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# Mass transfer kinetics of biologically active compounds from Propolis

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The present investigation is provoked by the increasing interest in propolis as a source of biologically active compounds (BAC) and the great differences in contact times, reported in literature, for their extraction by an ethanolic solvent. Two sets of kinetic investigations are performed:

- Liquid phase kinetic curves are obtained by spectrophotometric analysis of the extract (total polyphenols, flavones and flavonols, flavanones and dihydroflavonols). The total yield is determined gravimetrically.
- Size evolution of the propolis particles during extraction is continuously monitored by microscopy connected with a photo camera.

The effect of the liquid/solid ratio and the stirring intensity is studied. The results confirm that BAC release proceeds faster than usual solid-liquid extraction, because of the partial dissolution of the solid matrix, as well as the destruction to smaller particles, due to the particulate character of the propolis material. The effective mass transfer coefficient is of the order of $10^{-6}$ m/s. Favourable conditions for process performance are found.

**Keywords:** propolis, mass transfer, kinetics, biologically active compounds

## INTRODUCTION

Propolis-containing products have been intensely marketed by pharmaceutical industry and health-food stores. Propolis is composed of 45% resins, 30% waxes and fatty acids, 10% essential oils, 5% pollens and 10% organic compounds and minerals [1, 2]. More than 300 compounds, among which terpenoids, steroids, sugars and aminoacids have been detected in raw propolis. Important bioactive compounds in propolis are flavonoids and phenolic acids, as well as their derivatives, because of their antibacterial, antifungal, antiprotozoan, antiviral, antitumoral, immunomodulatory, anti-inflammatory and antioxidant activity [3–8]. The most common process for propolis extraction uses ethanol as solvent. There are very few investigations concerning the mass transfer of propolis constituents and the reported contact times vary in a wide range, as can be seen from Table 1. It is also seen from Table 1 that two concentrations of the ethanolic solvent are mainly used for extraction. As the extraction of biologically active compounds (BAC) in aqueous solutions is much lower [16], ethanolic solvents with higher water content are not in common use.

**Table 1.** Experimental conditions for extraction of BAC from propolis

| Time of extraction (days) | Origin of propolis/Reference | Pre-treatment | Solvent EtOH, % | Analytical control |
|---|---|---|---|---|
| 90 | Southeastern Brazil [9] | Powdered | 96 | TP* by Folin-Denis colorimetric method |
| 14 | European-Siberian and Irano-Turanian [10] | Grained | 96 | GC-MS analysis |
| 7 | Bulgaria and Brazil [11] | Ground | 70 | bactericidal activity |
| 7 | Beekeeping section of Lageado Farm (UNESP, Botucatu) [12] | Ground | 70 | immunomodulatory action |
| 3 | Greece, Aegean Sea islands and Cyprus [13] | Ground | 70 | TP by Folin-Ciocalteu colorimetric method |
| 2 | Turkey [14] | Grated | 70 | TP (Folin-Ciocalteu) |
| 1 | China [15] | – | 96 | TP (Folin-Ciocalteu) |
| 7 | Brazil [16] | In bench blender | EtOH or $H_2O$ | TP (Folin-Ciocalteu) total flavonoids by HPLC |

* TP – total phenolics

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In [17] different diffusion models have been tested to describe the release kinetics of selected polyphenols from propolis incorporated into polylactic acid (PLA) film. With ethanol as a solvent a very fast release has been observed whatever the polyphenol.

The object of the present investigation is the mass transfer kinetics of BAC from propolis into ethanol-water solvent.

## EXPERIMENTAL

Propolis was provided by the Centre of Phytochemistry of the Institute of Organic Chemistry, BAS, (Bulgaria); ethanol (99.9 %) and methanol (99.9%) were supplied by Valerus (Bulgaria); aluminium chloride anhydrous, potassium hydroxide (ISO), sodium carbonate anhydrous (ISO), sulfuric acid (96%), Folin-Ciocalteu's phenolic reagent and methanol Lichrosolv (99.8%), were supplied by Merck; pinocembrin was supplied by Extrasynthese (France); galangin was supplied by Fluka.

Before extraction the propolis material was cooled at 5°C and ground. The mean number-based diameter, obtained by ESEM micrographs and "Image-ProPlus 5" software, was 32 μm; 90% of the particles size was in the range of (15-52) ±2 μm [18]. Extraction was performed with 70% (v/v) EtOH-water solvent, as well as with pure ethanol at room temperature and different liquid/solid ratios (8 to 30 ml liquid/g solid). Contact times up to 2 days were used. Stirring (up to 300 rpm) was applied, using MM2A Lab. Pristroje Praha magnetic stirrer.

The decrease in the dimensions of propolis particles after contact with immobile liquid was continuously observed on an Axiovert 4OC microscope for transmitted-light brightfield and phase contrast with condenser 0.4, inclusive object traverser M, and optical micrographs from the particles taken by digital camera DeltaPix Invenio 3S, connected with the microscope. The undissolved solid collected after extract filtration, was determined gravimetrically.

UV-VIS analysis was performed on Hexiosy v 7.06 spectrophotometer:

- Flavones and flavonols were determined by aluminum chloride complex formation [19]. 20 ml methanol and 1 ml 5% $AlCl_3$ were added to 2 ml of the test solution and the volume was made up to 50 ml. After 30 min, the absorbance was measured at 425 nm. Blank: 2 ml methanol instead of test solution. Calibration with galangin was used in the concentration range 0.0052–0.052 mg/ml [18].

- Flavanones and dihydroflavonols were determined according to [20, 21]. 1 ml of the test solution and 2 ml of 2,4-dinitrophenylhydrazine (DNP) solution (1 g DNP in 2 ml 96% sulfuric acid, diluted to 100 ml with methanol) were heated at 50 °C for 50 min. After cooling to room temperature, the mixture was diluted to 10 ml with 10% (w/v) solution of KOH in methanol. 1 ml of the resulting solution was added to 10 ml methanol and diluted to 50 ml with methanol. Absorbance was measured at 486 nm. Blank: 1 ml methanol instead of the test solution. Calibration with pinocembrin was used in the concentration range 0.14–1.0 mg/ml [18].

- Total phenolics were quantified by the Folin–Ciocalteu's method [22]. 1 ml of the test solution was transferred into a 50 ml volumetric flask, containing 15 ml distilled water, and 4 ml of the Folin–Ciocalteu's reagent followed by 6 ml of a 20% sodium carbonate solution were added. The volume was made up to 50 ml with distilled water and kept for 2 h. Absorbance was measured at 760 nm. Blank solution: 1 ml methanol instead of test solution. Calibration with a 2:1 pinocembrin–galangin mixture was used in the concentration range 0.025–0.3 mg/ml [18].

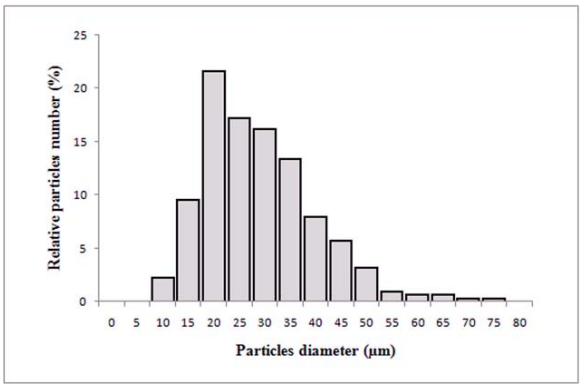
## RESULTS AND DISCUSSION

The ground propolis is characterized by a pronounced particle size distribution, shown in Fig.1.

By continuous observation of the particles in contact with the solvent (Fig. 2a), the time evolution of the particle size is obtained (Fig.2b). The latter undergoes essential alteration during the process, the final distribution being shifted to the left, corresponding to a decrease of the mean number-based particle diameter from 32 to 13 μm.

A certain correspondence is observed between the kinetic curves for polyphenolics release and the evolution of the mean particle size, as shown in Fig. 3. For comparison, the final concentration of polyphenols after 48 h is also given.

These results suggest a process involving BAC release, as well as partial dissolution of the solid matrix, as gravimetrically proven. The total solid content in the liquid phase after 15 min is 27 mg/ml and remains constant upon prolonging the time of extraction. The corresponding total phenolic concentration is 18.2 mg/ml, which constitutes about 67.6% of the dissolved solid substances



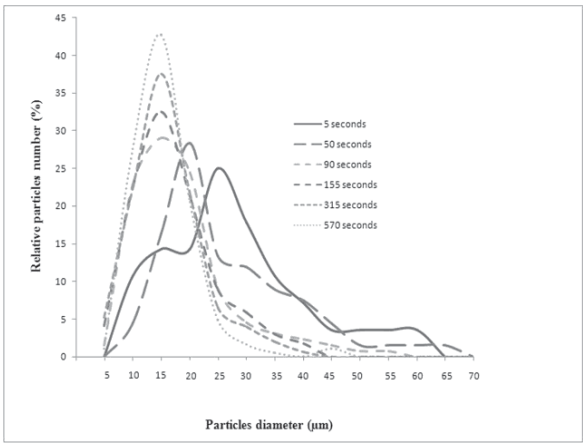




**Fig.1** Initial particle size distribution

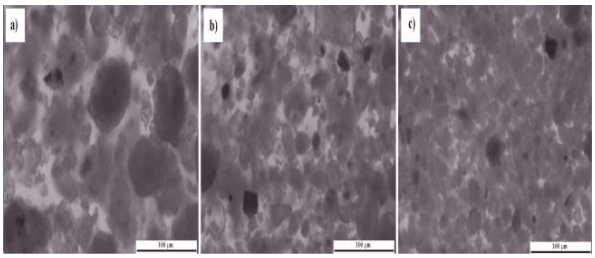



**Fig.2.** Optical micrographs for different time of extraction: a) 5s; b) 2 min; c) 15 min. Time evolution of the particle size distribution (70% ethanol, no mixing).

and 91.6% of the phenolics concentration after 48 h of contact. The use of pure ethanol slightly increases the total solid content (to 29 mg/ml after 15 min). Longer times of contact lead to a slight increase in the concentration of polyphenols - after 30 min the BAC are practically completely recovered (19.5 mg/ml).

As the mass transfer process is fast, no effect of stirring is observed, as shown in Fig.4. Few minutes are enough to reach the equilibrium concentration of the respective groups of extracted compounds,

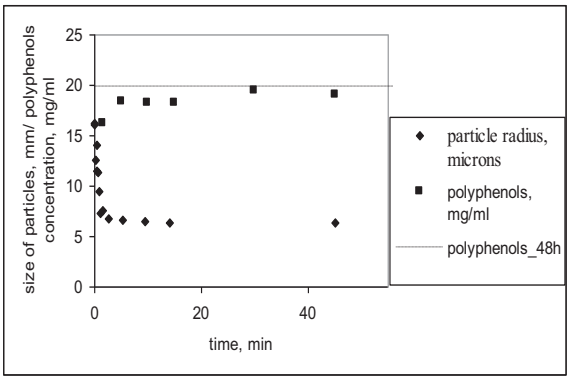



**Fig. 3** Time evolution of the mean particle radius (μm) and the total phenolics concentration (mg/ml) at a liquid/solid ratio of 20.

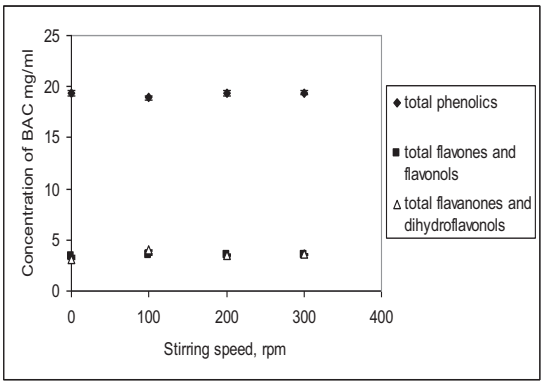



**Fig. 4**. Effect of the stirring speed on the extraction of BAC from propolis at a time of15 min and liquid/solid ratio of 20.

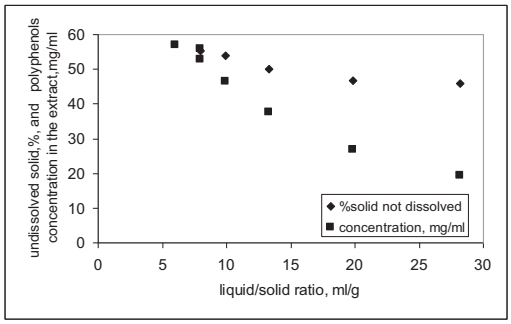



**Fig.5**. Undissolved propolis part and liquid phase concentration of total phenolics for different liquid/solid ratios.

which are (in mg/g propolis): total phenolics 386.4;flavones and flavonols 68.5; flavanones and dihydroflavonols 63.1.

Fig.5 shows the undissolved part (% of the solid mass) together with the liquid phase concentration (mg/ml) for increasing liquid/solid ratios. With liquid/solid ratios exceeding 20 ml/g the amount of undissolved solid remains practically constant (Fig.5). The insoluble part is about 46% of the initial mass of the propolis.

If we consider the BAC as diffusing species and the other propolis constituents as particle matrix, the above results show that part of the matrix also dissolves. In this way the mass transfer surface is renewed and the BAC extraction is accelerated.

The change in the particle mass is related to the mass balance of the extraction process:

$$\frac{dM_s}{dt} = \rho_s 4\pi R^2 N \frac{dR}{dt} = -\frac{dM_l}{dt} = -\frac{d\overline{C}_l}{dt} V_l \quad (1)$$

Here '*s*' and '*l*' denote the solid and liquid phase; *dR/dt* is known from Fig.3, the initial solid mass, particle radius and solid density are $M_{so}$ = 1g, $R_o$= 16μm and $\rho_s$=1180kg/m$^3$ [22-23]; *N* is the number of particles, considered constant during extraction. The slope of the initial linear part of the *R(t)* curve in Fig.3 (the first 1.5 min) gives $dR/dt$=1.09·10$^{-7}$m/s. Hence the average liquid phase concentration is evaluated to $\overline{C}_l$=20.69 mg/ml, i.e., about 77% of the final measured total concentration in the extract, which is a reasonable result.

Eq.(1) can be written with respect to the mass transfer from the particle surface (with concentration $C_R$) into the surrounding liquid, accounting for the mass transfer coefficient *k* [m/s].

$$\frac{dM_s}{dt} = -\frac{dM_l}{dt} = -k a\left(C_R - \overline{C}_l\right) V_l \quad (2)$$

The average liquid phase concentration $C_l = \left(M_s(t=0) - M_s(t)\right)/V_l$ is experimentally calculated (here $V_l$ is the liquid volume). The specific interface $a=6\varepsilon_s/(2R)=4\pi R^2 N/V_l$ decreases proportionally to the square of the particle radius, the initial value of $\varepsilon_s$ being $\varepsilon_s=V_{s0}/V_l=0.044$ and the volume of the solid – $V_{s0}=M_{s0}/\rho_s=(4/3)\,\pi R_0^3 N$ .

Eq.(2) supposes a linear plot of $dM_s/dt$ vs $a\left(C_R - \overline{C}_l\right)V_l$, which is confirmed by the results in Fig.6. The slope gives $k$=1·10$^{-6}$m/s, which is about one order lower than the usually observed values for dissolution processes. The latter can be easily checked, using the well known relation:

$$Sh = \frac{k2R}{D_m} = \left(4 + 1.2Pe^{2/3}\right)^{1/2}, \quad (3)$$

which tends to the limiting value of $Sh = 2$ in case of stagnant fluid [25].

The main components in the extract have molecular mass between 180 and 410 [15, 24]. Hence, the coefficients of molecular diffusion, estimated by Wilke-Chang equation, are of the order of 10$^{-10}$ m$^2$/s. For instance, with $D_{m,pinobanksin}$= 3.4·10$^{-10}$m$^2$/s we obtain $k$=1.9·10$^{-5}$m/s. This coefficient is time dependent and increases with decreasing particle size. The deviation from the origin of the coordinate system in Fig.6 can be due to errors either in the value of the saturation concentration, or in the number of particles. A short discussion of the latter is given below.

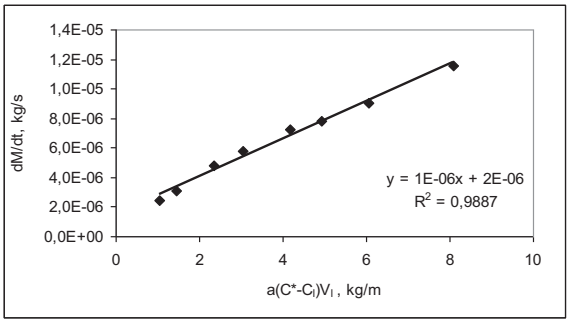



**Fig.6.** Determination of the effective mass transfer coefficient

From the analysis of the kinetic data (concentrations and particle size) the following question arises: is the particle size evolution due uniquely to mass transfer? For a positive answer the global balance with a constant number of particles should hold:

$$\Delta M = \rho_s N \frac{4\pi}{3}\left(R_0^3 - R_f^3\right) = V_l \overline{C}_l \quad (4)$$

Here '*0*' and '*f*' denote the initial and final volume averaged size of the particles (2*R*= 36 μm and 15 μm respectively). The initial number of particles ($N_0$) is:

$$N_0 = \frac{3M_{s0}}{\pi 4 R_0^3 \rho_s} \quad , \quad (5)$$

Combining eqs.(4) and (5) for $N=N_0$, the final liquid phase concentration is obtained:

$$\overline{C}_{l,f} = \frac{\left(R_0^3 - R_f^3\right)}{R_0^3 x} \quad (6)$$

where *x* stands for the initial liquid/solid ratio ($V_l/M_{s0}$=20).

Calculation by eq.(6) gives $\overline{C}_{l,f}$ =46.6 mg/ml, which is much higher than the experimentally obtained value $\overline{C}_l$=27 mg/ml and needs explanation. According to eq.(5) $N_0$ =3.55·10$^7$. As the final mass is $M_{sf}$ = 0.46$M_{s0}$ and 2$R_f$ = 15 μm, then the final number of particles is $N_f$ =23.9·10$^7$. A very probable reason for this difference lies in the destruction of mechanically unstable bigger

agglomerates of particles in contact with the solvent, which takes place in parallel to the dissolution process. This explanation is based on the particulate nature of the material, due to the way in which propolis is produced by the bees. The microscopic data, illustrated in Fig. 2, give some visual evidence for the increasing number of particles.

## CONCLUSIONS

The results, obtained from the measured particle size and BAC concentrations during extraction, prove that the release is a fast process and the preparation of ethanolic extracts from propolis might be essentially rationalized by decreasing the speed of rotation and the time of contact.

If we consider the BAC as diffusing species and the other propolis constituents as particle matrix, the above results show that part of this matrix also dissolves. The insoluble part is about 46% of the initial mass of the propolis. At a liquid-solid ratio of 20 (ml/g) practically all the soluble part of the solid is dissolved.

The kinetics of BAC release lies somewhat between dissolution and the usual liquid-solid extraction with internal diffusion control, the effective mass transfer coefficient being of the order of $10^{-6}$m/s. The partial dissolution of the solid phase leads to continuous renewal of the liquid-solid interface and to lower diffusion time in the particle, both resulting in an essential acceleration of the BAC extraction.

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# КИНЕТИКА НА МАСОПРЕНАСЯНЕТО НА БИОЛОГИЧНО-АКТИВНИ ВЕЩЕСТВА ОТ ПРОПОЛИС

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(Резюме)

Настоящето изследване бе провокирано от нарастналия интерес към прополиса като източник на биологично-активни вещества (БАВ), както и голямата разлика в публикуваните времена на контакт при тяхната екстракция с етанол-съдържащ разтворител. Бяха проведени два типа кинетични изследвания:

- Кинетичните криви в течна фаза бяха получени чрез спектрофотометричен анализ на екстракта (относно общи полифеноли, флавони и флавоноли, флаванони и дихридрофлавоноли). Общият извлек бе определян тегловно.
- Изменението на размера на частичките прополис във времето на екстракция беше непрекъснато следено микроскопски чрез свързана фотокамера.

Изследвано бе влиянието на хидромодула и скоростта на разбъркване. Получените резултати потвърждават, че извличането на БАВ протича по-бързо от обикновеното за екстракция твърдо-течност поради частично разтваряне на твърдата матрица, както и раздробяване на по-дребни частици поради зърнестия характер на изходния материал. Ефективният коефициент на масообмен е от порядъка на $10^{-6}$ m/s. Определени са благоприятните условия за провеждане на процеса.