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PVA and various diisocyanates based poly(urethane–urea) microcapsules for encapsulation of enzyme in water/butyl acetate emulsion: synthesis and study

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This study evaluates the impact of three different diisocyanates, (1,6-hexamethylene diisocyanate (HMDI), toluene-2,4-diisocyanate (TDI), or 4,4'-methylene diphenyl diisocyanate (MDI), on the structure and properties of PVA-based (poly(vinyl alcohol)) poly(urethane–urea) microcapsules (PUUMC). PUUMC consisting of an aqueous solution of maltogenic α-amylase (MG) were obtained via interfacial polyaddition in the proposed herein environment–friendly inverse water/butyl acetate emulsion at 30–70 °C temperature. It was shown that the reactivity of diisocyanates and the variations of synthesis time and temperature affected the encapsulation efficiency of MG and its release from PUUMC. It was found that the enzyme release from PUUMC mostly depended on the used diisocyanate.

Keywords: poly(urethane–urea), poly(vinyl alcohol), microencapsulation, porosity

INTRODUCTION

Amylases (α-amylase, β-amylase and glucoamylase) are of great importance in present day biotechnology with applications ranging from food, baking, brewing, fermentation, detergent applications, textile desizing, paper industries and analytical chemistry [1, 2]. β-amylases (or maltogenic α-amylases) are exo-amylases, which catalyse the successive removal of maltose from the non-reducing ends of glucose polymers with inversion of the anomeric configuration [3]. Hence, the spectrum of maltogenic α-amylase applications has expanded into many fields, such as clinical, medical, food industries and analytical chemistry [4]. There are many examples of α- and β-amylases immobilization on various matrices [1–2, 5–8].

The potential utilizations of polyurethanes and polyurea polymers have been discussed for a variety of pharmaceutical, medical and cosmetic applications, such as drug or other bioactive materials encapsulation and controlled release [9–13]. As immobilization carriers, polyurethanes based materials are used in various forms: foam, microspheres and microparticles, powder, layer and coatings [8]. Mostly enzymes are attached to a polyurethane carrier covalently and/or by physical adsorption [6, 8, 14–17]. Entrapment is a less common way for immobilizing enzymes into polyurethane microcapsules, film and microparticles [10, 18, 19]. There is no article, associated with enzyme encapsulation in the polyurethane or poly(urethane–urea) shell via interfacial polyaddition reaction or any other technique. Yow and Routh [20] argued that the use of monomers in both phases negatives the possibility of putting biologically active materials in the core when the interfacial polymerisation technique was used. Furthermore, as far as Yow and Routh [20] are aware, there are no reports on biological encapsulation using this method.

Poly(vinyl alcohol) (PVA) has attracted great attention in enzyme immobilization [21, 22]. However, only few reports were found where PVA was used instead of polyols in synthesis of polyurethane particles or films [6, 8, 23, 24]. In our previous work, the synthesis of microcapsules from PVA and HMDI in the water/toluene emulsion at 70 °C was described. It was estimated that physical and chemical properties of PUUMC can be controlled by varying synthesis conditions (concentration of catalyst and surfactants, initial molar ratio of PVA and HMDI, stirring rate and ratio of dispersed phase to external phase). A controlled release of MG could be observed in many cases when MG was encapsulated during PUUMC synthesis [25]. The originality of our study resides on the use of interfacial polyaddition technique (IFP) for synthesis of poly(urethane-urea)

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microcapsules in the proposed herein environment–friendly water/butyl acetate emulsion system (w/o) at low and high temperature. The influence of diisocyanate (1,6-hexamethylene diisocyanate (HMDI)), toluene-2,4-diisocyanate (TDI), 4,4'-methylene diphenyl diisocyanate (MDI) on the structure and properties of PVA-based PUUMC and encapsulation efficiency has been studied in detail. The effects of initial reaction conditions on the yield of shell, size distribution, surface area, pore size and the total pore volume of microcapsules, efficiency of MG encapsulation and the release ratio of encapsulated MG are studied. These capsules have potential application in biotechnology for saccharification of starch. The proposed method could be used for encapsulation of any enzyme or any water-soluble material.

EXPERIMENTAL

Materials

1,6-hexamethylene diisocyanate (HMDI), toluene-2,4-diisocyanate (TDI), 4,4'-methylene diphenyl diisocyanate (MDI) and poly(vinyl alcohol) (PVA, M_w = 100000, degree of hydrolysis 86–89%) which were used as shell-forming materials were obtained from Fluka, Switzerland. The non-ionic surfactant, Span 85® (sorbitane trioleate) from Fluka, Switzerland, was used as an emulsifier. The catalyst dibutyl tin dilaurate (BDTDL) was obtained from Merck, Germany. Diethylamine, sodium carbonate, potato starch, citric acid and butyl acetate were purchased from Reachem, Slovakia. Glycine, neocuproine hydrochloride hidrate, copper (II) sulfate pentahydrate, phthalic anhydride were purchased from Sigma-Aldrich, Chemical Co. Diethyl ether was purchased from Lachner, Czech Republic. Maltogenic α-amylase named maltogenase (MG) (EC 3.2.1.133) from *Bacillus stearothermophillus* and α-amylase (EC 3.2.1.1) from *Bacillus subtilis* were obtained from Novozymes, Denmark.

Preparation of poly(urethane–urea) microcapsules via IFP in w/o emulsion

PUUMC from PVA and diisocyanate (HMDI, TDI or MDI) were synthesized by the interfacial polyaddition in w/o emulsion. The aqueous phase was PVA solution in water. The organic phase was 5.0% (w/w) of Span 85® in butyl acetate with HMDI and MDI or 3.0% with TDI. 1.0% of the catalyst DBTDL (with respect to PVA) in the butyl acetate solution was used when HMDI was used as DI. The PVA solution was emulsified in the organic phase using a stirring rate of 400 rpm. The volume ratio of water to butyl acetate was 1:3. The initial concentration of PVA was varied from 0.1 to 0.6 M. The initial molar ratio of PVA and diisocyanates was also varied from 1:6 to 1:9. After emulsification, diisocyanate was added to the reaction mixture. The mixture was stirred continuously to complete the formation of the poly(urethane-urea) (PUU) shell. The reaction time and temperature were varied from 2 to 6 hours and from 30 to 70 °C, respectively. The PUUMC were separated by filtration using filter paper (pore size <2 μm) and washed with diethyl ether and distilled water. After that, the microcapsules were lyophilized by using the lyophiliser Labconco FreeZone Plus. The freezing temperature was –40 °C. The yield of microcapsules shell was determined from the mass of dried microcapsules as a percentage of the total mass of ingredients used. It was assumed that the yield of shell is the same as the yield of dried PUUMC.

MG encapsulation after formation of poly(urethane–urea) microcapsules

Approximately 1.75 g of the wet PUUMC (obtained by the method described in the previous section) were dispersed in 5 ml 0.1 M sodium citrate buffer (pH = 5) and 0.38 ml of MG diluted 4-fold solution in 0.1 M sodium citrate buffer (380 U) was added. The mixture was stirred for 30 min at 400 rpm at 40 °C. After that the reaction mixture was stored at 4 °C for 24 h. Next day the immobilized enzyme was thoroughly washed with the buffer and the efficiency of MG immobilization (encapsulation) was determined.

Preparation of poly(urethane–urea) microcapsules encapsulated with MG via IFP in w/o emulsion

The microcapsules were prepared in the same manner, as described in the 2nd section of *Experimental*, only the MG solution in the 0.1 M citrate buffer (pH = 5) was added to the aqueous phase with respect to its volume. The quantity of MG was assessed considering the amount of PUU microcapsules encapsulated with water under the same reaction conditions. For example, 0.38 ml of MG diluted 4-fold solution in the 0.1 M sodium citrate buffer (380 U) was used for 1.75 g of microcapsules encapsulated with water. The obtained microcapsules were separated by filtration using filter paper (pore size <2 μm) and washed with diethyl ether and 0.1 M citrate buffer. After that, the efficiency of MG immobilization (encapsulation) was determined.

Determination of enzymatic activity of MG

The catalytic activity of native and immobilized MG was determined by the reaction with 5% of liquefied potato starch solution and incubating the mixture at 40 °C for 20 min. The liquefied starch solution was prepared according to this procedure: 100 mL of the 5% potato starch suspension in the 0.1 M citrate buffer (pH = 5.0) was stirred with 0.5 mL (250 U) of α-amylase from the *Bacillus subtilis* solution for 5 min at 40 °C, and after that enzyme was inactivated by heating the solution for 30 min in a boiling water bath. The dextrose equivalent of liquefied starch was 5–10%. The dextrose equivalent is a measure of the amount of reducing sugars present in a sugar product, relative to glucose, expressed as a percentage on a dry basis. The activity of native

and immobilized MG was assayed by the Neocuproine method according to the procedure described in [26]. The Neocuproine method is applied to the determination of reducing sugars, which were formed after the starch hydrolysis by α-amylase or MG. The reducing carbohydrates are then determined by a colour reaction, which is measured at 450 nm. Copper ions are reduced by the reducing sugar; these react with neocuproine to form a coloured complex. During this reaction, values can be measured which depend on the amount of reducing sugar present after the starch hydrolysis. The activity unit of native or immobilized MG was defined as the amount of enzyme which under standard conditions (at 40 °C, pH = 5.0) produced 1 μmol of reduced sugars per minute. Four separate measurements of the native and immobilized MG were performed to check the reproducibility of the data.

The efficiency of MG encapsulation or immobilization (EI) was defined as the activity of immobilized MG in percentage from the activity of the native enzyme used for immobilization. In this study, we present the average of the 3–4 results of the EI of MG.

Characterization of PUU microcapsules

FT-IR analysis. The chemical structure of lyophilized PUUMC was characterized by using the Perkin Elmer FRONTIER FT-IR spectrometer, Waltham, USA, by the ATR method. The IR range was 4000–500 cm^{-1}, the scan number was 20, and the resolution was 4 cm^{-1}.

Determination of the functional groups of PUUMC and the elemental analysis. The amount of isocyanate groups was determined by a chemical method [27]. Determination was performed immediately after the synthesis of PUUMC and it was recalculated with respect to weight loss in the drying process. The method used to determine the isocyanate group amount consists of the reaction of dibutylamine excess with isocyanate, followed by back titration of the excess amine with hydrochloric acid. The elemental analysis was performed with the elemental analyser Flash 2000 series CHNS-O, Thermo Scientific, USA.

Determination of the size of PUU microcapsules. The size of PUUMC was investigated immediately after the synthesis by using the optical microscope Olympus BX51, Tokyo, Japan, and the image analysis software [28].

Determination of the surface area, total pore volume and pore size distribution of PUU microcapsules. The surface area, total pore volume and pore size distribution of the PUUMC were determined by using the Micromeritics Tristar II instrument, Norcross, GA. The samples were degassed at 140 °C for 2 hours prior to the experiments to eliminate any volatile compounds from the lyophilized PUUMC. The Brunauer–Emmett–Teller (BET) surface area was evaluated from the isotherm analysis in the relative pressure range of 0.05–0.22. The total pore volume was determined from the adsorption isotherm at the relative pressure of 0.98. The pore size distributions were derived from the desorption branch by using the Barret–Joyner–Halenda (BJH) model.

Thermal analysis. The TGA investigations were carried out by using the Perkin Elmer Pyris 1, Waltham, USA, in the temperature range between 30–600 °C in the 20 mL/min flow of nitrogen and the heating rate of 10 °C/min.

In all statistical analysis only p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Formation of the poly(urethane–urea) microcapsules

The synthesis of PUU microcapsules (PUUMC) was carried out by the interfacial polyaddition reaction of PVA and three different diisocyanates (HMDI, TDI or MDI) in a water/butyl acetate (w/o) emulsion. The butyl acetate/water emulsion is readily biodegradable and more environment-friendly in comparison to the toluene/water emulsion used in our previous investigations [25]. Moreover, butyl acetate is found in many types of fruit and is used as a synthetic fruit flavouring in foods. PVA with MG were enclosed in the emulsion drops. The emulsification step is of major importance as it determines the droplets size distribution and so the particles one. An important quantity of mechanical energy is maintained during few minutes to achieve the emulsification step [29]. The second step is the polyaddition step. The diisocyanate (HMDI, TDI or MDI) was added to the external butyl acetate phase of the emulsion and the polyaddition reaction took place at the liquid–liquid emulsion interface.

The PUUMC shell formation by polyaddition reaction is illustrated in Fig. 1a. Immediately after synthesis, PUUMC shells consist of macromolecules with four types of constitutional units: an unreacted hydroxyethylene constitutional unit of PVA (type I), a constitutional unit with one urethane group and a free isocyanate group at the end of the graft (type II), and/or a free amino group at the end of the graft (type III), and a constitutional unit with two urethane groups (type IV). The poly(urea) segments could present in constitutional units of type II–IV. The formation of poly(urea) segments was caused by the hydrolysis of the excess of diisocyanate to yield the corresponding primary diamine (Fig. 1b). The (di)amine formed reacts very fast with the (di)isocyanate forming a urea linkage.

PUU microcapsules characterization

The completion of the polyaddition reaction between PVA and diisocyanates was confirmed by FT-IR spectra and chemical analysis. The PVA spectrum shows a typical absorption band at 3302 cm^{-1} which is assigned to the O-H stretching from the intermolecular and intramolecular hydrogen bonds. The vibration band observed at 2918 cm^{-1} refers to the C-H stretching from alkyl groups and the peak at 1724 cm^{-1} is related to the C=O stretching from the acetate group remaining from PVA. The vibration bands at 1244 and 1083 cm^{-1} are related to ester C=O and C-O-C groups [30]. The PUUMC spectrum shows an absorption band at 1741 cm^{-1} of the C=O vibration

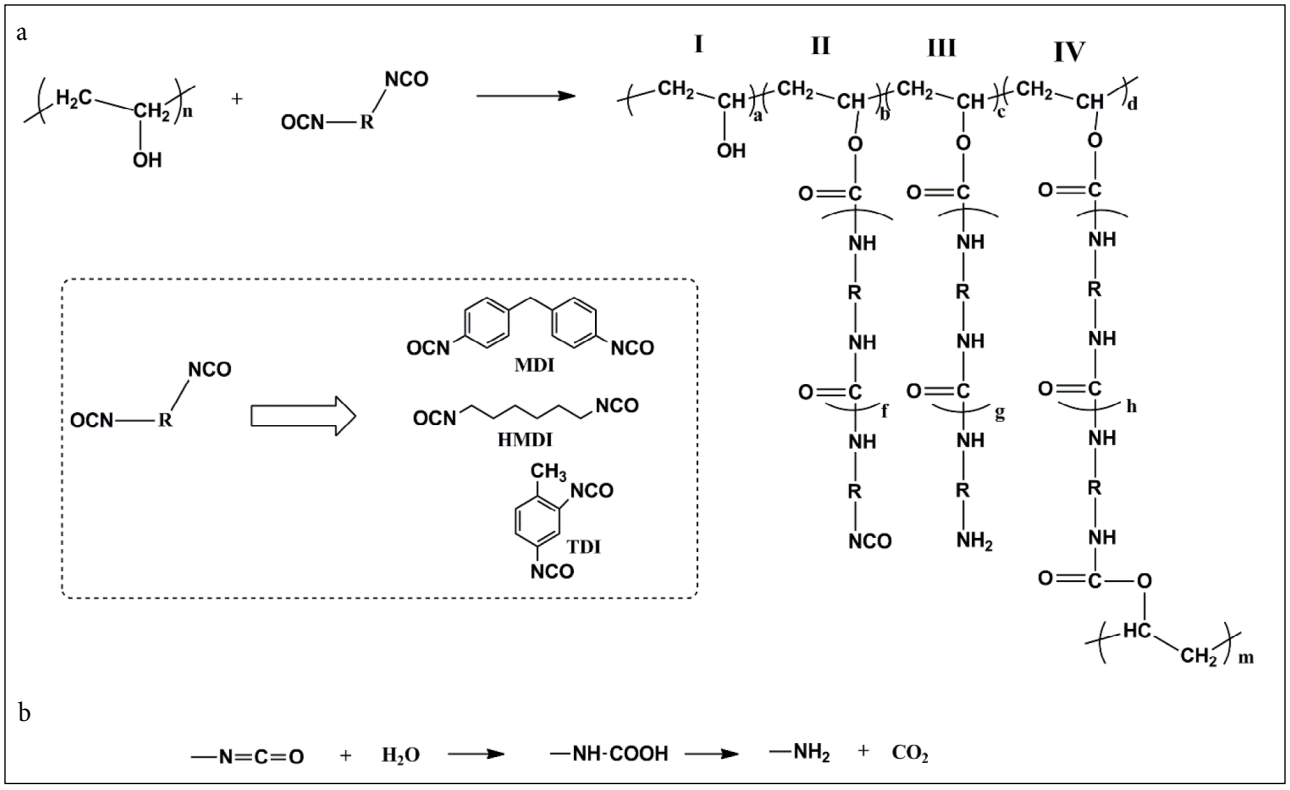

Fig. 1. The structure of shell of PUU microcapsules (a) and the reaction of isocyanate group with water (b)

of free urethane carbonyl. The N-H vibration was observed at 3320 cm^{-1}. The vibration band at 1615 cm^{-1} is caused by polyurea C=O groups. The peak of poly(urethane) and polyurea links at 1573 cm^{-1} is assigned to amide II (δ (N-H), ν (C=N)), peak at 1251 cm^{-1} is related to amide III (another type of δ (N-H), ν (C=N)), and peak at 1075 cm^{-1} (ν (C-O-C)) is related to ether linkage. The peak at 2265 cm^{-1} is assigned to the asymmetric stretching vibration of the –NCO group. The IR spectrum also indicates the completion of the reaction between diisocyanates and PVA by the disappearance of the NCO absorption band at 2257 cm^{-1} [29, 31]. According to the literature [32], diisocyanates diffusion is dynamically hindered with growing of the shell of microcapsules and isocyanate groups remained unreacted in the shell of the microcapsules.

Three different isocyanates were used in the PUUMC synthesis: HMDI is the liquid aliphatic diisocyanate, MDI and TDI are the solid aromatic diisocyanates. In general, aromatic isocyanates are more reactive than aliphatic ones, and this choice will change the final properties of polyurethanes. In the case of aromatic isocyanates the negative chap of the NCO group shifts in the aromatic group so aromatic isocyanates are more reactive than aliphatic or cycloaliphatic ones. The nature of the substituent also determines the reactivity, electron-acceptor substituents in the ortho position increase the reactivity, and electron-donor substituents reduce the reactivity of the isocyanate group [33]. According to the literature [34], K_1 (reactivities of –NCO groups against hydroxyl groups) of diisocyanates are 400, 320 and 1 with TDI, MDI and HMDI, respectively.

It was estimated that appropriate initial concentrations of PVA and molar ratios of PVA to diisocyanates were different in the synthesis of PUUMC and depended on the reactivity of DI. The molar ratio of PVA to DI was 1:6 and the concentration of PVA was 0.1 and 0.2 M with TDI and MDI, respectively. The synthesis of PUUMC at higher PVA and TDI concentrations was impossible due to the high reactivity of TDI, and crosslinked gels were obtained instead of microcapsules. The PVA concentration was 0.6 M and the molar ratio of PVA to HMDI was 1:9, because HMDI is less reactive than TDI and MDI. The reaction between PVA and HMDI was catalyzed by using DBTDL.

Preparation of PUUMC from PVA and diisocyanates HMDI, TDI or MDI depends not only on the reactivity of DI but also on the synthesis temperature and time. Increasing the PUUMC synthesis temperature from 30 to 70 °C resulted in increasing the yield of dried PUUMC when they were synthesized from PVA and any of diisocyanates for 3 hours (Fig. 2a). The prolongation of synthesis time from 2 to 8 hours resulted in increasing the yield of dried PUUMC in the reaction of PVA with all diisocyanates at 30 °C (Fig. 2b). The highest yields of dried PUUMC were in the case when MDI was used for the PUUMC synthesis and the lowest yields were obtained when TDI was used. Increasing the PUUMC synthesis temperature and time resulted in increasing the quantity of nitrogen in PUUMC. Therefore, it can be assumed that the formation of urea bonds and lengthening of the polyurea segments in PUUMC proceeded under these conditions (Table 1). According to the literature [35],

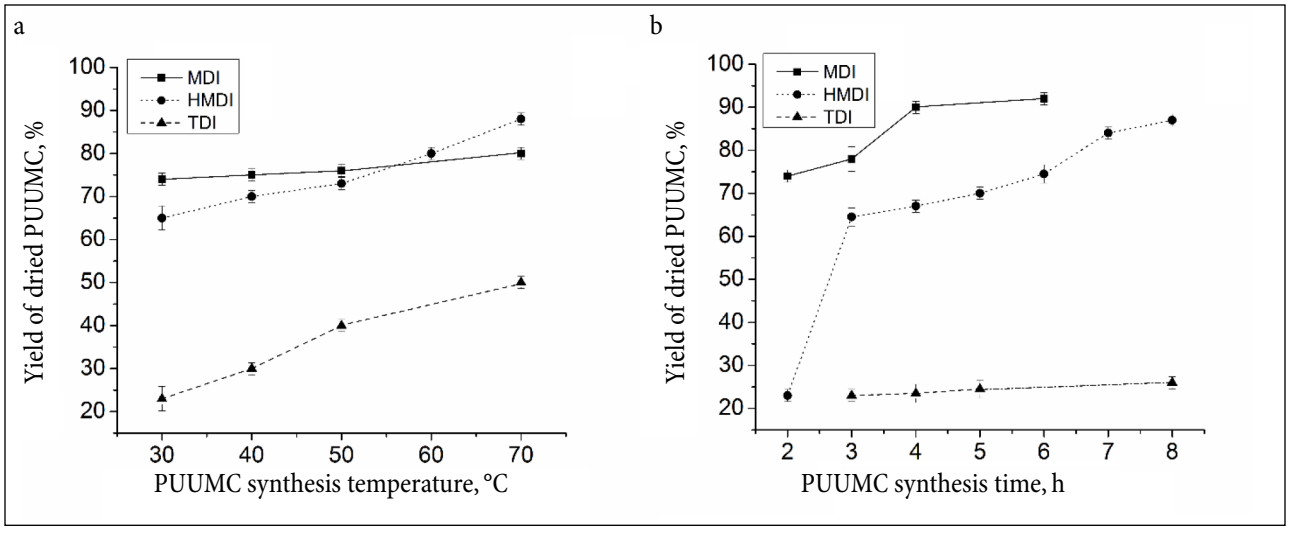

Fig. 2. The yield of PUUMC synthesized from PVA and different diisocyanates as a function of synthesis temperature (for 3 h) (a) and time (at 30 °C) (b)

the relative reaction rate of isocyanate with primary amine is 1000 times higher than the rate of isocyanate with primary hydroxyl. Therefore the reactions of formation of urea bonds dominated instead of crosslinking reactions and formation of urethane bonds.

The mean diameters of PUUMC were measured on optical microscope images by using image analysis according to the literature [28]. More than 100 microcapsules were counted. The mean diameters of PUUMC were 322 ± 16 μm, 334 ± 17 μm and 412 ± 20 μm when they were obtained from PVA and HMDI, TDI or MDI, respectively. Optical microscopy showed that the PUUMC were spherical, monocore when HMDI or TDI were used but multicore when MDI was used (3 h, 30 °C) (Fig. 3).

Table 1. **Results of the elemental analysis of PUUMC**

No.	Initial conditions of PUUMC synthesis			Quantity of element in PUUMC, %		
	DI	T, °C	t, h	N	C	H
1	TDI	30	3	13.3	65.8	6.7
2		70	3	13.5	56.3	6.3
3		30	8	13.8	56.6	6.2
4	MDI	30	3	8.7	72.8	6.7
5		70	3	9.5	70.7	6.3
6		30	6	9.5	70.3	6.2
7	HMDI	30	3	15.9	58.0	9.7
8		70	3	16.2	59.8	9.9
9		30	8	16.7	59.5	9.9

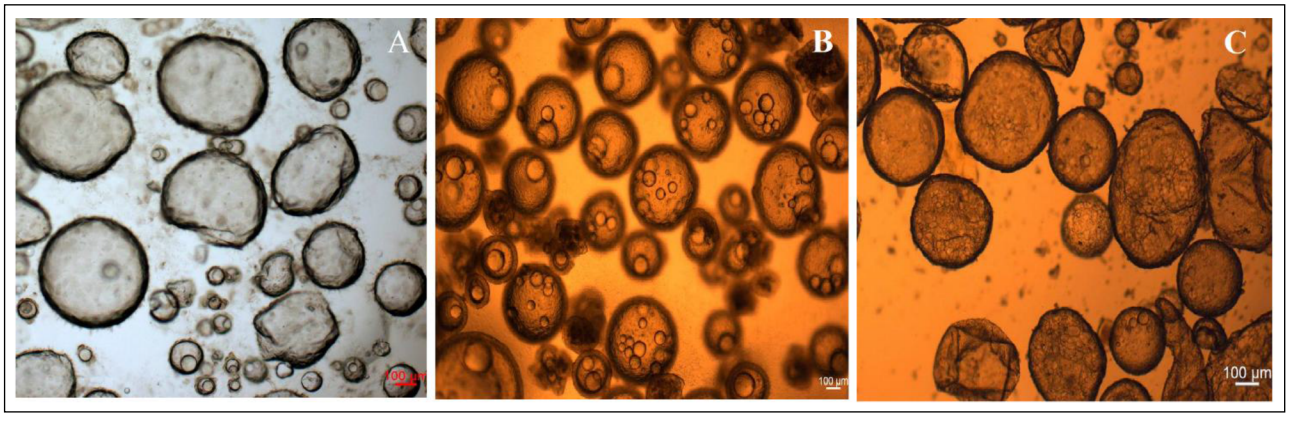

Fig. 3. Optical microscope images of PUUMC synthesized from PVA and various diisocyanates: A is HMDI, B is MDI, and C is TDI (3 h, 30 °C)

N_2 adsorption–desorption analysis

The surface area and porosity are important properties and contribute to understanding of the formation, structure and potential application of PUUMC. The instrument Tristar II used the Brunauner–Emmett–Teller (BET) equation to describe the surface area and the Barrett–Joyner–Halenda (BJH) equation to describe the pore size and volume. The approximation of the BET model yields the BET single point relationship

$$\frac{P}{V_a} = \left(\frac{1}{V_m}\right)\left(\frac{P}{P_0}\right),$$

where V_a is the quantity of gas adsorbed at pressure P, V_m is the quantity of gas adsorbed when the entire surface is covered with a monomolecular layer, and P_0 is the saturation pressure of gas [42].

The nitrogen adsorption–desorption isotherms and pore size distribution of PUUMC are shown in Fig. 4. The nitrogen adsorption–desorption isotherms of all samples corresponded to type IV of gas sorption isotherms, as categorized according to the IUPAC classification [36]. In addition, according to the IUPAC classification, H3 hysteresis loops are apparently for all samples of PUUMC, which were obtained from PVA and MDI or HMDI, whereas H1 hysteresis loops are apparently when TDI was used. A specific pore structure can be identified from hysteresis loops. According to the IUPAC, the H3 hysteresis loop is observed with aggregates of plate-like particles giving rise to slit-shaped pores. Type H1 is often associated with pores and hence has narrow distributions of the pore size. The PUUMC, which were obtained from PVA and TDI, have a narrower pore size distribution than those, which were obtained from PVA and MDI or HMDI (Fig. 4b). The results of the surface area, total pore volume and average pore size are summarized in Table 2. Increasing the PUUMC synthesis temperature from 30 to 70 °C resulted in increasing the length of polyurea segments (Fig. 1a, units II–III) and cross-links (Fig. 1a, units IV) in PUUMC, and increasing the surface area and total pore volume when PUUMC were synthesized from PVA and any of diisocyanates. As the synthesis temperature was increased, the rate of reaction between the isocyanate groups and water and yield

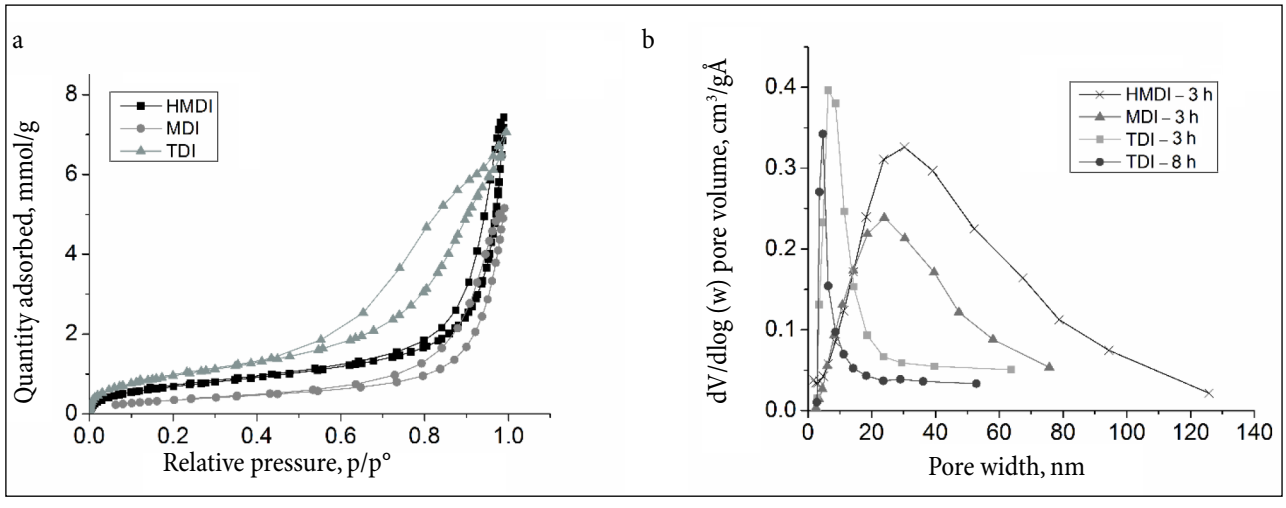

Fig. 4. Nitrogen adsorption/desorption isotherms (a) and BJH pore size distribution when PUUMC were synthesized from PVA and various diisocyanates at 30 °C (b)

Table 2. **The porosity properties and surface area of lyophilized microcapsules**

No.	Initial conditions of PUUMC synthesis			Surface area, $m^2 g^{-1}$	Total pore volume, $cm^3 g^{-1}$	Mean pore size, nm
	DI	T, °C	t, h			
1	TDI	30	3	79.5 ± 1.9	0.21 ± 0.013	9.46 ± 0.4
2		70	3	176.6 ± 4.1	0.48 ± 0.020	9.52 ± 0.4
3		30	8	58.4 ± 1.5	0.13 ± 0.010	8.51 ± 0.3
4	MDI	30	3	30.3 ± 0.9	0.16 ± 0.010	21.14 ± 1.4
5		70	3	162.9 ± 3.8	0.77 ± 0.030	18.91 ± 1.2
6		30	6	14.1 ± 0.4	0.06 ± 0.003	17.11 ± 1.3
7	HMDI	30	3	58.0 ± 1.7	0.22 ± 0.013	18.04 ± 1.3
8		70	3	77.2 ± 1.9	0.39 ± 0.018	20.18 ± 1.4
9		30	8	31.9 ± 1.0	0.14 ± 0.010	15.49 ± 0.9

of PUUMC were also increased (Fig. 1b). Therefore, more CO_2 was released and evaporated from the PUUMC shell. Consequently, the pore volume and surface area of PUUMC shells were increased. On the other hand, the surface area less depended on the PUUMC synthesis time. Prolongation of the PUUMC synthesis time resulted in decreasing the surface area, total pore volume and pore size. The narrower pore size distribution of PUUMC was also obtained (Fig. 4b). The highest surface area and pore volume of PUUMC were in the cases when MDI or TDI were used. However, the smallest pore size was in the case when TDI was used. The porosity parameters and surface area were less dependent on synthesis conditions when HMDI was used for the PUUMC synthesis.

Thermal degradation process

The TGA measurements were performed to determine the thermal stabilities of PUUMC as well as their compositional properties. The PVA and lyophilized samples of PUUMC were analyzed in the temperature range from 30 to 600 °C at a constant rate of 10 °C/min under nitrogen atmosphere (Table 3). Decomposition of PVA proceeded in two distinct weight loss stages. The first stage with the maximum of decomposition temperature at 298 °C mainly involves the elimination of H_2O and residual acetate groups, generates water, non-conjugated polyenes and acetic acid. The second decomposition stage with the maximum of decomposition temperature at 490 °C is dominated by chain-scission reactions [37]. According to the literature [38], it is obvious that the thermal degradation of poly(urethane–urea) occurs in two- to three-step process. The first step is due to degradation of the hard segments, which results in the formation of isocyanates and alcohol, primary or secondary amines and olefin, and carbon dioxide. The second and third steps correspond to the thermal decomposition of soft segments. After the first decomposition step, when the weakest bonds in the PU have been broken up, the second and third steps are much slower and depend on the soft segments structure and its three-dimensional arrangement. The results in Table 3 show the thermal degradation of PUUMC which is greatly dependent on reactivity of diisocyanates. In general, poly(urethane–urea) is more thermally stable than PU, probably because of the higher hydrogen-bonding capacity of this group when compared with urethane [39, 40]. The weight loss was higher in the first decomposition step when TDI or MDI were used in the PUUMC synthesis instead of HMDI. Therefore it is proposed that more multi-urea linkages were formed when HMDI was used and urethane and short urea linkages prevailed, when TDI or MDI were used. Increasing the reactivity of diisocyanates resulted in increasing the urethane linkages in the PUUMC shell. It is evident that the decomposition of PUUMC from PVA and TDI proceeded without the formation of multi-urea linkages and without the second step. The third decomposition step of PUUMC indicated the degradation of polyene residues of the main chains of PUUMC.

MG encapsulation and release from PUUMC

MG is one of the most important enzymes in starch industry, which is used for saccharification of starch for obtaining of high maltose syrups [8]. Encapsulation of MG was carried out by two ways: during the PUUMC synthesis or after it (3 and 4 experimental sections). In the first case, the MG encapsulation/immobilization onto/into the PUUMC shell proceeded by covalent attachment between amino and hydroxyl groups of enzyme and free isocyanate groups of the PUUMC shell or/and by physical adsorption. The reaction of free NCO groups with primary amines is much faster than the reaction with OH groups and water [8]. In the second case, MG was immobilized in PUUMC by entrapment, physical adsorption and by covalent attachment to the PUUMC shell. The activity of native MG and encapsulated MG in PUUMC was determined by the hydrolysis of 5% of the liquefied potato starch solution at 40 °C for 20 min. The amount of produced/liberated reduced sugar was assayed by the Neocuproine method according to the protocol presented in the Section *Determination of enzymatic activity of MG*.

The results show that the efficiency of encapsulation (EI) of MG was greatly dependent on the reactivity of diisocyanates (Fig. 5). Increasing the reactivity of diisocyanates (HMDI < MDI < TDI) resulted in increasing EI of MG after and during the PUUMC synthesis. At the same time, the EI of MG was higher in all cases, when MG was encapsulated during the PUUMC synthesis. However, the EI of MG in PUUMC also was influenced by PUUMC synthesis time and temperature. Increasing the PUUMC synthesis temperature

Table 3. **The TGA results of PVA and PUUMC shells (PUUMC were synthesized at 70 °C for 3 h)**

Sample code	DI	Decomposition steps							
		First			Second		Third		
		T_{inint}, C°	T_{max}, C°	Δm, %	T_{max}, C°	Δm, %	T_{max}, C°	T_{end}, C°	Δm, %
PVA		238	298	82.2	–	–	446	490	10.1
PUUMC-1	TDI	256	309	72.0	–	–	390	395	2.3
PUUMC-2	MDI	281	323	48.1	349	11.3	463	497	7.3
PUUMC-3	HMDI	262	305	10.5	352	78.7	450	494	3.6

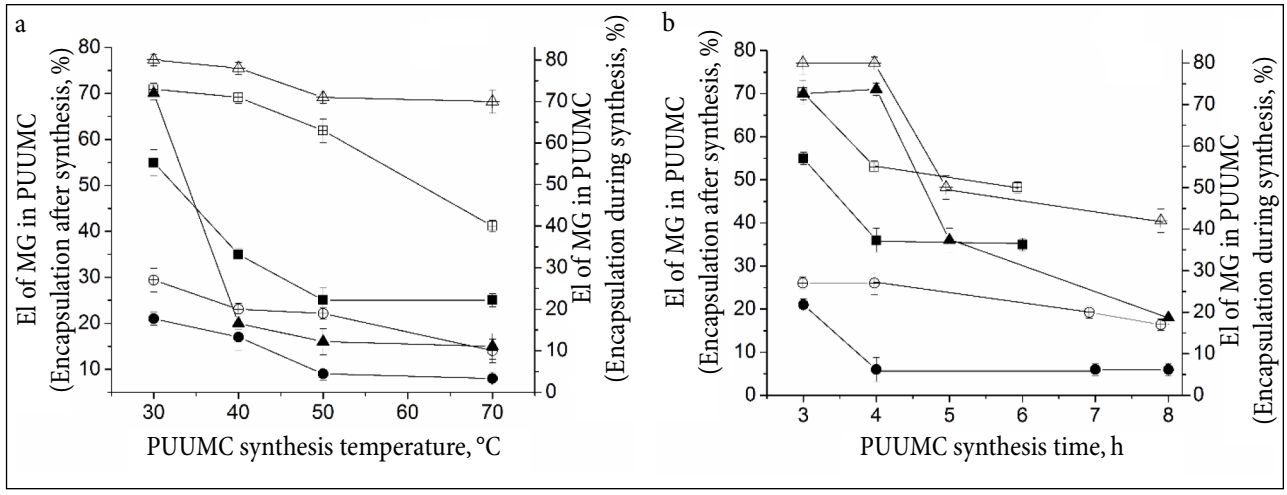

Fig. 5. The efficiency of MG encapsulation in PUUMC as a function of PUUMC synthesis temperature (a) and PUUMC synthesis time (b): a solid symbol is encapsulation after PUUMC synthesis, an open symbol is encapsulation during PUUMC synthesis, ■ is MDI, ● is HMDI and ▲ is TDI

resulted in decreasing the EI of MG in PUUMC, when MG was encapsulated after and during the PUUMC synthesis. The optimal activity temperature for native MG and encapsulated MG was determined in the same manner as presented in the Section *Determination of enzymatic activity of MG*, only the activity of MG was measured at temperature from 20 to 70 °C. Both native MG and encapsulated MG have the optimal temperature of 60 °C. Higher temperatures tended to speed up the PUUMC formation, and led to MG inactivation. Prolongation of the synthesis time resulted in decreasing the EI of MG. However, the EI of MG was almost unchanged, when PUUMC were synthesized from PVA and MDI or HMDI for 4–8 h. The porosity of the PUUMC shell is same of the major factor that determines MG encapsulation after the PUUMC synthesis, because the MG encapsulation onto/into PUUMC is proceeded basically by physical adsorption. The maximal EI of MG was achieved when PUUMC with the surface area in the range of 42–86 m^2/g, the total pore volume of 0.19–0.25 cm^3/g and the pore size of 9–40 nm were used for MG encapsulation after the PUUMC synthesis. When MG encapsulated during the PUUMC synthesis, the optimal PUUMC surface area was 42–90 m^2/g, the pore volume was 0.18–0.32 cm^3/g, and the size was 9–40 nm.

The release rate of encapsulated MG from PUUMC was investigated in the course of storage time in the sodium citrate buffer, pH 5.0, at 4 °C. The catalytic activity of encapsulated MG in PUUMC and filtrate was estimated according to the protocol presented in the Section *Determination of enzymatic activity of MG*. The catalytic activity of MG in PUUMC after the encapsulation was expressed as 100% and the initial release rate after the immobilization was expressed as 0%. The release rate of MG from PUUMC was expressed in percentages as the ratio of catalytic activity of MG in the filtrate to the initial catalytic activity of MG in PUUMC. The sum of the catalytic activity of MG in PUUMC and filtrate total was 100%.

The release rates of encapsulated MG from PUUMC obtained from PVA and different diisocyanates are shown in Fig. 6. The encapsulated MG release studies from PUMMC showed one initial burst release followed by a slow-release phase (extended release) in most cases. Burst release is a nonsteady-state and high-rate release of materials is mostly seen at the beginning of controlled release processes. The burst release can be caused by numerous reasons such as desorption of the materials entrapped on the surface, poor distribution of materials within the network during formation or storage, a heterogeneous nature of the polymer network or percolation-limited diffusion of entrapped materials [41]. The release of MG from PUUMC has burst release of 40–50 and 20–30% in the first 4 days when MDI and HMDI were used for PUUMC synthesis, respectively (Fig. 6 a–d). It is supposed that the MG burst release profile may be probably due to the desorption of MG that was adsorbed to the surface of PUUMC. The extended release phase of encapsulated MG may be possibly due to the MG that was closed in the pores of PUUMC and entrapped within the PUUMC.

Incomplete release of MG from capsules was obtained when HMDI was used for the synthesis of PUUMC. MG stability in PUUMC was higher in these cases and part of the residual activity of encapsulated MG remained after 44 days (Fig. 6 c–d).

The controlled release of MG could be observed when TDI was used for the PUUMC synthesis in all cases with the exception of MG which was encapsulated during the PUUMC synthesis at 30 °C for 3 h (Fig. 6 e–f). The PUUMC with a narrow pore size distribution were obtained in these cases to compare with PUUMC which were obtained using MDI and HMDI (Fig. 4b).

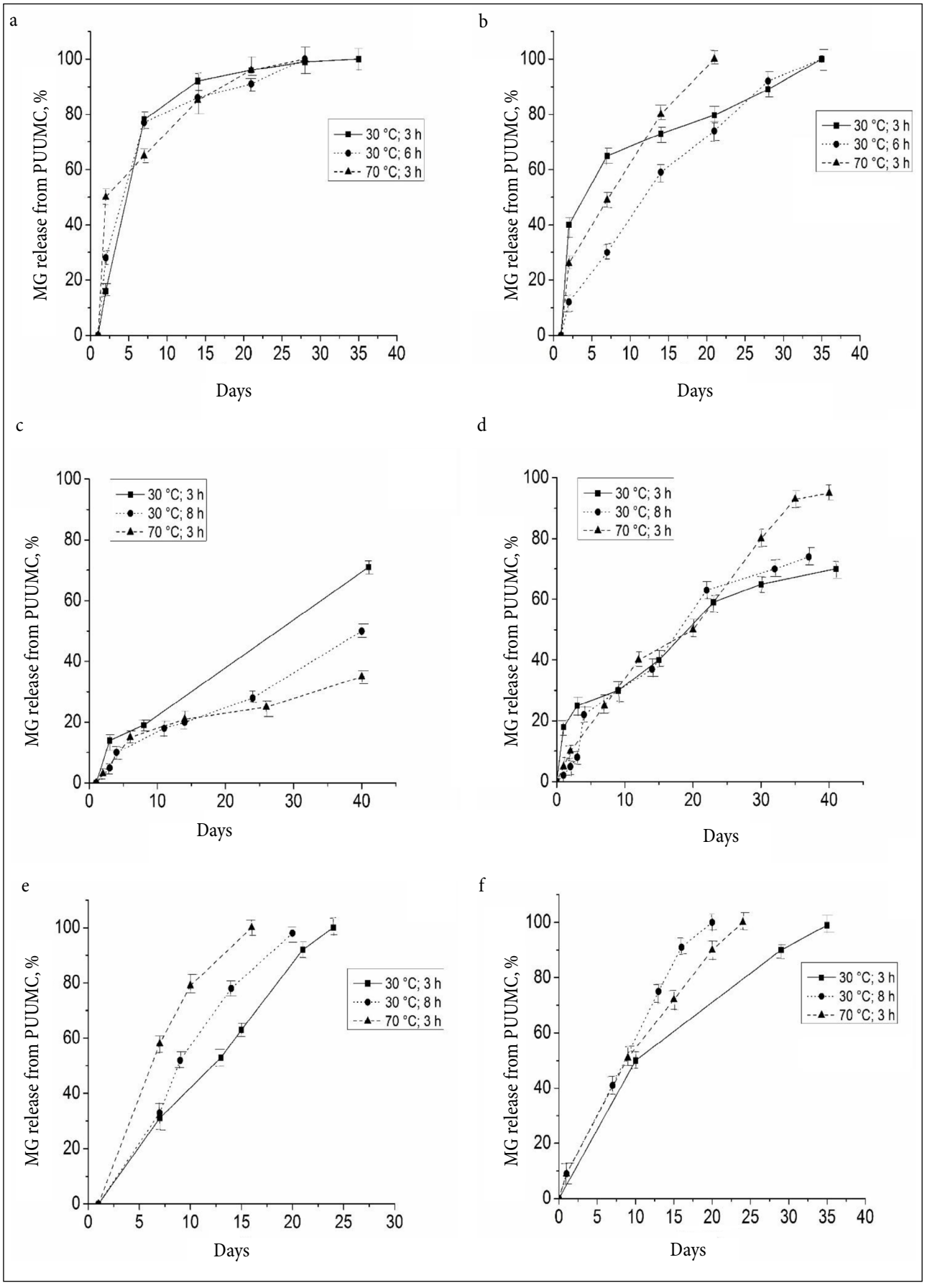

Fig. 6. The release rate of MG from PUUMC, which was encapsulated after (a, c, e) and during (b, d, f) the PUUMC synthesis using: MDI (a–b), HMDI (c–d) and TDI (e–f)

CONCLUSIONS

The poly(urethane–urea) microcapsules (PUUMC) containing the maltogenic α-amylase (MG) were prepared from poly(vinyl alcohol) and three different diisocyanates, TDI, MDI or HMDI, by the interfacial polyaddition technique in a new water/butyl acetate emulsion at temperatures from 30 to 70 °C. MG was chosen as the model of water-soluble biologically active material. Increasing the synthesis temperature and prolongation synthesis time resulted in increasing the yield of dried PUUMC. PVA and HMDI or MDI based PUUMC had slit-shaped pores. PUUMC from PVA and TDI had narrow distributions of pore size and the high surface area. Increasing diisocyanates reactivity (HMDI < MDI < TDI) resulted in increasing urethane linkages in the PUUMC shell. MG was efficiently encapsulated by two ways: during PUUMC synthesis or after it. The MG immobilization proceeded by covalent binding, entrapment and physical adsorption into PUUMC. EI of MG was increased when a more reactive diisocyanate was used for the synthesis of PUUMC and when MG was encapsulated during the PUUMC synthesis at lower temperature. The controlled release profile of MG was in most cases when TDI was used for the PUUMC synthesis.

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PVA IR ĮVAIRIŲ DIIZOCIANATŲ PAGRINDU GAUTŲ POLI(URETANKARBAMIDINIŲ) MIKROKAPSULIŲ SU INKAPSULIUOTU FERMENTU SINTEZĖ VANDENS / BUTILACETATO EMULSIJOJE IR TYRIMAS

Santrauka

Įvertinta trijų skirtingų diizocianatų: 1,6-heksametilendiizocianato (HMDI), 4,4'-difenilmetandiizocianato (MDI) ir toluen-2,4-diizocianato (TDI) įtaka poli(uretankarbamidinių) mikrokapsulių (PUUMC), kurių pagrindas yra poli(vinilalkoholis) (PVA), sandarai ir savybėms. PUUMC, į kurias įterptas vandeninis maltogeninės α-amilazės (MG) tirpalas, susintetintos tarpfazinės poliadicijos būdu 30–70 °C temperatūroje, naudojant naują mūsų pasiūlytą atvirkštinio tipo nekenksmingą aplinkai vandens / butilacetato emulsiją. Parodyta, kad diizocianato reakcingumas, sintezės temperatūra ir trukmė turi įtakos MG imobilizavimo efektyvumui ir jos pasišalinimui iš PUUMC. Fermento pasišalinimas iš PUUMC labiausiai priklauso nuo naudoto diizocianato.