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Novel technique for determination of toxicological character of synthesized drug carriers

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Abstract

Poly(ether urethane) (PEU) copolymers were chosen as drug carriers. The technique of polycondensation combined with spontaneous emulsification using lysine diisocyanate ester (LDI) and different ratio of diols-polyethylene glycol mixture, was used to synthesize PEU drug carriers. After the optimization of the work procedure, there were obtained nanostructures with diameters between 100-500 nm, which were characterized by pH, size and Zeta potential. The determination of toxicological character of these drug carriers was studied using a novel technique based on the skin mouse model. There were applied PEU drug carriers suspensions on C57BL/6 mice skin (topic application) and there were analyzed the changes of skin parameters using noninvasive procedures (tewametry, mexametry, skin-pH measurements). No significant modification was reported. According this data, these PEU materials can be used for further application in the field.

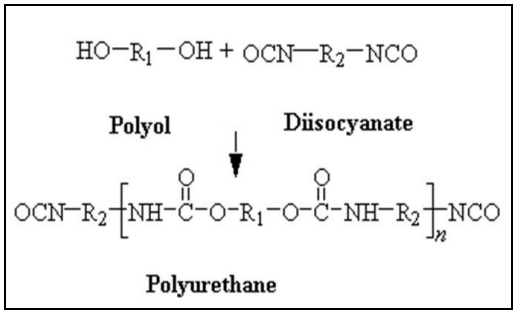
Keywords: poly(ether urethane), drug carrier, Tewameter, skin-pH-meter, Mexameter

1. Introduction

The polyurethanes are polymers containing significant number of urethane groups (–NH–COO–) in the molecular chains. The most common method of preparing polyurethane is the condensation reaction of di- or tri-isocyanates with polyols (polyethers and/or polyesters) in the presence of diols with low molecular weight used as chain extenders and tertiary amines or tin organometallic compounds used as catalysts. Unlike conventional polycondensation, this polymerization reaction does not eliminate any by-product [1].

The polyurethane structures are complex and diverse containing "hard" and "soft" segments, which contribute to the balance between rigid and elastomeric properties. Polyurethanes are synthesized by two methods namely, prepolymer method and one-shot method.

In the prepolymer method, the polyisocyanate and polyol are reacted to form an intermediate polymer called "prepolymer".



It is then converted into final high molecular weight polymer by further reaction with diol or diamine chain extenders. In the "one-shot" method, the polymer formation is done by simultaneous reaction of polyol, polyisocyanate and chain extenders [2].

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In medicine history, the first medicinal polyurethane containing polyester polyols used polyesters as soft segments [3], but it was hydrolytically unstable due to the structure that provides powerful hydrophilic features [4]. Therefore there was extended the popularity of polyurethanes based on polyether, which has a high modulus value, good biocompatibility, mechanical strength and excellent stability for long-term implants [5].

The polyurethane applications include materials such as injectable bone cements [6] or the production of vascular stents and stent coatings [7]. Polyurethanes have been studied as materials for dressings of wounds [8] or biodegradable porous matrices for tissue engineering or injecting. In the vascular tissue engineering, there were synthesized polycaprolactone-polyurethane matrix composites [9], poly(ester-ether-urethane), L-linear biodegradable block copolymers based on lysine diisocyanate and poly(ε-caprolactone)-poly(ethylene) [10]. They also presented some type of biodegradable poly(urethane-urea) based on diol chain extenders as polycaprolactone and amino acids [11] and lysine-based polyurethanes for bone cement applications [12].

There were synthesized PEU nanostructures with different sizes and stabilities. After a physico-chemical characterization, it was chosen a recipe that involves a reactants ratio and stirring speed to obtain structures that include a larger quantity of drug, but to penetrate the skin easily. In this study, there were evaluated the effects of carrier suspensions application on C57BL/6 mice skin. There were chosen PEU nanostructures based on lysine diisocyanate ester and three different aqueous phase components ratios, which present good values for pH, size and stability. After the applications on the skin, there were measured the values of transepidermal waterloss (TEWL), skin-pH, melanin, and erythema using a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany.

2. Materials and methods

The following substances were used to synthesize the PEU nanostructures: lysine diisocyanate ester (LDI) was furnished by Hangzhou ImagineChem Co., Ltd (China). Mono-ethylene glycol (MEG) is from Lach-Ner s.r.o. (Czech Rep.), Aldrich 1,3-propanediol (PD), polyethylene glycol M = 200 (PEG), the solvent (acetone) and surfactant (Tween®20) were obtained from Merck (Germany).

2.1. Synthesis protocol

The interfacial polycondensation technique combined with spontaneous emulsification used to obtain PEU nanostructures, presents the following steps:

1. Preparation of the organic phase - 1.5 ml lysine diisocyanate ester was mixed with 20 ml acetone in a Berzelius beaker and heated at 40 ^{o}C;
2. Preparation of the aqueous phase - different ratios of MEG, PD, PEG and 2.0 ml Tween®20 were mixed with 40 ml distilled water in an Erlenmeyer flask and heated at 40 ^{o}C;
3. The organic phase was injected into the aqueous phase under magnetic stirring (500 rpm) and heated (40 ^{o}C). It was chosen this mixing speed after another study made by our team when there were observed the size and zeta potential of the reaction products vs. magnetic stirring speed. This is the moment when PEU nanostructures precipitate instantaneously.
4. The stirring is still maintained for four hours at 40 ^{o}C in order to ensure the maturation of the carrier wall.
5. The acetone and the water were removed by slow evaporation, keeping the suspension as thin layers (approx. 3 mm) in Petri dishes at 80 ^{o}C in oven for 24 hours.
6. The products were purified by three times cycle of centrifugation and redispersion in a mixture (water-acetone 1:1 v/v) in order to eliminate secondary products (amines) and aqueous phase components excess.
7. The samples were dissolved in water in order to obtain suspension with the same concentration for the pH, size and Zeta potential determination and application on the mice skin.

Three experiments were done by using the same procedure already described. It was varied the diol/polyol ratio as is presented in Table 1 in order to obtain nanostructures with different size and/or stability.

Table 1. Raw materials for PEU carrier synthesis

Raw materials	Quantity / Sample		
	A	B	C
LDI, ml	1.5	1.5	1.5
MEG, ml	0.0	0.5	1.5
PD, ml	0.0	0.5	1.5
PEG, ml	3.0	2.0	0.0
Tween®20, mL	2.0	2.0	2.0

2.2. Animal model

C57BL/6, often referred to as "C57 black 6" or just "black 6" (standard abbreviation: B6), is a common inbred strain of laboratory mice. It is probably the most widely used "genetic background" for genetically modified mice for use as models of human disease. They are the most widely used mouse strain, due to the availability of congenic strains, easy breeding, and robustness.

C57BL/6 mice have a dark brown, nearly black coat, and an easily irritable temperament. They have a tendency to bite, and cannot be handled like a typical pet mouse or more docile laboratory strains such as BALB/c.

Group-housed B6 mice display barbering behavior, in which the dominant mouse in a cage selectively removes hair from its subordinate cage mates. Mice that have been barbered have large bald patches on their bodies, commonly around the head, snout, and shoulders, although barbering may appear anywhere on the body. Both hair and vibrissae may be removed. Barbering is more frequently seen in female mice; male mice are more likely to display dominance through fighting [13, 14].

C57BL/6 female mice were kindly obtained from Biobase of "Victor Babes" University of Medicine and Pharmacy Timisoara and were acquired from Charles River. At the beginning of experiment they were eight weeks old. The work procedure followed all National Institute of Animal Health (NIAH) rules: animals were maintained during the experiment in standard conditions such as 12 hours light-dark cycles, food and water *ad libidum*, temperature 24±2 ºC, humidity above 55%.

In order to observe the changes of the mice skin, the three types of PU nanostructures suspensions were applied on the C57BL/6 mice skin for four weeks (50 µL suspension / application).

For this study there were used 15 mice: group 0 (3 mice as blank, treated with water), group A (4 mice, treated with A sample), group B (4 mice, treated with B sample) and group C (4 mice, treated with C sample). The mice were shaved in the first, tenth, and the sixteenth day and the applications and measurements were done twice a week.

After applications, each determination was performed within 30 minutes. All the measurements on the mice skin were carried out with a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany (Figure 1): the measurements of transepidermal waterloss (TEWL) were carried out with a Tewameter®TM 300 probe, the pH with a Skin-pH-meter®PH 905 probe, and the melanin and erythema measurements with a Mexameter®MX 18 probe.

Figure 1. The multiprobe Adapter System (MPA5), Courage-Khazaka used for skin measurements

3. Results and Discussions

The three types of drug carrier powders were dissolved in water in order to obtain suspensions with the same concentration (1:5000 v / v). It was measured the pH of these suspensions with a Schott TitroLine by simply plunging the electrode into the aqueous suspensions (Figure 2). The samples present slightly acid pH values due to the characteristics of its components (free hydroxyl groups on the polyurethane chains). The absence of secondary products (amines) is demonstrated by the low acid character of the suspensions.

Besides, these pH values (close to 5) are appropriate for products intended for cutaneous administration.

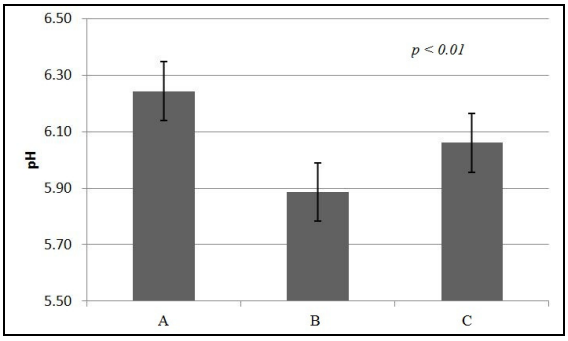

Figure 2. pH values of PEU durg carriers suspensions

The particles size and charge were measured using a Zetasizer Nano series equipment Nano-Zs, Malvern Instruments (Table 2). The samples were diluted at a ratio of 1:5000 (v / v) and the measurements were repeated three times for each sample.

Table 2. PEU sizes and Zeta potentials

Raw materials	Values / Sample		
	A	B	C
Size mean, nm	501	387	192
polydispersity index	0.3	0.5	0.2
Zeta potential mean, mV	31.0	29.5	27.4

The values for the diameter of each sample can be correlated with the polyol molecular weight. The Zeta potential values are important because if all the particles have a Zeta potential which is more negative than -30 mV or more positive than +30 mV the dispersion should remain stable. This is the reason why it was considered that the A sample is the most stable.

The evolutions of average values that included main parameters such as TEWL, melanin, and erythema for each mice group are shown in the Figure 3.

It could be observed in Figure 3 the good results of this research: the transepidermal waterloss (TEWL) decreased in each group during the four weeks of application. The melanin shows a slight increase for each mice group, the same as in the case of erythema which indicate a slight redness due to the skin stress. The pH determinations are not relevant because of the chaotic evolutions.

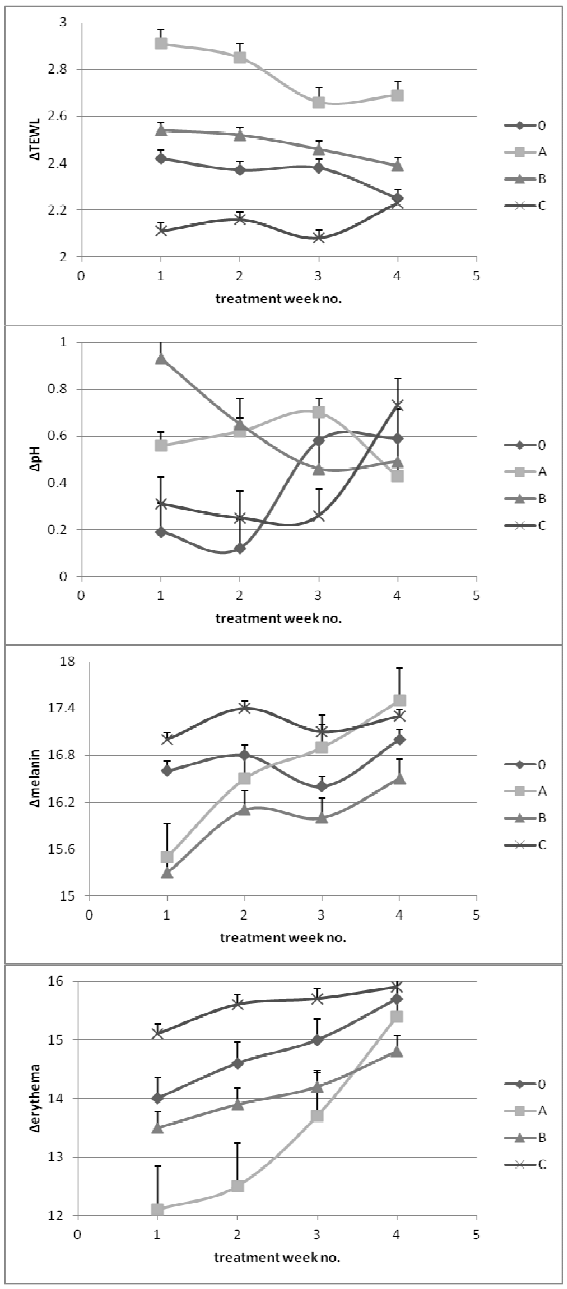

Figure 3. Skin parameters evolutions

The transdermal drug administration has been studied extensively because of the *stratum corneum* (SC) barrier property [15]. This study tried to avoid any such problems by creating nanostructures with the size in the range 100-500 nm. Another experiment was done by our team in order to synthesize drug carriers with different size and stability by changing the speed of stirring during the synthesis.

Now it was studied the modification of the carrier size by using different diol/polyol ratio.

The mice skin is very important for such of these studies because it is very sensitive and it has a penetration degree a few times greater than human skin [16, 17]. So, the sensitivity of the mice skin is an advantage as it can be used as a parameter characterizing the investigational product harmfulness. In the polyurethane domain it is known that the diisocyanates great reactivity due to the two double bonds from its specific group (–N=C=O) is a danger to human health [18, 19], but on the other hand it is well-established that the polyurethane products did not present any toxicity. In present work it was used a polyol component excess in order to avoid this problem, but also because this excess is easily removed by washing and it is a way to prevent formation of secondary products (amines).

An important reagent for actual synthesis is the surfactant, Tween®20. The synthesis needs a surfactant to create droplets that grow structures. In the literature this reagent is relative non-toxic one but many studies have dealt with this topic [20, 21]. That important feature of the mice skin that we already describe, the sensitivity is a way to test the toxicity of our products based on this reagent. The mice skin presents a very rapid change of the erythema values in case of toxic agents assaulted. The erythema values recorded in our four weeks experiment have an easily growing trend which is a normal one for any skin treatment. This is the reason why it could be considered that these products are not toxic.

4. Conclusion

The obtaining of PEU nanostructures using the interfacial polycondensation technique combined with spontaneous emulsification is an easy applicable procedure. This study demonstrated that these PEU nanostructures are a proper transdermal drug delivery system because of the pH values which are appropriate for products intended for cutaneous administration. They decreased TEWL values but not significant. The nanostructures based on PEG present the best values for drug encapsulated amount and stability.

Acknowledgements

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Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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