VILNIUS UNIVERSITY
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Sandra
Mačiulytė

Synthesis and Investigation of Poly(urethane urea) Microcapsules with Immobilized Maltogenic α-Amylase

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Academic supervisor:

Prof. Dr. Saulutė Budrienė (Vilnius University, Physical Sciences, Chemistry – 03P).

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Chairman – **Prof. Dr. Ričardas Makuška** (Vilnius University, Physical Sciences, Chemistry – 03P).
Members:
Prof. Habil. Dr. Albertas Malinauskas (Center for Physical Sciences and Technology, Physical Sciences, Chemistry – 03P);
Prof. Dr. Jolita Ostrauskaitė (Kaunas University of Technology, Technological Sciences, Materials Engineering – 08T);
Dr. Teresė Rauckytė-Žak (University of Sciences and Technology in Bydgoszcz, Poland, Physical Sciences, Chemistry – 03P);
Doc. Dr. Aušvydas Vareikis (Vilnius University, Physical Sciences, Chemistry – 03P).

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Address: Naugarduko 24, LT-03225, Vilnius, Lithuania.

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FIZINIŲ IR TECHNOLOGIJOS MOKSLŲ CENTRAS

Sandra
Mačiulytė

Poliuretankarbamidinių mikrokapsulių su imobilizuota maltogenine α-amilaze sintezė ir tyrimas

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Mokslinė vadovė:
prof. dr. Saulutė Budrienė (Vilniaus universitetas, fiziniai mokslai, chemija – 03P)

Gynimo taryba:
Pirmininkas – **prof. dr. Ričardas Makuška** (Vilniaus universitetas, fiziniai mokslai, chemija – 03P).
Nariai:
prof. habil. dr. Albertas Malinauskas (Fizinių ir technologijos mokslų centras, fiziniai mokslai, chemija – 03P);
prof. dr. Jolita Ostrauskaitė (Kauno technologijos universitetas, technologijos mokslai, medžiagų inžinerija – 08T);
dr. Teresė Rauckytė-Žak (Bydgoščiaus technologijos mokslų universitetas, Lenkija, fiziniai mokslai, chemija – 03P);
doc. dr. **Aušvydas Vareikis** (Vilniaus universitetas, fiziniai mokslai, chemija – 03P).

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SUMMARY

1. INTRODUCTION

Relevance of the work. Due to the extremely broad applicability, the enzyme immobilization by microencapsulation method is a very promising field because of the use of this method allows to immobilize high amounts of enzyme and control their selective and dosage release. The immobilized enzyme can be characterized by high resistance to changes in pH, temperature and ionic strength. The most popular methods of immobilization of enzymes are encapsulation in liposomes and gelatin-agar or other complexes. However, each of these methods has its drawbacks, for example, when complexation is used, the capsules are sensitive to changes in the environment. When liposomes are used the small amount of enzyme is immobilized, furthermore such capsules are characterized by low thermal and mechanical stability, so they can not be adapted to industrial processes. These drawbacks can be removed by encapsulation of enzyme in polyurethane (PU) or poly(urethane-urea) (PUU) microcapsules (PUUMc) which have good mechanical and thermal stability and are biocompatible.

The articles on the enzyme encapsulation in the PU or PUU microcapsules were not found. Maybe because there are several challenges when enzymes are encapsulated. One of the disadvantages of PUU is its hydrophobicity, so such microcapsules are not congenerous to the most enzymes. But this problem can be solved by using poly(vinyl alcohol) (PVA) instead of polyol for synthesis of PUUMc, which increases the hydrophilicity of the PUUMc. The synthesized PUUMc shell is porous with free isocyanate groups, so enzyme can be immobilized not only by entrapment and adsorption but also by covalent attachment, so increasing the enzyme stability in the PUUMc.

The aim of this work was to synthesize poly(urethane-urea) microcapsules (PUUMc) by inverse emulsion method, to study their properties and the suitability for immobilization of maltogenic α-amylase (MG).

The objectives of the research are the following:

1. To find optimal conditions of PUUMc synthesis from PVA and 1,6-hexamethylene diisocyanate (HMDI) in water/toluene emulsion.
2. To find optimal conditions of PUUMc synthesis from PVA and diizocyanate (HMDI, 4,4'-methylene diphenyl diisocyanate (MDI), 2,4-toluene diisocyanate (TDI)) or blend of diizocyanate (HMDI, MDI or TDI) and diamine (ethane-1,2-diamine (EDA), propane-1,3-diamine (PDA) and butane-1,4-diamine (BDA)) in water/butyl acetate emulsion.
3. To find optimal conditions of PUUMc synthesis from PVA modified with 3-aminopropyltriethoxysilane (PVA/APTES) and HMDI in water/butyl acetate emulsion.
4. To investigate the structure of PUUMc, their thermal properties, surface area and porosity.
5. To immobilize MG in PUUMc and to determine the efficiency of MG immobilization and its stability in capsules.

Scientific novelty and practical value of the dissertation. For the first time the thermostable and mechanically resistant PUUMc were synthesized by the interfacial polyaddition reaction between biocompatible and hydrophilic PVA and various diisocyanates (HMDI, MDI or TDI) or blend of diisocyanate (HMDI, MDI or TDI) and diamine (EDA, PDA or BDA) in inverse emulsion: water/toluene or water/butyl acetate. Also, for the first time PUUMc were synthesized from PVA modified with APTES and HMDI. The PUUMc were thoroughly characterized including yields of PUUMc shell, quantity of isocyanate and amine groups and elements (Si, C, N, H), porosity and thermal stability. The influence of various reaction conditions such as initial molar ratio of starting materials, catalyst concentration, synthesis time and temperature and stirring rate on the

PUUMc formation was evaluated. For the first time MG as the model enzyme was immobilized in this type of PUUMc. The MG was immobilized by two ways: during the PUUMc synthesis or after it. Enzymatic activity and stability of immobilized MG were studied. The PUUMc are suitable to immobilize other enzymes, because of their synthesis is possible at low temperature and PUUMc shell is porous with free isocyanate groups. The PUUMc have potential application in biotechnology, especially when dosage of enzyme release and industrial processes requiring thermal stability and mechanical strength of the PUUMc are needed.

Defensive statements:

- The structure of PUUMc shell, synthesized from PVA or PVA/APTES and various diisocyanate and blend of diisocyanate and diamine, is crosslinked.
- The PUUMc shell is porous, the pores are slit-shaped or cylindrical, their size is suitable for immobilization of enzyme.
- The decomposition of PUUMc shell proceeds in two stages: at the first stage urethane and urea linkages are decomposed, while at the second stage the residues of polyene chain.
- The PUUMc are suitable to immobilize MG by entrapment and both physical sorption and covalent methods, because of the shell is porous and has free isocyanate groups.
- The release of immobilized MG from the PUUMc is gradual.

Approbation of the research results. Results of the research were presented in 2 scientific papers in the journals included into the database of *Clarivate Analytics Web of Science.* Results were also reported in 9 international conferences.

Structure of the doctoral dissertation. The doctoral dissertation is written in Lithuanian and contains the following parts: Introduction with the motivation of the research objectives, Literature review, Experimental Part, Results and discussion, Conclusions, List of references (245) and List of Scientific Publications. The material of

the doctoral dissertation is presented in 189 pages, including 5 schemes, 50 figures and 33 tables.

MATERIALS AND METHODS

Main materials. Poly(vinyl alcohol) (PVA) (Mw = 100000, the degree of hydrolisation 86-89 mol %, viscosity (4 % in water, 20 ºC) is 34-45 mPa·s), 1,6-hexamethylene diisocyanate (HMDI) and ethane-1,2-diamine (EDA) were obtained from Fluka. 4.4'-Methylene diphenyl diisocyanate (MDI), 2,4-toluene diisocyanate (TDI), propane-1,3-diamine (PDA) and butane-1,4-diamine (BDA), were obtained from Aldrich. The catalyst dibutyl tin dilaurate (DBTDL) was obtained from Merck. 3-aminopropyltriethoxysilane (APTES) was obtained from Sigma-Aldrich.

Poly(vinyl alcohol) modification with APTES. The PVA solution (0.6 M) was modified with APTES (molar ratio of PVA to APTES was 1:0.05-1:0.55) in acidic conditions. The solution was stirred at 40-80 ºC for 1-24 h. to complete hydrolization reaction.

Synthesis of the PUU microcapsules. Poly(urethane-urea) (PUU) microcapsules (PUUMc) from PVA and various diisocyanates (HMDI, MDI or TDI) or diisocyanate (HMDI, MDI or TDI) blend with diamine (EDA, PDA or BDA) or PVA/APTES and HMDI were synthesized by the interfacial polyaddition method in w/o (water/toluene or water/butyl acetate) emulsion.

Characterization of PUU microcapsules. Synthesized PUUMc were examined by FT-IR spectroscopy (Perkin Elmer FT-IR spectrometer FRONTIER), elemental analysis (Thermo Scientific Flash 2000 series CHNS-O), surface area and total pore volume (Micromeritics Tristar II) and functional group chemical analysis. Size and morphology of synthesized microcapsules were evaluated by Scanning Electron Microscopy (Hitachi SU 70) and optical microscopy (Olympus BX 51). Thermal properties of PUU were determined thermogravimetrically (Perkin Elmer Pyris 1 or STA 6000).

Enzymes. Maltogenic α-amylase (MG) from *Bacillus stearothermophilus* (specific activity 4000 MANU/mg) was obtained from Novozymes.

Immobilization. The immobilization of MG was carried out by two ways: during the PUUMc synthesis or after it. In the first case, the MG solution in the sodium citrate buffer (0.1 M, pH=5) was added to the aqueous phase. In the second case, the mixture of the enzyme, buffer and PUUMc immediately after synthesis was stirred at 40 ºC for 30 min and then left at 4 ºC overnight.

Enzyme assay. Activity of native MG was assayed by incubation of enzyme sample with liquefied potato starch (Dextrose equivalent ≈ 2-5 %) as substrate in 0.1 M sodium citrate buffer (pH = 5.0) at 40 ºC for 20 min. Activity unit of native or immobilized MG was defined as the amount of enzyme which under standard conditions (at 40 ºC, pH = 5.0) produced 1 μmol of reduced sugars per minute. Amount of reducing sugars produced in this reaction was determined spectrophotometrically by Neocuproine method. Activity of the immobilized MG was determined in the same way except that immobilized enzyme was added by weight (0.1 g) to the substrate solution and incubation was carried out under stirring (300 rpm). Efficiency of immobilization (EI) was defined as percentage ratio of immobilized enzyme activity vs native enzyme activity.

Determination of release rate of MG immobilized onto PUUMc. Immobilized preparations were held in sodium citrate buffer (pH = 5.0) at 4 ºC to determine their stability. The catalytic activity of MG in PUUMc was expressed as 100 % and the initial release rate after the immobilization was expressed as 0 %.

3. RESULTS AND DISCUSSION

The present study focuses on a detailed investigation of synthesis of PUUMc from PVA and diisocyanate (DI) or blend of diisocyanate and diamine (DA), or PVA/APTES and HMDI. The synthesis of PUUMc was carried by interfacial polyaddition reaction of PVA (or PVA/APTES) and DI (or blend of DI and DA) in W/O emulsion. The microencapsulation process can be divided into two consecutive steps: the first emulsification step governs the size and the size distribution of the microcapsules, in the second step shells of the microcapsules are formed by interfacial polyaddition reaction of DI (or blend of DI and DA) and PVA in the dispersed phase. The effects of the reaction conditions, such as an initial molar ratio of components, reaction time and temperature on the yield, surface area, porosity and structure of the PUUMc shell were particularly studied. Possibility to apply obtained PUUMc for immobilization of MG was investigated. Properties and release rate of immobilized MG were also evaluated.

3.1 Synthesis and study of PUUMc obtained from PVA and HMDI in water/toluene emulsion

PUUMc were synthesized from PVA and HMDI by polyaddition reaction in water/toluene emulsion when initial molar concentration of PVA was 0.2 M (mole of repeating unit of PVA). The reaction time was 2 hours and temperature was 70 ºC. The stirring rate of reaction mixture was varied from 400 to 1500 rpm ([PVA]:[HMDI]=1:9) and it resulted in decreasing the mean diameter of PUUMc from 425 to 30 μm and a slight decreasing of yields of PUUMc shell from 66 % to 62 %. In all other cases the stirring rate was 400 rpm.

Changing the initial molar ratio of PVA and HMDI from 1:1 to 1:9 resulted in increasing yield of PUUMc shell from 52 % to 66 % (Table 1) and quantity of isocianate groups from 2 % to 6 % (determination of the quantity of isocyanate groups was performed immediately after synthesis of PUUMc).

Table 1

Results of yield and porous properties of PUUMc shell and EI of MG in PUUMc

Molar ratio of PVA and HMDI	DBTDL (%)	Yield of PUUMc shell (%)	Surface area (m^2g^{-1})	Total pore volume (cm^3g^{-1})	EI^1 of MG (%)	EI^2 of MG (%)
1:1	-	52	-	-	8 ± 0.4	9 ± 0.5
1:3	-	53	3.6	0.01	19 ± 1.0	12 ± 0.6
1:6	-	55	12.3	0.04	31 ± 1.6	13 ± 0.7
1:9	-	66	20.1	0.03	19 ± 1.0	15 ± 0.9
1:9	0.2	67	58.0	0.23	60 ± 3.0	33 ± 1.7
1:9	0.4	74	56.9	0.26	58 ± 2.8	22 ± 1.1
1:9	0.6	75	57.0	0.25	54 ± 2.8	23 ± 1.3
1:9	1.0	77	-	-	23 ± 1.1	20 ± 1.0

EI^1 – MG immobilization after PUUMc synthesis

EI^2 – MG immobilization during PUUMc synthesis

Aliphatic diisocyanates are less reactive than aromatic ones [1], and for this reason, we used the catalyst DBTDL. This has specific catalytic effect on the reaction of isocyanate group with hydroxyl group and no measurable effect on the reaction of isocyanate group with amino group [2]. The thickness of shell was increased by using higher concentration of DBTDL and the yield of PUUMc shell increased (Table 1) but the quantity of isocianate groups was decreased from 6 % to 1.5 % at the same time. The size of PUUMc was in the same range and was independent on DBTDL amount.

Surface area and porosity are important physical properties and contribute to understanding the formation, structure and potential application of microcapsules. The instrument Tristar II used the Brunauner–Emmett–Teller (BET) equation to describe the surface area and Barrett–Joyner–Halenda (BJH) equation to describe the pore size and volume. The nitrogen adsorption and desorption isotherms were determined at -196º C. PUUMc display the type IV of gas sorption isotherm with the H3 hysteresis loops wich provided distribution of slit-shaped pores width [3]. Change in PVA to HMDI initial molar ratio in synthesis of PUUMc from 1:1 to 1:9 resulted in

increasing urea segments and cross-links in PUUMc and surface area from 3.6 to 20.1 $m^2 g^{-1}$ (Table 1).The dominant pore size (i.e. the pore size that occupies the largest volume in the PUUMc shell) were 35-40 nm when PVA and HMDI molar ratios were 1:6 and 1:9 and 54-60 nm, when ratio was 1:3. Influence of catalyst DBTDL on the porous properties and surface area of PUUMc was more significant. The surface area was increased from 20.1 to 58.0 m^2g^{-1} and the total pore volume was increased from 0.03 to 0.23 cm^3g^{-1}, when 0.2 % of DBTDL was used for microcapsules synthesis in comparison to results without catalyst. However, the further increasing of DBTDL amout does not affect the PUUMc porosity (Table 1). In these cases dominant pore size was 30-38 nm.

Maltogenic α-amylase (MG) is one of the most important enzyme in the dairy industry that catalyses the conversion of starch into maltose which is an important sugar for many applications in the food and pharmaceutical industry [4]. This enzyme exhibits both an endo and an exoaction pattern, with a high degree of multiple attack action and with endoaction increasing with increases in temperature [5]. MG was immobilized in PUUMc by entrapment, physical adsorption and by covalent attachment between amino and hydroxyl groups of MG and free isocyanate groups of PUUMc shell. The reaction of free isocyanate groups with primary amines is much faster than the reaction with hydroxyl groups and water. Immobilization efficiency of enzyme by physical adsorption depends on the surface area and porosity of PUUMc shell, when shell has a high surface area and total pore volume, the enzyme could easily adsorb into pores without diffusion limits and steric hindrance [6, 7]. According to the literature, enzyme can easily get into pores when diameter is larger than 30 nm [8] or smaller than 100 nm [6].

The best results of EI of MG were when 0.2 % catalyst DBTDL was used for PUUMc synthesis (Table 1). This is due to DBTDL which accelerated isocyanate/hydroxyl reaction and as a result increasing in surface area, pore volume and pore size of shell of PUUMc was obtained. The entrapment and adsorption of MG in

PUUMc was increased in such cases. The increasing quantity of catalyst from 0.2 % to 1.0 % resulted in decreasing EI of MG, when MG was encapsulated after and during PUUMC synthesis, due to decreasing quantity of free isocyanate groups in PUUMC from 6.0 % to 1.5 %, and less probability of covalent attachment of MG to the PUUMc.

Investigation of MG release from PUUMc partially disclosed by which way MG was immobilized into PUUMc: covalent attachment or physical adsorption. The immobilized MG release studies from PUUMc showed one initial burst release followed by a slow-release phase (extended release) in some 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 [9].

The MG release from PUUMc was slowly, when it was immobilizated during PUUMc synthesis. It is supposed, that main part of MG was entrapped in PUUMc rather than adsorbed into PUUMc shell and as a result the shell of PUUMc was retarded the MG mobility through the network. The burst release can be indentified in two cases, when the PUUMc synthesis was carried out with 0.2 % DBTDL either initial molar ratio of PVA and HMDI was 1:1, because of about 30 % of immobilized MG was released in the first 2-3 days (Fig.1.). The high storage stability exhibited the immobilized MG in PUUMc, which were obtained when initial molar ratio of PVA and HMDI was 1:9 and reaction was not catalyzed. In this case only 36 % of the immobilized MG was released from PUUMc after 23 days (Fig.1).

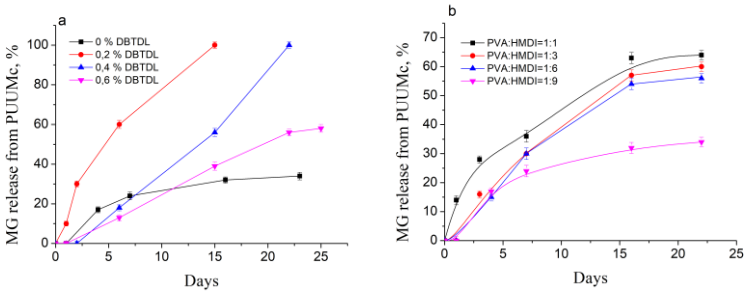


Fig. 1. Release rate of MG from PUUMc as a function of DBTDL concentration (a) and initial molar ratio of PVA and HMDI (b), when MG was immobilizated during PUUMc synthesis.

3.2 Synthesis and study of PUUMc from PVA and various diisocyanates in water/butyl acetate emulsion

Second series of PUUMc were synthesized from PVA and three different diisocyanates (HMDI, MDI and TDI) in water/butyl acetate emulsion. The volume ratio of water to butyl acetate was 1:3, initial molar concentration of PVA was 0.6 M, 0.2 M and 0.1 M when for PUUMc synthesis was used HMDI, MDI and TDI, respectively. The initial molar ratio of PVA and HMDI, MDI or TDI was 1:9 and 1:6, respectively. The organic phase was 5.0 % of Span 85 with HMDI or MDI and 3.0 % of Span 85 with TDI. 1.0 % of DBTDL (with respect to PVA) was used when HMDI was used as DI. Increasing the PUUMc synthesis temperature from 30 to 70 ºC (for 3 h) and prologation synthesis time from 2 to 8 hours (at 30 ºC) resulted in increasing the yield of PUUMc shell when they were synthesized from PVA and any of DI (Fig. 2). The highest yields of PUUMc shell were in the case when MDI was used for PUUMc synthesis and the lowest yields were obtained when TDI was used.

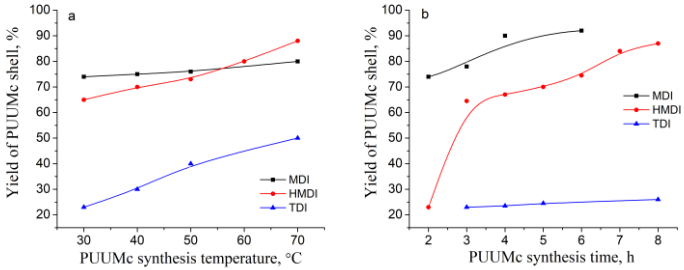


Fig. 2. The yield of PUUMc shell synthesized from PVA and different DI as a function of synthesis temperature (for 3 h) (a) and time (at 30 ºC) (b).

Table 2

The porosity properties and surface area of lyophilized PUUMc

No.	Initial conditions of PUUMc synthesis			Surface area (m^2g^{-1})	Total pore volume (cm^3g^{-1})	Pore size* (nm)
	DI	T (°C)	t (h)			
1		30	3	58.0	0.22	30
2	HMDI	70	3	77.2	0.39	32
3		30	8	41.9	0.14	32
4		30	3	30.3	0.16	24
5	MDI	70	3	162.9	0.77	19
6		30	6	14.1	0.06	24
7		30	3	79.5	0.21	6
8	TDI	70	3	176.6	0.48	6
9		30	8	58.4	0.13	5

* Dominant pore size, i.e. the pore size that occupies the largest volume in the PUUMc shell.

As in the previous case, the PUUMC from PVA and DI corresponded to type IV of gas sorption isotherms. The H3 hysteresis loops were typical for all samples of PUUMc, which were obtained

from PVA and MDI or HMDI, whereas H1 hysteresis loops, were obtained when TDI was used. Increasing the PUUMc synthesis temperature from 30 to 70 ºC resulted in increasing the surface area and total pore volume when PUUMc were synthesized from PVA and any of DI (Table 2). The surface area and total pore volume less depended on the PUUMc synthesis time. Prolongation synthesis time resulted in decreasing the surface are and total pore volume (Table 2) and also pore size distribution of PUUMc became narrower.

As in 3.1 chapter, MG was immobilized in PUUMc from PVA and various DI. EI of MG was greatly dependent on the reactivity of DI, and increasing in reactivity of DI (HMDI<MDI<TDI) resulted in increasing EI of MG after and during the PUUMc synthesis. Increasing the PUUMc synthesis temperature from 30 to 70 ºC resulted in decreasing EI of MG from 21 to 8 % and from 27 to 10 %, when PUUMc were obtained from HMDI; from 55 to 25 % and from 73 to 40 %, when MDI was used, from 70 to 15 % and from 80 to 70 %, when PUUMc were obtained from TDI, and MG was immobilized after PUUMc synthesis or during it, respectively. Prolongation of the PUUMc synthesis time from 3 to 8 h resulted in decreasing EI of MG from 21 to 6 % and from 27 to 17 % when PUUMc were obtained from HMDI, from 55 to 35 % and from 73 to 50 %, when MDI was used, from 70 to 18 % and from 80 to 42 %, when TDI was used, and MG was immobilized after PUUMc synthesis or during it, respectively.

The release rates of immobilized MG from PUUMc obtained from PVA and different DI are shown in Fig. 3. The release of MG from PUUMc has burst release of 40-50 % and 20-30 % in the 4 days when MDI and HMDI were used for PUUMc synthesis, respectively (Fig. 3 a-d). Incomplete release of MG from PUUMc was obtained when HMDI was used for PUUMc synthesis. The controlled release of MG

could be observed when TDI was used for the PUUMc synthesis in all cases (Fig. 3 e-f).

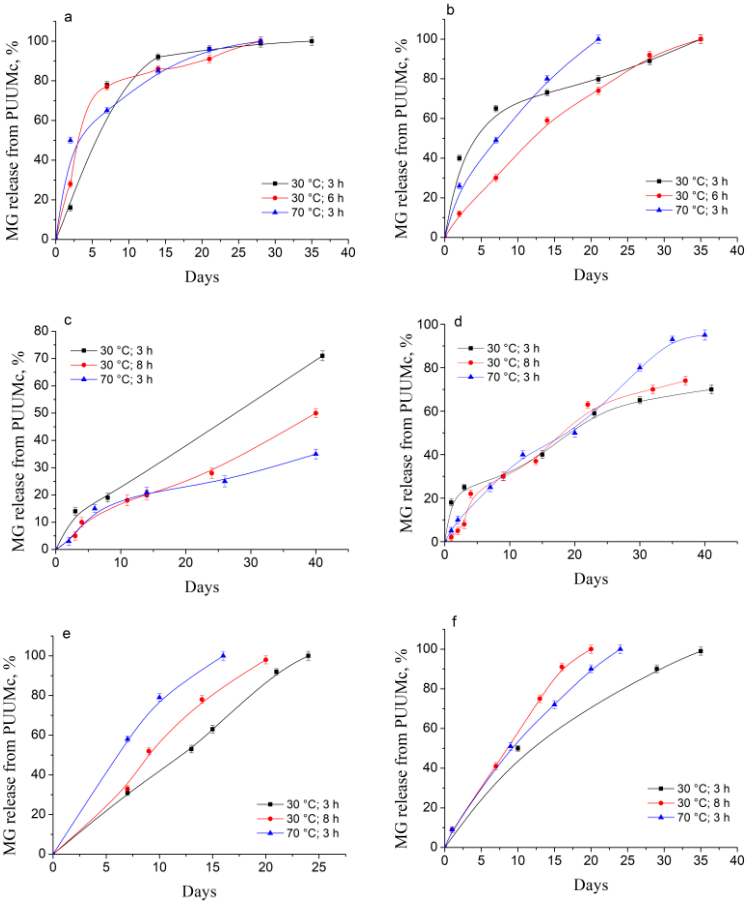


Fig.3. The release rate of MG from PUUMc, which was immobilized after (a, c, d) and during (b, d, f) the PUUMc synthesis using: MDI (a, b), HMDI (c, d) and TDI (e, f).

3.3 Synthesis and study of PUUMc from PVA and blend of diizocyanate and diamine

Other series of PUUMc were synthesized from PVA and blend of one of three different diisocyanates (HMDI, MDI and TDI) and one of three diamines (EDA, PDA and BDA) in water/butyl acetate emulsion. The synthesis conditions were the same as in 3.2 section, only one of three diamines (DA) was added into synthesis mixture. The DA was added together with DI or after 30 minutes from DI insertion. The initial molar ratio of PVA and DA was 1:1, 1:3 and 1:6.

Increasing the molar amount of DA resulted in increasing yield of PUUMc shell (Table 3) and decreasing quantity of isocyanate groups. Also the yield of PUUMc was higher when DA was added after 30 min from DI insertion. The PUUMc obtained from PVA and blend of DI and DA display the type IV gas sorption isotherm with the H3 hysteresis loops. Increasing amount of DA in synthesis mixture resulted in decreasing surface area of PUUMc, when HMDI or TDI were used. However, when the PUUMc were synthesized from PVA and blend of MDI and DA, the surface area was increased when initial molar ratio of PVA and DA was changed from 1:1 to 1:3, but it was decreased, when molar ratio was 1:6 (Table 3).

In the most cases increasing the molar amount of DA resulted in increasing EI of MG irrespective on MG immobilization way (Table 4). The highest EI of MG was in PUUMc with TDI and the lowest was with HMDI. Comparing the PUUMc from PVA and DI with PUUMc from PVA and blend of DI and DA, it could be observed that the EI of MG was increased only in PUUMc from PVA and blend of HMDI and DA, but in other cases EI of MG was decreased.

The release rate of MG depends on DA and DI used for the synthesis and immobilization way of MG. The release rate of MG was slower from PUUMc which were synthesized from PVA and blend of HMDI and DA. After 30 days, 10-89 % and 37-89 % of immobilized MG were left, when MG immobilized after PUUMc synthesis or during it, respectively. The release rate of MG from PUUMc was

faster, when PUUMc were synthesized from PVA and blend of TDI and DA.

Table 3

Yield, porosity properties and surface area of PUUMc obtained from PVA and blend of DI and DA

Molar ratio of PVA and DA	DI used for PUUMc synthesis								
	HMDI	MDI	TDI	HMDI	MDI	TDI	HMDI	MDI	TDI
	Yield of PUUMC (%)			Surface area (m^2g-1)			Total pore volume (cm^3g-1)		
EDA									
1:1	60	71	12	49,8	35,5	80,7	0,26	0,18	0,27
1:3	66	81	13	22,6	92,8	42,3	0,15	0,45	0,14
1:6	81	81	13	22,0	87,2	7,0	0,14	0,39	0,03
1:1*	73	83	15	29,4	46,3	53,9	0,19	0,23	0,14
1:3*	81	86	26	29,3	82,7	33,9	0,20	0,24	0,15
1:6*	88	87	34	25,4	53,4	5,3	0,16	0,36	0,01
PDA									
1:1	54	69	15	18,8	99,6	24,5	0,10	0,36	0,13
1:3	76	81	19	17,3	106,3	16,7	0,11	0,43	0,11
1:6	79	85	26	6,8	61,5	14,6	0,04	0,31	0,06
1:1*	67	74	24	17,7	70,7	34,3	0,07	0,35	0,13
1:3*	82	85	31	17,5	86,7	21,2	0,11	0,40	0,12
1:6*	85	88	49	13,7	61,4	15,1	0,09	0,31	0,05
BDA									
1:1	64	78	19	16,9	76,9	41,0	0,08	0,33	0,18
1:3	69	75	25	13,3	78,8	39,8	0,08	0,36	0,16
1:6	81	83	35	12,9	56,6	9,6	0,07	0,29	0,04
1:1*	78	82	17	24,3	77,4	67,8	0,11	0,34	0,20
1:3*	85	85	18	23,4	85,6	13,1	0,10	0,34	0,07
1:6*	89	89	55	21,9	70,6	16,4	0,09	0,34	0,08

* The DA was added after 30 minutes from DI insertion.

Table 4

Results of immobilization of MG in PUUMc obtained from PVA and blend of DI and DA

Molar ratio of PVA and DA	DI used for PUUMc synthesis					
	HMDI	MDI	TDI	HMDI	MDI	TDI
	EI^1 of MG (%)			EI^2 of MG (%)		
			EDA			
1:1	19 ± 1,0	23 ± 1,2	39 ± 2,0	13 ± 0,7	38 ± 1,9	39 ± 2,0
1:3	23 ± 1,2	45 ± 2,3	61 ± 3,1	27 ± 1,4	40 ± 2,0	74 ± 3,7
1:6	44 ± 2,2	48 ± 2,4	63 ± 3,2	44 ± 2,2	44 ± 2,2	34 ± 1,7
1:1*	27 ± 1,4	26 ± 1,3	43 ± 2,2	36 ± 1,8	24 ± 1,2	51 ± 2,6
1:3*	33 ± 1,7	26 ± 1,3	92 ± 4,6	38 ±1,9	27 ± 1,4	82 ± 4,1
1:6*	35 ± 1,8	29 ± 1,5	56 ± 2,8	41 ± 2,1	39 ± 2,0	48 ± 2,4
			PDA			
1:1	26 ± 1,3	34 ± 1,7	48 ± 2,4	24 ± 1,2	32 ± 1,6	54 ± 2,7
1:3	29 ± 1,5	36 ± 1,8	49 ± 2,5	26 ± 1,3	40 ± 2,0	74 ± 3,7
1:6	31 ± 1,6	41 ± 2,1	56 ± 2,8	35 ± 1,8	43 ± 2,2	78 ± 3,9
1:1*	22 ± 1,1	30 ± 1,5	48 ±2,4	17 ± 0,9	40 ± 2,0	84 ± 4,2
1:3*	27 ± 1,4	40 ± 2,0	50 ± 2,5	22 ± 1,1	45 ± 2,3	92 ± 4,6
1:6*	36 ± 1,8	47 ± 2,4	54 ± 2,7	25 ± 1,3	46 ± 2,3	93 ± 4,7
			BDA			
1:1	23 ± 1,2	13 ± 0,7	33 ± 1,7	30 ± 1,5	30 ± 1,2	42 ± 2,1
1:3	24 ± 1,2	13 ± 0,7	37 ± 1,9	33 ± 1,7	32 ± 1,6	56 ± 2,8
1:6	31 ± 1,6	49 ± 2,5	48 ± 2,4	45 ± 2,3	38 ± 1,9	88 ± 4,4
1:1*	22 ± 1,1	34 ± 1,7	28 ± 1,4	22 ± 1,1	36 ± 1,8	63 ± 3,2
1:3*	29 ± 1,5	35 ± 1,8	48 ± 2,4	22 ± 1,1	38 ± 1,9	91 ± 4,6
1:6*	36 ± 1,8	52 ± 2,6	69 ± 3,5	34 ± 1,7	50 ± 2,6	77 ± 3,9

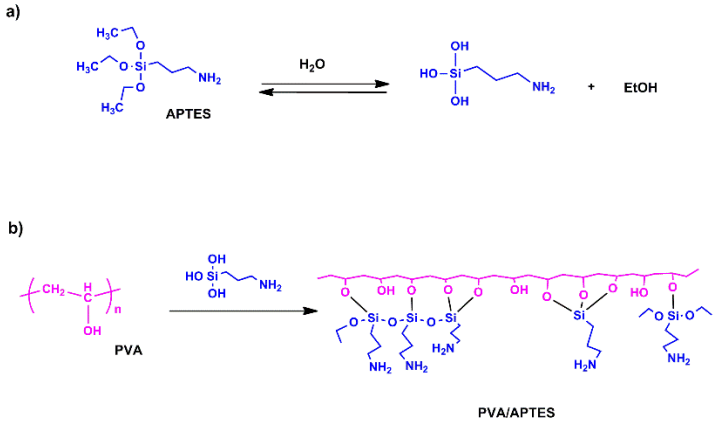
* The DA was added after 30 minutes from DI insertion.

EI^1 – MG immobilization after PUUMc synthesis

EI^2 – MG immobilization during PUUMc synthesis

3.4 Synthesis and study of PUUMc from PVA/APTES and HMDI

First of all, PVA has been modified with APTES in acidic conditions and only then the PUUMc were synthesized from PVA/APTES and HMDI in water/butyl acetate emulsion. PVA modification process took place in two stages: in the first stage silanol groups were formed during hydrolysis of APTES (Scheme 1a), the second stage involved polycondensation of PVA and hydrolyzed APTES (Scheme 1b).



Scheme 1. APTES hydrolysis (a) and modification of PVA (b).

The completion of the polycondensation reaction between PVA and APTES was confirmed by FT-IR spectra and TGA analysis. The modified PVA FT-IR spectrum shows a typical absorption band of PVA/APTES at 944 and 917 cm^{-1} which is assigned to the Si-OH stretching vibration. The vibration band observed at 1046 cm^{-1} refers to the stretching vibration of Si-O-C bonds and the peak at 1242 cm^{-1} is related to the Si-CH_x-. The C-N vibration was observed at 1473, 1463 and 1142 cm^{-1} and the vibration band at 1576 cm^{-1} is caused by NH group. The decomposition of the pure and modified PVA (PVA/APTES) samples were analyzed in the temperature range from 30 to 600 ºC with a constant rate of 10 ºC/min under nitrogen atmosphere. Decomposition of pure PVA proceeded in two distinct

weight loss stages according to TGA and DTGA results (Fig. 4). The first stage of decomposition is the elimination of water and residual acetate groups and the second stage is dominated by chain-scission reactions. The first decomposition stage of PVA/APTES was splitted into two consecutive steps (Fig. 4). The first step is the elimination of water and residual acetate groups and the second step corresponded to the removal of amino groups from APTES segments. The second stage of decomposition of PVA/APTES mainly involves decomposition of chain-scission reaction and C-Si bond.

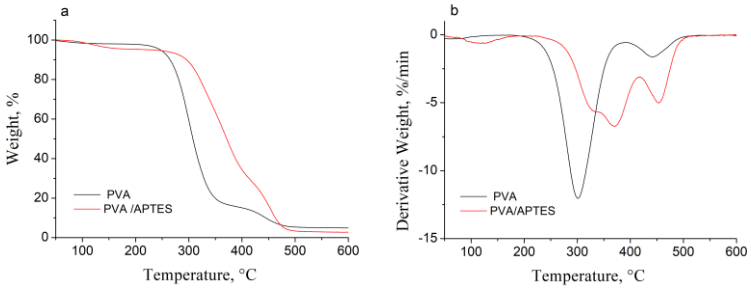


Fig.4. TGA (a) and DTGA (b) curves of PVA and PVA/APTES (PVA modification conditions: 40 ºC, 1 h, [PVA]:[APTES]=1:0.05).

The PUUMc synthesis conditions were the same as those given in 3.2 section, with the exception that PVA/APTES was used for synthesis. Changing the initial moral ratio of PVA and APTES from 1:0.05 to 1:0.55 resulted in increasing yield of PUUMc (Table 5), also increasing quantity of isocyanate (from 3.1 % to 4.0 %) and amino groups (from 0.3 % to 0.8 %) and Si (from 0.1 % to 1,4 %) in the PUUMc shell. Increasing the PVA modification temperature and prolongation of PVA modification time resulted in increasing the yield of PUUMc (Table 5).

Table5

Yield, porosity properties and surface area of PUUMc obtained from PVA/APTES and HMDI

Initial conditions of PVA modification			Yield of PUUMc (%)	Surface area (m^2g^{-1})	Total pore volume (cm^3g^{-1})	Pore size* (nm)
[PVA]:[APTES]	T (°C)	t (h)				
1:0.05	40	1	36	26,1	0,10	61
1:0.10	40	1	41	29,8	0,13	51
1:0.25	40	1	38	27,1	0,18	54
1:0.35	40	1	44	11,4	0,07	26
1:0.55	40	1	60	1,9	0,01	36
1:0.25	50	1	40	50,9	0,26	43
1:0.25	60	1	45	53,5	0,28	50
1:0.25	70	1	42	54,8	0,31	41
1:0.25	80	1	65	57,2	0,28	51
1:0.25	40	2	35	19,3	0,08	66
1:0.25	40	4	35	47,7	0,25	52
1:0.25	40	5	39	43,7	0,21	49
1:0.25	40	12	47	51,5	0,31	48
1:0.25	40	24	50	54,3	0,30	58

* Dominant pore size, i.e. the pore size that occupies the largest volume in the PUUMc shell.

The profile of nitrogen adsorbtion/desorption isotherms of all PUUMc samples is assigned to the IV type with the H3 hysteresis loops. Increasing the amount of APTES in the PVA modification reaction resulted in decreasing the surface area, total pore volume and pore size (Table 5), because of the shell crosslink density resulting from reactions between -NCO and -NH_2 groups at the end of the grafts of PVA is increased as the amount of APTES is increased and as a result the open pores in the PUUMc shell become closed. The PUUMc porosity properties and surface area were less dependent on PVA modification temperature (Table 5). Surface area and total pore volume of PUUMc shell were increased when PVA modification time was longer (Table 5).

Table 6

Results of EI of MG in PUUMc obtained from PVA/APTES and HMDI

Initial conditions of PVA modification			EI^1 of MG (%)	EI^2 of MG (%)
[PVA]:[APTES]	T (ºC)	t (h)		
1:0.05	40	1	22 ± 1,0	15 ± 0,9
1:0.10	40	1	18 ± 1,0	24 ± 1,2
1:0.25	40	1	19 ± 1,0	16 ± 0,8
1:0.35	40	1	18 ± 0,9	28 ± 1,4
1:0.55	40	1	10 ± 0,6	19 ± 1,0
1:0.25	50	1	15 ± 0,8	18 ± 0,9
1:0.25	60	1	12 ± 0,6	20 ± 1,0
1:0.25	70	1	12 ± 0,6	18 ± 0,9
1:0.25	80	1	18 ± 0,9	17 ± 0,9
1:0.25	40	2	17 ± 0,9	18 ± 0,9
1:0.25	40	4	15 ± 0,8	20 ± 1,0
1:0.25	40	5	17 ± 0,9	18 ± 0,9
1:0.25	40	12	21 ± 1,0	17 ± 0,8
1:0.25	40	24	14 ± 0,7	22 ± 1,1

EI^1 – MG immobilization after PUUMc synthesis

EI^2 – MG immobilization during PUUMc synthesis

The EI of MG in PUUMc depended on PVA modification conditions (Table 6). Changing initial molar ratio of PVA and APTES from 1:0,05 to 1:0.55 resulted in decreasing EI of MG after PUUMc synthesis, because of surface area, total pore volume and pore size of PUUMc shell were decreased so probability of physical adsoption of MG into PUUMc shell was decreased. (Table 5). The EI of MG was varied from 15 to 28 %, when it was immobilized during PUUMc synthesis. The EI of MG was less dependent on PVA modification temperature and time and it was in the range of 12-22 % and 14-22 %, respectively (Table 6).

In the most cases, the major part of immobilized MG was released for the first 15-20 days, and then its release was slowed down or stopped at all (Fig. 5). The MG release from PUUMc was slower, when it was immobilized during PUUMc synthesis, in most cases.

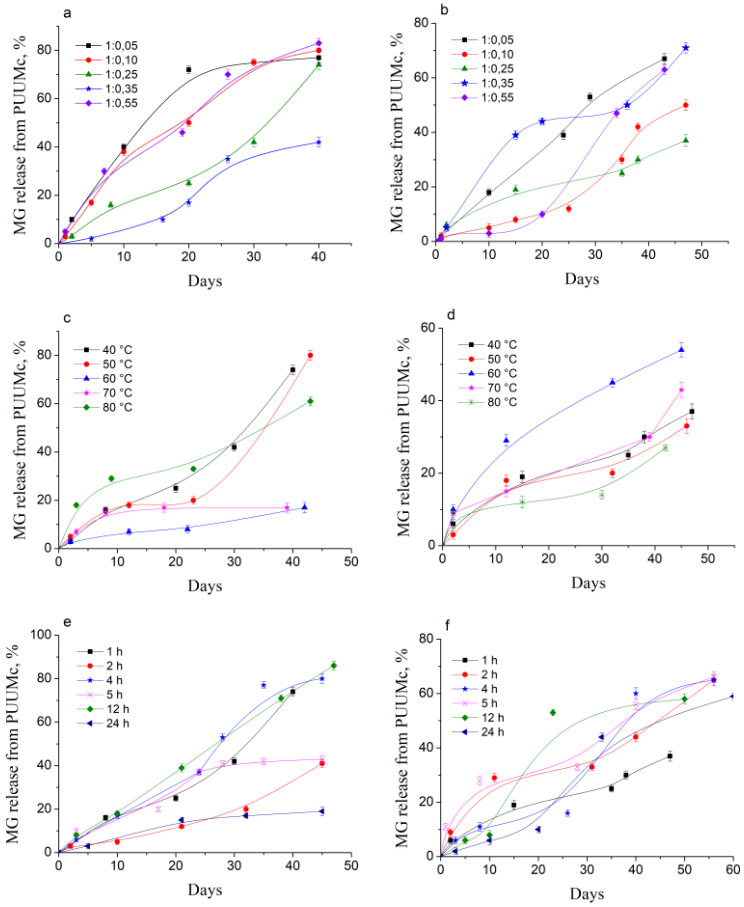


Fig. 5. The release rate of MG from PUUMc as a function of initial molar ratio of PVA:APTES (a and b (1 h, 40 ºC); synthesis temperature (c and d (1 h, [PVA]:[APTES]=1:0.25)) and time (e and f (40 ºC, [PVA]:[APTES]=1:0.25)) used for PVA modification, when the MG was encapsulated after (a, c, e) and during (b, d, f) the PUUMc synthesis.

3.5 Structure analysis of PUUMc

The structure of PUUMc, which were synthesized in this work, has been proven by FT-IR spectra, elemental, chemical and thermogravimetric analysis.

Table 7

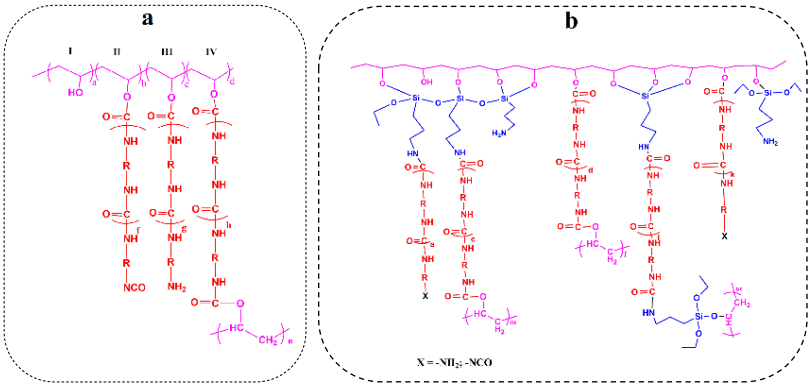
Assignments of absorption bands in FT-IR spectra of PUUMc obtained from PVA and various DI

Frequency (cm^{-1})			Assignment
PUUMc from PVA and HMDI	PUUMc from PVA and MDI	PUUMc from PVA and TDI	
3323	3301	3275	(OH) ir (NH)
2931	2920	2923	(C-H) alkyl group
2855	2855	2855	(C-H) alkyl group
2275	2273	2273	(O=C=N)
1740–1743	1752	1741	(C=O) urethane group
1615	1638	1638–1641	(C=O) urea group
1558	1588	1542–1551	(N-H), (C=N) amide II
1249–1251	1229	1221–1225	(N-H), (C=N) amide III
1077	1180	1017	(C-O-C)

According to FT-IR spectra, PUUMc have urea and urethane linkages, and the spectra show almost the same bands independently on PUUMc synthesis conditions. The main absorption bands are summarized in Table 7. When for PUUMc synthesis was used PVA and blend of DI and DA, absorption bands of polyurea –C=O groups were moved to lower wave numbers. In FT-IR spectra of the PUUMc obtained from PVA/APTES and HMDI the additional absorption bands of –CN group of APTES segments were observed at 1478 and 1462 cm^{-1}. The peak at 1076 cm^{-1} is assigned to the overlap of Si-O-Si and C-O-C bonds.

The TGA of PUUMc showed that thermal decomposition mainly occurs in two stages. According to TGA and DTGA results, the first

stage of decomposition of PUUMc was splitted into two or three steps. The first of all the decomposition of urethane bonds and then short lengths of polyurea segments and lastly long length polyurea segments, which were stabilized by hydrogen bonds were occurred. The second stage indicated the decomposition of polyene residues.



Scheme 2. Structure of PUUMc obtained from PVA and DI or blend of DI and DA (a) and from PVA/APTES and HMDI (b)

According to results of chemical analysis, the amount of isocyanate groups in the PUUMc was increased with increasing the amount of diisocyanates in initial mixture and it was decreased with increasing reaction temperature and prolongation of synthesis time. According to results of elemental analysis, it was obtained, that amount of nitrogen in the PUUMc was higher, when the PUUMc were synthesized from PVA and blend of DI and DA to compare with PUUMc obtained from PVA and DI. It is supposed, that adding diamine into the reaction mixture, resulted in increasing length and amount of urea segments in the PUUMc. However, the amount of isocyanate groups in these PUUMc was decreased. The amounts of isocyanate and amino groups and Si in the PUUMc were increased, with increasing the amount of APTES in the PVA modification mixture. The amount of Si in

PUUMc has not been changed with increasing the PVA modification temperature and prolongation of time.

The expected structure of PUUMc obtained from PVA and DI or blend of DI and DA is provided in Scheme 2 a and the structure of PUUMc from PVA/APTES and HMDI is presented in Scheme 2 b. PUUMc were consisted of macromolecules with four types of constitutional units: non-reacted hydroxyethylene constitutional unit of PVA (type I), unit with one urethane group after reaction of hydroxyethylene monomeric unit with one isocyanate group from either of diisocyanates and free isocyanate (type II) or amino (type III) group at the end of branch, and constitutional unit with two urethane groups after crosslinking reaction of two hydroxyethylene monomeric units with two isocyanate groups from either of diisocyanate (type IV). The polyurea segments could present in constitutional units of type II, III and IV. In PUUMc from PVA/APTES and HMDI can be the same constitutional units as shown in the Scheme 1a but they can contain the APTES residue, too (Scheme 2 b).

CONCLUSIONS

1. Poly(urethane-urea) microcapsules (PUUMc) from poly(vinyl alcohol) (PVA) and various diisocyanates (DI): 1,6-hexamethylene diisocyanate (HMDI), 4,4'-methylene diphenyl diisocyanate (MDI) and 2,4-toluene diisocyanate (TDI), or blend of one of three DI and one of three diamines (DA): ethane-1,2 diamine (EDA), propane-1,3-diamine (PDA) and butane-1,4-diamine (BDA) were synthesized by inverse emulsion method. The yield of PUUMc was higher when microcapsules were synthesized from PVA and MDI. Increasing synthesis time and temperature resulted in increasing yield of PUUMc and decreasing quantity of isocyanate groups. The yield of PUUMc was higher when higher amount of DA was used and when it was added later than DI.
2. PUUMc from PVA modified with APTES (PVA/APTES) and HMDI were synthesized for first time. Increasing PVA modification temperature, time, pH and amount of APTES in modification mixture resulted in increasing yield of PUUMc. Quantity of Si in PUUMc shell increased with increasing the amount of APTES in modification mixture.
3. The PUUMc shell had crosslinked structure. There were hydroxyl groups, urethane linkages and various length polyurea chains which were formed hydrogen bonds. PUUMc having more and longer polyurea chains stabilized by hydrogen bonds were formed when PVA and blends of DI and DA instead of PVA and DI were used for synthesis of PUUMc. Decomposition of PUUMc shell proceeded in two stages independently from conditions of synthesis. At the first stage of PUUMc decomposition urethane and short urea segments were decomposed at first while later longer polyurea segments stabilized by hydrogen bonds were decomposed. At the second stage, the residues of polyene were decomposed.

4. The PUUMc have slit-shaped pores with the exception of capsules from PVA and TDI, where the pores are cylindrical. Increasing synthesis temperature resulted in increasing surface area and total pore volume of PUUMc shell, while the prolongation of synthesis time resulted in their decreasing and also narrower pore size distribution in the shell of PUUMc. The highest surface area (up to 176.6 m^2g^{-1}) have PUUMc from PVA and TDI, smaller (up to 162.9 m^2g^{-1}) have PUUMc from PVA and MDI, and the smallest (up to 77.2 m^2g^{-1}) have PUUMc from PVA and HMDI. The smallest size of dominant pores (4-9 nm) was when PVA and TDI were used for PUUMc synthesis and the biggest size (30-41 nm) was when PVA and HMDI were used. When blends of HMDI or TDI and any of DA were used for PUUMc synthesis, surface area and total pore volume were decreased but when MDI and DA were used they were increased. When PVA/APTES and HMDI were used for PUUMc synthesis, the surface area of capsules decreased and the size of dominant pores increased to compare with PUUMc obtained from PVA and HMDI. The pore size of PUUMc was suitable for immobilization of enzyme by using sorption method.
5. Maltogenic α-amylase (MG) was immobilized by entrapment, covalent attachment and physical adsorption into PUUMc shell during the capsules synthesis or after it. The EI of MG was the highest (80 %) when PUUMc were synthesized from PVA and TDI and MG was immobilized during PUUMc synthesis. The smallest EI was when PUUMc were synthesized from PVA and HMDI (27 %). Increasing PUUMc synthesis time and temperature resulted in decreasing EI of MG. Addition of DA into reaction mixture resulted in higher EI only when HMDI was used for synthesis of PUUMc, but in the cases of MDI or TDI EI was lower to compare with results without DA. Increasing amount of DA in PUUMc synthesis mixture resulted in increasing EI of MG in most cases. When PVA/APTES was used for PUUMc synthesis, the EI of MG did not increase.

6. The MG release from PUUMc was depended on DI used for PUUMc synthesis. When HMDI or MDI were used, MG was released in two stages, at first burst release and later on – slow release. When TDI was used, release of MG was controlled. The slowest or incomplete release of MG was from capsules, when HMDI was used for synthesis and the fast release was when TDI was used. The stability of MG in capsules was increased when DA or PVA/APTES were used for PUUMc synthesis. After 30 days, the least release of immobilized MG (11 %) was obtained when PVA, HMDI and PDA ([PVA]:[PDA] = 1:1) were used for synthesis of PUUMc.

LIST OF SCIENTIFIC PUBLICATIONS ON THE THEME OF DISSERTATION

Publications in the journals included into the database of Clarivate Analytics Web of Science

1. S. Maciulyte, T. Kochane, S. Budriene. Microencapsulation of maltogenic α-amylase in poly(urethane-urea) shell: Inverse emulsion method. *J. Microencapsul.* 2015, 32 (6), p. 547-558.
2. S. Maciulyte, G. Gutauskiene, J. Niedritis, T. Kochane, S. Budriene. PVA and various diisocyanates based poly(urethane–urea) microcapsules for encapsulation of enzyme in water/butyl acetate emulsion: synthesis and study. *Chemija*, 2017, 28 (1), p. 74-84.

Proceedings and abstracts of international scientific conferences

1. I. Gerasimcik, G. Valungeviciute, S. Maciulyte, T. Kochane and S. Budriene. Influence of types of isocyanates on synthesis of poly(vinyl alcohol) based microcapsules. *Baltic Polymer Symposium 2012.* Programme and proceedings. Liepaja, Latvia, 2012, p. 223.
2. S. Maciulyte, T. Kochane, S. Budriene. Controlled encapsulation of water soluble biocatalyst in poly(urethane-urea) microcapsules. *European Polymer Congress EPF 2013.* Book of Abstracts. Pisa, Italy, 2013, P1-18.
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8. S. Maciulyte, T. Kochane, S. Budriene. Preparation of poly(urethane-urea) microcapsules using poly(vinyl alcohol) as the polyol. *Chemistry and Chemical Technology*. Proceedings of the International Conference. Vilnius, 2015, p. 311-314. (ISBN 978-609-459-461-8)
9. S. Mačiulytė, G. Gutauskienė, J. Niedritis, T. Kochanė, S. Budrienė. Preparation and characterization of poly(urethane-urea) microcapsules with different diisocyanates and poly(vinyl alcohol). *Baltic Polymer Symposium 2018.* Programme and abstracts. Jurmala, Latvia, 2018, p. 65.

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CURRICULUM VITAE

Name, Surname	Sandra Mačiulytė
Birth date and place	9 August, 1987, Rokiškis
Education	
2012-2018	Doctoral degree studies at the Departament of Polymer Chemistry, Faculty of Chemistry and Geosciences of Vilnius University;
2010-2012	Studies at Vilnius University, Faculty of Chemistry, Master of Science in Chemistry;
2006-2010	Studies at Vilnius University, Faculty of Chemistry, Bachelor of Science in Chemistry;
2006	Graduation from Romuvos gymnasium, Rokiškis;
Practical experience	
2012-2015	Participation in the project "*Synthesis and Investigation of the Possibility of Applying of Organic and Inorganic Carriers and Matrices for Suitable Selected Biocatalyzators"* of the National Integrated Programme "Biotechnology and Biopharmacy", No. VP1-3.1-ŠMM-08-K-01-005.

2013-2015	Participation in the project *"New Flexible Structure Bifluoren Compounds for Optoelectronics (BiFluorene)"*. This work was supported by the European Structural Fund., No. VP1-3.1-ŠMM-10-V-02-023.
2014-2015	Participation in the project „*Investigation of Encapsulation of Bacteriocines as Bioconservants*". This work was supported by Lithuanian State, No. SVE-03/2014.
Foreign languages	English, Russian
For contacts	maciulyte.sandra@gmail.com

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Vilniaus universiteto leidykla
Universiteto g. 1, LT-01513 Vilnius
El. p. info@leidykla.vu.lt,
www.leidykla.vu.lt
Tiražas 50 egz.