flowers of Calendula officinalis, leaves of Urtica dioica and etc.

Reagents and chemicals

Assay test of BAS in raw material was carried out with the help of RP HPLC instrumentation method using the reference samples: arbutin, glyceram, viscous extract of Chlorophyllipt, isosalipurposide, licuroside, salipurposide, rutin, chlorogenic acid in accordance to the State Pharmacopoeia of Ukraine and etc.

Method of extraction

The process of equilibrium establishment was studied at temperature levels such as 4, 20, 40 and 60±1°C, wherein the method of simple maceration was implemented for 24 hours of decoction. Interphase distribution of BAS was studied using the proportion of raw material weight / extractant volume as 1:5, 1:10, 1:20, 1:40. The watery ethanol solution of 70 or 80±1 %vol.

Reverse phase high performance liquid chromatography (RP HPLC) instrumentation

RP HPLC analysis was carried out using the chromatographic equipment by «Agilent Technologies», «Agilent 1200 Infinity» series, manufactured in the USA. The detailed description is available highlighted in the following research [24].

Theoretical part

In order to explain the possible mechanism of equilibrium state in extraction system and development of the mathematical model, which quantitatively describes this state, the following hypothesis was used – mechanism of equilibrium state is related to classic Boltzmann distribution for discrete values of molecular energy of BAS (or Fermi-Dirac quantum distribution), which is presented below as an equation (1):

$$\frac{n}{n_0} = \frac{1}{1 + \exp\left(\dfrac{\Delta G}{kT}\right)} = \frac{1}{1 + \exp\left(\dfrac{\Delta G}{RT}\right)} \quad (1)$$

where n is quantity of BAS in solvent with energy equals ΔG, mol;

n_0 is overall quantity of BAS molecules in extraction system, mol;

ΔG is molecular energy of BAS, J or J/mol;

exp is the basement of natural logarithm, constant value that approximately equals 2,72;

k is Boltzmann constant value, $1,38 \cdot 10^{-23}$ J/K;

R is gas constant value, 8,314 J/(mol·K);

T is absolute Kelvin's temperature, K.

This hypothesis is promoted and based upon an assumption that extraction system consisted of sum of large number of BAS molecules, backbone of vegetal raw material and extractant, should obey to the law of quantum and statistical physics, thermodynamics, physical and colloidal chemistry.

Thereafter, in the extraction system with the invariable value of dielectric constant of solvent (concentration of solvent is unchanged), this is possible to assume that value of intermolecular energetic processes is kept invariable as well, i.e. $\Delta G = const$. Taking into account that $C = n/V$ and marking $\exp(\Delta G/kT) = K_H$, from equation (1), the equation (2) is obtained, which expresses the desired dependence of equilibrium concentration of BAS in extraction system on temperature and proportion of phases (volume of extractant):

$$\frac{1}{C} = \frac{M}{m_0} \cdot V + K_H \cdot \frac{M}{m_0} = a \cdot V + b \quad (2)$$

where C is concentration of BAS in extract, g/ml;

m_0 is initial content of BAS in raw material, g;

M is weight of raw material, g;

V is volume of extractant in extraction system, ml;

a is constant value, equals to reversed value of overall BAS content in extraction system g/g;

b is constant value, equals to multiplicity of Henry's constant and (K_H) and reversed value of overall BAS content in raw material (M/m_0), ml/g.

As it's seen, the equation (2) in coordinates $1/C=f(V)$ wherein *T*=const and $\Delta G=const$, predicts the rectilinear dependence ($1/C=a \cdot V+b$), wherein the rate of conformity between the mathematical model and experimental data is possible to check using the method of regression analysis.

Using the empiric constant values (*a*, *b*) from equation (2), it's possible to calculate the constant values

III. RESULTS AND DISCUSSION

Results for studies of concentration dependence of arbutin, glyceram, isosalipurposide, licuroside, salipurposide, chlorogenic acid and euglobal with

characterizing this theoretical model (ΔG и m_0/M). By this way, from empiric constant value (*a*), the overall (initial) quantity of BAS in the extraction system (m_0/M) can be calculated, which gives an additional opportunity to check the theoretical model and experiment. By use of both empiric constant values (*a*, *b*) it's possible to calculate the Gibbs energy (ΔG) for BAS at various temperature levels from regression equation in coordinates $\ln(b/a)=f(1/T)$.

Mathematical methods

For assessment of adequacy of the proposed hypothesis, authors used the regression analysis of experimental data in theoretically predicted coordinates. The regression analysis was carried out using the preset for data analysis in MS Excel 2010.
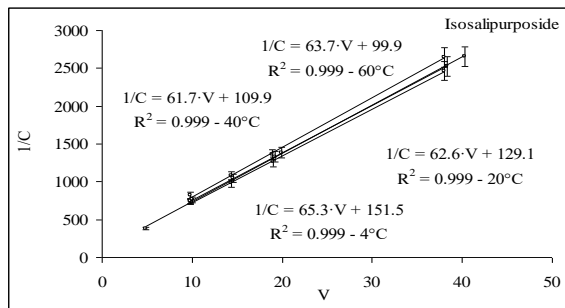
recalculation to viscous extract of Chlorophyllipt in extractions on extractant volume in coordinates $1/C=f(V)$ for various types of vegetal raw materials at temperature levels 4, 20, 40, 60 °C, are shown on Fig.1-7.
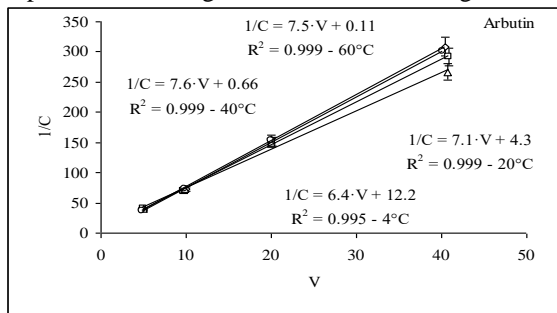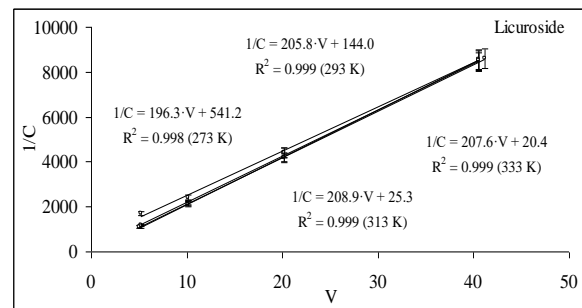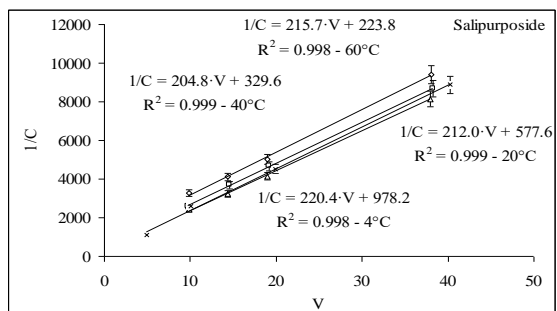

Arbutin from leaves of Uva-ursi
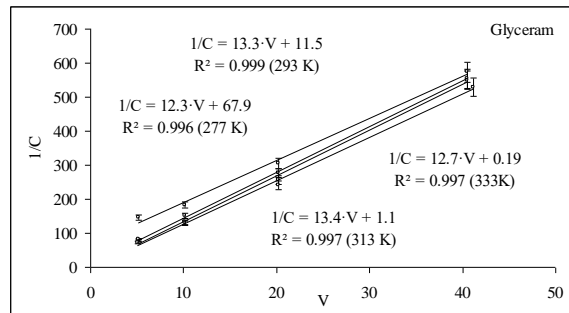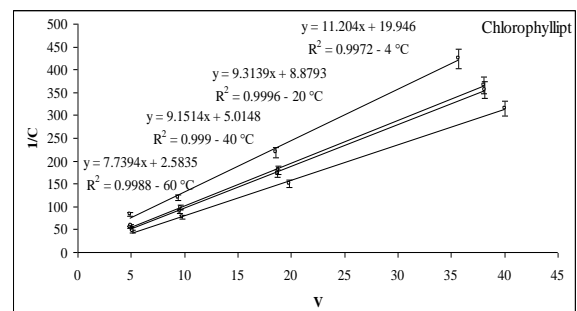

Glyceram from roots of Glycyrrhiza

Fig.1. Regression dependence equations of some BAS concentrations in extraction on extractant volume in coordinates $1/C=f(V)$

Isosalipurposide from flowers of Helichrysum

Licuroside from roots of Glycyrrhiza

Fig.2. Regression dependence equations of some BAS concentrations in extraction on extractant volume in coordinates $1/C=f(V)$

Salipurposide from flowers of Helichrysum

Euglobal recalculated to viscous extract of Chlorophyllipt from leaves of Eucalyptus

Fig.3. Regression dependence equations of some BAS concentrations in extraction on extractant volume in coordinates $1/C=f(V)$
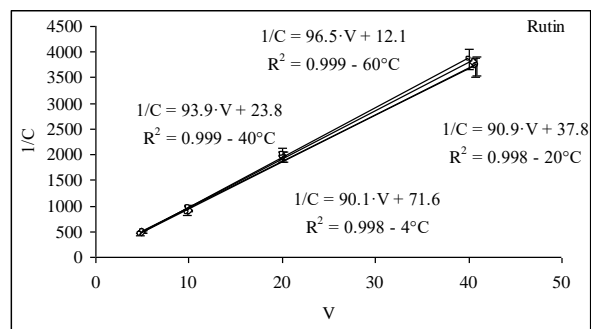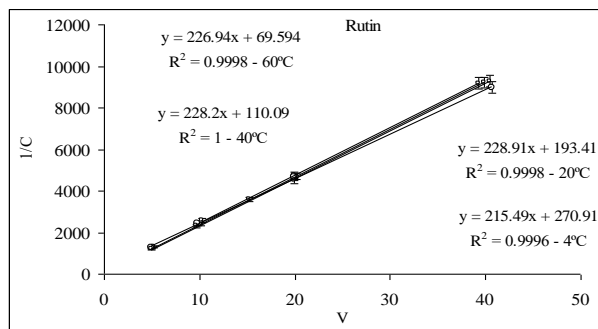
Rutin from flowers of Calendula

Rutin from leaves of Uva-ursi

Fig.4. Regression dependence equations of some BAS concentrations in extraction on extractant volume in coordinates $1/C=f(V)$

Rutin from leaves of Eucalyptus
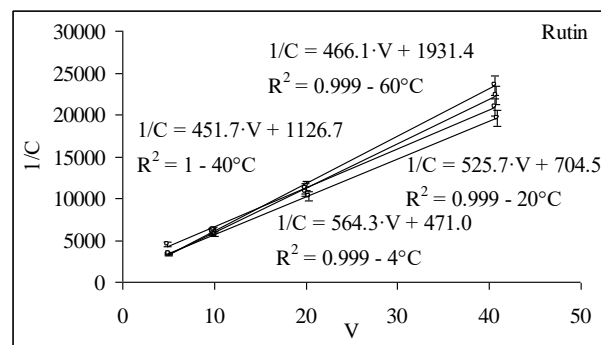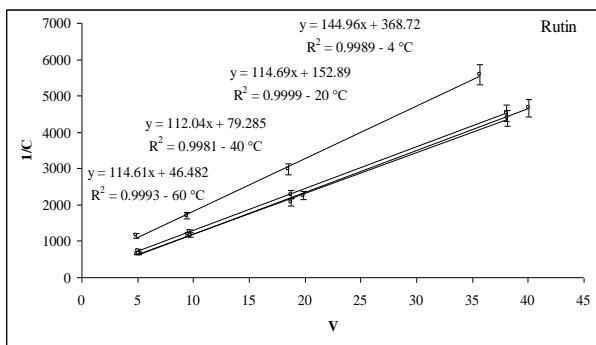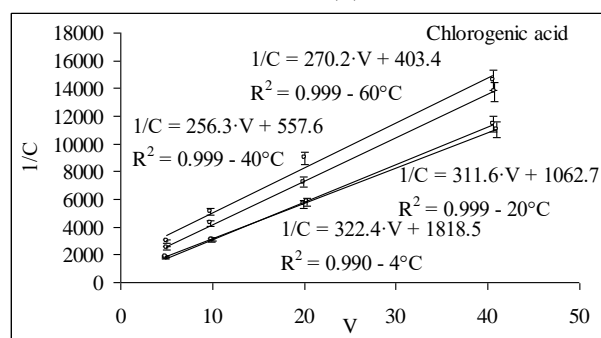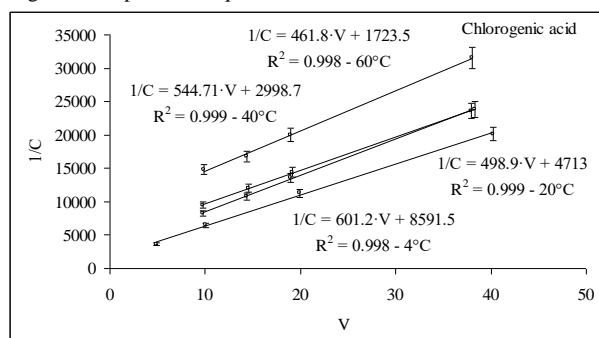

Rutin from leaves of Urtica

Fig.5. Regression dependence equations of Rutin concentration in extraction on extractant volume in coordinates $1/C=f(V)$

Chlorogenic acid from flowers of Helichrysum

Chlorogenic acid from leaves of Urtica

Fig.6. Regression dependence equations of some BAS concentrations in extraction on extractant volume in coordinates $1/C=f(V)$

Chlorogenic acid from leaves of Eucalyptus

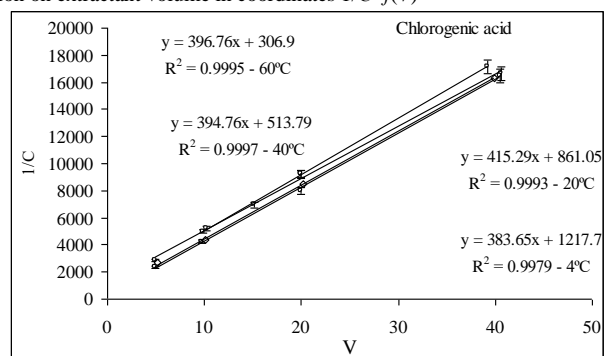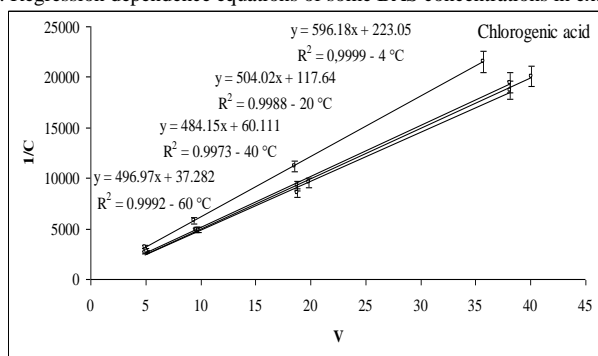Chlorogenic acid from flowers of Calendula

Fig.7. Regression dependence equations of chlorogenic acid concentration in extraction on extractant volume in coordinates $1/C=f(V)$
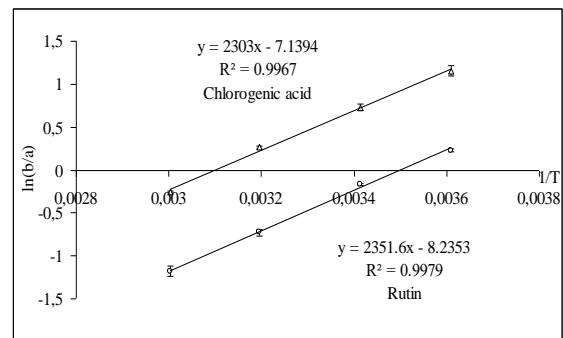
As it's seen from data provided on pictures 1-3, experimental points for all the studied BAS from various types of vegetal raw materials are well-approximated by regression lines built up in theoretically predicted coordinates in the view of equation (2), which is confirmed by the high value of determination coefficient $R^2 \geq 0,99$.

After that, using the obtained empiric constant values (*a*, *b*), the dependence was built up in coordinates $\ln(b/a)=f(1/T)$, results of which are provided on Fig.8-10.
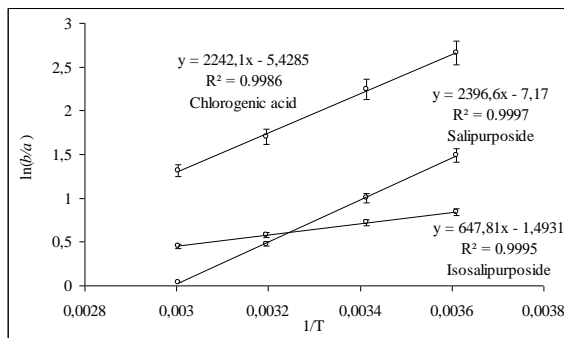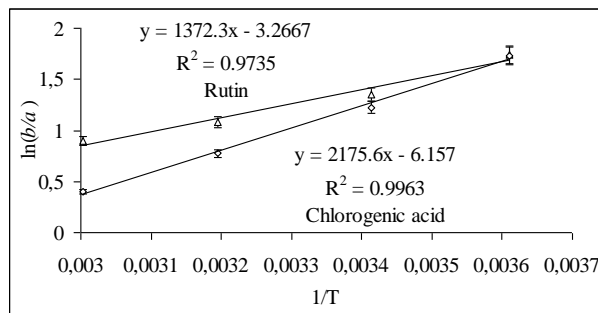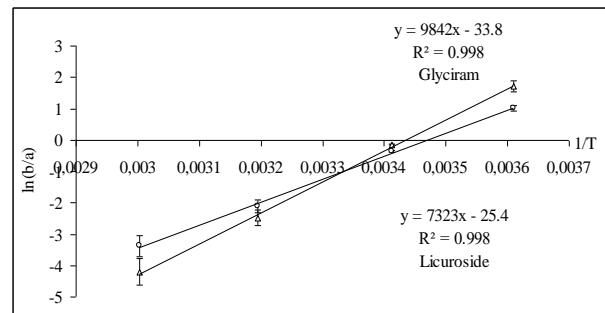
BAS from flowers of Helichrysum

BAS from flowers of Calendula

Fig.8. Regression dependence equations of empiric constant values (*a*, *b*) on temperature in coordinates ln(*b/a*)=*f*(1/*T*)
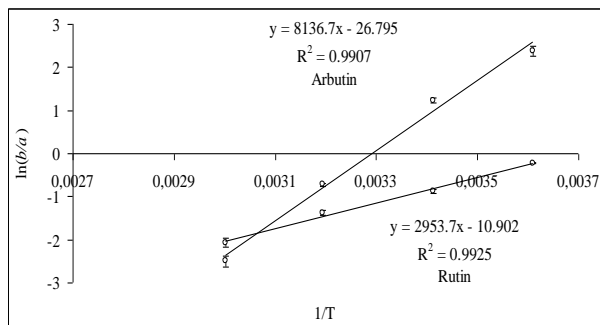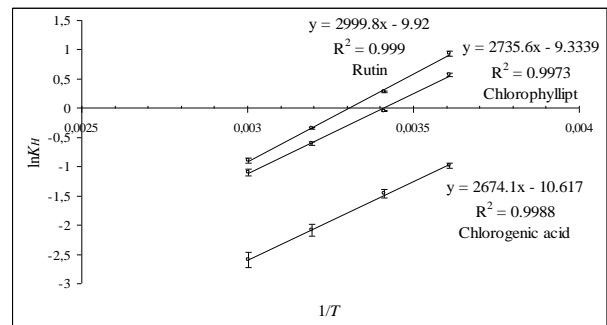

BAS from leaves of Urtica

BAS from roots of Glycyrrhiza

Fig.9. Regression dependence equations of empiric constant values (*a*, *b*) on temperature in coordinates ln(*b/a*)=*f*(1/*T*)

BAS from leaves of Uva-ursi

BAS from leaves of Eucalyptus

Fig.10. Regression dependence equations of empiric constant values (*a*, *b*) on temperature in coordinates ln(*b/a*)=*f*(1/*T*)

As it is seen from the data provided on picture 4, experimental points for all the studied BAS from various types of vegetal raw materials are also well-approximated by regression lines built up in theoretically predicted coordinates in the view of equation (2), which is confirmed by the high value of determination coefficient $R^2 \geq 0,99$.

The obtained results are well-confirmed by the proposed mathematical model in the view of equation (2). Hence, the hypothesis, which was promoted to explain the mechanism and quantitative description of interphase molecular BAS distribution in extraction system, wherein its equilibrium state is reached, does not contradict the experimental data (not deviating), and can be approved to be used as a theoretical statement.

The developed mathematical model successfully explains the relation between such general parameters in extraction system as initial/overall BAS content, equilibrium concentration of BAS in extractant, temperature and volume

of extractant, and this allows to develop the rational technology of tincture or extracts manufacturing.

IV. CONCLUSION

The new approach to description of equilibrium state in extraction system vegetal raw material-extractant has been proposed, which is based on the promotion of hypothesis, according to which the mechanism of interphase molecular distribution of BAS in extraction system is explained and described by classic Boltzmann distribution for discrete values of molecular energy (or Fermi-Dirac quantum distribution).

The developed and experimentally confirmed mathematical model was based on the promoted hypothesis, and it successfully described the relation of the main parameters of extraction system, which allows to develop the rational technology of tincture or extracts manufacturing.

V. FINANCIAL SUPPORT AND SPONSORSHIP

VI. REFERENCES

[1] D.J. Newman, G.M. Cragg, "Natural products as sources of new drugs over the 30 years from 1981 to 2010", J. Nat. Prod., vol.75(3), pp.311–335, 2012.

[2] G.M. Cragg, D.J. Newman, "Natural products: a continuing source of novel drug leads", Biochim Biophys Acta Gen Subj., vol.1830(6), pp.3670–3695, 2013.

[3] D.J. Newman, G.M. Cragg, "Natural products as sources of new drugs from 1981 to 2014", J. Nat. Prod., vol.79(3), pp.629–661, 2016.

[4] A.G. Atanasov, B. Waltenberger, E.M. Pferschy-Wenzig, T. Linder, C. Wawrosch, P. Uhrin, "Discovery and resupply of pharmacologically active plant-derived natural products: a review", Biotechnology Advances, vol.33(8), pp.1582–1614, 2015.

[5] V. Brower, "Back to Nature: Extinction of Medicinal Plants Threatens Drug Discovery", JNCI Journal of the National Cancer Institute, vol.100(12), pp.838–839, 2008.

[6] D.G.I. Kingston, "Modern natural products drug discovery and its relevance to biodiversity conservation", Journal of Natural Products, vol.74(3), pp.496–511, 2011.

[7] A.V. Akamova, O.D. Nemyatykh, I.A. Narkevich, "Multiple view marketing analysis of the russian plant-based drugs market", Drug development & registration, №4, pp.276-280, 2017 (In Russ.).

[8] N.N. Boyko, A.V. Bondarev, E.T. Zhilyakova, "Phytopreparations, analysis of the pharmaceutical market of the russian federation Research Result", Medicine and Pharmacy, vol.3(4), pp.30-38, 2017 (In Russ.).

[9] A.R. Silva, C. Grosso, C. Delerue-Matos, J.M. Rocha, "Comprehensive review on the interaction between natural compounds and brain receptors: Benefits and toxicity", Eur. J. Med. Chem., vol.174, pp.87-115, 2019.

[10] L. Wagner, H. Cramer, P. Klose, R. Lauche, F. Gass, G. Dobos, J. Langhorst, "Herbal medicine for cough: a systematic review and meta-analysis", Forsch. Komplementmed, vol.22, pp.359-368, 2015.

[11] R. Blazejova, J. Hosek, "Plant-derived drugs and their applications in the anticancer therapy", Ceska Slov Farm., vol.68(1), pp.3-11, 2019.

[12] A. Seca, D. Pinto, "Plant Secondary Metabolites as Anticancer Agents: Successes in Clinical Trials and Therapeutic Application", International journal of molecular sciences, vol.19(1), pp.263, 2018.

[13] K.B. Ishnava, "Role of herbal medicine in dental health", J. Environ. Chem. Toxicol., vol. 2(1), pp.28-29, 2018.

[14] M.E.A. Dawoud, Y.A. Mawgoud, T.M. Gouda, "Dawoud Synergistic interactions between plant extracts, some antibiotics and/or their impact upon antibiotic-resistant bacterial isolates", African Journal of Biotechnology, vol.12(24), pp.3835-3846, 2013.

[15] A.C. Abreu, A.J. McBain, M. Simoes, "Plants as sources of new antimicrobials and resistance-modifying agents", Nat. Prod. Rep., vol.29, pp.1007-1021, 2012.

[16] S.A. Minina, I.E. Kauhova, Chemistry and technology of phytodrugs. Manual. 2nd Ed. Revised. and add. Moscow: GEOTAR-Media, 2009, 560 p.

[17] Q.W. Zhang, L.G. Lin, W.C. Ye, "Techniques for extraction and isolation of natural products: a comprehensive review", Chinese medicine, vol.13, №. 20, 26 p. 2018.

[18] Technology and standardization of drugs. Collection of scientific works. Kharkov: LLC "Rireg", 1996, 784 p. (In Russ.).

[19] L.A.P. De Freitas, C.C.C. Teixeira, C.M. Zamarioli, Recent developments in phytomedicine technology. New York: Nova Science publishers, Inc. 2017, 283 p.

[20] S.S. Handa, S.P.S. Khanuja, G. Longo, D.D. Rakesh, Extraction technologies for medicinal and aromatic plants. Trieste: International centre for science and high technology, 2008, 260 p.

[21] F. Chemat, M.A. Vian, Alternative solvents for natural products extraction. Green chemistry and stainable technology, Berlin Heidelberg: Springer-Verlag, 2014. 315 p.

[22] A. Konichev, P. Baurin, N. Fedorovskiy, A. Marakhova, L. Yakubovich, M. Chernikova, "Traditional and modern methods of exstraction of biology active substances from plant materials: perspective, dignities, limitations", Bulletin MSRU. Series: Natural Science, vol.3, pp.49-54, 2011 (In Russ.).

[23] B. Bouillot, S. Teychené, B. Biscans, "An evaluation of thermodynamic models for the prediction of drug and druglike molecule solubility in organic solvents", Fluid Phase Equilibria, vol. 309(1), pp.36-52, 2011.

[24] E.T. Zhilyakova, O.O. Novikov, D.I. Pisarev, A.Y. Malyutina, N.N. Boyko, "Studying the polyphenolic structure of Laurus nobilis L. leaves", Indo Am. J. Pharm. Sci., vol.4(9), pp.3066-3074, 2017.