VILNIUS UNIVERSITY
CENTER FOR PHYSICAL SCIENCES AND TECHNOLOGY

ANTANAS STRAKŠYS

POLY(URETHANE UREA) MICROPARTICLES: SYNTHESIS, INVESTIGATION AND APPLICATION FOR IMMOBILIZATION OF MALTOGENIC α-AMYLASE

Summary of doctoral dissertation
Physical Sciences, Chemistry (03 P)

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The official defence of the doctoral dissertation will be held at the open meeting of the Council of Chemical Sciences trend at 12 p.m. on December 11th, 2015 in the Auditorium of Inorganic Chemistry of the Faculty of Chemistry of Vilnius University, Naugarduko 24, LT-03225, Vilnius, Lithuania.

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VILNIAUS UNIVERSITETAS
FIZINIŲ IR TECHNOLOGIJOS MOKSLŲ CENTRAS

ANTANAS STRAKŠYS

POLI(URETANKARBAMIDINĖS) MIKRODALELĖS: SINTEZĖ, TYRIMAS IR PANAUDOJIMAS MALTOGENINEI α-AMILAZEI IMOBILIZUOTI

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Su disertacija galima susipažinti Vilnius universiteto ir Fizinių ir technologijos mokslų centro Chemijos instituto bibliotekose *ir VU interneto svetainėje adresu: www.vu.lt/lt/naujienos/ivykiu-kalendorius*.

1. INTRODUCTION

Relevance of the work. At the present time a lot of carriers for immobilization of enzymes are produced. Most of enzymes are immobilized by the physical adsorption such as hydrophobic and *Van der Walls* interactions, hydrogen and ionic bonds. Enzymes could be immobilized by covalent attachment, however most carriers must be modified by difunctional compounds, usually. High interest in application of immobilized enzymes for biotechnological, biomedical and pharmaceutical industry is remained. Scientific area of synthesis of carriers for immobilization of enzymes is still under development due to novel synthesis methods.

There are a lot of studies based on immobilization of enzymes onto polyurethane (PU) matrix, foams, powders, layers, membranes, however the most of immobilized enzymes were bound to carrier by physical methods, which are not effective and the obtained immobilized preparations are unstable. The number of scientific publications about immobilization of enzymes onto PU and poly(urethane-urea) (PUU) microparticles by the covalent attachment is sparse and insufficient. Enzyme immobilization onto porous PUU microparticles by covalent binding has a heightened interest and potential application.

It is important that synthetic carrier would be universal for most enzymes. Universal carrier should have such properties as: a good mechanical resistance, biocompatibility and biodegradation. However, the number of such carriers is minimal. Poly(vinyl alcohol)-based PUU microparticles can be considered as suitable carriers for enzyme immobilization. It is suspecting that the use of poly(vinyl alcohol) (PVA) for synthesis of PUU could enhance their hydrophilicity, and as a result affinity to the most of enzymes. Moreover, better carrier and enzyme interaction is achieved in comparison to hydrophobic carriers. Due to free isocyanate groups in the structure of porous PUU microparticles no additional modification of carrier with difunctional compounds, which could inactivate enzyme, is needed. Immobilization of enzyme onto new porous PUU microparticles could be preceded by both covalent attachment and physical adsorption methods.

The aim of this work was to synthesize porous PUU microparticles from poly(vinyl alcohol) and various diisocyanates, to study their properties and to use for immobilization of maltogenic α-amylase (Ma) from *Bacillus stearothermophilus*.

The objectives of the research are the following:

- ✓ To synthesize porous poly(urethane-urea) (PUU) microparticles from poly(vinyl alcohol) (PVA) and diisocyanates: binary blend of diisocyanates (1,6-hexamethylene diisocyanate (HDI) and 2,4-toluene diisocyanate (TDI)), isophorone diisocyanate (IPDI) or 4,4'-methylenebis(cyclohexyl isocyanate) (HMDI) and to optimize synthesis conditions;
- ✓ To investigate the structure of PUU microparticles, their thermal properties, surface area and porosity;
- ✓ To use the synthesized PUU microparticles as a carriers for immobilization of Ma and to investigate the efficiency of immobilization of Ma and stability of immobilized preparations;
- ✓ To determine optimal temperatures for native and immobilized Ma, *Michaelis-Menten* constants and maximum reaction rates, to investigate the influence of NaCl on activity of Ma and its desorption from PUU microparticles.

Scientific novelty and practical value of the dissertation. New porous PUU microparticles from biocompatible, biodegradable and hydrophilic PVA and various diisocyanates: blend of HDI-TDI, IPDI or HMDI were synthesized and particularly investigated. The influence of various reaction conditions such as initial molar ratio of PVA and diisocyanate, synthesis time and temperature on the yield of PUU microparticles, quantity of functional groups, porosity and thermal stability was evaluated. PUU microparticles can be used as carriers for immobilization of Ma and other enzymes due to free isocyanate groups and porosity of microparticles. Enzymatic activity, stability and optimal temperatures of immobilized preparations were particularly studied. Ma immobilized onto PUU microparticles synthesized from PVA and IPDI have shown good enzymatic activity and stability after 28 days storage and after 7 cycles of starch hydrolysis in batch operation. These immobilized preparations

can be used for starch hydrolysis. They were successfully used for immobilization of urease and biosensors design to determine concentration of urea, too.

Defensive statements:

- ✓ Porous PUU microparticles synthesized from PVA and diisocyanates: blend of HDI-TDI, IPDI or HMDI have crosslinked structure that contain hydroxyl groups, urethane linkages and poly(urea) chains of various lengths which could have the free isocyanate groups. With increasing of amount of diisocyanate, the poly(urea) chains become longer, more ordered and stabilized by hydrogen bonds.
- ✓ PUU microparticles have plate-like shapes with slit-shaped pores which are suitable for immobilization of enzymes. The highest surface area and total pore volume are obtained when PUU microparticles are synthesized from PVA and IPDI.
- ✓ Porous PUU microparticles are suitable for covalent immobilization of Ma. Efficiency of Ma immobilization is higher when PUU microparticles have larger surface area, total pore volume and more free isocyanate groups.
- ✓ Optimal temperature of Ma immobilized onto PUU microparticles is higher compare with native Ma. Preparations of immobilized Ma are stable and can be used for starch hydrolysis in batch reactors. PUU microparticles are appropriate for immobilization of urease.

Approbation of the research results. The results of the research were presented in 18 scientific publications, 2 of them as articles have been published in the journal included into the Thomson Reuters Web of Science database, 1 article is submitted, 2 publications in the issues, which correspond to the list of database of Institute of Scientific Information (Proceedings etc.), 3 publications – in reviewed proceedings of international scientific conferences.

Structure of the doctoral dissertation. The doctoral dissertation is written in Lithuanian and contains the following parts: Introduction, Literature review, Material and methods, Results and discussion, Conclusions, List of references (302 entries) and

List of scientific publications. The material of the doctoral dissertation is presented in 168 pages, including 7 schemes, 46 figures and 14 tables.

2. MATERIALS AND METHODS

Main materials. Poly(vinyl alcohol) (PVA) (M_w = 100000, the degree of hydrolisation 86-89 mol %, viscosity (4 % in water, 20 ^oC) is 34-45 mPa·s), 1,6-hexamethylene diisocyanate (HDI) and 2,4-toluene diisocyanate (TDI) were obtained from *Fluka*. Isophorone diisocyanate (IPDI) and 4,4'-methylenebis(cyclohexyl isocyanate) (HMDI), were obtained from Aldrich. All materials were used as purchased.

Synthesis of the PUU microparticles. Poly(urethane-urea) (PUU) microparticles from PVA and various diisocyanates (blend of HDI-TDI, IPDI or HMDI) were synthesized by one-step polyaddition reaction in dimethyl sulfoxide-water (98-99 %/ 1-2 vol %) solution.

Characterization of PUU microparticles. Synthesized PUU microparticles 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 analysis. Size and morphology of synthesized microparticles was evaluated by Scanning Electron Microscopy (Hitachi SU 70) and optical microscopy (Olympus BX 51). Thermal properties of PUU were determined thermogravimetrically (Perkin Elmer STA 6000).

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

Immobilization. The immobilization of Ma onto PUU microparticles was carried out in sodium citrate buffer (0.1 M pH = 5.0). The mixture of the enzyme, buffer and carrier immediately after synthesis was stirred at 40 ^oC for 30 min and then left at 4 ^oC overnight.

Enzyme assay. Activity of native Ma 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 Ma 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 Ma 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. Protein content in native enzyme solution or left in solution after immobilization was assayed by bicinchinonic acid method (BCA kit, Germany). Immobilization yield by protein (YP) was defined as the percentage ratio of immobilized protein *vs* protein used for immobilization.

Determination of stability of Ma immobilized onto PUU microparticles. Immobilized preparations were held in sodium citrate buffer (pH = 5.0) at 4 ^{o}C for 14 and 28 days to determine their stability.

Treatment of liquefied starch in batch operation. The hydrolysis of liquefied starch was carried out in flask in the presence of 10 ml of liquefied starch and Ma immobilized onto PUU microparticles under stirring (300 rpm) for 20 min at 40 ^{o}C. After each batch reaction immobilized Ma was filtered and washed with sodium citrate buffer (0.1 M, pH = 5.0), and then reused for the next batch reaction.

Determination of optimal temperature of native and immobilized Ma. The value of optimal temperature for native and immobilized enzyme was obtained by performance of starch hydrolysis reaction with native or immobilized enzyme at temperature range of 20-80 °C for 20 min.

Evaluation of NaCl effect on enzymatic activity of native and immobilized Ma. Effect of NaCl on enzymatic activity of native and immobilized enzyme onto PUU microparticles were carried out for 20 min at 40 ^{o}C in 0.1 M sodium citrate buffer solution (pH = 5.0) containing 0.01-0.10 M NaCl. After incubation, the immobilized Ma was washed several times with 0.1 M sodium citrate buffer (pH = 5.0). Activity of Ma after treatment was assayed by incubation of sample with liquefied potato starch (Dextrose equivalent 2-5 %) as substrate in 0.1 M sodium citrate buffer (pH = 5.0) at 40 ^{o}C.

3. RESULTS AND DISCUSSION

The present study focuses on a detailed investigation of synthesis of PUU microparticles from PVA and diisocyanates: blend of HDI-TDI, IPDI or HMDI. 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 PUU microparticles were particularly studied.

Possibility to apply obtained PUU microparticles for immobilization of Ma was investigated. Properties and reusability of immobilized Ma were also evaluated.

3.1 Synthesis and study of PUU microparticles from PVA and blend of diisocyanates.

PUU microparticles were synthesized from PVA and various diisocyanates by one step polyaddition reaction in DMSO-water solution. The reaction between isocyanate and water leads to production of gaseous carbon dioxide and an amino group which can react with free isocyanate group to form urea linkages. The reactivity of isocyanate in reaction with primary amine is greater than in reaction with primary alcohol or water. Isocyanate groups are around three times more reactive in reaction with primary hydroxyl groups or water than with secondary hydroxyl groups, which present in PVA. In this study PUU microparticles were obtained from PVA and diisocyanates without using diamines.

PUU microparticles were synthesized from PVA and blend of HDI-TDI in

Table 1. Yield of PUU and quantity of isocyanate and hydroxyl groups in PUU microparticles from PVA and blend of HDI-TDI (t = 90 min, T = 90 ^{o}C)

No.	[PVA]:([HDI]:[TDI])	Yield of PUU (%)	Quantity of NCO groups (%)	Quantity of OH groups (%)
1	1:(0.25:0.25)	47	2.8	6.6
2	1:(0.35:0.35)	50	3.2	5.2
3	1:(0.50:0.50)	67	7.3	2.7
4	1:(0.40:0.60)	55	5.4	4.0
5	1:(0.75:0.25)	72	9.1	2.6

DMSO/H_2O (99/1 vol. %) solution when initial molar concentration of PVA was 0.1 M (mole of repeating unit of PVA). The initial molar ratio of PVA and HDI-TDI in reaction mixture was varied from 1:0.5 to 1:1 and initial molar ratio of HDI and TDI in the blend of diisocyanates was also varied (Table 1). Increasing the molar amount of diisocyanates in initial molar ratio of PVA and (HDI:TDI) from 1:0.5 to 1:1.0 and molar amount of HDI in the blend of diisocyanates resulted in increasing yield of PUU, quantity of isocyanate groups (determination of the quantity of isocyanate groups was performed immediately after synthesis of PUU microparticles and it was recalculated with respect to weight loss in the drying process)and decreasing quantity of hydroxyl groups. The highest yield of PUU microparticles (72 %) and the highest quantity of isocyanate groups (9.1 %) were in the case when initial molar ratio of PVA:(HDI:TDI) was 1:(0.75:0.25) (t = 90 min, T = 90 ^oC) (Table 1, No. 5).

According to synthesis route, PUU microparticles may have porous surface, because of the reaction between isocyanates and water resulted in production of gaseous

Table 2. Porous properties of the PUU microparticles from PVA and blend of HDI-TDI (t = 90 min, T = 90 ^oC)

No.	[PVA]:([HDI]:[TDI])	Surface area (m^2/g)	Total pore volume (cm^3/g)	Mean pore width (nm)
1	1:(0.25:0.25)	4	0.016	21
2	1:(0.35:0.55)	5	0.021	20
3	1:(0.50:0.50)	5	0.015	15
4	1:(0.40:0.60)	5	0.012	23
5	1:(0.75:0.25)	4	0.017	21

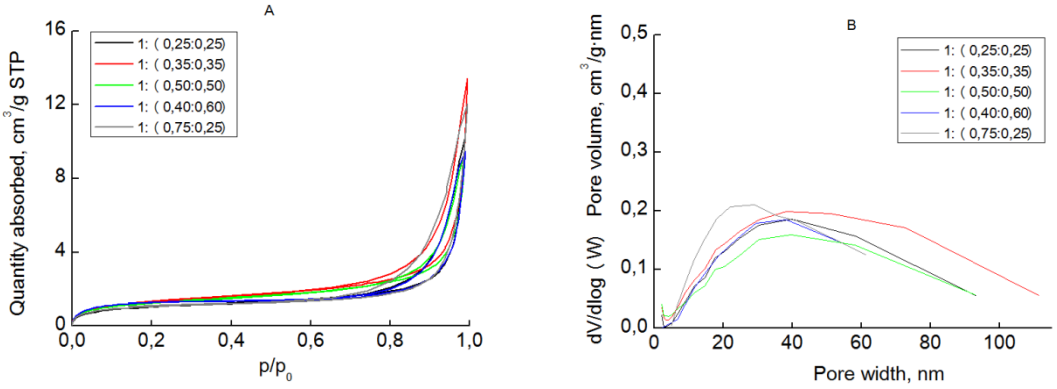


Fig. 1. Sorption hysteresis (A) and pore width distribution (B) of the PUU microparticles from PVA and blend of HDI-TDI (t = 90 min, T = 90 ^oC).

carbon dioxide, which foamed PUU microparticles and solvent occupied formed empty spaces. When the solvent is removed from the system, the space that it had previously occupied becomes the pore volume and network of intercommunicating pores left behind. The information about the surface area, the total pore volume, mean pore width and width distribution is summarized in Table 2 and Fig. 1. PUU microparticles display the type IV of gas sorption isotherm with the H3 hysteresis loops which provided aggregates of plate-like particles with distribution of slit-shaped pores width (Fig. 1 A). According to results, initial molar ratio of PVA and diisocyanates had minor influence on the surface area and total pore volume. However, increasing the molar amount of diisocyanates in initial molar ratio of PVA and (HDI:TDI) from 1:0.5 to 1:1.0 resulted in decreasing mean pore width from 21 to 15 nm. It is supposed, that decreasing of pore width is related with increasing crosslinks in PUU. According to Fig. 1 (B), pore width distribution in PUU microparticles has a broad curve and predominantly pore width is from 20 to 80 nm with a maximum at 40 nm in most cases.

3.2 Synthesis and study of PUU microparticles from PVA and IPDI

Another series of PUU microparticles were synthesized under similar conditions from PVA and IPDI ([PVA] = 0.06 M, DMSO/H_2O = 99/1 vol %). Changing the initial molar ratio of PVA and IPDI from 1:2.0 to 1:4.0 (t = 90 min, T = 90 ^oC) resulted in increasing yield of PUU microparticles from 49 to 67 %, increasing the quantity of isocyanate groups from 0.6 to 1.7 % and decreasing quantity of hydroxyl groups from 11.6 to 6.8 % (Table 3). A gel was formed immediately when the initial molar ratio of

Table 3. Yield of the PUU and quantity of isocyanate and hydroxyl groups in the PUU microparticles from PVA and IPDI (t = 90 min, T = 90 ^oC)

No	[PVA]:[IPDI]	Yield of PUU (%)	Quantity of NCO groups (%)	Quantity of OH groups (%)
1	1:2.0	49	0.6	11.6
2	1:2.5	53	1.3	10.5
3	1:3.0	62	1.5	8.1
4	1:3.5	67	1.6	7.3
5	1:4.0	67	1.7	6.8

PVA and IPDI was 1:5.0 or when concentration of PVA was higher than 0.06 M. The highest yield of PUU microparticles and the highest quantity of isocyanate groups were in the case when initial molar ratio of PVA and IPDI was 1:4.0 (Table 3, No. 5). Similarly to PUU microparticles form PVA and blend of TDI-HDI, PUU microparticles from PVA and IPDI display the type IV gas sorption isotherm with the H3 hysteresis loops (Fig. 2 A). The nitrogen adsorption and desorption isotherms show

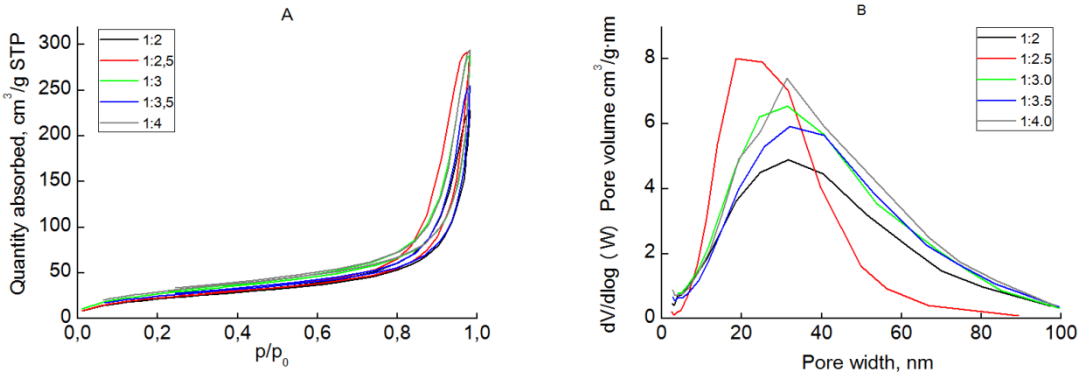


Fig. 2. Sorption hysteresis (A) and pore width distribution (B) of the PUU microparticles from PVA and IPDI (t = 90 min, T = 90 ^{o}C).

the clear hysteresis loops. The remarkable rise to medium (p/p_o=0.4-0.8) and high pressure region (p/p_o=0.8-1) of the nitrogen adsorption and desorption isotherms implies the presence of mesopores and macropores in PUU microparticles. The information about the surface area and the total pore volume is summarized in Table 4. According to

Table 4. Porous properties of the PUU microparticles from PVA and IPDI (t = 90 min, T = 90 ^{o}C)

No.	[PVA]:[IPDI]	Surface area (m^2/g)	Total pore volume (cm^3/g)	Mean pore width (nm)
1	1:2.0	83	0.36	16
2	1:2.5	88	0.45	18
3	1:3.0	94	0.45	16
4	1:3.5	104	0.45	18
5	1:4.0	113	0.46	17

results, changing the initial molar ratio of PVA and IPDI from 1:2.0 to 1:4.0 (t = 90 min, T = 90 ^{o}C) in the synthesis of PUU microparticles, resulted in increasing the surface area from 83 to 113 m^2/g, total pore volume from 0.36 to 0.46 cm^3/g and mean pore width

from 16 to 18 nm. According to Fig. 2 (B), pore width distribution in PUU microparticles has a broad curve and predominantly pore width is from 20 to 60 nm with a maximum at 32 nm. However when initial molar ratio of PVA and IPDI was 1:2.5 maximum pore width was 19 nm. Also, when initial molar ratio of PVA and IPDI is 1:4.0, the curve of pore width distribution has two maximum values where the first is at 20 nm and the second is at 32 nm. The pore width distribution is bimodal in this case.

3.3 Synthesis and study of PUU microparticles from PVA and HMDI

The third series of PUU microparticles were synthesized from PVA and HMDI ([PVA] = 0.06 M, DMSO/H_2O = 98/2 vol %). Changing the initial molar ratio of PVA and HMDI from 1:2.0 to 1:4.0 (t = 90 min, T = 90 ^oC) resulted in increasing yield of PUU microparticles from 38 % to 54 %. Further increasing initial molar ratio of PVA and HMDI to 1:6 resulted in slightly increasing the yield of PUU microparticles to 55 % (Table 5). A gel was formed immediately when the initial molar ratio of PVA and HMDI

Table 5. Yield of the PUU and quantity of isocyanate and hydroxyl groups in the PUU microparticles from PVA and HMDI (t = 90 min, T = 90 ^oC)

No.	[PVA]:[HMDI]	Yield of PUU (%)	Quantity of NCO groups (%)	Quantity of OH groups (%)
1	1:2	38	0.2	2.6
2	1:3	50	0.7	1.2
3	1:4	54	0.5	0.9
4	1:5	55	0.3	0.3
5	1:6	55	0.2	2.9

was 1:7.0. It was determined, that the highest quantity of isocyanate groups was 0.7 % when PUU microparticles were synthesized using initial molar ratio of PVA and HMDI 1:3. Changing initial molar ratio of PVA and HMDI to 1:6 resulted in decreasing quantity of isocyanate groups to 0.2 %.

As in previous cases, synthesized PUU microparticles from PVA and HMDI displayed the type IV gas sorption isotherm with the H3 hysteresis loops (Fig 3 A). The information about the surface area, the total pore volume and mean pore width is

Table 6. Porous properties of the PUU microparticles from PVA and HMDI (t = 90 min, T = 90 °C)

No.	[PVA]:[HMDI]	Surface area (m^2/g)	Total pore volume (cm^3/g)	Av pore width (nm)
1	1:2	38	0.22	21
2	1:3	68	0.37	22
3	1:4	67	0.24	16
4	1:5	54	0.08	14
5	1:6	28	0.08	14

summarized in Table 6. As in previous cases, synthesized PUU microparticles from PVA and HMDI have mesopores and macropores structure. According to results presented in Table 6, changing the initial molar ratio of PVA and HMDI from 1:2.0 to 1:3.0 in the

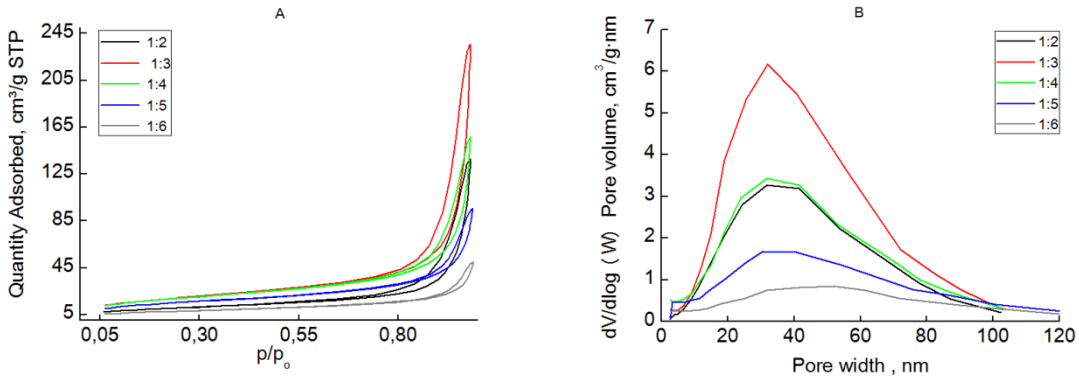


Fig. 3. Sorption hysteresis (A) and pore width distribution (B) of the PUU microparticles from PVA and HMDI (t = 90 min, T = 90 °C).

synthesis of PUU microparticles, resulted in increasing the surface area from 38 to 68 m^2/g, and the total pore volume from 0.22 to 0.37 cm^3/g. Further increasing amount of HMDI during synthesis of PUU microparticles resulted in decreasing the surface area to 28 m^2/g, and the total pore volume to 0.08 cm^3/g. It is supposed that more closed pores were formed than opened ones in these cases. Synthesis of PUU from PVA and HMDI using initial molar ratio 1:7 resulted in gel formation. Changing initial molar ratio of PVA and HMDI from 1:2 to 1:6 in the synthesis of PUU microparticles resulted in decreasing mean pore width from 22 to 14 nm. According to Fig. 3 (B), pore width distribution in PUU microparticles has a broad curve with a maximum at 32 nm in all cases with exception of microparticles which were obtained when molar ratio of PVA

and HMDI was 1:6. Pore width distribution curve has very broad profile without dominated pore width in this case

3.4 Structure analysis of PUU microparticles

The structure of PUU microparticles, which were synthesized from PVA and blend of HDI-TDI, IPDI, or HMDI, have been proven by FT-IR spectra (Fig. 4), elemental and

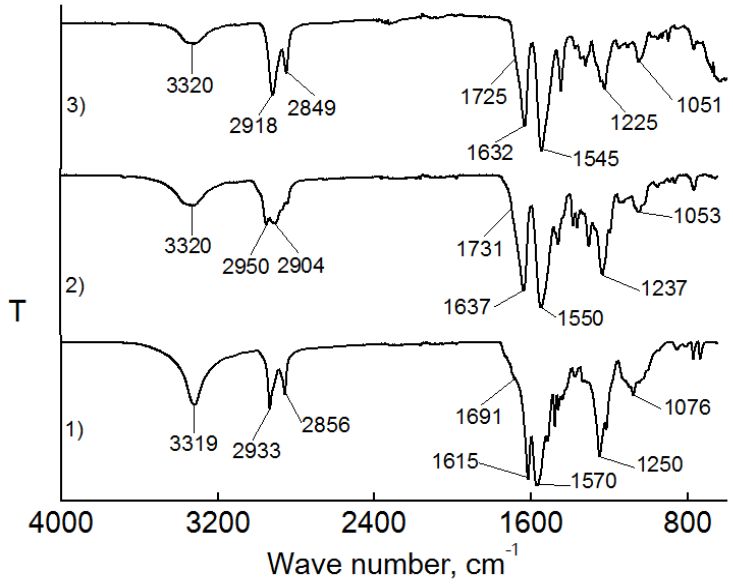


Fig 4. FT-IR spectra of PUU microparticles: 1 – [PVA]:([HDI]:[TDI]) = 1:(0.75:0.25); 2 – [PVA]:[IPDI] = 1:4.0; 3 – [PVA]:[HMDI] = 1:3 (t = 90 min., T = 90 ^{o}C).

thermogravimetric analysis.

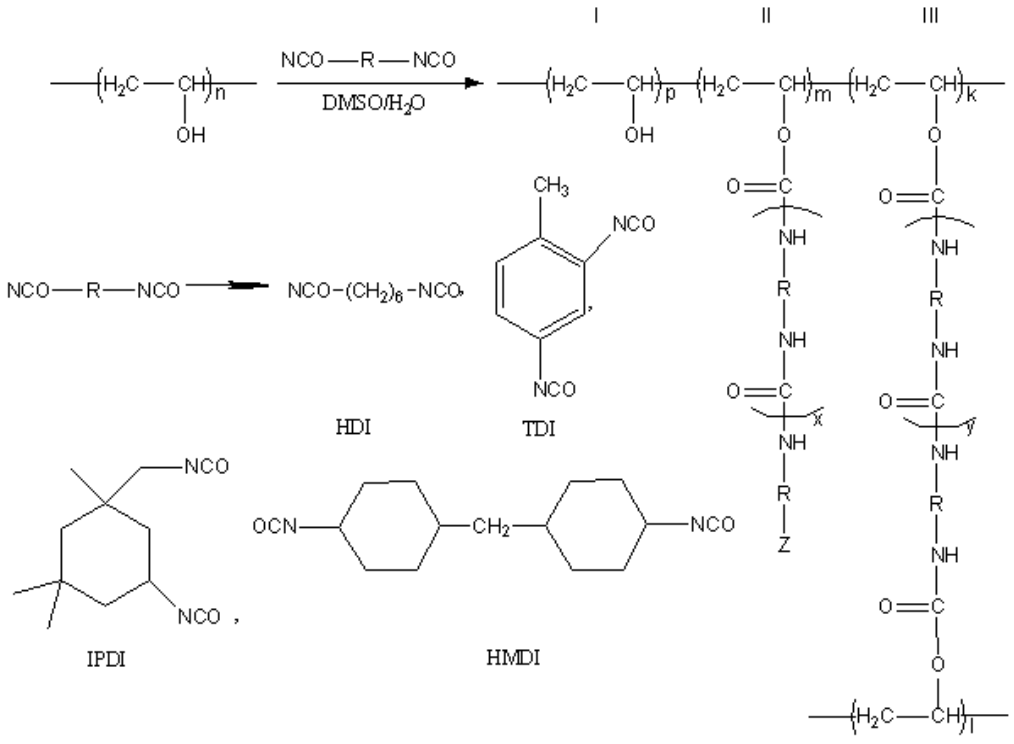
FT-IR spectra of the PUU microparticles, prepared from PVA and blend of HDI-TDI, IPDI, or HMDI show almost the same bands at 2933 cm^{-1} and 2856 cm^{-1}, 2950 cm^{-1} and 2904 cm^{-1}, 2918 cm^{-1} and 2849 cm^{-1} related to C-H from alkyl groups, band at 1615 cm^{-1}, 1637 cm^{-1} and 1632 cm^{-1} related to C=O of the urea group, band at 1570 cm^{-1}, 1550 cm^{-1} and 1545 cm^{-1} related to amide II (δ N-H, ν C=N), band at 1250 cm^{-1}, 1237 cm^{-1} and 1225 cm^{-1} related to amide III (another type of δ N-H, ν C=N), band at 1076 cm^{-1}, 1053 cm^{-1} and 1051 cm^{-1} (ν C-O-C) is assigned to ether linkage and small shoulder at 1691 cm^{-1}, 1731 cm^{-1} and 1725 cm^{-1} is related to C=O of the urethane group. The FT-IR spectra did not show bands at 2237-2250 cm^{-1} related to isocyanate group.

According to FT-IR spectra, synthesized PUU microparticles have urea and urethane linkages.

According to results of elemental analysis, it was obtained, that content of nitrogen in the PUU microparticles was increased with increasing the amount of diisocyanates in initial mixture. It is supposed, that increasing the amount of diisocyanates in initial molar ratio, resulted in increasing amount of urea segments in the PUU microparticles.

TGA of PUU microparticles showed that thermal decomposition mainly occurs in two stages. First stage indicated the thermal decomposition of urethane and urea linkages. In the second stage, the polyene residue was decomposed. According to DTGA results, the first stage of decomposition of PUU microparticles was splitted into two peaks. The First peak indicated the decomposition of urethane linkages and short length of poly(urea) segments and the second peak indicated decomposition of long length poly(urea) segments, which were stabilized with hydrogen bonds.

The Scheme 1 shows the expected structure of PUU. PUU microparticles were consisted of macromolecules with three types of constitutional units: non-reacted hydroxyethylene constitutional unit of PVA (type I), constitutional unit with one urethane group after reaction of hydroxyethylene monomeric unit with one isocyanate

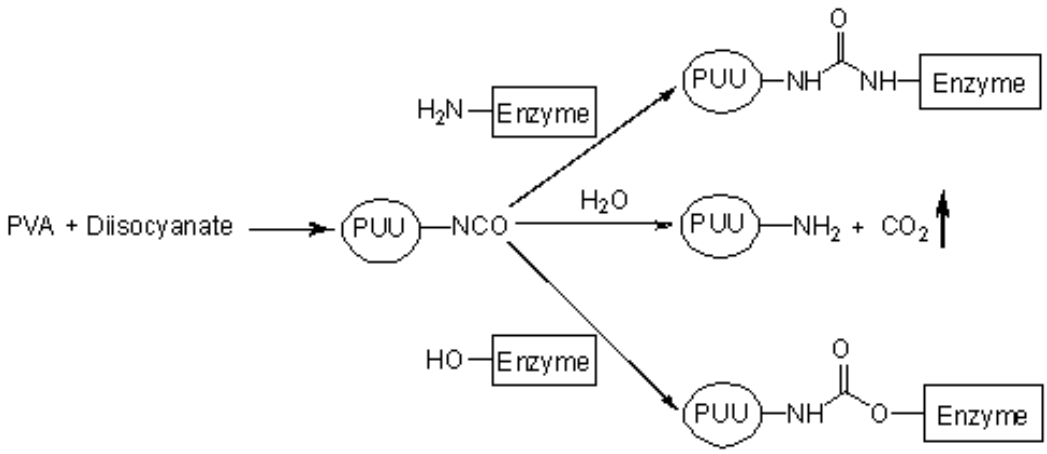


Scheme 1. Structure of PUU (Z = NCO or NH_2).

group from either of diisocyanates and free isocyanate or amino group at the end of branch (type II) and constitutional unit with two urethane groups after crosslinking reaction of two hydroxyethylene monomeric units with two isocyanate groups from either of diisocyanates (type III). The poly(urea) segments could present in constitutional units of type II and III.

3.5 Immobilization of maltogenic α-amylase onto PUU microparticles

Maltogenic α-amylase (Ma) is one of the most important enzymes in starch industry, which is used for saccharification of starch for obtaining high-maltose syrups. Ma from *Bacillus stearothermophilus* EC 3.2.1.133) is an exo-acting enzyme as known as glucan 1,4-α-maltohydrolase, which randomly cleaves off 1,4-α-D-glucosidic linkages of starch and other big carbohydrate molecules to produce shorter oligomaltose molecules and which are hydrolysing from non-reducing end to finally product of α configuration maltose units. Also Ma is used in transglycolysation reactions, too. Enzyme, which has amino and hydroxyl groups, can be immobilized onto PUU microparticles, which have unreacted NCO groups and form urea or urethane linkages



Scheme 2. Covalent immobilization of maltogenic α-amylase.

(Scheme 2). NCO groups of PUU react faster with amino than with primary alcohol groups or with water. Whereas the immobilization procedure followed in the aqueous media, the remaining free NCO groups reacted with water by formation of CO_2 and did not have any inactivation effect on enzyme.

PUU microparticles which were synthesized from PVA and blend of HDI-TDI, IPDI or HMDI (t = 90 min., T = 90 ^{o}C) were used for immobilization of Ma. It was estimated that the initial molar ratio of PVA and diisocyanate during the synthesis of PUU has an obvious influence on the efficiency of immobilization (EI) and yield of immobilization by protein (YP) of Ma (Table 7). Ma could be immobilized by covalent binding and physical adsorption. Efficiency of enzyme immobilization by physical adsorption depends on carrier surface area and porosity, because of when carrier has high surface area and total pore volume, enzyme could easily adsorb in the pores without diffusion limit and steric hindrance. As it was known, enzyme can easily get into pores

Table 7. Results of immobilization of maltogenic α-amylase onto PUU microparticles

No.	[PVA]:[diisocyanate]	EI (%)	YP (%)
[PVA]:([HDI]:[TDI])			
1	1:(0.25:0.25)	45 ± 2	47 ± 2
2	1:(0.35:0.35)	51 ± 3	52 ± 3
3	1(0.40:0.60)	60 ± 3	62 ± 3
4	1:(0.50:0.50)	74 ± 4	76 ± 4
5	1:(0.75:0.25)	96 ± 5	100 ± 5
[PVA]:[IPDI]			
6	1:2.0	46 ± 2	51 ± 3
7	1:2.5	59 ± 3	63 ± 3
8	1:3.0	62 ± 3	67 ± 3
9	1:3.5	67 ± 3	71 ± 3
10	1:4.0	68 ± 3	74 ± 4
[PVA]:[HMDI]			
11	1:2	60 ± 3	63 ± 3
12	1:3	88 ± 4	90 ± 5
13	1:4	68 ± 3	71 ± 4
14	1:5	51 ± 3	53 ± 3
15	1:6	49 ± 2	51 ± 3

which diameter is in the range of 30-100 nm.

The results show that the initial molar ratio of PVA and blend of HDI-TDI during the synthesis of PUU has an obvious influence on the EI and YP of Ma. EI and YP of Ma increased when the amount of diisocyanates in the initial molar ratio of PVA and the blend of HDI-TDI increased from 1:0.5 to 1:1. Increasing initial molar amount of HDI in the blend of HDI-TDI resulted in increasing quantity of NCO groups and as a result

increasing EI and YP of Ma (Table 7, No. 3 and 5). The highest EI was 96 % when initial molar ratio PVA:(HDI:TDI) = 1:(0.75:0.25) (t = 90 min., T = 90 ^{o}C) was used for synthesis of carrier.

Ma was successfully immobilized onto PUU microparticles which were synthesized from PVA and IPDI. Increasing amount of IPDI in the initial reaction mixture resulted in increasing quantity of free isocyanate groups, surface area and total pore volume of PUU microparticles. Changing initial molar ratio of PVA and IPDI from 1:2.0 to 1:4.0 (t = 90 min., T = 90 ^{o}C) during synthesis of PUU microparticles resulted in increasing EI of Ma onto PUU microparticles from 46 % to 68 % and YP from 51 to 74 %.

Similarly Ma was immobilized on third series of PUU microparticles which were synthesized from PVA and HMDI. Results show that changing initial molar ratio of PVA and HMDI from 1:2.0 to 1:3.0 (t = 90 min., T = 90 ^{o}C) during synthesis of PUU microparticles resulted in increasing EI of Ma onto PUU microparticles from 60 % to 88 % and YP from 63 to 90 %. Further increasing the amount of HMDI during synthesis of PUU resulted in decreasing EI and YP of Ma. EI was 49 % and YP was 51 % when initial molar ratio of PVA and HMDI was 1:6.

The immobilization of Ma onto PUU microparticles could proceed by covalent fixation and physical adsorption. Analysis of storage stability of Ma onto PUU microparticles could confirm which immobilization way is predominant.

Stability of native and immobilized Ma onto PUU microparticles was investigated after 14 and 28 days storage in sodium citrate buffer (0.1 M, pH = 5.0) at 4 ^{o}C (Fig. 5). According to results, residual activity of native Ma after storage for 28 days remained 56 %. The residual activity of immobilized Ma onto PUU microparticles remained from 6 % to 16 % after 28 days when initial molar ratios of PVA:(HDI:TDI) were from 1:(0.25:0.25) to 1:(0.40:0.60). High storage stability exhibited the immobilized Ma onto PUU microparticles, which were obtained when initial molar ratios of PVA:(HDI:TDI) were 1:(0.50:0.50) and 1:(0.75:0.25) and the residual activity of immobilized enzyme after 28 days remained 72% and 95%, respectively. When Ma was immobilized onto PUU microparticles which were synthesized from PVA and IPDI and their molar ratios were from 1:2.0 to 1:4.0, the residual activity of immobilized Ma after storage for 28

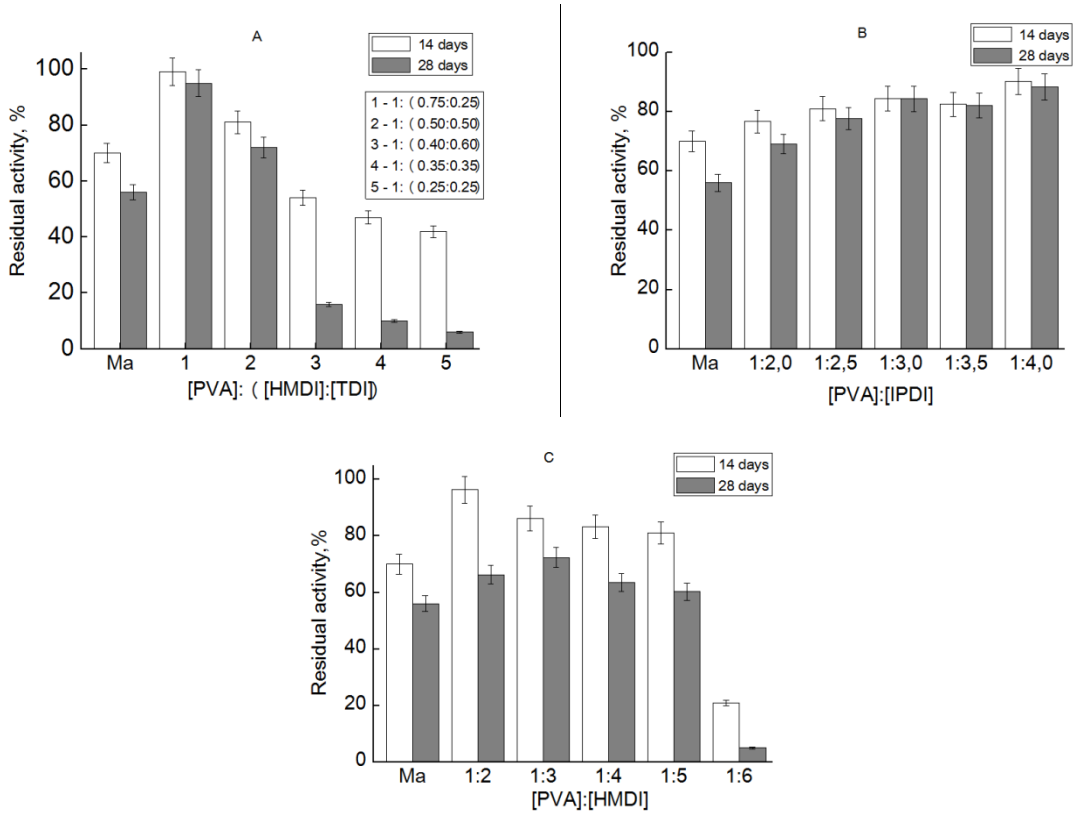


Fig. 5. Residual activity of native and immobilized Ma onto PUU microparticles after 14 and 28 days of storage (in sodium citrate buffer, pH = 5.0 at 4^{o}C) as a function of initial molar ratio of PVA and diisocyanates used for synthesis of PUU: A – PVA:(HDI:TDI); B – PVA:IPDI; C – PVA:HMDI (t = 90 min, T = 90 ^{o}C).

days remained from 69 to 88 %. Low storage stability exhibited then immobilized Ma onto PUU microparticles, which were obtained from PVA and HMDI when initial molar ratio was 1:6. In this case only 5 % of initial activity was remained after 28 days. The residual activity of immobilized Ma after 28 days storage remained from 60 to 72 %, when molar ratios were from 1:2 to 1:5.

It is supposedly, that high residual activity of Ma onto PUU microparticles were obtained in cases when mostly covalent attachment between amino and hydroxyl groups of enzyme and isocyanate groups of PUU microparticles proceeded. Low residual activity was obtained when enzyme was attached to PUU microparticles by physical adsorption which is caused by hydrophobic and *Van der Wall's* interactions, and hydrogen bonds.

3.6 Hydrolysis of starch in batch operation

As the immobilization process enables the reusability of the enzymes in industrial applications, the immobilized Ma was examined for the repeated catalysis immediately after immobilization onto various PUU microparticles (Fig. 6). It was estimated, that the relative activity of immobilized Ma onto PUU microparticles, which were synthesized

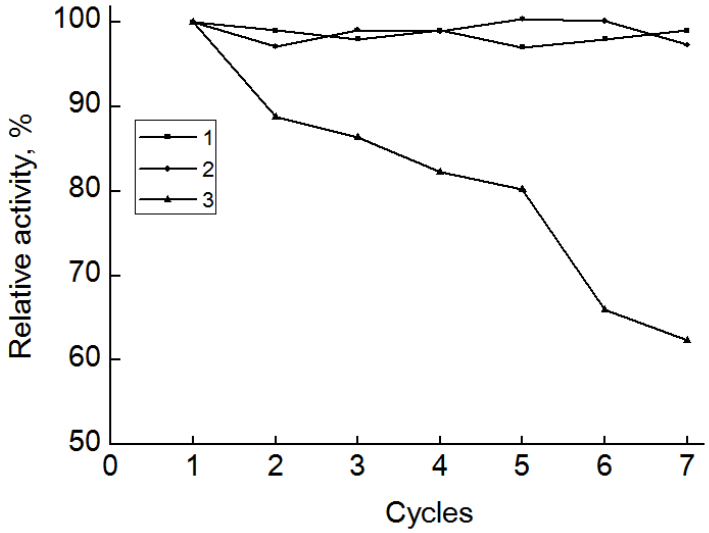


Fig. 6. The relationship between the cycle number and the relative activity of immobilized Ma onto PUU microparticles when bath temperature was 40 ^{o}C and incubation time was 20 min. (1 – [PVA]:([HDI:TDI]) = 1:(0.75:0.25); 2 – [PVA]:[IPDI] = 1:4.0; 3 – [PVA]:[HMDI] = 1:3.0; (t = 90 min, T = 90 ^{o}C)).

from PVA and blend of HDI-TDI ([PVA]:([HDI:TDI]) = 1:(0.75:0.25), PVA and IPDI ([PVA]:[IPDI] = 1:4.0) or PVA and HMDI (([PVA]:[HMDI] = 1:3.0) was decreased by 1 %, 3 % and 38 %, respectively, after 7 cycles of use for starch hydrolysis. A large decrease in relative activity of Ma immobilized onto PUU obtained from PVA and HMDI, showed that a part of physically adsorbed enzyme molecules was leached from PUU microparticles during use. Ma immobilized onto PUU microparticles obtained from PVA and blend of HDI-TDI or IPDI could be used for saccharification of starch.

3.7 Effect of NaCl on enzymatic activity and immobilized Ma

Enzyme could be attached onto support by covalent attachment or physical interaction. Similar data of leaching of enzyme from immobilized carrier to the aqueous media can show whether enzyme was immobilized *via* hydrophobic and *Van der Walls* interactions, hydrogen or ionic bonds and high salt concentration can lead to ion exchange and washing out of immobilized enzyme.

Relative activity of native Ma was increased by 10 % when NaCl amount was increased until 0.02 M, further increasing salt concentration to 0.1 M resulted in decreased activity of native enzyme by 10 % compare with Ma activity in buffer solution (Fig. 7 A). Ma immobilized onto various PUU, which were showed high stability, was used for sodium chloride effect experiments (Fig. 7 B). It was estimated, that influence of NaCl on relative activity of Ma immobilized onto PUU microparticles from PVA and IPDI was minimal, when NaCl concentration was in the range of 0.01-0.10 M. However, in both other cases, the relative activity of Ma immobilized onto PUU microparticles,

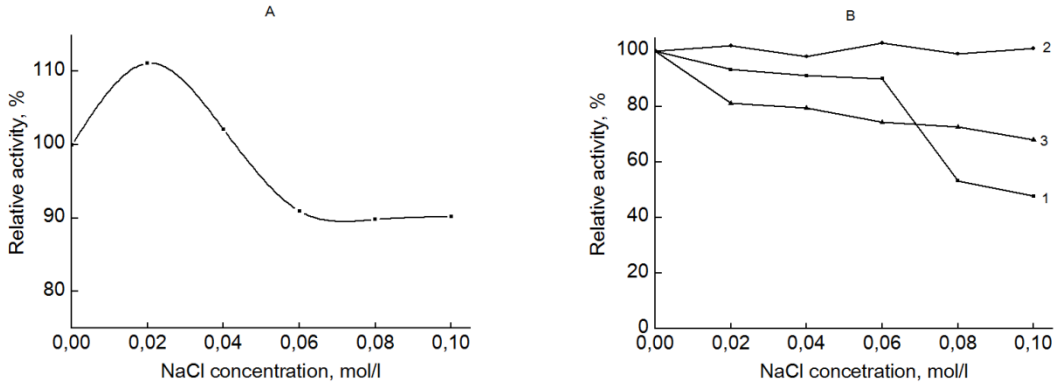


Fig. 7. Influence of NaCl concentration on relative activity of native (A) and immobilized Ma onto PUU microparticles (B): 1 – [PVA]:([HDI:TDI]) = 1:(0.75:0.25); 2 – [PVA]:IPDI = 1:4.0; 3 – [PVA]:HMDI = 1:3.0 (t = 90 min, T = 90 ^{o}C).

synthesized from PVA and blend of HDI-TDI or PVA and HMDI, was decreased with increasing salt concentration. Moreover, relative activity of Ma immobilized onto PUU microparticles, synthesized from PVA and blend of HDI-TDI had two decreasing steps, when initial NaCl concentration was increased from 0.01 to 0.1 M. Ma immobilized onto PUU microparticles, which were synthesized from PVA and IPDI, has higher resistance

to desorption compare with another two systems. Therefore, it can be concluded that mainly covalent attachment of BsMa proceeded onto PUU, which were obtained when synthesis was carried out using molar ratio of PVA and IPDI 1:4 at 90 ^{o}C for 90 min.

3.8 Determination of optimal temperature and kinetic parameters of native and immobilized Ma

The effect of temperature on the enzymatic activity of the native and immobilized Ma was investigated. For this purpose, immobilized Ma preparations with high initial activity were used for experiments. Ma was immobilized onto PUU microparticles, which were synthesized from PVA and blend of HDI-TDI ([PVA]:([HDI]:[TDI] = 1(0.75:0.25)), PVA and IPDI ([PVA]:[IPDI] = 1:4.0) and PVA and HMDI ([PVA]:[HMDI] = 1:3.0), when synthesis was carried out at 90 ^{o}C for 90 min (Fig. 8). Liquefied starch solution was used as a substrate. The highest relative activity of native

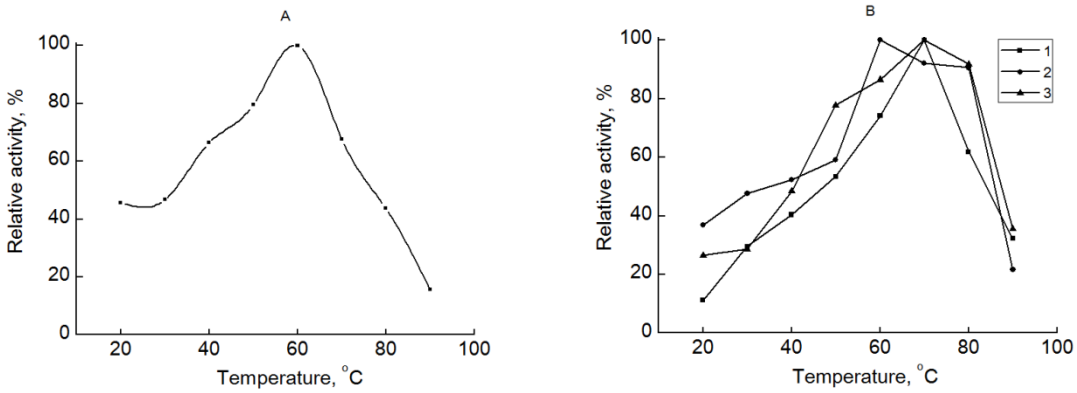


Fig. 8. Relative activity of native (a)) and immobilized (b)) Ma onto PUU microparticles as a function of bath temperature (pH = 5.0) with 20 min incubation time:1 – [PVA]:([HDI]:[TDI] = 1:(0.75:0.25); 2 – [PVA]:[IPDI] = 1:4.0; 3 – [PVA]:[HMDI] = 1:3.0.

Enzyme was observed, when starch hydrolysis was performed at optimal 60 ^{o}C temperature. Starch hydrolysis by Ma immobilized onto PUU microparticles obtained from PVA and blend of HDI-TDI is could be carried out at higher temperature in comparison to native enzyme. The highest relative activity of Ma immobilized onto those PUU microparticles was obtained when starch hydrolysis was performed at 60-80 ^{o}C. It may lead to the conclusion that immobilization of Ma on various PUU

microparticles protects enzyme from denaturation and enzymatic reaction could be carried out at higher temperatures.

Michaelis-Menten (K_M) constants and maximum reaction rates of starch hydrolysis were determined for native and Ma immobilized onto PUU microparticles which were

Table 8. K_M and $V_{MAX}/2$ values of native and immobilized Ma onto PUU microparticles (t = 90 min, T = 90 ^oC)

No	Ma immobilized onto PUU	K_M (%)	$V_{MAX}/2$ (mol/min·l)
1	Native Ma	1.8	$4.8 \cdot 10^{-4}$
2	[PVA]:([HDI]:[TDI]) = 1:(0,75:0,25)	2.1	$4.1 \cdot 10^{-4}$
3	[PVA]:[IPDI] = 1:4,0	1.2	$4.7 \cdot 10\text{-}^{4}$
4	[PVA]:[HMDI] = 1:3	2.9	$6.3 \cdot 10^{-4}$

synthesized from PVA and blend of HDI-TDI, IPDI, or HMDI (t = 90 min., T = 90 ^oC). The lowest value of K_M was achieved when Ma was immobilized onto PUU microparticles, which were synthesized form PVA and IPDI. It means that immobilized enzyme is more affinitive to a starch solution as a substrate than a native enzyme.

PUU microparticles obtained under optimal conditions ([PVA]:[IPDI] = 1:4, t = 90 min, T = 90 ^oC) were used for immobilization of urease (EC.3.5.1.5, obtained from *Jack beans* seeds, activity 77 U/mg, *Sigma*) to construct the biosensor to determine the concentration of urea. According to received results, characteristics of biosensor are promising and in future could be used as biosensor in medical, food or biotechnological industries. Moreover, this method will be useful for the development of immobilized enzymes for industrial applications.

CONCLUSIONS

1. Porous poly(urethane urea) (PUU) microparticles from poly(vinyl alcohol) and binary blend of diisocyanates (1,6-hexamethylene diisocyanate (HDI) and 2,4-toluene diisocyanate (TDI)), isophorone diisocyanate (IPDI) or 4,4'-methylenebis(cyclohexyl isocyanate) (HMDI) were synthesized and studied in detail for first time. The yield and quantity of isocyanate groups of PUU microparticles were found to increase in the following order: PVA-HMDI < PVA-IPDI < PVA and blend of HDI-TDI. Increasing synthesis time and temperature resulted in increasing yield of PUU and decreasing quantity of isocyanate groups with exception of PVA-IPDI when highest yield of PUU microparticles was obtained at 90 ^{o}C.
2. PUU microparticles have crosslinked structure, that contain hydroxyl groups, urethane linkages and poly(urea) chains of various length which could have the free isocyanate groups. Decomposition of PUU microparticles proceeded in two stages. Using excess of diisocyanate for synthesis of PUU microparticles, the first decomposition stage was splitted in 2 steps. In the first step, firstly urethane linkages and poly(urea) chains of short length were decomposed. In the second step, longer and more ordered poly(urea) chains, which were stabilized by hydrogen bonds, were decomposed. In the second stage, the polyene residue was decomposed.
3. PUU microparticles have plate-like shapes with slit-shaped pores. PUU microparticles from PVA and blend of HDI-TDI have the smallest surface area (4-5 $m^2 \cdot g^{-1}$). The higher surface area was obtained when PUU were synthesized from PVA and HMDI (28-68 $m^2 \cdot g^{-1}$). The highest surface area have microparticles from PVA and IPDI (71-122 $m^2 \cdot g^{-1}$). Change in total pore volume of PUU microparticles was the same as the surface area. The pores in the range of 32-40 nm width were dominated in PUU microparticles.
4. Ma immobilization onto PUU microparticles was proceeded by covalent binding mostly. Efficiency of immobilization (EI) of Ma was 72 % when PUU microparticles synthesized from PVA and IPDI were used as carriers. The highest

EI were achieved when Ma was immobilized onto PUU synthesized from PVA and HMDI or PVA and blend of HDI-TDI (EI ≈ 98 %).

5. Ma immobilized onto PUU microparticles obtained from PVA and IPDI under optimal synthesis conditions ([PVA]:[IPDI] = 1:4, t = 90 min, T = 90 ^{o}C) has shown the highest stability. Immobilized Ma was retained all initial catalytic activity after 28 days storage and after at least 7 cycles in bath operation. Ma desorption from PUU was not observed when 0.1 M NaCl was added to buffer solution.
6. Optimal temperatures of immobilized Ma were 10-20 ^{o}C higher compare with native enzyme. PUU microparticles from PVA and IPDI obtained under optimal synthesis conditions were preferable for Ma immobilization. In this case the lower value of *Michaelis-Menten* constant was obtained compare with native enzyme and maximum enzymatic reaction rates were almost the same.
7. Ma immobilized onto PUU microparticles obtained from PVA and IPDI under optimal conditions can be used for starch hydrolysis. These PUU microparticles were successfully used for immobilization of urease and biosensors design to determine concentration of urea, too.

LIST OF SCIENTIFIC PUBLICATIONS ON THE THEME OF DISSERTATION

Publications in the journals included into the Thomson Reuters Web of Science (WOS) database

1. Strakšys, A., Kochanė, T., Budrienė, S. Synthesis and characterization of poly(urethane-urea) microparticles from poly(vinyl alcohol) and binary blends of diisocyanates and their application for immobilization of maltogenic α-amylase. *Chemija*, 2013, 24(2), p. 160-169.
2. Strakšys, A., Kochanė, T., Budrienė, S. Preparation and characterization of porous poly(urethane-urea) microparticles from poly(vinyl alcohol) and isophorone diisocyanate. *Chemija*, 2015, 26 (2), p. 132-140.
3. Strakšys, A., Kochanė, T., Budrienė S., Catalytic properties of maltogenic α-amylase immobilized onto PUU microparticles, (submitted to *Biotechnology Journal*).

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2. Razumiene, J., Sakinyte, I., Kochane, T., Maciulyte, S., Straksys, A., Budriene, S, Barkauskas, J. Carbon electrode based urea sensor modification of graphite and new polymeric carriers for enzyme immobilization. *BIODEVICES 2013 - International Conference on Biomedical Electronics and Devices 2013*. Barselona, Spain, 2013, p. 197-201. (ISSN:1476-4687).

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3. Budriene, S., Straksys, A., Kozlovskaja, A., Romaskevic T. Synthesis of poly(vinyl alcohol) based polyurethanes: comparison of different diisocyanates. *Baltic Polymer Symposium 2010: Programme and Book of Abstracts*. Palanga, Lithuania, 2010, p. 75.
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6. Razumiene, J., Sakinyte, Gurevičienė V., Kurtinaitienė B., Strakšys A., Kochane, T., Budriene, S. Polymeric carriers for immobilization of biocatalysts. *Fifth International Symposium: Advanced micro- and mesoporous materials.* Golden Sands, Bulgaria, 2013, p. 80.
7. Straksys, A., Dulko, A., Razumiene, J., Sakinyte, J., Kochane, T., Budriene, S. Immobilization and catalytic properties of maltogenic α-amylase on PUU microparticles. *Baltic Polymer Symposium 2013: Programme and abstracts*. Trakai, Lithuania, 2013, p. 166.
8. Strakšys, A., Kochane, T., Mačiulytė, S., Dulko, A., Budrienė, S. Polyurethane microparticles bound Maltogenase: preparation and study. *Chemistry 2013: 11th international conference of Lithuanian chemists: Abstracts*. Vilnius, Lithuania, 2013, p. 94.
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POLI(URETANKARBAMIDINĖS) MIKRODALELĖS: SINTEZĖ, TYRIMAS IR PANAUDOJIMAS MALTOGENINEI α-AMILAZEI IMOBILIZUOTI

SANTRAUKA

Fermentai yra brangūs, juos sunku išgryninti, o jų panaudojimas yra vienkartinis. Jie jautrūs temperatūros, pH ir joninės jėgos pokyčiams. Norint panaikinti arba dalinai pašalinti išvardintus trūkumus, fermentus būtina imobilizuoti.

Labai svarbu, kad nešikliai būtų kuo universalesni ir tiktų imobilizuoti daugumą fermentų. Pageidautina, kad jie būtų mechaniškai patvarūs, pasižymėtų biosuderinamumu ir bioskalumu, o tokių nešiklių labai trūksta. Šias savybes galima suteikti, PUK mikrodalelių sintezei naudojant poli(vinilo alkoholį) (PVA). Be to, PVA padidina nešiklių hidrofiliškumą, todėl jie gimininges ni daugumai fermentų, o imobilizavimo metu yra geresnė tarpusavio sąveika, lyginant su hidrofobiniais nešikliais. Susintetintose akytose PUK mikrodalelėse yra laisvų izocianato grupių, todėl nešiklių nereikia papildomai modifikuoti difunkciniais junginiais, kurie gali deaktyvuoti fermentą. Fermentas ant naujų akytų PUK mikrodalelių gali imobilizuotis ne tik kovalentiniu, bet ir fizikinės adsorbcijos būdu.

Publikacijų skaičius apie fermentų įterpimą į PU matricas, putplasčius ar imobilizavimą ant plėvelių ir membranų yra didelis, deja, dauguma išvardintų imobilizavimo būdų ant PU/PUK yra neefektyvūs, o gauti imobilizuoti preparatai – mažai stabilūs.

Pagrindinis šio darbo tikslas buvo susintetinti akytas PUK mikrodaleles iš PVA ir diizocianatų: HDI ir TDI mišinio, IPDI arba HMDI, ištirti jų savybes ir įvertinti tinkamumą Ma imobilizuoti.

Svarbiausi šio darbo rezultatai, atspindintys naujumą, originalumą ir svarbą:

Pirmą kartą susintetintos akytos, termostabilios ir temperatūrai atsparios PUK mikrodalelės iš biosuderinamo, bioskalaus ir hidrofilinio poli(vinilo alkoholio) (PVA) ir diizocianatų: 1,6-heksametilendiizocianato (HDI) ir 2,4-toluendiizocianato (TDI) mišinio, izoforondiizocianato (IPDI) arba 4,4- dicikloheksilmetandiizocianato (HMDI). Iki šiol nebuvo publikuota darbų, kuriuose PVA būtų naudojamas akytoms PUK dalelėms sintetinti. Susintetintos PUK mikrodalelės yra įvairiapusiškai ištirtos:

nustatytos mikrodalelių išeigos, funkcinių grupių, akytumo, terminio stabilumo priklausomybės nuo pradinių reakcijos sąlygų (pradinių medžiagų molinių santykių, trukmės, temperatūros). PUK mikrodalelės yra tinkamos maltogeninei α-amilazei (Ma) ir potencialiai kitiems fermentams imobilizuoti dėl jose esančių laisvų izocianatogrupių ir akytumo. Detaliai ištirtas Ma imobilizuotų preparatų (IP) aktyvumas ir stabilumas, Ma IP optimalios temperatūros. Ant PUK mikrodalelių, susintetintų iš PVA ir IPDI, imobilizuota Ma išlieka aktyvi po 7 krakmolo hidrolizės ciklų arba laikant 28 paras, todėl gali būti panaudota krakmolo hidrolizei. Šios PUK mikrodalelės panaudotos ureazei imobilizuoti ir biojutikliams, skirtiems karbamido kiekiui nustatyti, kurti.

PUK mikrodalelių struktūra yra tinklinė. Joje yra hidroksigrupių, uretaninių grandžių ir įvairaus ilgio polikarbamido grandinių, kurios gali baigtis laisva izocianatogrupe. PUK mikrodalelės yra plokštelių pavidalo, akutės – plyšinės formos. PUK mikrodalelių paviršiaus plotas yra mažiausias, sintezei naudojant PVA ir HDI-TDI mišinį (4-5 $m^2 \cdot g^{-1}$), didesnis – PVA-HMDI (28-68 $m^2 \cdot g^{-1}$), o didžiausias – PVA ir IPDI (71-122 $m^2 \cdot g^{-1}$). Bendro akučių tūrio kitimo tendencija yra ta pati. PUK mikrodalelėse dominuoja 32-40 nm pločio akutės. PUK mikrodalelės, nepriklausomai nuo naudoto diizocianato, skyla per dvi stadijas.

Didžioji dalis Ma ant PUK mikrodalelių imobilizuota kovalentiniu būdu. Ma imobilizavimo efektyvumas yra didesnis, esant didesniam PUK mikrodalelių paviršiaus plotui, bendram akučių tūriui ir izocianatogrupių kiekiui. IE yra didžiausias, kai Ma imobilizuota ant PUK mikrodalelių, sintezę vykdant iš PVA ir HMDI arba iš PVA ir HDI-TDI mišinio (IE ≈ 98 %), o IE yra mažesnis – kai iš PVA ir IPDI (IE = 72 %).

Didžiausias IP stabilumas gautas, kai Ma imobilizuota ant PUK mikrodalelių, kurios susintetintos iš PVA ir IPDI, esant optimalioms sąlygoms ([PVA]:[IPDI] = 1:4, t = 90 min., T = 90 ^oC), nes IP išlieka stabilūs po 28 parų saugojimo, Ma santykinis aktyvumas nekinta, naudojant IP 7 ciklų krakmolo hidrolizei, o Ma desorbcija nuo PUK mikrodalelių nevyksta, pridėjus 0,1 M NaCl.

Ma IP optimali temperatūra yra 10-20 ^oC didesnė už tirpaus fermento optimalią temperatūrą. Ma imobilizuoti tinkamiausios yra optimaliomis sąlygomis iš PVA ir IPDI susintetintos PUK mikrodalelės, nes sumažėja *Michaelis-Menten* konstantos vertė, palyginus su tirpia Ma, o maksimalūs reakcijos greičiai yra praktiškai vienodi.

Optimaliomis sąlygomis iš PVA ir IPDI susintetintos PUK mikrodalelės sėkmingai panaudotos ureazei imobilizuoti ir biojutikliams, skirtiems karbamido kiekiui nustatyti, kurti.