Comparative Study on Polyurethane and Cyclodextrin Carriers for Triclosan

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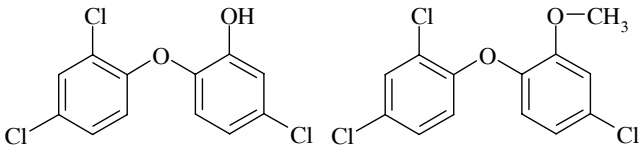
Triclosan (Tr), an antibacterial and antifungal agent, provides an extra benefit to health beyond its anti-gingivitis effect in toothpaste. Polyurethane(PU) microstructures and cyclodextrins(CD) inclusion complexes with and without methyl-triclosan were synthesized in order to study any improvement of the drug efficacy and release. Physical and chemical properties of the products were evaluated using pH, scanning electron microscopy, differential scanning calorimetry, size and Zeta potential measurements. Highly optimized results were obtained for CD inclusion complexes which can be used as drug carriers in toothpastes and mouthwashes.

Keywords: triclosan, polyurethane (PU), cyclodextrin (CD), Zeta potential

Oral health problems such as dental caries and periodontal diseases are very important worldwide [1]. The oral cavity of healthy persons includes hundreds of bacterial and fungal species which can be associated in biofilms resistant to mechanical tooth cleaning or antibiotic treatments [2]. A lot of bacteria have become resistant to antibiotic effects because of their abusive prescription worldwide [3]. Oral microorganisms adhere to the tooth surface, form a bacterial biofilm recognized as dental plaque and then continue to grow in this environment [4].

2,4,4'-Trichloro-2'-hydroxy-diphenyl-ether, known as triclosan (Tr) (scheme 1), is a synthetic, broad-spectrum antimicrobial agent that has been used in a wide variety of antibacterial products in the last decades (soaps, deodorants, toothpastes and cosmetics) [5].

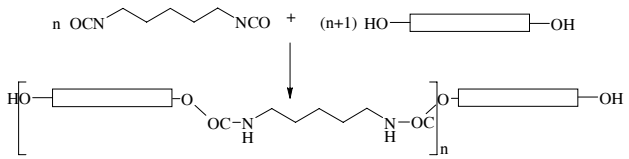


Scheme 1. Structural formulae of triclosan and methyl-triclosan

Drug carriers used to deliver antibacterial substances to the site of infection (periodontal pocket) have gained interest in the last decades; this kind of treatment has the advantage of delivering the drug in the specific site, sustaining and/or controlling the drug concentration [6].Oral delivery of different substances is available for a small number of drugs but it has revealed promising results when compared to the conventional intravenous administration [7]. Takeuchi *et al.* [8] examined the effect of a dental drug delivery system on *S. mutans* and other oral bacterial flora; the results showed that dental drug delivery system used with a professional mechanical tooth cleaning selectively killed *S. mutans* on the tooth surface and the process was considered safe in terms of disturbing the oral flora.

The potential of liposomes as carriers for the oral cavity has been investigated and the results revealed that saliva constituents may interact with liposomes. An appropriate liposomal drug delivery system intended for the use in the oral cavity seems to be dependent on the liposomal formulation [9].

Biodegradable polymers used as drug carriers are represented by polyglycolide, polylactide, polyanhydride, poly(propylene fumarate), polycyanoacrylate, polycaprolactone and polyurethanes (PU) [10]. PU chains are formed by a chemical reaction between diisocyanates and diols to yield polymers with urethane bonds (–NH–CO–O–) in their main chains (scheme 2) [11].



Scheme 2. Synthesis of polyurethane chains

Cyclodextrins or cycloamyloses are cyclic oligomers of glucose which can form water-soluble inclusion complexes with small molecules or side chains of large compounds. These cyclic oligosaccharides do not give elicit immune responses and present low toxicities in animals and humans [12]. CD are used in drug delivery and pharmaceutical industry due to their complex ability; some of the most common applications are the increase of the solubility, stability, safety and bioavailability of drug molecules [13]. External associations between CD and pentacyclictriterpenes increase the water solubility of these active substances, but the best results are achieved by

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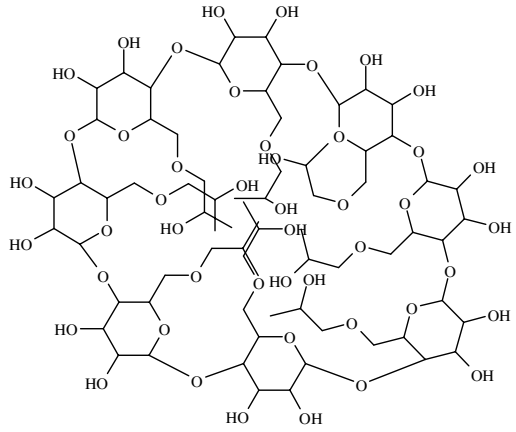


Scheme 3. Structural formula of hydroxypropyl-gamma-cyclodextrin (HP-γ-CD)

preparing real inclusion complexes. Therefore, gamma-cyclodextrin and its derivatives (scheme 3) are used because their inner cavity allows the accomodation of large molecules [14].

The aim of this investigation was to develop and study PU microstructures and CD inclusion complexes as carriers for triclosan; their use is considered safe because of the minimal disturbance of the oral flora.

Experimental part

Chemicals and Reagents

Lysine diisocyanate ester (LDI) was obtained from China. Ethylene glycol (EG) was purchased from Czech Rep., and 1,4 - butanediol (BD) was purchased from Germany. Polyethylene glycol, M = 200 (PEG)and solvent (acetone) were obtained from Merck (Germany). Emulsifier (Cremophor A6, known as polyethylene glycol 260 mono(hexadecyl/octadecyl)ether and 1-octadecanol), was kindly donated by our colleagues from University of Szeged (Hungary) and methyl-triclosan was purchased fromFluka.Hydroxypropyl-gamma-cyclodextrin (HP-γ-CD) was purchased from Hungary.

Methods of preparation

The procedure used to obtain the PU microstructures was already described in detail in a series of papers (fig. 1) [15-17]: 1. The organic phase and 0.2 g emulsifier (Cremophor A6)were injected into the aqueous phase at 40°C under magnetic stirring at 700 rpm (PU chains were formed in this phase) (table 1); 2. Stirring was continued for four hours at 40°C to ensure the maturation of structures walls; 3. The solvent (acetone) and water were removed by keeping the obtained suspensions in Petri dishes at 60°C in the oven for 12 h; 4. The final powders were purified by repeated dispersion in a 1:1 (v/v) mixture of water-acetone, followed by centrifugation.

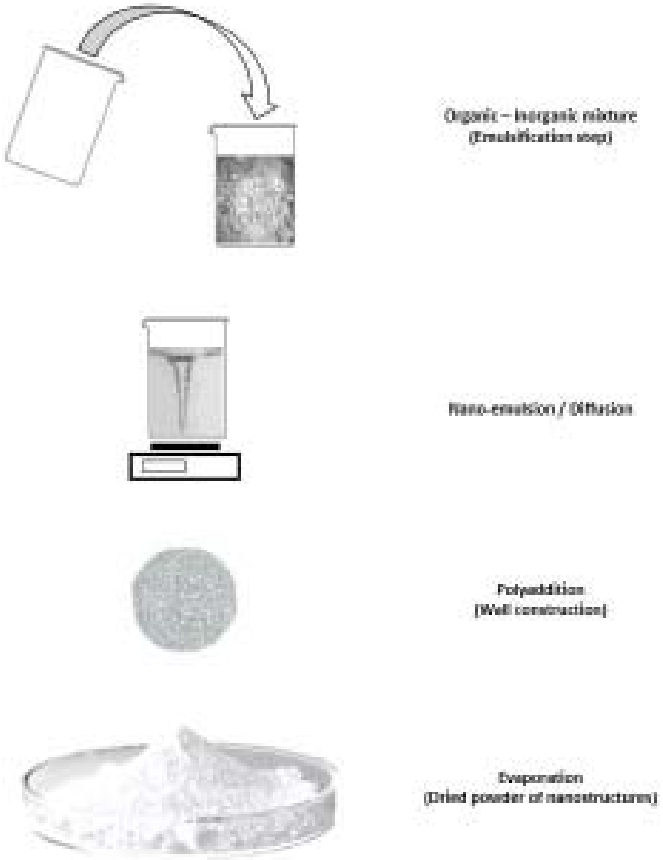


Fig. 1. The procedure used to obtain the PU microstructures

Two types of PU carrier samples were obtained using this procedure; the recipe of the synthesis is based on data presented in table 1.

The kneading procedure was applied for the preparation of two CD inclusion complexes (CD_EmS for the empty sample and CD_TrS for the sample with methyl-triclosan)using hydroxypropyl-gamma-cyclodextrin; the molar ratio betweenTr and HP-γ-CD was 1:2 as suggested by previous physical and chemical evaluations [18]. Firstly, a powder mixing using a mortar and a pestle was performed, followed by kneading with ethanol 50% in water (v/v) until the bulk of solvent evaporated.The mixture was finally dried at room temperature for 24 h and then at 105°C in the oven, for several hours, until constant weight. The final products were pulverized and sieved.

Analysis

The *p*H of all samples was measured in triplicate at the same concentration with a TitroLine alpha plustitrator (SI Analytics, Germany), by simply plunging the electrode into the aqueous solutions (1:5000 v/v). Carrier morphology was investigated with a scanning electron microscope Hitachi 2400S (Hitachi Scientific Ltd., Japan) at a voltage of 10 kV. The size and the charge of samples were

Samples codes	Raw materials - type, volume (ml)		
	Methyl-triclosan (sol.2% in EtOH 50%,w/w)	Organic phase	Aqueous phase
PU_EmS (empty sample)	-	LDI, 1.6 acetone, 20.0	EG, 0.6 BD, 0.6 PEG, 1.2 water, 40.0
PU_TrS (sample with triclosan)	0.3		

Table 1
THE RECIPE OF PU CARRIER SAMPLES

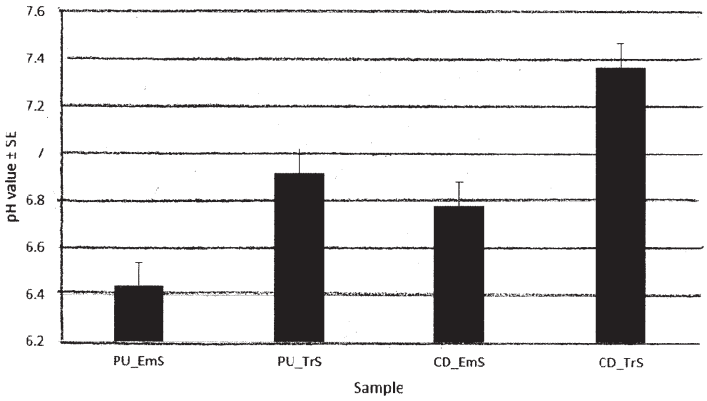
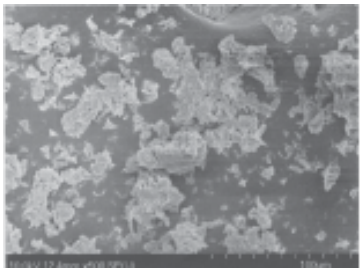
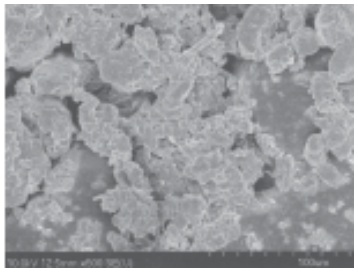


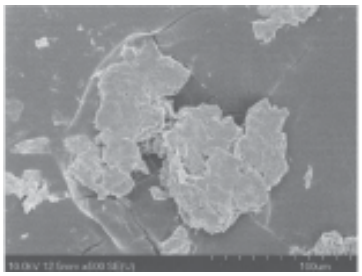
Fig. 2. The *p*H values of the sample



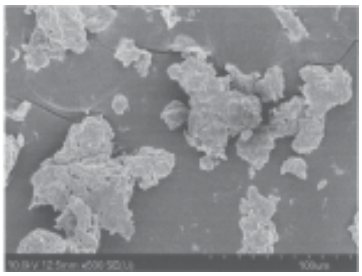
PU_Ems



PU_TrS



CD_EmS



CD_TrS

Fig.3.SEM micrographs of the sample

measured with a Zetasizer Nano series equipment Nano-Zs (Malvern Instruments, UK). For this purpose, the same aqueous solutions (1:5000 v/v) were used; the measurements were carried out three times for each sample.The thermal analysis was carried out on a differential scanning calorimeter model 821e (Mettler-Toledo, Switzerland) in dynamic air atmosphere (100 mL min^{-1}); the heating rate was 11.1 degree min^{-1}, up to 250°C.

Results and discussions

Diluted aqueous solutions of PU microstructures and CD inclusion complexes were obtained in order to evaluate their *p*H, size and Zeta potential values. Experiments revealed that CD complexes are 100-200 fold more soluble in water than PU samples. The 1:5000 dilution was chosen because it is the limit where all samples can be solubilized in water.The *p*H values of aqueous solutions are presented in figure 2. One can notice that PU samples are situated in a low acidic range which indicate the absence or very low content of secondary synthesis products (amines). The samples containingTr present higher *p*H values (a difference of 0.5-0.6 units) than the empty carrier samples; pKa for Tr has already been reported in the range of 7.9-8.1 [19]. All the samples present appropriate *p*H values for products intended for oral cavity use.

The SEM micrographs (fig. 3) revealedno important differences between the samples with and without Tr.The PU microstructures seem to have a predominant amorphous character which is indeed a feature of many polymeric materials. On a larger scale of SEM technique one can noticethe presence of elongated particles, probably due to particle aggregation.

The particle aggregation was also indicated by the data provided by theZetasizer (table 2). This technique indicated the formation of carrier structures with sizes between 500-1000 nm. There is a balance between the advantages and disadvantages of particle size in nanotechnology. The nanometer range is considered to be below 100 nm [20]. This is the field of structures with a particular behavior and toxicity because these very small particles can penetrate the cells walls [21, 22]. On the other hand, it was proven that larger particles can include larger amounts of

Sample	Particle size (nm)		Zeta Potential (mV)
	Mean ± SD	Polydispersity index	Mean ± SD
PU_EmS	495 ± 19	0.2	22.3 ± 4.1
PU_TrS	538 ± 45	0.4	21.8 ± 3.3
CD_EmS	959 ± 42	0.6	28.4 ± 4.2
CD_TrS	994 ± 17	0.5	27.7 ± 3.3

Table 2
THE ZETASIZER CHARACTERIZATION

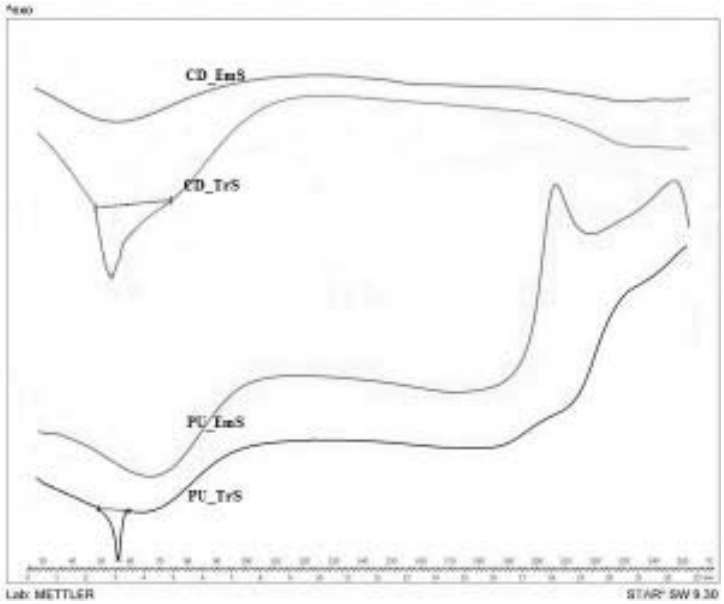


Fig. 4. DSC curves of the samples

biologically active substances; these "giant" structures present a lower transfer through different membranes[23].

The Zeta potential values indicate that the PU microstructures tend to cluster in order to stabilize themselves. The particles with a Zeta potential within the range of -30 ÷ -20 mV and +20 - +30 mV, respectively,have a medium stability degree and present a high tendency to agglomerate [24].

The first analyzed thermal phenomenon is the Tr melting (fig. 4); the maximum of the corresponding endothermic peak is in agreement with the value indicated by the producer (56-58°C). The degradation of Tr carriers is due to a thermooxidative process (exothermic effect) and takes place at significant higher temperatures than the melting point of the active substance. Both drug carriers are heat resistant up to 250°C. The glass transition of CD complexes was recorded above 230°C. The PU microstructures show a crystallization process around 210-240°C,specific for partially crystalline polymers.

Conclusions

Two different drug carriers were synthesized: PU microstructures and CD inclusion complexes. The PU carrier presented lower values of *p*H, size and Zeta potential, meaning that the amount and stability of the encapsulated triclosan are lower. The best results were obtained for the hydroxypropyl-gamma-cyclodextrin complexes. The size of these complexes was around 1µm, a proper size for a carrier with dental application. For the CD inclusion complexes, the Zeta potential values indicated a moderate tendency to aggregate. The synthesized carriers are heat resistant up to 250 °C.

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