Biophysical Assessment and Impact of Catalyst on the Properties of Polyurethane Drug Carriers

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The metal complexes and amine compounds are the most frequent categories of catalysts used in polyurethanes synthesis. Unfortunately, all auxiliary substances added to a synthesis increase the toxicological risk of final products used in a therapy. In the present study, polyether-urethane (PU) microstructures with and without catalyst were obtained. These structures were characterized by the measurement of samples' pH, of their size and stability, were obtained and the skin irritation potential. The results show that samples with a neutral pH, PU microstructures with sizes between 120 and 165 nm and with a medium stability. It was found that the microstructures from syntheses with catalyst present increased values of skin irritation.

Keywords: DABCO, drug delivery system, skin irritation, Zeta potential

Polyurethanes are macromolecular compounds obtained in the reaction between di- and/or tri-isocyanates and polyols (polyesters and/or polyethers). They were discovered by Otto Bayer before the beginning of World War II and they became the most important material used to obtain foams for the furniture industry and elastomers for the automotive industry [1].

Chain extenders (functionality f=2) such as diols (ethylene glycol, 1,4-butanediol, 1,6-hexanediol) and diamines (ethylene-diamine) with short chains and low molecular weights are used to prolong the macromolecular chains. The cross linkers (functionality fe ≥ 3) are hydroxyl and/or amine terminated compounds with low molecular weights such as glycerin, 1,2,6-trihydroxyhexane, 2,4,6-trihydroxybenzaldehyde etc. [2].

The obtaining of PU foams is based on the reaction between isocyanates and water with formation of amines and carbon dioxide which swells the material. Chloro-fluoro-carbons (CFC) were used before 2000 to swell the foams, but they contribute to a thinner ozone layer and this is the reason why they are not used presently (Vienna Convention, 1985 and Montreal Protocol, 1987) [3].

Other important auxiliary substances added to the synthesis of polyurethane materials are the catalysts. It is well-known the fact that the duration of polyurethane foams' synthesis is about few minutes and this is due to the use of catalysts. Tertiary amines such as tri-ethylene-diamine (DABCO), di-methyl-ethanol-amine (DMEA) and di-methyl-cyclohexyl-amine (DMCHA) represent some of the traditional compounds used as catalysts in PU synthesis [4]. Modern solutions are based on different urea derivatives. Organometallic compounds based on tin, lead, bismuth or mercury are frequently used for obtaining of coating and sealant applications and of elastomers [5].

The aim of this research was to compare the characteristics of PU microstructures based on syntheses with and without a catalyst.

Experimental part

Materials

Hexamethylene diisocyanate (HMDI), isophorone diisocyanate (IPDI), polyethylene glycol (PEG 200), acetone and Tween®20 were obtained from Merck (Germany); ethylene glycol (EG) was purchased from Lach-Ner s.r.o. (Czech Rep.), 1,4-butanediol (BD) from Carl Roth GmbH (Germany) and 1,4-Diazabicyclo[2.2.2]octane (DABCO) from Sigma-Aldrich Co. All substances were used without any previous purification.

Synthesis of PU microstructures

We already described in detail the multi-step procedure used to synthesize PU microstructures in our previous papers [6-12]:

The formulation of non-aqueous phase: 20 mL solution 10% HDMI/IPDI (1:1, v/v) in acetone was magnetically stirred at 450 rpm and 40°C for 20 min;

The formulation of the aqueous phase: 40 mL aqueous solution 10% of EG, BD and PEG (2:2:1, v/v) mixture was magnetically stirred at 400 rpm and 40°C for 20 min (it was used an excess of the aqueous component in order to consume all the isocyanate and to wash easier the final products);

The mixing of the two phases: the non-aqueous phase was injected into the aqueous phase under magnetically stirring at 650 rpm and 50°C (PU chains start to form in this phase) (fig. 1);

The samples' maturation: stirring was continued for 4 hours at 50°C to ensure the maturation of PU chains in the case of sample without a catalyst (S1) and only 45 min for the other samples;

The purification of the products: the samples were repeatedly washed using a water-acetone mixture (1:1, v/v), centrifuged, and dried; acetone and water were removed by keeping the obtained suspensions as thin layers at 55°C in a lab oven for approx. 12 h, until the weight of the sample was constant.

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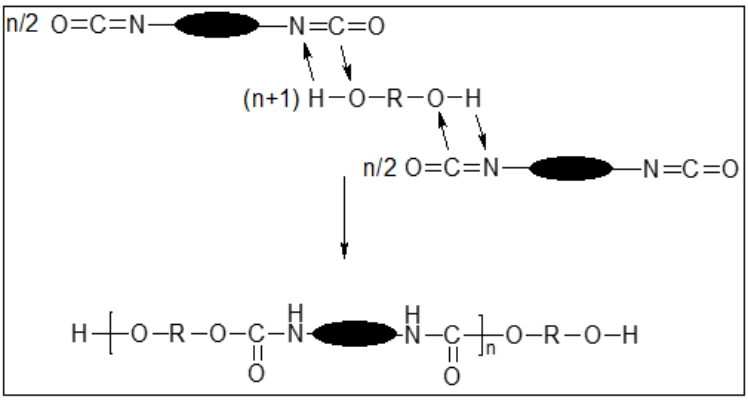


Fig. 1. Synthesis of PU chains

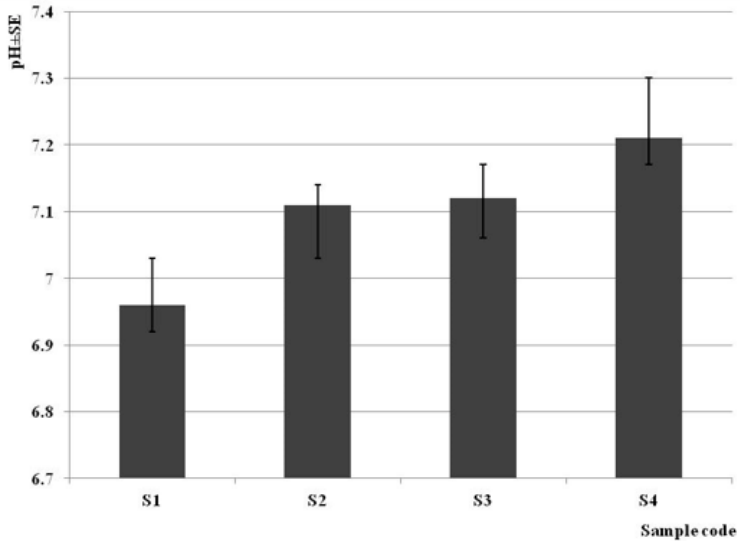


Fig. 2. The *p*H values of the samples

The previously described procedure was repeated 4 times in order to obtain different samples of PU microstructures with and without catalysts: sample S1 (without catalyst), sample S2 (with 50 mg DABCO), sample S3 (with 100 mg DABCO), and sample S4 (with 150 mg DABCO).

Analysis

The *p*H values were determined in triplicate in diluted aqueous solutions (1:200 w/v) with a TitroLine alpha plus titrator (SI Analytics, Germany), by simply plunging the electrode into the solution. The size and the charge of the PU microstructures were measured with a Cordouan system including a Vasco Size Analyzer and a Wallis Zeta Potential Analyzer (Cordouan Technol., France). For this purpose, the same aqueous solutions (1:200 w/v) were used; the measurements were carried out three times for each sample. The following Vasco Particle Size Analyzer parameters were set: temperature (25°C), time interval (20μs), and number of channels (450), laser power (95%), acquisition mode (continuous), and analysis mode (Pade-Laplace). The following Wallis Zetapotential Analyzer parameters were chosen: plastic cuvette, temperature (25°C), laser power (90%), applied field (automatic), medium resolution (0.8 Hz), 3 measures/sequence, and Henry function (Smoluchowski).

Six-week old Balb/c nude male mice (2 mice/sample and other 2 as control) were purchased from Charles River (Sulzfeld, Germany). Mice were kept in standard conditions of 12 h light-dark cycle, food and water *ad libitum*, 24±1°C, humidity above 55% (rules of the National Institute of Animal Health). The samples were applied on the skin on the back of the mice every three hours and the changes of skin parameters were determined in triplicate by the same investigator, in a narrow range of temperature (24±1°C) and air humidity (55±3%). The measurements of skin changes were carried out with a Multiprobe Adapter System (MPA5) from Courage&Khazaka Electronics, Germany, equipped with a Mexameter®MX18 probe and a Corneometer®CM825 probe.

Statistics

All statistical analyses were performed using a trial version of IBM SPSS. Numerical data were presented as mean ± standard errors. Student t test was used to determine the statistical difference between various experimental groups. Correlations between different variables were detected using the Pearson correlation coefficient r. Statistical significance was considered at a p-value less than 0.05.

Results and discussions

The influence of catalyst addition on the reaction rate was evaluated using a protocol described by S. Niyogi *et al.* [13]: 1 mL of the reaction mixture was taken out every 15 minutes from the reaction flask and was quenched with a solution of trichlorobenzen and dibutylamine. Unreacted amine was titrated by a standard methanolic HCl using bromocresol green as an indicator. This protocol indicate that reactions were finished after: around 4 h in the case of sample S1, less than 45 min for samples S2 and S3, and less than 30 min for sample S4.

The PU microstructures were dispersed in distilled water to evaluate their *p*H, size and Zeta potential values. Figure 2 presents the *p*H values recorded for the diluted aqueous solutions (1:200, w/v).

The samples of PU microstructures synthesized in the presence of a catalyst present an increase of *p*H values. S. Arai et al. [14] mention that amine catalysts react with any free acidic groups from PU chains (carboxyl, aliphatic and/or phenolic hydroxyl groups) and form organic salts.

Sample code	Particle size (nm)		Zeta Potential (mV)
	Mean ± SD	Polydispersity index	Mean ± SD
S1	143 ± 11	0.3	21.7 ± 4.2
S2	122 ± 18	0.3	26.5 ± 3.9
S3	149 ± 16	0.5	24.9 ± 4.8
S4	164 ± 21	0.4	28.8 ± 3.6

Table 1
THE ZETASIZER CHARACTERIZATION

This process modifies the *p*H by reducing the acidity level of samples.

The diluted aqueous solutions of PU microstructures (1:200, w/v) were analyzed by a zetasizer to examine the size distribution (table 1). The zetasizer results implied that the mean particle size was in micro scale (above 100 nm). An easier aggregation of PU microstructures is shown by the recorded Zeta Potential values which are situated at the border between stable and unstable particles. We already mentioned in our previous papers [15-20] that samples containing colloidal particles with a Zeta potential within the range of 20-30 mV, have a medium stability degree and present a slight trend to agglomerate.

The irritation potential of synthesized samples can be evaluate by visual observations, but some investigation tools are currently available to reduce the inter-observer variability. Thus, tewameter, skin pH-meter, sebumeter, mexameter, and corneometer measurements are non-invasive techniques used to evaluate the skin changes [21].

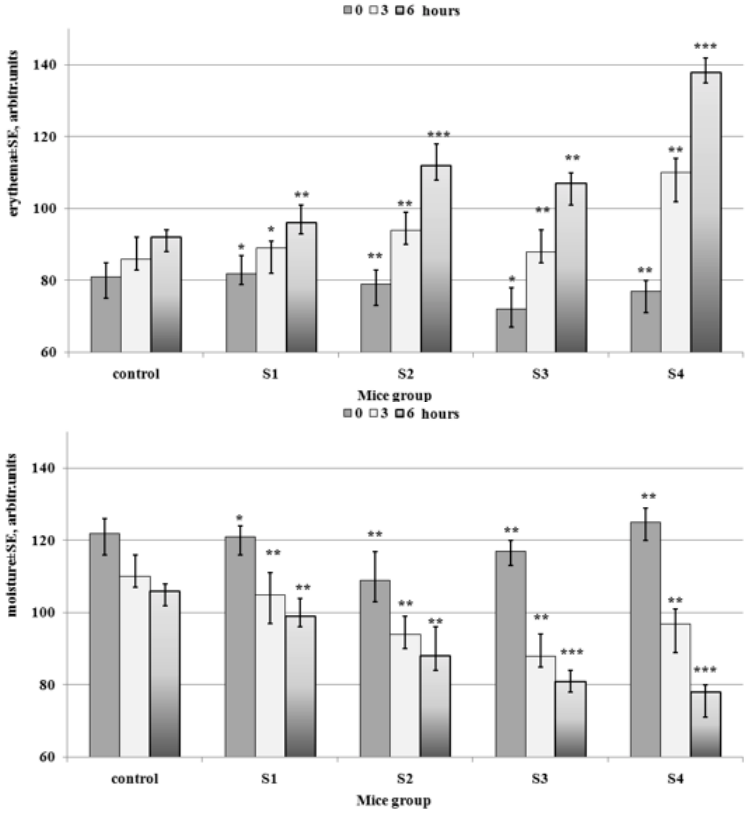
An important number of animal models to assess different types of melanoma, irritation of skin and pigmentation behavior are available today. The development of sciences and the progress of animal models are two inter-dependent factors which determine good correlations between the reality and the modeling [22].

The hairless mice represent the best animal model used in experiments on skin due to its increased sensitivity and because it has a penetration degree a few times greater than human skin [23]. Thus, the sensitivity of hairless mice skin is an important advantage as it can be used as a tool for assessing the toxicological risk of new synthesized samples.

The evolutions of average values of erythema and hydration state of skin for mice groups are shown in figure 3. Mexameter®MX18 emits 3 specific light wavelengths and a detector which evaluates the reflected light to measure the melanin and haemoglobin levels. The erythema presents an important increase for sample S4 as it is shown in figure 3(A).

The measurement of moisture of skin surface is based on capacitance measurement of a dielectric medium. In this experiment it was observed that a treatment with PU microstructures with large amounts of catalyst lead to an important decrease of moisture level of *stratum corneum* as it is shown in figure 3(B). It can be assumed that the effects of PU microstructures with catalyst on mice skin are dose-dependent.

On correlating the DABCO dose with all the studied skin parameters (both erythema and moisture of skin), a significant negative correlation was revealed with respect to the moisture of skin after 6 h of experiment (table 2 and fig. 4).



A

B

Fig. 3. Evolution of (A) erythema and (B) moisture of *stratum corneum* *, ** and *** indicate $p<0.05$, $p<0.01$ and $p<0.001$ vs. control

Skin parameter	Correlation with DABCO dose	Pearson correlation coefficient, r	p
Erythema (0 hours)	-	-0.676	0.324
Erythema (3 hours)	-	0.723	0.277
Erythema (6 hours)	-	0.877	0.123
Moisture (0 hours)	-	0.378	0.622
Moisture (3 hours)	-	-0.548	0.452
Moisture (6 hours)	Negative	-0.969*	**0.031**

** Correlation is significant at the 0.05 level (2-tailed)*

Table 2
CORRELATION OF DABCO DOSE WITH CHANGES OF SKIN PARAMETERS

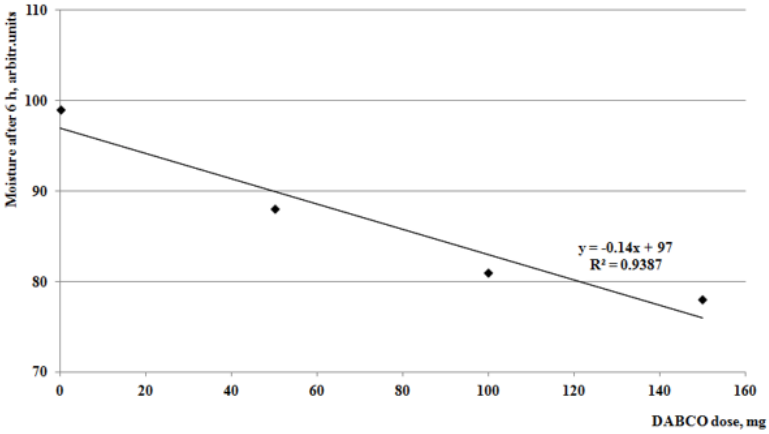


Fig. 4. Dependence between DABCO dose and skin moisture after 6 h

Conclusions

Polyurethane industry is still one of the most important parts of the manufacturing of plastic materials. Foams, bricks, architectural columns, car seats, air ducts, floor mats, steering wheels, adhesives and coatings are the main applications of polyurethanes. One important category of raw materials in the polyurethane synthesis is represented by the catalysts; two main categories are used: tertiary amines and organometallic compounds. In this study, the influence of a catalyst (tri-ethylene-diamine or 1,4-Diazabicyclo[2.2.2]octane, DABCO) dose on the characteristics of a polyurethane drug delivery system was evaluated. Samples of polyurethane microstructures with sizes between 120 and 165 nm, medium stability and neutral *p*H with different amounts of catalyst were synthesized. It was found a correlation between the increase of DABCO dose and the decrease of skin moisture.

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