Optimization of Hollow Aliphatic Polyurethane Particles Used as a Drug Delivery System

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Drug delivery systems are used to protect unstable pharmaceutical active substances, to modify the characteristics of the loaded drug or to deliver the active ingredient to its specific receptor. In this study, polyurethane-based particles were obtained and the influence of stirring speed during the synthesis, the drying speed of products as well as the chain extender/polyol ratio were determined in order to describe the characteristics of the new particles. The final particles were elongated in shape and stable up to 280°C. We established that the drying speed did not influence in a noticeable manner the particles size and led to the formation of a dispersed system in which the constituents presented a high tendency to form clusters or conglomerates. The results suggested that the stirring speed during the synthesis and the chain extender/ polyol ratio may be used as two important parameters which can influence the particles sizes without affecting other characteristics. In vivo evaluation on mice skin model did not reveal any damaging properties of the newly synthesized particles.

Keywords: drug delivery system, polyurethane, zeta potential, skin irritation

The development of nano- and bio-sciences in the last decades led to the discovery of novel and important applications in medicine and pharmaceutical industry. A very important target in nanopharmacy is the discovery and characterisation of novel and improved drug delivery systems consisting of particles with sizes below 100 nm. The main goals of drug delivery systems are 1) to transport the loaded active substance to a specific receptor and 2) to provide its delivery for a prolonged period of time and in the same concentration. The carrier may also serve as protective shield for the active compound and may modify its physical and chemical properties such as aqueous solubility, pH, thermal and/or spectral properties [1, 2].

Nanosized drug delivery systems represent an interesting and versatile platform for a variety of poorly water-soluble drugs [3]. In recent years, one of the main goals in the antimicrobial and antiparasitic research is the preparation of new improved pharmaceutical formulations in order to expedite the need of new drug discovery. One of the main issues in public health is the helminthic-caused diseases in which the therapeutic arsenal is rather poor. One of the most recommended drugs, albendazole, has a broad spectrum activity against human and animal helminth parasites, but its effectiveness is limited by its poor water- solubility and the consequent low bioavailability [4]. Many studies were focused on overcoming the disadvantage of the unpredictable therapeutic response of albendazole, numerous including nanoparticle formulations [5-9].

The drug delivery systems are classified in two main types: inorganic compounds such as metallic nanostructures (Ag nanoparticles, gold-coated capsules, hydroxy-apatite or zeolites-based carriers) and organic or macromolecular compounds such as polymers or lipids - the latter being more oftenly used in therapeutic delivery and imaging [10]. Drug delivery systems with different shapes were developed and studied in order to determine which is the most suitable for a pre-established purpose: carbon nanotubes, fibbers or wires, spherical or elongated capsules and dendrimers, etc. [11]. The shape and size of particles used as drug delivery systems seem to be the most important parameters which may influence the hydrodynamic force on drug release [12]. Citu *et al.* [13] described the influence of particles' shapes and sizes over their movement through the blood vessels: larger particles tend to attach to the wall of the vessel while spherical particles flow with a higher speed than the particles with elongated shapes.

Our team already studied the influence of the surfactant on the size of a polyurethane-based drug delivery system [14, 15] finding that larger amounts of surfactant lead to the formation of smaller particles with increased stability; however, the surfactant excess is difficult to be removed. The purpose of this research was to develop polyurethane nano- and micro-particles with different sizes based on the modification of synthesis parameters (temperature, stirring speed and drying speed of products) as well as of chain extenders/polyols ratio. We also studied the toxicological potential of such polyurethane-based particles in order to collect valuable data about the possibility of their use as drug delivery systems.

Experimental part

Materials

Isophorone diisocyanate (IPDI), polyethylene glycol (PEG, average M_n ~200) and acetone were supplied by Merck (Germany); polycaprolactone diol (average M_n ~530) and 1,6-hexanediol by Aldrich (USA). The surfactant (Cremophor RH40) was kindly donated by colleagues from the Faculty of Pharmacy - University of Szeged (Hungary), monoethylenglycol was purchased from Lach-Ner s.r.o. (Czech Rep.) and 1,4-butanediol (1,4-BD) from Carl Roth GmbH (Germany). All storage conditions were followed and the substances (analytical grade purity) were used without any further purification.

Synthesis of polyurethane particles

The polyurethane particles were prepared through a multi-step process based on interfacial polyaddition

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Sample code	Isophorone diisocyanate, mL	Aqueous phase						Stirring speed, rpm	Drying speed
		MEG, mL	BD, mL	HD, mL	PEG, mL	PCL, mL	Surfactant, mL		
01	1.75	0.25	0.40	0.40	0.20	1.25	1.60	350	N
02	1.75	0.25	0.40	0.40	0.20	1.25	1.60	500	N
03	1.75	0.25	0.40	0.40	0.20	1.25	1.60	650	N
11	1.75	0.45	0.40	0.40	0.20	1.05	1.60	350	N
12	1.75	0.45	0.40	0.40	0.20	1.05	1.60	500	N
13	1.75	0.45	0.40	0.40	0.20	1.05	1.60	650	N
21	1.75	0.45	0.40	0.40	0.20	1.05	1.60	350	F
22	1.75	0.45	0.40	0.40	0.20	1.05	1.60	500	F
23	1.75	0.45	0.40	0.40	0.20	1.05	1.60	650	F

N= normal program; F= forced program

Table 1
THE RAW MATERIALS RATIO FOR THE POLYURETHANE PARTICLES SYNTHESIS

combined with spontaneous emulsification [16]. The protocol was already described in detail in our previous papers [17-20]; briefly, an organic phase (isocyanate dissolved in acetone) is heated with an aqueous phase (mixture of diols, polyols and surfactant) under magnetic stirring for 4-5 h. The resulted products were dried and washed several times with a mixture of water/acetone (1.5:1 (v/v)); the procedure was repeated in order to obtain various polyurethane-based particles (table 1).

Petri dishes with thin layers of final products were maintained inside a PolEko SL115 laboratory oven with the following heating program: 8 hours at 65°C and forced air convection daily (for a normal program, N) and 12 h at 105°C and forced air convection daily (for a forced program, F). The samples were dried until constant weight (between 4 and 5 days for the normal drying program and around 3 days for the forced drying program).

Physical and chemical characterization

The products' solubility was tested in distilled water and several organic solvents such as methanol, ethanol, acetone, dimethyl-sulfoxide (DMSO) and anhydrous tetrahydrofuran (THF) at 25°C. The *p*H values were determined using 50 mL solution and a portable HI 98103 (Hanna Instruments, USA) at 25°C. Three standard buffer solutions (*p*H=4.50, 7.00, and respectively 9.50) were used to calibrate the instrument.

The size and stability of polyurethane particles were measured using a Vasco Size Analyzer and a Wallis Zeta potential Analyzer (Cordouan Technol., France). The following parameters were selected for the Size Analyzer: temperature (25°C), time interval (between 20-24 μs), number of channels (480-560), laser power (between 85-90%), DTC position: UP, acquisition mode (continuous), and analysis mode (Pade-Laplace). The following parameters were selected for the Zeta potential Analyzer: cuvette type (plastic), temperature (25°C), resolution (medium, 0.8 Hz), and Henry function (Smoluchowski).

The UV/VIS spectra of samples were assessed at 25°C using a SI Analytics UViLine 9400 spectrophotometer; diluted samples and plastic cuvettes were used.

In vivo evaluations

Twenty-two SKH1 female mice (ten weeks old) were purchased from Charles River (Sulzfeld, Germany). Mice were divided into numerically equal groups (2 mice for every sample, 2 mice as blank and 2 mice for positive control). Sodium lauryl sulphate (SLS) was used as positive control due to its well-known irritation potential.

Concentrated aqueous suspension of each sample and both positive and negative control (same concentration, 1.5 mg/mL) were gentle applied on the back skin of mice every third day (1 ml / application) for three weeks. The determination of skin parameters was performed at 30 minutes after each application. The measurements were carried out following the specified protocols from the manufacturer guidelines [21] using a Multiprobe Adapter System (MPA5) from Courage&Khazaka Electronics, Germany, equipped with a Tewameter®TM300 probe, a Mexameter®MX18 probe and a Corneometer®CM825 probe. The evaluations were carried out at the same moment of the day, by the same operator, in a narrow range of temperature (25±1°C) and air humidity (50±5%).

Compliance with Ethics Requirements

Authors declare that all procedures involving animal subjects respect the specific regulations and standards; this study was firstly evaluated and approved by the Ethical Committee of the *Victor Babes* University of Medicine and Pharmacy Timisoara, Romania. The work protocol followed the rules of the National Institute of Animal Health: during the experiment animals were maintained in standard conditions of 12 h light-dark cycle, food and water *ad libitum*, 25±1°C, humidity above 55%.

Statistics

All the measurements from this research were done in triplicate for each sample; the results were expressed as mean ± standard error. The one-way Anova followed by the Bonferroni method were used to determine the statistical parameters of this study. $p<0.05$ was considered statistically significant; *, ** and *** indicate $p<0.05$, $p<0.01$ and <0.001, respectively.

Results and discussions

The preparation of polyurethane-based drug delivery systems by our team was reported for the first time in 2012 in a study emphasizing the influence of aliphatic diisocyanates over the particles' characteristics [22] in which we reported their elongated shape as well as heat resistance up to 280°C.

The synthesis conducted in the current study is based on the following innovations: (i) the absence of any catalyst - which may contribute to alleviation of the irritation potential; (ii) the replacement of a part of polyethylene glycol with polycaprolactone - diol which may lead to a significant shortening of particles' degradation time [23].

The solubility values found for the polyurethane-based particles fall between 1.1 and 1.9 mg/mL at 25°C (Table 2). Higher solubility values were recorded when organic solvents were used, particularly DMSO and THF.

The recorded *p*H values were situated in a narrow range between 6.5 and 6.9 units.

Table 3 exhibits the size and the Zeta potential of the resulted particles. The first three samples (01, 02 and 03) show the lowest values of particles' diameter the result being attributable to the minimum chain extender/polyol ratio used. Comparing the samples 11, 12 and 13 with samples 21, 22 and 23, one can notice that the drying speed slightly influences the size values; thus, a forced drying program leads to the formation of smaller particles but the recorded differences were quite small. Another interesting aspect was the influence exerted by the stirring speed during the synthesis; one can notice that samples 03, 13 and 23 resulted after a stirring speed of 650 rpm contain particles with significantly smaller diameters than samples 01, 11 and 21 where the stirring speed was only 350 rpm.

The samples are quite homogeneous with polydispersity index values around 0.2-0.3 units, except for those samples which were dried using a forced program (samples 21, 22 and 23) with a polydispersity index between 0.4 and 0.5 (medium homogenity).

Sample code	Solubility in various solvents, mg/mL						Average pH value ± SE
	distilled water	methanol	ethanol	acetone	DMSO	THF	
01	1.1	1.6	1.6	1.3	1.8	1.7	6.78 ± 0.11
02	1.2	1.6	1.5	1.4	1.7	1.7	6.71 ± 0.09
03	1.2	1.5	1.6	1.4	1.9	1.8	6.91 ± 0.08
11	1.1	1.4	1.4	1.3	1.5	1.6	6.59 ± 0.17
12	1.1	1.5	1.6	1.4	1.6	1.6	6.60 ± 0.12
13	1.2	1.6	1.6	1.5	1.7	1.5	6.56 ± 0.14
21	1.1	1.7	1.4	1.4	1.9	1.8	6.61 ± 0.16
22	1.1	1.5	1.5	1.6	1.8	1.8	6.67 ± 0.13
23	1.2	1.6	1.7	1.5	1.7	1.9	6.59 ± 0.09

Table 2
THE SOLUBILITY AND PH VALUES OF POLYURETHANE-BASED PARTICLES

Sample code	Average particle size (nm) ± SE	Polydispersity index	Average Zeta potential (mV) ± SE
01	89 ± 14	0.3	27.8 ± 5.3
02	81 ± 11	0.3	28.4 ± 4.4
03	76 ± 13	0.2	27.1 ± 3.9
11	211 ± 19	0.3	29.9 ± 4.1
12	180 ± 17	0.2	29.0 ± 3.7
13	175 ± 21	0.3	25.9 ± 4.8
21	203 ± 24	0.5	21.8 ± 6.5
22	184 ± 21	0.5	22.5 ± 5.7
23	171 ± 18	0.4	22.8 ± 5.2

Table 3
THE SIZE AND CHARGE OF POLYURETHANE-BASED PARTICLES

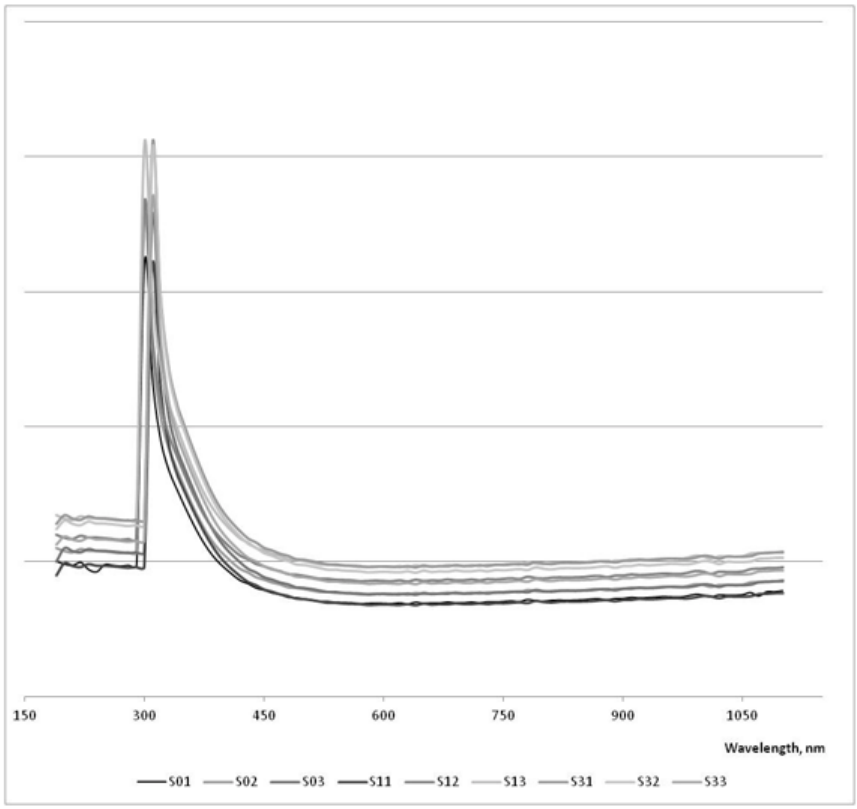

Fig. 1. UV/Vis spectra of obtained samples

In the field of colloidal suspensions the Zeta potential is used in order to predict the stability of final particles; the particle mobility inside an electrical field is used to indicate their stability depending on their tendency to form clusters or conglomerates. Scientific literature describes that unstable particles present Zeta potential values between -30 and +30 mV [24]. During the current study polyurethane-based particles with an average stability were reported for samples 01, 02, 03, 11, 12 and 13 and with a low stability for samples 21, 22 and 23.

The UV/Vis spectra (fig. 1) show very similar curves with a maximum absorption peak around 300-310 nm.

Animal models are increasingly used alongside the *in vitro* tests on cell lines for the purpose of assessing the toxicity of newly synthesized products [25, 26]. Many instruments such as tewa-meter, mexameter, corneometer, skin-pH meter were developed in the last decades in order to evaluate any changes of skin parameters (trans-epidermal water loss, level of melanin and haemoglobin/erythema, moisture of *stratum corneum*, skin *p*H, etc.) induced by potentially harmful compounds.

In our study, average evaporation rates (AER) were estimated according to the equation:

$$TEWL = 0.92xAER + 1.37,$$

where TEWL is the trans-epidermal water loss (fig. 2).

Given that TEWL is strongly related to the ambient relative humidity (ARH), the resulted values were corrected to an ARH of 50% (TEWL50) using the equation:

$$TEWL50 = 50xTEWL/(100-ARH),$$

as was described in previous papers [27].

Figure 2 reveals the increasing tendency of TEWL50 values for all mice groups, which is a normal process for the hairless mice that were the subject of our experiment. Thus, a very slight increase of TEWL50 values was noticed for the blank group; however, it is important to note the much higher values recorded for the SLS group used as positive control. For all the others mice groups the increases of TEWL50 values cannot be associated with the presence of toxic compounds.

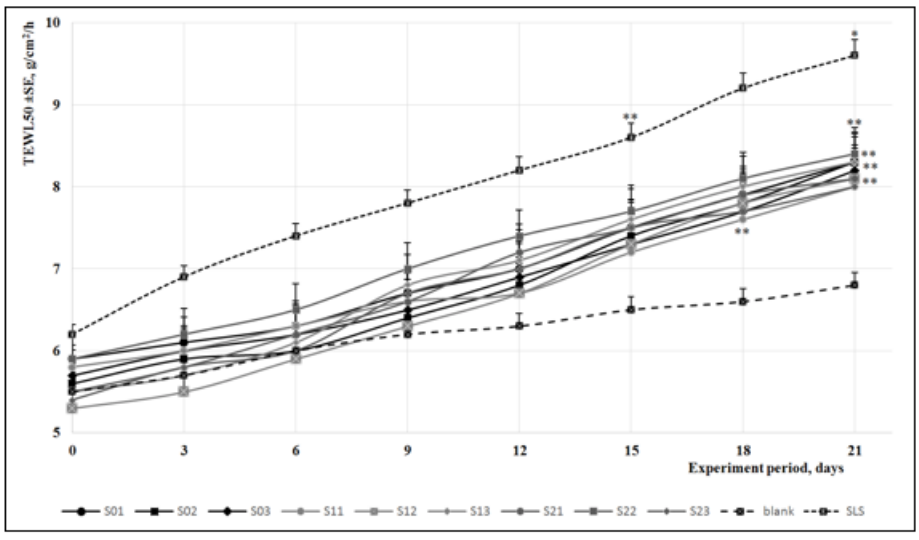

Fig. 2. Evolution of TEWL50 values

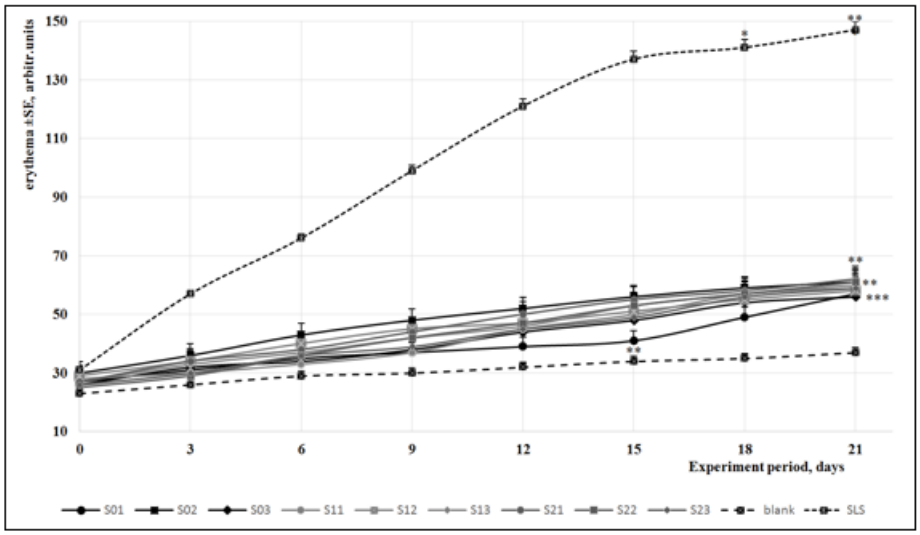

Fig. 3. Evolution of mice erythema during the experiment

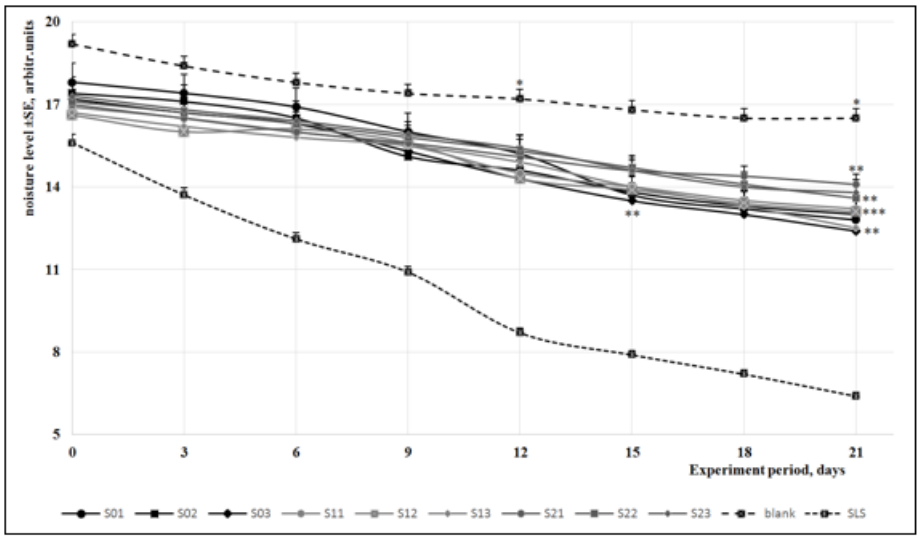

Fig. 4. Evolution of *stratum corneum* moisture

The haemoglobin/erythema value can be considered as the most important skin parameter during the assessment of the irritation potential. Figure 3 shows an important increase of the erythema values for the SLS treated mice. On the other hand, the slight increase of erythema level for the mice treated with polyurethane-based particles is comparable to the increase recorded for the blank group. Once again, the polyurethane-based particles may be considered as non-irritating compounds.

The evolution of moisture levels is displayed in Figure 4 indicating that the most important decrease was reported for the SLS treated mice; all the other groups (including the blank) exhibit mild decreases of the moisture level.

Conclusions

Interfacial polyaddition combined with spontaneous emulsification were involved in the synthesis of polyurethane-based particles which can be used as drug delivery system. An organic phase (IPDI in acetone) and an aqueous phase (a mixture of MEG, BD and HD as chain extenders, PEG and PCL as polyols and Cremophor RH40 in water) were preheated and mixed. The stirring speed, drying speed and chain extender/polyol ratio were modified in order to assess their influence over the particle properties. In this experiment elongated and thermally stable (up to 280°C) particles were prepared. Comparative analysis revealed that increased drying speed leads to smaller but more dispersed and unstable particles; the increasing of stirring speed and/or decreasing of chain extender/polyol ratio may lead to smaller particles without affecting other characteristics. No irritating potential was reported for the final particles.

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