Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2018.22631

128 (2018) 170–178
October

A method for investigating transport properties of partly biodegradable spherical membranes using vitamin B_{12} as the marker

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Received 30 March 2018; Accepted 21 June 2018

ABSTRACT

The paper presents a method of assessing the transport properties of spherical, semipermeable synthetic membranes, in which the diffusion of a low-molecular weight marker, vitamin B_{12}, was examined. To determine the mass transfer coefficient *h*, experimentally obtained curves of the decrease in marker concentration in the solution (stirring of capsules in a solution of the marker and spectrophotometric measurement of concentration in a flow-through cuvette) were approximated using a suitably transformed model equation based on the general equation for mass transfer between two phases given by Radcliffe. The studied microcapsule membranes contained 5% admixture of the biodegradable polymer. The *h* was determined for both directions – from the outside into microcapsules and from their inside into the surrounding liquid. Next, membranes were chemically degraded and mass transfer coefficients *h* were again determined for them. Mass transfer coefficients *h* were established to be significantly higher after the membrane degradation than *h* values before the degradation.

Keywords: Polymer microcapsules; Transport properties; Vitamin B_{12}; Electrostatic method

1. Introduction

Microcapsules, in which a semipermeable synthetic or natural polymer membrane surrounds an encapsulated active substance, are increasingly often studied with respect to their applications in protecting mankind's natural environment. Two basic directions of the research may be distinguished here. The first relates to the capsules' application in the water treatment processes for the removal of various types of organic [1–7] and inorganic pollutants, for example, heavy metal ions [8]. The second direction involves the introduction of microcapsules in technological processes, which results in reduction of water usage through multiple use of wastewaters produced in a process [9]. The authors found that using a dye encapsulated in polyurethane-urea microcapsules allowed a dyeing bath to be reused with no detriment to the effectiveness of the polyester fiber dyeing process. Furthermore, the sewage produced in such a process did not contain other additives that were harmful to the environment (surfactants) and contained far less dye than sewage manufactured in a process, in which the dye was dissolved directly in the dyeing bath. Studies on the application of capsules for the remediation of soil (land) contaminated with heavy metals [10] are also available.

Irrespective of whether the active substances are enzymes [5], cells [2,6,7], or other chemical substances [1,4,9,10] encapsulated in microcapsules, their performance is directly dependent on the transport properties of their membranes.

These properties are most frequently characterized using the diffusion (permeability) coefficient [11–14] and molecular mass cut-off [15] values. To determine these values, measurements for several markers with differing molecular weights such as dextrans, proteins, and polyethylene glycols have to be made. This is both, laborious and difficult

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Presented at the XII Scientific Conference "Membranes and Membrane Processes in Environmental Protection" – MEMPEP 2018 13–16 June 2018, Zakopane, Poland.

due to the small number of available high-molecular weight markers and often inconvenient methods of measuring their concentration.

In this situation, finding a universal, simple, and cheap method for assessing microcapsule membrane permeability seems advantageous. Such method should characterize membrane permeability in a rapid and objective way, like the ultrafiltration rate characterizes flat and capillary membranes. This information is necessary during designing of new membranes, for testing possible changes in transport properties of microcapsules occurring during their use (e.g., during work in bioreactors), and also during studies on new methods of microcapsules' formation.

These conditions may be met by the method proposed by us in 2002, which was dedicated for studying of transport properties using a low-molecular weight (Mw 1,355 Da) globular marker, that is, vitamin B_{12} [16]. Using Radcliffe's [17] model describing the phenomenon of the mass transfer between two phases, the mass transfer coefficient *h* for vitamin B_{12} was determined in a simple experiment involving stirring of the microcapsules on a magnetic stirrer, in a marker solution. As our previous studies indicated, this parameter was a very sensitive tool allowing to distinguish not only membranes with different structures, but also alginate gels with different degrees of cross-linking [16,18]. Unfortunately, attempts to apply this method to study transport properties of alginate microcapsules covered by comparatively thick (50–300 μm) membranes formed of a synthetic polymer, polyethersulfone [19], did not give the expected results. One of the reasons of the failure was the way of measuring vitamin B_{12} concentration. This was done outside the experimental setup (stationary bath), from which samples for spectrophotometric measurement of the marker concentration were taken.

The aim of the study was to improve our method including experimental setup in order to increase the accuracy of the mass transfer coefficient *h* determination and to examine whether the proposed method allowed for the detection of changes in membrane structure.

2. Materials and methods

In the study, following Legallais and colleagues [20], the experimental setup was modified and direct measurement of vitamin B_{12} concentration using a spectrophotometric flow-through cuvette was used. This enabled to significantly increase the number of measurements, particularly in the initial period of the experiment. To verify whether the proposed method of studying of membrane transport properties may be used to describe membrane permeability, alginate-polyethersulfone microcapsules, of modifiable structure were studied. For modifying purposes, a small quantity of biodegradable polymer was added to membrane-forming solution containing polyethersulfone. Several batches of microcapsules with varying diameter and membrane thickness were formed, and their coefficients *h* were determined. Next, microcapsules were subjected to chemical degradation, involving the removal of the biodegradable polymer from their membranes, and their mass transfer coefficients *h* were measured again. To verify the correctness of the conducted measurements, *h* was determined by detecting the speed of the marker's penetration into the capsules from the outside (the marker was dissolved in the liquid surrounding the capsules – IN experiment) and by measuring the speed of the marker's release from inside the microcapsules into the surrounding water (OUT experiment).

2.1. Microcapsule production and characterization

The membranes of microcapsules intended for transport studies were formed using membrane-forming solution containing 8.8% (w/w) polyethersulfone type PE2020, Mw = 75 kDa (BASF, Poland) – base polymer; 5.0% poly (glycolide-*co*-ε-caprolactone), Mw = 14 kDa (Corbion Purac, The Netherlands) – biodegradable polymer; and 4.4% polyvinylpyrrolidone type PVP40 with a molecular weight of 40 kDa (Merck, Poland) – as a pore precursor agent. For the better mixing of polymers, a small addition (0.7% w/w) of a hydrophilic nonionic surfactant Pluronic f127 (BASF, Poland) was applied. As the solvent, 1-methyl-2-pyrrolidone (Sigma, Poland) was used. The dynamic viscosity of the solution at 25°C was 250 mPa s.

All the batches were obtained in a one-stage method of capsule formation in an electrostatic field designed by the Laboratory of Electrostatic Bioencapsulation Methods [19]. The method used a three-nozzle head with nested nozzles of our own design. During the microcapsule formation process, 100% glycerol (Avantor, Poland) was pushed through the inner and middle nozzles, and the membrane forming solution through the outer nozzle. The two-layer droplets forming at the tip of the nozzle were dropped into the gelling bath placed below, containing 0.25% Tween 80 surfactant (Avantor), where the spherical semipermeable polymer membranes were formed via gelling. To manufacture microcapsules of various sizes, the electrical voltage applied to the head in the form of electrical impulses was changed during the process. The impulses were characterized by voltage *U*, frequency *f*, and duration τ as provided in the data in Table 1.

After formation, microcapsules from each batch were photographed in reflected and transmitted light using an Olympus CKX 41 optical microscope equipped with a digital camera and CellSens Standard software. The diameters of 20 microcapsules from each batch were measured and the average diameter of microcapsules in a batch D_A and the variation coefficient of diameter VC (VC = (SD × 100)/D_A) were calculated.

The internal microcapsule structure was analyzed on the basis of microscope images of microcapsule specimens formed using a method designed in previous years – embedding microcapsules in synthetic resin and cutting into thin sections (10 μm) with an RM 2265 Leica rotary microtome. For analysis of microcapsule appearance and thickness, sections taken from the geometric center of capsules were used. The membrane structure was also imaged using photographs taken under a Hitachi TN 1000 electron microscope.

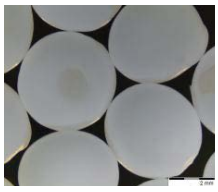
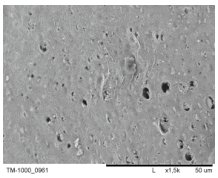
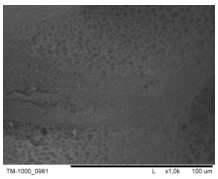
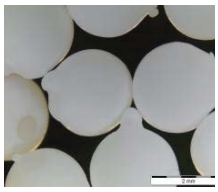
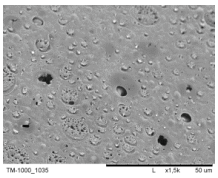
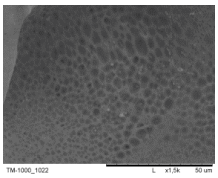
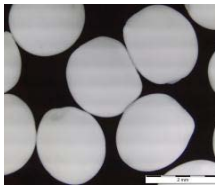
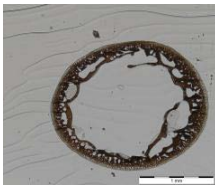
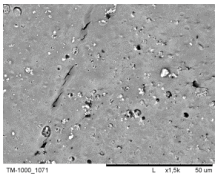
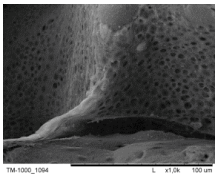
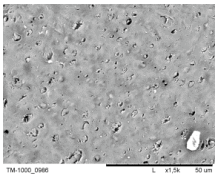
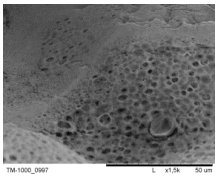
The average membrane thickness in microcapsules d_A was measured using photographs of their sections using the APEK program [21]. This program is equipped with an algorithm for carrying out automatic measurements of a membrane thickness around its circumference every 2°. The appearance and structural parameters of the microcapsules obtained in the above studies are presented in Table 2.

Table 1
Process parameters for microcapsule formation

Batch symbol	Electric parameters			Liquid flow in nozzles			L (mm)
	U (kV)	f (Hz)	τ (ms)	v_1 (mL/min)	v_2 (mL/min)	v_3 (mL/min)	
PPL-1	5	50	6	0.031	0.018	0.163	60
PPL-2	8	50	6	0.033	0.016	0.174	60
PPL-3	11	50	6	0.036	0.027	0.218	60
PPL-4	13	50	6	0.038	0.021	0.191	60

PPL – microcapsule batch symbol; v_1, v_2 – volumetric flow of glycerol in inner and middle nozzle, respectively; v_3 – volumetric flow of membrane-forming solution in outer nozzle; L – distance between nozzle tip and gelling bath surface.

Table 2
Appearance, D_A, and d_A of the studied microcapsules

Batch symbol	General view of the batch[a]	Microcapsule cross section[b]	External surface SEM	Internal surface SEM	D_A (mm)	VC (%)	d_A (μm)
PPL-1					2.65 ± 0.04	1.5	272 ± 56
PPL-2					2.39 ± 0.07	2.9	263 ± 63
PPL-3					1.98 ± 0.11	5.5	255 ± 65
PPL-4					1.63 ± 0.41	25.1	182 ± 37

[a]Magnification 20×.
[b]magnification 40×.

Three batches of well-formed spherical microcapsules (PPL-1–3) and one batch with a slightly elongated, egg-like shape were obtained. Manufactured batches characterized with a large uniformity in terms of size – the variation coefficients of average microcapsule diameter VC changed within a narrow range from 1.5% to 5.5%. Only the PPL-4 batch, due to oval shape, had a greater VC = 25%. Microcapsule membranes had a fairly dense, porous structure typical for polymer membranes formed using the wet phase inversion method. The average membrane thickness decreased from 272 ± 56 to 182 ± 32 μm together with the decrease in microcapsule diameter.

2.2. Chemical degradation of microcapsules

After measurement of their transport properties, microcapsules from each of the batches were subjected to chemical degradation, which was conducted in 3% aqueous NaOH (Avantor) solution at room temperature. The capsules were then rinsed with 8 × 400 mL deionized water at pH = 7.

The duration of the degradation process was 4 weeks for all batches. The aim of the degradation was to remove the biodegradable polymer from the microcapsules' membrane structure. After degradation of membranes, another measurement of mass transfer coefficients was carried out for capsules from individual batches.

2.3. Mass transfer coefficients h determination

Experimental determination of coefficient h (expressed in cm/min) is a two-stage method: the first stage involves determining the curve of the decrease in a marker concentration in the solution during stirring of a known amount of microcapsules in it. In the second stage, the obtained curve is approximated with a model equation. The mass transfer coefficient h_{IN} is determined using parameters of a fitting curve and experimental data.

By conducting a reverse experiment, in which after the experiment involving marker penetration into the microcapsules (IN experiment), the kinetics of the marker's exit from the interior of the microcapsule out into the solution (OUT experiment) is studied, we can calculate the coefficient of the marker's exit from the microcapsule – h_{OUT}.

2.3.1. Determination of mass transfer coefficient h – theory

The derivation of formulas describing the mass transfer phenomenon has been discussed in Ref. [16]. In the case of a study of a marker penetration into microcapsules from the outside (marker solution), the changes in marker concentration in the solution surrounding the microcapsules are described by Eq. (1) as follows:

$$C = \left(C_0 - C_{eq}\right) \times e^{-\left(\frac{1}{V_0}+\frac{1}{V_c}\right)hAt} + C_{eq} \tag{1}$$

where C (mg/mL) – concentration of the marker in the solution, C_{eq} (mg/mL) – concentration of the marker in equilibrium state, C_0 (mg/mL) – initial concentration of marker in solution $C_0 = C(t = 0)$, V_0 (cm^3) – volume of the solution, V_c (cm^3) – volume of capsules available to marker, h (cm/min) – mass transfer coefficient, t (min) – time (independent variable), and A (cm^3) – interface surface area, calculated with Eq. (2).

$$A = 6 \times \frac{V_c}{D_A} \tag{2}$$

where D_A – average diameter of microcapsules.

Fitting the exponential curve described by formula (1) to experimentally obtained measurement points enables to establish, on the basis of the formula coefficients, the mass transfer coefficient h expressed in cm/min. In our studies, the approximation of formula (1) to the experimental results was done using Origin Pro V.8. The ExpDec1 mathematical model present in the Origin calculation environment, described by equation (the original form is retained), was used as follows:

$$y = A_1 e^{-\left(\frac{x}{t_1}\right)} + y_0 \tag{3}$$

where A_1, t_1, y_0 – Origin model equation coefficients and x – independent variable.

Comparing Eqs. (1) and (3), we obtain:

$$\left(\frac{1}{V_0}+\frac{1}{V_c}\right)hA = \frac{1}{t_1} \tag{4}$$

Transforming Eq. (4), we obtain an expression describing the mass transfer coefficient as follows:

$$h = \frac{V_0 V_c}{t_1 A\left(V_0 + V_c\right)} \tag{5}$$

By calculating the differential of Eq. (5) for each variable, we obtain a formula describing the maximum calculation error of the mass transfer coefficient Δh, while the total differential of Eq. (2) gives the maximum calculation error of the interface surface area ΔA expressed by Eq. (7).

$$\begin{aligned}\Delta h = &\left|\frac{V_c^2}{At_1\left(V_0+V_c\right)^2}\right|\Delta V_0 + \left|\frac{V_0^2}{At_1\left(V_0+V_c\right)^2}\right|\Delta V_c + \\ &\left|\frac{V_0 V_c}{A^2 t_1\left(V_0+V_c\right)}\right|\Delta A + \left|\frac{V_0 V_c}{At_1^2\left(V_0+V_c\right)}\right|\Delta t_1\end{aligned} \tag{6}$$

$$\Delta A = \left|\frac{6}{D}\right|\Delta V_c + \left|\frac{6V_c}{D^2}\right|\Delta D \tag{7}$$

When the values of individual components of the error are known, it is possible to calculate the maximum errors for every established mass transfer coefficient h. Thus, the range of the values $h \pm \Delta h$ was determined, which had to contain the real value, while Δh was the maximum uncertainty.

An ANOVA (one-way analysis of variance) test was carried out to compare transport coefficients h_{IN} and h_{OUT}, as well as h_{IN} before degradation and h_{IN} after degradation, and h_{OUT} before degradation and h_{OUT} after degradation. The ANOVA tests were carried out assuming a significance level of $p = 0.05$.

2.3.2. Determination of mass transfer coefficient h – experimental setup

As a low-molecular weight marker, the method uses vitamin B_{12} (Mw 1,355, Avantor), which demonstrates light absorption at wavelength λ = 361 nm in spectrophotometric studies. This property was used in measurements of a current concentration of the marker in the measurement system. The study of transport properties was carried out using a setup comprised of an external tank (Fig. 1), in which the liquid containing capsules was stirred using a magnetic stirrer. A peristaltic pump forced the liquid in the system to flow between the tank and the spectrophotometer's flow-through measurement cuvette. The system used automatic triggering of light absorbance measurements at 1-min intervals.

The decrease in marker concentration caused by the adsorption on components of the measurement system did not exceed 1.7% of the initial value. The measurement (Fig. 2) was carried out in the experimental setup without capsules.

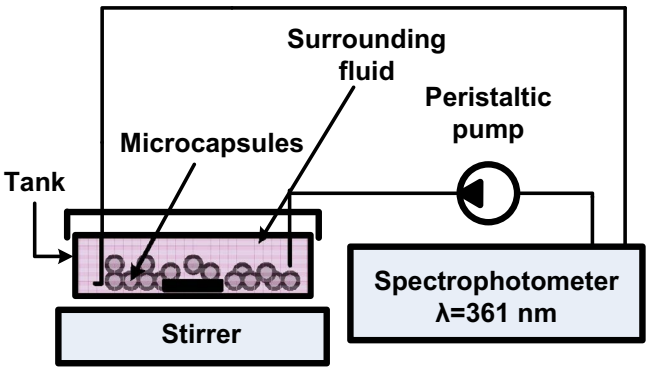


Fig. 1. The scheme of the experimental setup for spectrophotometric measurements of the marker concentration.

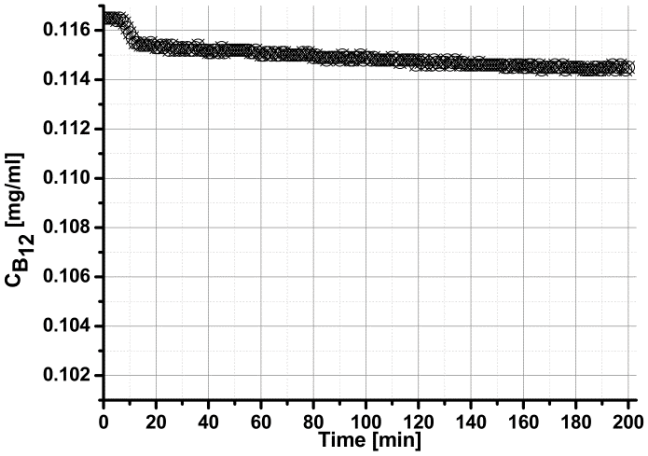


Fig. 2. The decrease in the marker concentration registered for experimental setup without capsules.

The study of transport properties of microcapsule membranes consisted of two parts. The first part of the experiment involved measurement of the speed of the marker's penetration into the capsules (experiment designated by the "IN" symbol). To establish the rate of penetration, a measured volume V_0 = 8.00 ± 0.05 mL of vitamin B_{12} solution with a concentration of 0.12 mg/mL was poured over a known amount of studied capsules with a volume of V_c = 1.50 ± 0.05 mL. Next, during stirring, the marker penetration experiment was conducted up to the point of reaching equilibrium concentration C_{eq} (200 min).

The total interface surface area – A was calculated on the basis of measurements V_c and D_A, by substituting the obtained data into Eq. (2). The maximum uncertainty ΔA, based on the total differential method, is given in Eq. (7).

Next, using the same experimental setup, the rate of the marker's release from the inside of the microcapsule into the surrounding liquid was studied (experiment marked as "OUT"). Deionized water with a measured volume V_0 = 8.00 ± 0.05 mL was poured over capsules saturated with vitamin B_{12} solution from the first part of the experiment.

After establishing h_{IN} and h_{OUT} transport coefficients, microcapsules were quantitatively transferred to conical flasks and subjected to chemical biodegradation. Experiments for studying the transport properties of vitamin B_{12} after the membrane degradation process were conducted using the same procedure.

3. Results and discussion

3.1. Study of vitamin B_{12} penetration/release

Exemplary results of experiments for batch PPL-2 showing changes in the marker concentration in the circulating liquid over time are presented in Figs. 3 (before degradation) and 4 (after degradation). Data obtained via the measurement in the experimental setup are marked with black dots. Theoretical curves, to which the experimental curve was fitted, are marked with a continuous line. Each experiment consisted of two stages: marker penetration into the capsule (IN) (Figs. 3(a) and 4(a)) and its release from the inside of the capsule (OUT) (Figs. 3(b) and 4(b)).

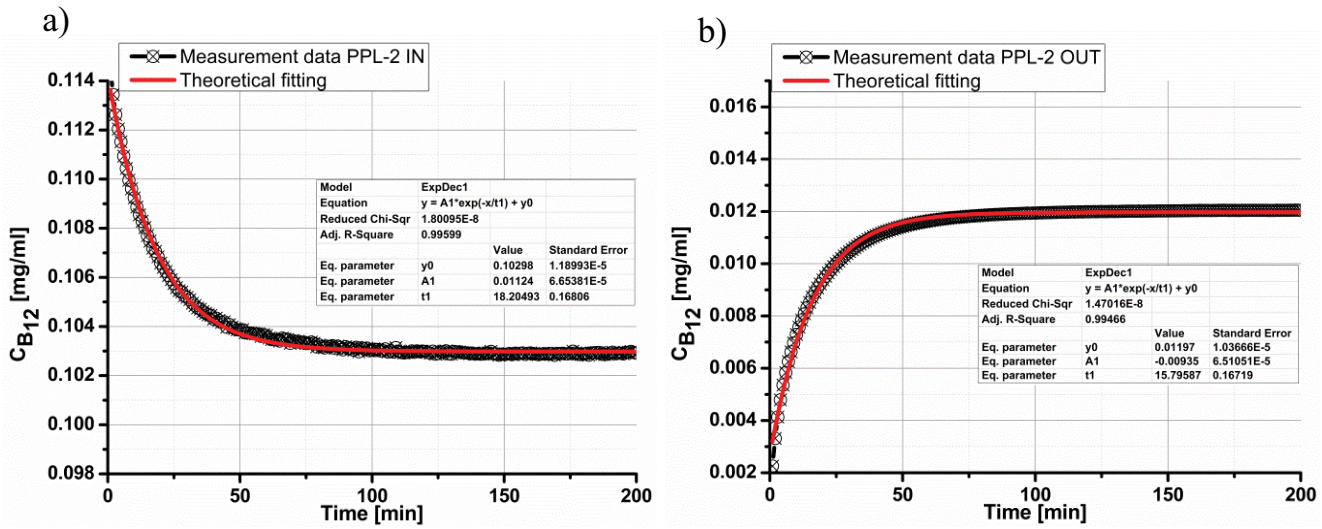


Fig. 3. Changes in vitamin B_{12} concentration in experiment with capsules PPL-2 before degradation: (a) IN and (b) OUT.

One should note that the initial concentration of the marker (for $t = 0$) in the OUT experiments (Figs. 3(b) and 4(b)) was nonzero. This was because the complete removal/drying of the marker solution residuals from the first stage of the experiment (IN) was impossible. An analogous situation takes place in the case of the marker penetration study (Figs. 3(a) and 4(a)). Furthermore, residuals of the water, in which the capsules were stored before the experiment, caused slight initial dilution of the marker solution – $C_0 < 0.12$ mg/mL. The mathematical model applied in the calculations takes into account the initial conditions mentioned: the model contains the component $C_0 = C\ (t = 0) \neq 0$. Therefore, the phenomenon does not affect the correctness of the calculations of mass transfer coefficient h, which appears in the expression that is an exponent in Eq. (1).

A very good fit of the theoretical curve to measurement points ($R^2 > 0.98$) was obtained in all experiments. Values of the corrected coefficient of determination R^2 for individual cases of approximation of the measurement points with an exponential curve are presented in Table 3. The overall error of the method, despite the strong impact of the experimental measurement system with a spectrophotometer and error of numerical fitting of a theoretical curve to measurement data (Origin), did not exceed 3.5%.

3.2. Mass transfer coefficients h determination

All the calculated mass transfer coefficients h are presented in Table 4.

A graphical representation of the calculated coefficients h with values of the maximum total error Δh marked is presented in Fig. 5.

The coefficients of vitamin B_{12} penetration into microcapsules before biodegradation h_{IN} for batches with varying diameter changed slightly (maximum by 12%) in a range between 1.75×10^{-3} and 1.96×10^{-3} cm/min. The coefficients of the

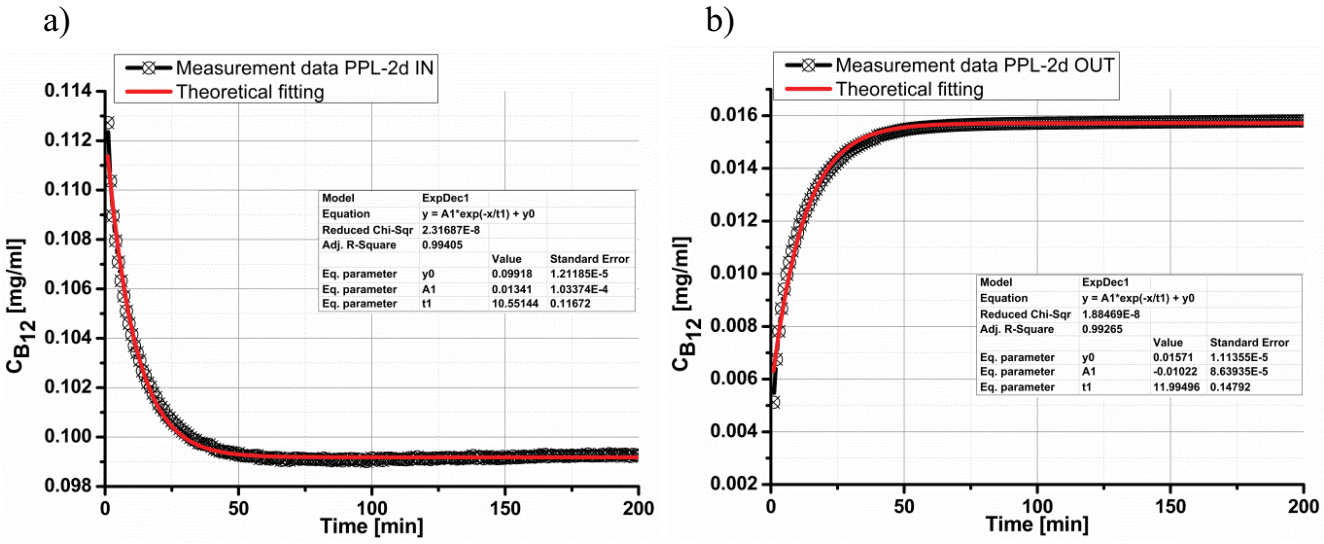


Fig. 4. Changes in vitamin B_{12} concentration in experiment with capsules PPL-2 after degradation: (a) IN and (b) OUT.

Table 3
Parameters of fitted theoretical equation

	Symbol							
	PPL-1		PPL-2		PPL-3		PPL-4	
	IN	OUT	IN	OUT	IN	OUT	IN	OUT
	Before degradation							
y_0	0.1032	0.0131	0.1030	0.0120	0.1062	0.0104	0.1016	0.0139
A_1	0.0114	–0.0098	0.0112	–0.0094	0.0084	–0.0081	0.0088	–0.0094
t_1	19.1 ± 0.2	18.4 ± 0.2	18.2 ± 0.2	15.8 ± 0.2	16.6 ± 0.1	16.8 ± 0.2	11.7 ± 0.1	10.3 ± 0.1
R^2	0.994	0.993	0.996	0.995	0.998	0.996	0.998	0.997
	After degradation							
y_0	0.1029	0.0139	0.0992	0.0157	0.1040	0.0138	0.0938	0.0173
A_1	0.0087	–0.0010	0.0134	–0.0102	0.0115	–0.0093	0.0162	–0.0109
t_1	15.2 ± 0.2	13.7 ± 0.1	10.6 ± 0.1	12.0 ± 0.1	10.4 ± 0.2	11.3 ± 0.1	6.8 ± 0.1	6.9 ± 0.1
R^2	0.995	0.995	0.994	0.993	0.989	0.992	0.992	0.997

Table 4
Transport coefficients h

Symbol	$h \times 10^6$ (cm/min)			
	Before degradation		After degradation	
	IN	OUT	IN	OUT
PPL-1	1,948 ± 180	2,022 ± 190	2,452 ± 220	2,721 ± 240
PPL-2	1,862 ± 200	2,146 ± 230	3,213 ± 350	2,826 ± 310
PPL-3	1,745 ± 260	1,724 ± 270	2,796 ± 440	2,578 ± 340
PPL-4	1,957 ± 630	2,229 ± 720	3,346 ± 1,100	3,315 ± 1,100

marker release from the inside of capsules h_{OUT} for individual batches were 4%–15% higher and ranged from 1.72×10^{-3} to 2.23×10^{-3} cm/min. This was related to the initial rapid release of vitamin B_{12} molecules located on the outer surface of the capsules to water. The exception was batch PPL-3, for which the penetration coefficient was 1% higher than the release coefficient. However, all the discussed results were within the calculated maximum error. Obtained values corresponded well with data obtained for alginate capsules surrounded by thin poly(methylene-*co*-guanidine) membranes, for which the penetration coefficient h_{IN} was 8.00×10^{-3} cm/min for a 59 μm thick membrane and 13.00×10^{-3} cm/min for a 62 μm thick membrane [18]. Obtained results were also in agreement with data published by other authors [20].

The chemical degradation of membranes caused an increase in the transport coefficient of vitamin B_{12} as expected. The coefficients of vitamin B_{12} penetration h_{IN} into the microcapsules, after the chemical degradation, range from 2.45×10^{-3} to 3.35×10^{-3} (difference of 37%) and in three cases (PPL-2–PPL-4) were 1%–12% higher than the coefficients of release h_{OUT}. Only for batch PPL-1 the reverse behavior was observed – h_{OUT} was 11% higher than h_{IN}. The observed difference could be caused not only by an increase in number/size of membrane pores, but also by an increase in total internal surface area of the membrane, leading to the increased marker adsorption. Statistical analysis did not show significant differences either within the two studied groups (h_{IN} and h_{OUT} coefficients of individual microcapsule batches) or between them (h_{IN} vs h_{OUT} coefficients). As expected, these values were statistically different from the h_{IN} and h_{OUT} values of the respective microcapsule batches before degradation. The loss of the polymer caused changes in the membrane structure (Fig. 6), and the porosity increase allowed for the more rapid penetration of the marker into capsules. Within this group, the penetration coefficients did not differ from each other.

No statistically significant differences in coefficient value were ascertained for microcapsules with different membrane thickness, which, taking into account the fact that all capsules were formed with membrane-forming solution of the same composition, was a correct result. The slight differences were within the maximum error Δh.

The maximum relative error of the calculated transport coefficients was within the range from 9% to 16% (batches PPL-1–PPL-3), and equaled 33% in the case of batch PPL-4. Such a high maximum error Δh for batch PPL-4 resulted from the greatest, in this case, standard deviation (SD) of the capsule diameter measurement (Table 2). Batch PPL-4 was characterized by the greatest deformation of spherical shape (elongated, egg-shaped capsules), which translated

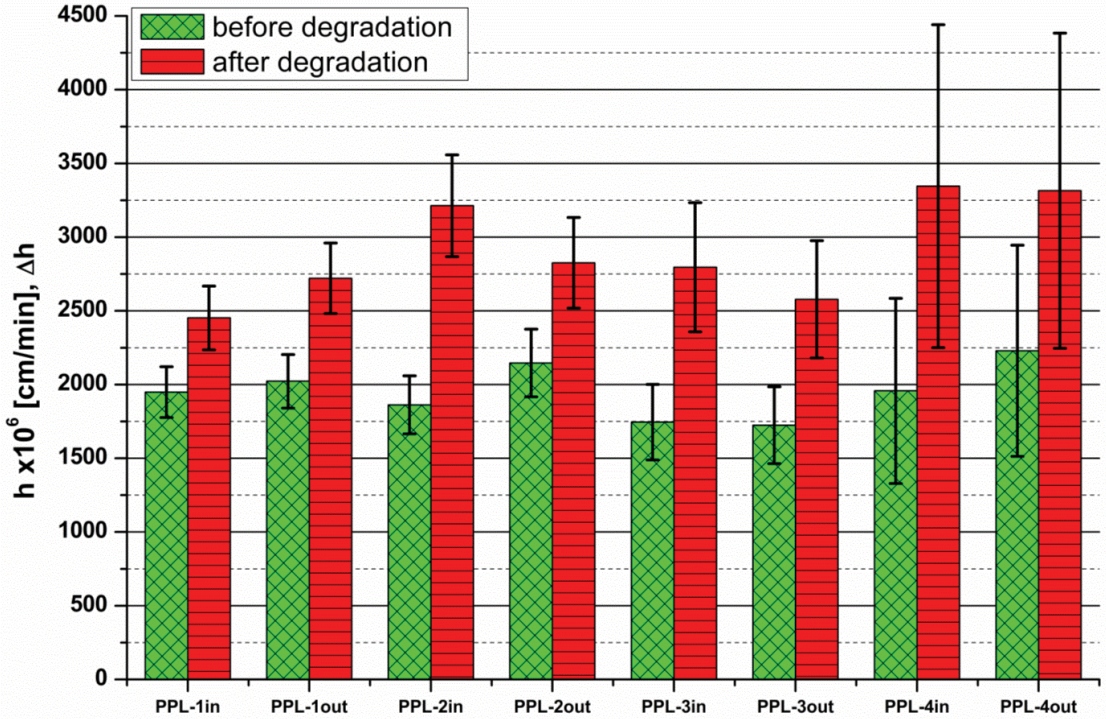


Fig. 5. Graphical presentation of the h for individual experiments.

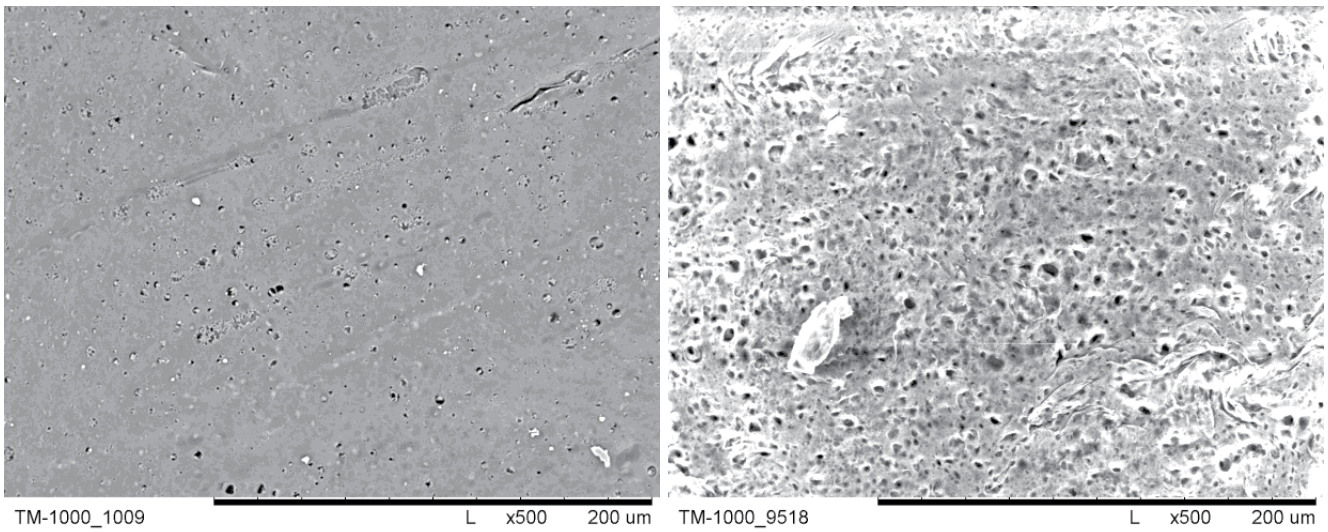


Fig. 6. SEM images of external surface in sample PPL-2: (a) before degradation (sputtered sample) and (b) after degradation (no sputter coating).

into greater SD of diameter measurements, increasing the component of Eq. (6).

$$\left|\frac{V_0 V_c}{EA^2\left(V_0 + V_c\right)}\right|\Delta A \tag{8}$$

4. Conclusions

The presented method for studying the transport properties of membranes in polymer microcapsules is highly accurate. The precise determination of the moment in time of the light absorbance measurement and the sufficiently short measurement interval, together with the high sensitivity and accuracy of the spectrophotometric method, allow for very good fit of the theoretical curve to experimental data ($R^2 > 0.98$) to be achieved. Moreover, in this method, measurements of the marker concentration are conducted in a closed system, from which no samples are taken "outside," which guarantees stability of conditions (constancy of marker mass and volume of the liquid surrounding the capsules) for the conducted measurements.

Furthermore, a lack of correlation between the value of the transport coefficient and capsule diameter or membrane thickness is observed. For both, capsules with a diameter of 2.65 (membrane thickness 272 μm) and 1.63 mm (membrane thickness 182 μm), h_{IN} values are very close and equal to 1.95×10^{-3} and 1.96×10^{-3} cm/min, respectively. Expressing the marker's mass transfer coefficient in cm/min characterizes the internal structure of the membrane. The speed of the marker's movement in the membrane is independent of its thickness (physical dimensions), but it depends on its porosity and pores structure.

It has been established that removing a small, 5% addition of biodegradable polymer from the membrane material causes a change in membrane porosity, reflected in a statistically significant increase in vitamin B_{12} transport coefficients.

The lack of statistically significant differences in the mass transfer coefficients of the studied marker from the outside into microcapsules h_{IN} and out of the microcapsule from the inside h_{OUT} may indicate that the studied membrane has a symmetrical structure. However, determining whether the described method is sufficiently sensitive to allow for the detection of such subtle differences in membrane morphology still requires examination.

The presented method is comparatively simple, fast, and cheap. Easy to master, it may be implemented in daily research practice in most laboratories.

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