Extraction and Liquid-Membrane Preconcentration of Vincamine from Periwinkle (*Vinca Minor* L.) Leaves. Process Modelling

B. Yordanov,[a] K. Dimitrov,[a,b] and L. Boyadzhiev[a,*]

[a]Institute of Chemical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev str., Bl. 103, 1113 Sofia, Bulgaria

[b]Laboratoire ProBioGEM EA 1026, Polytech'Lille, USTL, Lille, France

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An integrated process coupling solid-liquid extraction and liquid-membrane preconcentration of vincamine was studied. The effect of the ratios solid phase/extractant volume and extractant volume/liquid membrane volume on the mass transfer rate and efficiency of vincamine removal were estimated. It was found that the transfer rate and removal efficiency of the alkaloid were higher with smaller quantities of solids and membrane volume. A mathematical description of the overall integrated process was proposed, and the values of the model parameters – mass transfer coefficients – were evaluated. An agreement between the model-predicted results and the experimental data obtained was demonstrated. The four-phase integrated process provides simultaneously almost complete extraction and appreciable enrichment of the extracted product.

Key words:
Liquid membranes, process integration, pertraction, vincamine, modelling

Introduction

Herbs are always considered an important source of various valuable bioactive substances. With their mild action and low-allergic effects, the latter are comparatively well tolerated by the human organism. In most cases, they are in low or extremely low concentrations in the herbs, making their extraction as well as their subsequent preconcentration and purification difficult. Usually, this is a complicated multistage procedure accompanied by significant energy consumption, product losses, and ecological problems. For such cases, the concept of process integration could offer an adequate solution free of these drawbacks.

An integration of solid-liquid extraction and liquid-membrane separation (or pertraction of the extracted species) seems to be an attractive solution especially for recovery of some natural compounds such as alkaloids. This approach can increase the extraction efficiency and reduce the number of operation steps, the amount of the solvent used and the product losses.

Although the number of publications devoted to liquid-membrane separations is very large,[1] few of them consider the separation of alkaloids applying these techniques[2–11] and only three of them[9–11] present data on the integration of solid-liquid extraction and pertraction of such substances.

The aim of this work is to throw more light on this kind of process integration as well as to propose a mathematical process description for the case of simultaneous vincamine extraction and liquid membrane purification.

Mechanism of vincamine recovery and model description

Vincamine as an alkaloid that exists in two forms: water-soluble, protonized $VinH^+$ in acidified aqueous solutions molecule and a basic molecule Vin, existing in alkaline aqueous solutions. The alkaloid, being in its basic form, can be extracted by means of organic solvents, usually chloroform.[12,13] Jusiak[13] demonstrated that when an acetate buffer solution with pH 4.0–4.2 is used as a leaching solution, the alkaloid can pass into chloroform directly without alkalization of the acetate solution. This fact was used for integrated process implementation because otherwise necessary consecutive changes in the pH values of the native extract, before and after pertraction, were avoided thus preventing accumulation of inert products, formation of precipitates, complication of the installation, etc. Therefore, this study used the acetate buffer solution with pH 4.2 as an extracting agent as well as a donor (feed) phase (F) in the liquid-membrane system.

As we determined earlier[8] by testing eight organic solvents as membrane liquids, trichloroethylene proved the most appropriate for this purpose. Finally, hydrochloric acid solution with pH < 1.0 was used as receiving phase since vincamine forms in this medium a stable water-soluble hydrochloric

*Corresponding author: e-mail: lboyadzh@bas.bg,
Fax: ++(359) (2) 870 7523, Phone ++(359) (2) 871 00 29

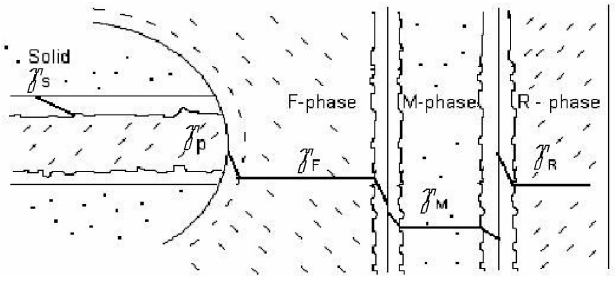

Fig. 1 – *Concentration profiles of vincamine during its transfer in the case of the integrated process*

complex, insoluble back into the organic membrane. Fig. 1 presents the above-mentioned four-phases system, a subject of this study.

In order to simplify the model presentation, the following assumptions were made:

1. The solid particles are considered spherical with uniform size and isotropic porous structure.

2. The liquid swelling during the process provokes an increase of the particle size.

3. The solute is uniformly scattered in the particle tissue.

4. The concentration of vincamine in the herb γ_S can be obtained from the quotient m/V_S, where m is vincamine mass in volume V_S, which is the solid phase volume excluding the pores.

After the initial loading of the pores with liquid, the solute begins to dissolve in that liquid. Since a uniform distribution of the alkaloid in the solid phase is assumed, the vincamine concentration γ_P in the liquid filling the pores will be the same at every point of the pore space. This concentration varies because of two fluxes. It increases due to the vincamine dissolution and decreases due to the vincamine diffusion through the hypothetical boundary layer at the particle surface where vincamine leaves the liquid, immobilized in the pores.

Therefore, one can write:

$$\frac{\mathrm{d}\gamma_P}{\mathrm{d}t} = \frac{k_{SP} A_P (\gamma_S - \gamma_P)}{V_p} - \frac{k_S A_{PF} (\gamma_P - \gamma_F)}{V_P} \qquad (1)$$

where:

k_{SP} – is the mass transfer coefficient for the boundary layer S/P (solid/liquid in pores) and accounts for the rate of solute dissolution;

A_P – is the contact surface, i.e. the internal pore surface;

V_P – is pore volume;

k_S – is the mass transfer coefficient of the solute leaving the solid particles and entering the feed solution (F);

A_{PF} – is the mass transfer area, namely, that portion of the total particle surface, corresponding to the pore apertures;

γ_F – is the vincamine concentration in the bulk of the feed phase (F).

Since the distribution of the pores on the particle surface was hard to determine, it was assumed:

$$A_{PF} = \varphi A_{ST} \qquad (2)$$

where, A_{ST} is the total surface of the particles and φ is the particle volume fraction:

$$\varphi = V_P / V_{ST} \qquad (3)$$

In eq. (3) V_{ST} represents the total volume of the particles.

However, in the course of the process, the particle size, as already mentioned, increased due to the liquid swelling, which led to an increase of the contact area between the liquid and the solid phases inside the particles A_P. The relationship between the surface area of a cylindrical pore and its volume is given by:

$$A_P = B\sqrt{V_P} \qquad (4)$$

It was found experimentally[14] that the pore volume increased relatively fast up to almost 9 times its initial volume, i.e.:

$$\frac{V_P^*}{V_{P,0}} = 9 \qquad (5)$$

where V_P^* is the pore volume at saturation and $V_{P,0}$ is the initial pore volume. Then:

$$A_{P,0} = B\sqrt{V_{P,0}}; \qquad A_P^* = B\sqrt{9V_{P,0}} \qquad (6)$$

Eqs. (6) show that until the complete soaking and saturation of the solid particle with liquid, the internal pore area increased thrice. Therefore, the following expression can be given:

$$A_P = cA_{P,0} \qquad (7)$$

where c is a coefficient which value varies from 1 to 3.

Further, this coefficient c can be expressed as a linear function of V_P.

$$c = aV_P + b \qquad (8)$$

The coefficients a and b can be determined knowing the values of $V_{P,0}$ and V_P^*.

Describing the variation of the amount of liquid penetrated into the porous material, Seikova and Simeonov[14] used the following relationship:

$$V_P = V_{SP}(1 - e^{-pt}) \qquad (9)$$

The coefficient p is empirically determinable and depends on the mean particle size and the environmental temperature, and t is time. In this case, however, in the initial moment, $V_{P,0} \neq 0$ and eq. (9) is modified to:

$$V_P = V_{P,0} + (V_{SP} - V_{P,0})(1 - e^{-pt}) \quad (10)$$

Taking into consideration eq. (7), eq. (1) becomes:

$$\frac{\mathrm{d}\gamma_P}{\mathrm{d}t} = \frac{cQ_{SP}(\gamma_S - \gamma_P)}{V_P} - \frac{k_S A_{PF}(\gamma_P - \gamma_F)}{V_P} \quad (11)$$

where:

$$Q_{SP} = A_{P,0} k_{SP} \quad (12)$$

Thus, in this case, Q_{SP} = const.

The particle number is estimated from:

$$N = \frac{V_{ST,0}}{V_{ST(1),0}} \quad (13)$$

In eq. (13), $V_{ST,0}$ is the total initial volume of the solid phase and $V_{ST(1),0}$ is the volume of a particular particle. The latter can be calculated as volume of sphere with radius r_0 – the initial particle radius:

$$V_{ST(1),0} = \frac{4}{3}\pi r_0^3 \quad (14)$$

Moreover, the following relation can be written:

$$V_{ST} = V_S + V_P \quad (15)$$

Here, V_S is the solid-phase volume of the solid phase only, excluding the pore volume.

The volume of an individual particle can be estimated at every moment from the relationship:

$$V_{ST(1)} = V_{ST} / N \quad (16)$$

The surface area of a separate particle $A_{ST(1)}$ can be estimated from the relationship between the area of the sphere and its volume:

$$A_{ST(1)} = 4.83 V_{ST(1)}^{0.67} \quad (17)$$

Thus, the total surface area of all particles is:

$$A_{ST} = N A_{ST(1)} \quad (18)$$

Tracking out the solute transfer in each formed boundary layer, the rates of the solute concentration variations in the bulk of the feed phase (F), the liquid membrane (M) and the receiving phase (R) can be expressed by eqs. (19), (20) and (21), respectively:

$$\frac{\mathrm{d}\gamma_F}{\mathrm{d}t} = \frac{k_S A_{PF}(\gamma_P - \gamma_F)}{V_F} - \frac{k_F A_{MF}}{V_F}\left(\gamma_F - \frac{k_F\gamma_F + k_M\gamma_M}{k_M D_{MF} + k_F}\right) \quad (19)$$

$$\frac{\mathrm{d}\gamma_M}{\mathrm{d}t} = \frac{k_M A_{MF}}{V_M}\left(\frac{D_{MF}(k_F\gamma_F + k_M\gamma_M)}{D_{MF}k_M + k_F} - \gamma_M\right) - \frac{k_M A_{MR}}{V_M}\left(\gamma_M - \frac{D_{MR}(k_M\gamma_M + k_R\gamma_R)}{D_{MR}k_M + k_R}\right) \quad (20)$$

$$\frac{\mathrm{d}\gamma_R}{\mathrm{d}t} = \frac{k_R k_M A_{MR}}{V_R(k_M D_{MR} + k_R)}(\gamma_M - D_{MR}\gamma_R) \quad (21)$$

In the last three equations γ_M is the solute concentration in the liquid membrane; k_F is the mass transfer coefficient for the boundary layer in the F-phase located on the liquid membrane surface; k_M is the mass transfer coefficient for the boundary layers in the membrane phase formed at the interfaces of this phase and the two adjacent aqueous phases; D_{MF} is the distribution coefficient of the solute between the liquid membrane and the extracting phase; A_{MF} is the interface area between these two phases; γ_R is the solute concentration in the receiving phase; k_R is the mass transfer coefficient for the boundary layer formed in this phase and located on the interface with the liquid membrane; D_{MR} is the distribution coefficient of the solute between the liquid membrane and the receiving phase; A_{MR} is the interface area between these two phases; and V_F is the free volume of the extracting phase and can be estimated from:

$$V_F = V_{FT} - V_P \quad (22)$$

where V_{FT} is the total volume of the feed phase (F).

The overall mass balance for the five phases formed is given by:

$$\gamma_S = \frac{m_0 - \gamma_P V_P - \gamma_F V_F - \gamma_M V_M - \gamma_R V_R}{V_S} \quad (23)$$

where m_0 is the initial mass of the solute in the solid.

In the case when solid phase is not used, e.g. the system is reduced to three liquid phases or to a bulk liquid-membrane process, the solute transfer in this ordinary pertraction system will be described by the simplified eq. (19) or:

$$\frac{\mathrm{d}\gamma_F}{\mathrm{d}t} = -\frac{k_F A_{MF}}{V_F}\left(\gamma_F - \frac{k_F\gamma_F + k_M\gamma_M}{k_M D_{MF} + k_F}\right) \quad (24)$$

and the solute concentration in the membrane phase (M) will be found from the overall mass balance:

$$\gamma_M = \frac{V_F(\gamma_{F,0} - \gamma_F) - \gamma_R V_R}{V_M} \qquad (25)$$

where $\gamma_{F,0}$ is the initial vincamine concentration in the native extract (feed phase (F)).

In this case, eq. (21) is used again to describe the change of the solute concentration in the receiving phase.

Experimental

Materials used and analysis

Periwinkle leaves – *Vinca minor* L., gathered in June in the Sofia region, were used as a source of vincamine. Before usage, they were dried at temperature of 40 °C and then ground. The fraction $0.1 < d_p < 0.3$ mm was collected and further used. Dried particles of the plant contained $w = 0.028$ % vincamine.

As a feed solution in the pertraction studies filtered native extracts of *Vinca minor* L. with pH 4.2 were used. As a liquid membrane trichloroethylene, p.a. grade (Merck®) was used. The receiving phase was solution of hydrochloric acid, purrum (Fluka®) in distilled water. Acetate buffer, prepared of sodium hydroxide, p.a. grade, (Fluka®) and acetic acid, p.a., (Chemicals®, Dimitrovgrad), was used as extracting (feed) solution in the studies of integrated process. Vincamine concentrations in the feed (F) and membrane (M) phases were determined directly by HPLC analysis. Details are given elsewhere.[8] In the receiving solution (R) vincamine, masked as a hydrochloric complex Vin.HCl_w, had to be destroyed prior to the analysis of the solution. For this purpose, a sample of this solution was alkalized with 25 % solution of ammonia. Then the alkalized solution, pH 9.0–9.5 was extracted twice with chloroform. The collected chloroform extracts were evaporated to dryness and the residue dissolved in methanol was analyzed applying the same HPLC procedure as for the other two phases. The eluent was prepared of methanol, "super gradient" grade (Labscan®), ammonium carbonate, p.a. (Boron®) and distilled water. Pure vincamine (> 99 %), kindly supplied by Covex®, Spain, was used as external standard.

Experimental procedure

Fig. 2 shows the laboratory bulk-type pertraction apparatus used in the experiments. In the studied case, the organic phase (M) is heavier than the aqueous phases (F) and (R), and therefore fills the bottom part of the apparatus. Its level is above the lower edge of the inner vessel, a bottomless tube (1) separating the two compartments above.

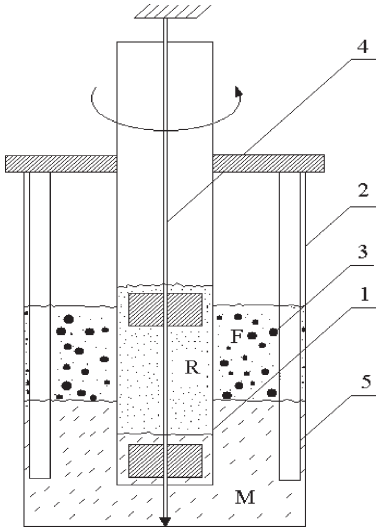

Fig. 2 – *Laboratory glass pertractor: (1) Inner mobile vessel; (2) Outer vessel; (3) Plant material (in the case of the integrated process, only); (4) Fixed, immobile stirrer; (5) Immobile baffles*

The inner vessel contains the receiving phase (R) and the outer, annular space, is filled with the donor (feed) phase (F) or, in the cases of integrated process, with the solid-liquid dispersion. During the process, the inner cylinder is set in rotation by means of an electric motor. The immobile stirrer (4) is fixed along the vertical axis and the baffles (5) mounted in the annular compartment favor the mixing, intensifying in this way the solute transfer through each liquid interface. The interfacial areas were $A_{MF} = 49.7$ cm^2 and $A_{MR} = 10.8$ cm^2, respectively. Two rotation speeds of the inner cylinder were applied: $n = 150$ min^{-1} and $n = 200$ min^{-1}. The amount of the three liquid phases in all experiments were $V_{FT} = 200$ mL, $V_M = 230$ mL and $V_R = 60$ mL, respectively. The mass of the dry herb, added to the feed phase (F) was 10.0 or 20.0 g, respectively, in the experimental runs. By means of two sampling capillary tubes, samples of the two aqueous phases (F) and (R) were taken periodically.

In order to reduce the number of unknown parameters in the model of the integrated process, parallel runs without solid phase (simple pertraction process) were carried out to evaluate the mass transfer coefficients k_F, k_M, and k_R.

The duration of all experiments was 16 hours and they were carried out at a temperature of 25 °C.

Determining the volume and the mass of unbroken plant leaves, the density of the dried solid phase can be obtained. For the material studied this was $\rho_h = 0.775$ g cm^{-3}. The ground herb particles, previously weighed, were placed into a measuring cylinder. The volume of the ground herb is larger than the volume of the unbroken structure having

the same weight due to the void between the separate particles. Thus, a coefficient taking into account the chaotic position of the particles can be obtained – $\psi = 0.527$, thus by multiplying the measured volume of the ground herb by ψ, the volume of the unbroken solid phase can be obtained. The herb was then soaked with a portion of the extracting liquid. Four days later, the volume of the whole system was registered and after simple calculations the value of the particle volume fraction was obtained: $\varphi = 0.34$.

Comparing the mass of a portion of dried herb and the same portion of impregnated herb after a four-day soaking, the coefficient l can be obtained so that:

$$m_h l = V_P^* \tag{26}$$

where m_h is the mass of the dried herb. The obtained value for l was $l = 3.906$ mL g^{-1}.

The coefficient p was calculated using the following procedure: A portion of the extracting liquor was poured onto an amount of dried herb with known mass. After five-minute mixing, the system was centrifuged for 30 s and the upper liquid layer was removed. The mass of the impregnated solid phase was then measured. By solving the reversed problem – eq. (10) – the value of the coefficient p was obtained: $p = 0.065$ min^{-1}.

Results and discussion

Vincamine distribution coefficients D_{MF} and D_{MR} were determined in our previous study.[8] Their values under the same experimental conditions were $D_{MF} = 0.76$; $D_{MR} = 0.02$, respectively. Coefficients a and b in eq. (8) are easily calculated if the values of $V_{P,0}$ and V_{SP} are known. In the studied cases, the value of the intercept b is 0.75 and that of the slope a, which varies depending on the amount of the solid phase in the system, is 0.058 and 0.028 mL^{-1}, respectively. The remaining unknown parameters in the model are the mass transfer coefficients Q_{SP}, k_S, k_F, k_M, k_R. In order to reduce their number to guarantee more precise results applying the identification procedure, the last two parameters, k_M and k_R, were assumed the same as those obtained in the parallel pertraction experiment, as mentioned above, under the condition that both experimental conditions are the same. This substitution is based on the assumption that the hydrodynamics of the membrane (M) and the receiving (R) phases in the vicinity of the two phases are slightly affected by the presence of solid particles in the feed (F). Therefore, when the model parameters in the case of an integrated process were evaluated, the values of k_M and k_R were not the subject of identification. The parameter evaluation was carried out applying the TUTSIM® dynamic simulator and its built-in identification procedure, based on the Nelder and Mead optimization method.[15]

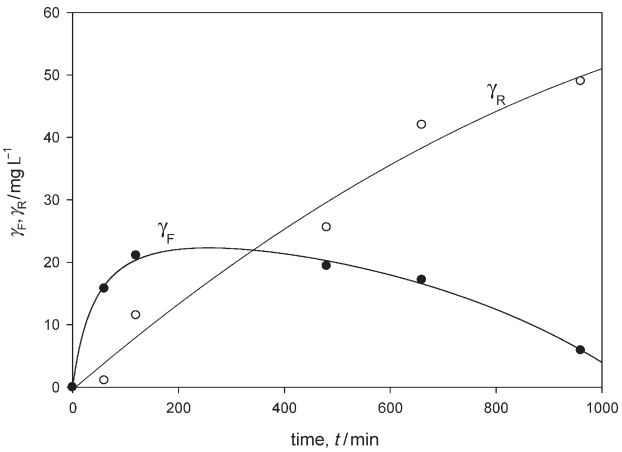

Fig. 3 – *Evolution of vincamine concentrations in feed phase (γ_F) and receiving phase (γ_R), respectively. Lines are calculated by the model. The points represent the experimental concentrations. Phase volumes were: V_F = 200 mL; V_M = 230 mL; V_R = 60 mL and the herb mass was 20.0 g.*

The data obtained as well as the computed lines clearly demonstrate the logical concentration maximum and the retarded concentration rise of the alkaloid in the feed phase (F) and the receiving solution (R), respectively. The retarded rise is due to the feed being free of the solute in the initial period. During accumulation of the solute in the feed, its transfer to the membrane liquid intensifies. Then, as the source becomes exhausted, the transfer flux decreases.

At the end of this experiment, the distribution of the alkaloid is as follows: 59.5 % in the receiving phase, 22.1 % in the membrane liquid, 16.3 % in the feed solution, and 2.1 % in the extracted herb. Obviously, this result is not very satisfactory, except for the almost complete extraction of the alkaloid from the treated herb. However, one should consider that in the studied system, the two equilibria (D_{MF} and D_{MR}) do not imply complete extraction and that the mass transfer areas and fluxes in the used laboratory device are rather low.

All values of the evaluated mass transfer coefficients are summarized in Table 1.

The results obtained show that an increased amount of solid phase leads to a slight increase in the mass transfer coefficient between the solid particles and surrounding liquid – feed phase (F). The increased number of solid particles has a slightly negative effect on the solute transfer rate between feed and membrane phases.

Table 1 – *Values of computed mass transfer coefficients*

Run No.	Process	m	n	a (eq. (8))	Q_{FP}	k_S	k_F	k_M	k_R
		g	min^{-1}	mL^{-1}	$m^3\ s^{-1} \cdot 10^{-10}$	$m\ s^{-1} \cdot 10^{-5}$	$m\ s^{-1} \cdot 10^{-5}$	$m\ s^{-1} \cdot 10^{-5}$	$m\ s^{-1} \cdot 10^{-5}$
1	Pertraction process (3-phases)	10	150	–	–	–	3.33	9.11	11.8
2	Integrated process (4-phases)	10	150	0.058	1.67	0.97	4.17	9.11*	11.8*
3	Integrated process (4-phases)	20	150	0.028	1.89	1.18	3.85	9.11*	11.8*
4	Pertraction process (3-phases)	10	200	–	–	–	4.44	9.70	18.1
5	Integrated process (4-phases)	10	200	0.058	1.81	1.22	4.75	9.70*	18.1*

Note: Values denoted with an asterisk were subject of evaluation in the parallel pertraction runs only, and once obtained they were included in the integrated process model as known parameters.

As expected, more pronounced is the effect of agitation intensity: an increase of 33 % in rotation speed, from 150 to 200 min^{-1}, provokes a similar rise in the values of mass transfer coefficients, particularly those responsible for the last transfer step.

An important characteristic of the integrated process is its ability to provide selective recovery and enrichment of the product, especially when the concentration of the desired species in the source material is very low, as it is in the studied case. As mentioned above, the vincamine content in the dried periwinkle leaves was w = 0.028 %. This amount in the residue, obtained after drying the native extract – the feed phase (F) was raised to w = 0.091 %. The alkaloid content in the final product of the integrated process – the dried residue of the receiving solution (R), obtained in the above-mentioned experimental runs, varied between 6.1 and 9.0 %; therefore 60 – 100 times more than in the native dry extract.

Conclusions

An integrated process, comprising a combination of solid liquid extraction and pertraction was applied successfully for recovery of the indole alkaloid vincamine from dried and ground periwinkle leaves (*Vinca minor* L.). The experiments were carried out in a simple laboratory glass pertractor, in which the feed phase compartment was filled with a suspension of ground herb particles in an extracting liquor – an aqueous acetic buffer. It was found that the ratio of the solid phase and the solution has a slight but noticeable effect on the rate of mass transfer, while the influence of phase agitation intensity is much more pronounced.

A diffusion kinetic model describing the integrated process was proposed. Assuming fast saturation of the solvent penetrated into the particles, the model takes into account all solvent mass transfer steps, namely the rates of solute dissolution, its transfer into the bulk of the extracting liquid, its transfer from the latter into the membrane liquid, and finally, the alkaloid recovery in the receiving acidic phase. By means of the dynamic simulator TUTSIM® and its incorporated optimization procedure, the mass transfer coefficients in all transport steps were evaluated.

Besides the alkaloid recovery, a considerable purification effect was achieved. The vincamine content in the used dried plant tissues was w = 0.028 %. Applying this integration scheme, the vincamine fraction in the dried residues of the receiving phases was increased to w = 6.1–9.0 %.

ACKNOWLEDGEMENTS

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List of symbols

A – mass transfer area, m^2
a – coefficient in eq. (8), m^{-3}
B – coefficient in eqs. (4) and (6)
b – coefficient in eq. (8)
c – coefficient in eqs. (7–8)
D – vincamine distribution coefficient between the liquid membrane and the corresponding aqueous phase, –
d – diameter, m

F – feed (extracting) phase
k – local mass transfer coefficient, m s^{-1}
l – coefficient in eq. (26), mL g^{-1}
M – membrane phase
m – mass, g
N – particles number
n – rotation speed, min^{-1}
p – coefficient in eqs. (9–10), s^{-1}
Q – volume mass transfer coefficient ($Q = kA$), m^3 s^{-1}
R – receiving phase
r – radius, m
t – time, s
V – volume, m^3
Vin – vincamine
w – mass fraction, %
γ – vincamine concentration, mg L^{-1}
ρ – density, g cm^{-3}
φ – particle volume fraction
ψ – coefficient of chaotic position of the particles

Subscript

F – in the free feed (extracting) phase
FT – in the total feed (extracting) phase
h – of the herb
M – in the membrane phase
MF – at the interface between liquid membrane and extracting (feed) phase
MR – at the interface between liquid membrane and receiving phase
P – in the pores
PF – at the interface between the pores of the particles and the feed solution
p – of the particles
R – in the receiving phase
S – in the solid phase
SP – in the boundary layer S/P (solid/liquid in the pores)
ST – of total solid phase
(1) – particular particle
0 – initial

Superscript

* – at equilibrium

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