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Original scientific paper

PREPARATION AND CHARACTERIZATION OF SOL-GEL PROCESSED SPRAY DRIED SILICA XEROGEL MICROPARTICLES AS CARRIERS OF HEPARIN SODIUM

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A spray drying technique was used for xerogel microparticles preparation from acetic acid catalyzed tetraethoxysilane (TEOS) – based silica sol, TEOS co-hydrolyzed with methyltriethoxysilane (METES), or α,ω-silane-terminated poly(ethylene glycol). The effect of TEOS modification on the heparin dissolution rate, particle degradation rate, as well as particle size and morphology was investigated. Smooth, spherical microparticles in the range of 1.02 – 13.51 μm, were produced by the spray drying method, which showed slow degradation rate and controlled release of heparin over prolonged period of time, dependent on the polymer's composition.

Key words: silica xerogel; microparticles; spray drying; controlled release; heparin

ПОДГОТОВКА И КАРАКТЕРИЗАЦИЈА НА МИКРОЧЕСТИЧКИ ОД СИЛИКА-КСЕРОГЕЛ КАКО НОСАЧИ НА ХЕПАРИН ДОБИЕНИ СО СОЛ-ГЕЛ-ПРОЦЕС И СУШЕЊЕ СО СПРЕЈУВАЊЕ

Микрочестички од ксерогел се добиени од раствор на тетраетоксисилан (TEOS), TEOS ко-хидролизиран со метилтриетоксисилан (METES) или полиетиленгликол (PEG) со крајни триетоксисилански групи, со оцетна киселина како катализатор, со сол-гел-процес и сушење со спрејување. Испитуван е ефектот на овие модификации во формулацијата на микрочестичките базирани на TEOS врз степенот на ослободување на хепаринот и нивната деградација, како и врз големината на честичките и нивната морфологија.

Добиените микрочестички се мазни и сферични, со димензии во интервалот од 1,02 μm до 13,51 μm. Тестовите за дисолуција и деградација на трите формулации на микрочестички покажаа дека се карактеризираат со бавен процес на деградација и со контролирано ослободување на хепарин во подолг временски период, зависно од употребениот прекурсор за ксерогелот.

Клучни зборови: силика-ксерогел; микрочестички; сушење со спрејување; контролирано ослободување; хепарин

INTRODUCTION

The sol-gel process, one of the fastest growing fields in material chemistry, has opened new possibilities for linking ceramics, glasses, and other inorganic materials on one hand, with bioactive molecules on the other hand, forming a novel, wide-scope family of biochemical reactive materials [1].

The sol-gel process involves the manufacture of inorganic matrices through the formation of a colloidal suspension, which is called sol. After gelation, the wet gel forms a globally connected solid matrix, which after drying forms the dry gel state, called xerogel. Drug molecules incorporated into the sol state are located within the porous silica xerogel network [2].

Using this basic technique and specific preparation processes, one can obtain bio-doped hydrogels or xerogels in various configurations (e.g. monoliths, sheets, granulates, microparticles and

thick and thin films) [3–4]. These silica matrices are chemically inert, hydrophilic, and inexpensive to synthesize. They also exhibit higher mechanical strength, enhanced thermal stability, and negligible swelling in organic solvents compared to most organic polymers. Other advantages of silica supports include biocompatibility and resistance to microbial attack [5].

The sol-gel process has been used as matrix for catalysts in hybrid materials such as ceramic-polymer, ceramic-metal composites or biosensors in diagnostic applications [6]. In the field of biosensors, Narang et al. [7], encapsulated glucose oxidase for a period of over 2 months. Growth factor TGF-β_1 [8], as well as several enzymes has been shown to retain their activity in dried silica xerogel matrices [9]. Conventional drug molecules have also been incorporated into silica gel matrices [10].

A major concern with the use of artificial organs and biomedical devices is the untoward interactions of blood upon contact with foreign surface. Local, controlled release of an appropriate anticoagulant at the site of trauma would be a good approach that would prevent thrombus formation [11].

For inhibition of blood coagulation in extracorporeal circulation procedures, the anticoagulant heparin is of interest due to its specificity, low collateral responses, and good tolerance by the organism [12].

The purpose of the present study was to evaluate the suitability of sol-gel produced microparticles as a carrier material for a controlled release of heparin. TEOS based silica xerogels were designed as carriers for heparin, but organically modified systems were evaluated as well.

EXPERIMENTAL

Materials

Silica xerogel particles were prepared by using the following reagents: tetraethoxysilane (TEOS, Merck), deionised water, acetic acid (CH_3COOH, Sigma), ammonium hydroxide (NH_4OH, Merck) and heparin sodium salt (Dongcheng biochemicals). Particular heparin sodium injectable grade is derived from porcine intestinal mucosa with declared potency of 175 IU/mg. Organically modified silica xerogels included methyltriethoxysilane (METES, Fluka) and α,ω-silane-terminated PEG (synthesized for the purpose of this work using isocyanate chemistry) [13].

Two moles of γ-isocyanatopropyltriethoxysilane (Fluka) were reacted with 1 mol α,ω-hydroxy-terminated poly(ethylene glycol) (PEG, Fluka, M_w = 600 g/mol; dried for 48 h at 40 ˚C) in vacuum oven, at 80 ˚C under dry nitrogen, until complete disappearance of the absorptions of the hydroxyl and isocyanate groups in the IR spectrum, at cca 3500 cm^{-1} and cca 2280 cm^{-1}, respectively, in favor of the urethane linkage (3384 cm^{-1}). The reaction was completed in 24 h.

Preparation of silica xerogels

TEOS based silica xerogels

Silica sol containing heparin was prepared by a two-step sol-gel process using acetic acid as a catalyst. The molar ratio of the silica sol was TEOS: H_2O:CH_3COOH = 0.05:5:0.004.

After the hydrolysis of TEOS had proceeded to a point where a homogenous solution was formed, pH was adjusted up to 4.5-4.6 using 1 M solution of NH_4OH and heparin sodium was dissolved directly into the sol. The concentration of heparin in the silica sol was 1.39 % corresponding to 36.36 % in spray-dried silica xerogel. Hydrolyzed silica sol was spray dried with a mini spray dryer (B-290, Büchi Labortechik AG; Switzerland). The spray drying process parameters were: inlet temperature 135 ˚C; pump 15; aspirator setting 90; spray flow 600 NL/h; pump setting 3–4 ml/min; outlet temperature: 60 ˚C. A 0.77 mm nozzle was used throughout the experiments.

Organically modified silica xerogels

Modification of the TEOS based xerogel was made by co-hydrolysis of TEOS with METES or α,ω-silane-terminated PEG. Partial substitution of TEOS was made with 10 % of the organically modified alkoxy silanes.

Organically modified silica xerogels were prepared using the procedure described in the previous section.

Evaluation of the heparin loaded silica xerogel microparticles

Particle size and particle size distribution determination

Volumetric particle size and size distribution of the microparticles was obtained by laser diffractometry using Malvern mastersizer 2000S

tometry using Malvern mastersizer 2000S Hydro. The samples were dispersed under pressure in a special gas chamber intended for particle size determination in a dry state.

Morphology

The shape and surface morphology of the microparticles was determined from micrographs taken with scanning electron microscope (Jeol SEM 6400 Japan).

Determination of heparin entrapment in silica xerogels

The percent of the incorporated heparin in silica xerogels was determined by dispersing 50 mg sample in 25 ml 0.02 M NaOH, stirred with magnetic stirrer at temperature not exceeding 60 ˚C until clear solution was obtained.

Toluidine blue test

The total amount of heparin was measured by a colorimetric toluidine blue method [14] modified for the present study; 0.005 % toluidine blue solution was prepared in 0.01 M HCl containing 0.2 % NaCl.

A 2 ml of sample in 0.02 N NaOH were pipetted in test tubes, 2 ml of toluidine blue solution were added, and the mixture was vigorously vortexed for 30 s. Four ml of hexane was then added to the tubes and they were shaken for another 30 s to separate the heparin-dye complex formed. The aqueous layers were sampled for measurement and diluted if necessary. The absorbance was measured at 631 nm within 30 minutes with UV-Vis-Spectrophotometer – Perkin Elmer Lambda 16.

Release of heparin in vitro

The dissolution test from different silica xerogel matrices was carried out in a shaking water bath at 37 ˚C. The average weight of the evaluated samples was 50 mg.

Simulated body fluid (SBF) was used as a dissolution medium. SBF was prepared by dissolving the following reagents: NaCl (136.8 mM); $NaHCO_3$ (4.2 mM); KCl (3.0 mM); $K_2HPO_4 \times 3H_2O$ (1.0 mM); $MgCl_2 \times 6H_2O$ (1.5 mM); $CaCl_2 \times 2H_2O$ (2.5 mM); Na_2SO_4 (0.5 mM) in deionised water.

The solution was buffered with tris(hydroxymethyl)aminomethane (TRIZMA) and hydrochloric acid (HCl) at pH 7.40. The composition of inorganic ions corresponded to that of human blood plasma [15].

Silica xerogel microparticles were immersed in 10 ml SBF in a polyethylene tube with a screw cap. Alternatively, a 5ml sample was removed from each tube and replaced immediately with fresh SBF in predefined time intervals.

Toluidine blue test

The total amount of heparin dissolved was also measured by the colorimetric toluidine blue method. Standard heparin solution was prepared by diluting 20 mg heparin to 20 ml deionised water.

The standard dilutions were between 5 and 30 μg of heparin in SBF.

A 1.3 ml of toluidine blue solution and 1.3 ml of sample in SBF were pipetted in test tubes, then vigorously vortexed for 30 s. A 2.6 ml of hexane was then added to the tubes and they were shaken for another 30 s to separate the heparin-dye complex formed. The aqueous layers were sampled for measurement and diluted if necessary. The absorbance was measured at 631 nm within 30 minutes with UV-Vis-Spectrophotometer.

Degradation of xerogel

The degradation process of the silica xerogel microparticles was evaluated using unloaded microparticles immersed in SBF, by measuring the dissolved $Si(OH)_4$ as a molybdenum blue complex by UV-Vis-Spectrophotometer at 815 nm [16].

RESULTS AND DISSCUSION

Preparation of microparticles

At first, unloaded microparticles were obtained by spray-drying method. Examination by scanning electron microscopy showed that unloaded silica microparticles were spherical, with no visible pores on the surfaces.

The microparticle yields of three different xerogel formulations (n = 3 batches for each formulation) were calculated from the theoretical amount of silica gel (SiO_2). Unloaded microparticles were produced with the following yields: TEOS based: 39.16 %; TEOS/METES based: 27.50 %; and TEOS/α,ω-silane-terminated PEG based microparticles: 47.89 %.

Heparin loaded microparticles were prepared by dissolving the active ingredient in the sol before

spray-drying. Examination by SEM showed that loaded silica microparticles were with spherical shape and smooth surfaces, regardless of the composition of the silica network. (Figs. 1, 2).

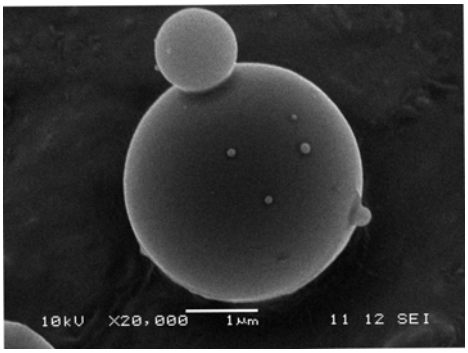

Fig.1. Scanning electron micrograph of TEOS based loaded microparticles

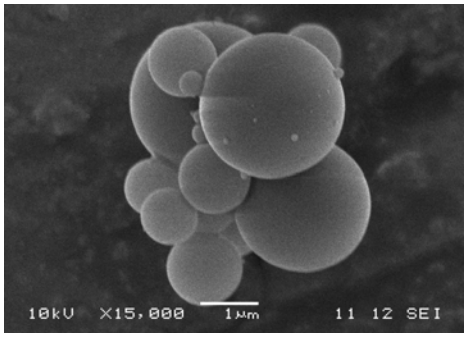

Fig. 2. Scanning electron micrograph of TEOS/α,ω-silane-terminated PEG loaded microparticles.

Particle size measurement showed that TEOS based microparticles have Dv (0.1) bellow 1.78 μm and Dv (0.9) bellow 13.51 μm. As for the TEOS/METES based microparticles, Dv (0.1) was bellow 1.08 μm and Dv (0.9) was bellow 7.68 μm. For TEOS/α,ω-silane-terminated PEG, Dv (0.1) was bellow 1.02 μm and Dv (0.9) was bellow 4.89 μm.

It has been shown that hydrogen bonds between organic polymers prone to hydrogen bonding with the silanol functions formed by hydrolysis of tetraethoxysilane have a decisive effect on the formation and final preparation of the obtained hybrid materials [17]. Thus, it can be expected that PEG increases the average particle size in the silica sols due to hydrogen bonding, but also hinders in some way the polycondensation process during spray-drying process, resulting in formation of smaller particles.

The yields of loaded microparticles were as follows: TEOS based: 48.26 %; TEOS/METES based: 40.48 %; and TEOS/α,ω-silane-terminated PEG based microparticles: 71.19 %.

Experimentally determined loading efficiency of heparin sodium in the microparticles was 84.11 % in TEOS based, 98.63 % in TEOS/METES based, and 90.88 % in TEOS/α,ω-silane-terminated PEG based microparticles.

The differences in loading efficiency of heparin sodium into the silica microparticles are probably due to the incorporation of different precursors in the silica sol. In fact, incorporation of ormosils into hydrolysis combined with TEOS results in decreased hydrophilicity of the network and changes in porosity [18]. Organic groups linked to the oxide network by stable chemical bonds change the inner structure by reducing the degree of cross-linking [19]. Hence, higher loading efficiency would be expected in the organically modified systems. Also, according to Ahola et al. [2] when pure PEG was incorporated as an additive into an acetic acid catalyzed TEOS based silica xerogel, the pore volume and the specific area of pores in silica xerogel samples were decreased, which is a factor involved in the efficacy of loading of the active substance during preparation.

Incorporation of heparin into the microparticles was confirmed by means of FTIR spectroscopy, comparing the O–H bending mode of heparin $SO_3^-(H_3O)^+$ groups located at 1623 cm^{-1} [20], as well as by TG analysis and comparison of the weight losses of unloaded and loaded microparticles at temperatures above 180 °C.

Release of heparin and degradation of silica microparticles

The *in vitro* release of heparin from the three different formulations is shown in Fig. 3.

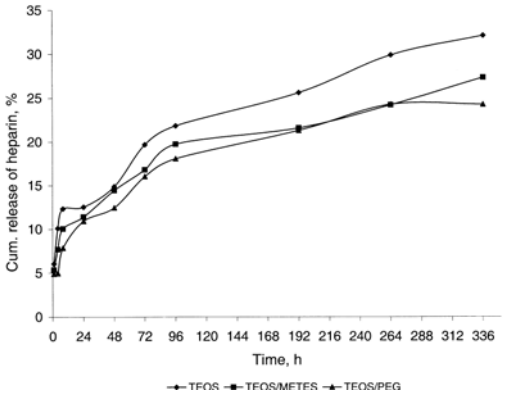

Fig. 3. Cumulative release of heparin from different xerogel formulations

After 14 days the cumulative percent of released heparin was: 32.11 % from TEOS microparticles, 27.34 % from TEOS/METES microparticles, and 24.27 % from TEOS/α,ω-silane-terminated PEG microparticles.

Dissolution curves show an initial burst release of heparin sodium (in the first hour) followed by a slower release pattern in all three systems. The plot of the release was linear with respect to the square root of time for TEOS, TEOS/METES and TEOS/α,ω-silane-terminated PEG (R: 0.989; 0.988; 0.971), respectively.

The increased initial burst may be associated with sufficiently high amount of drug close to the surface of the gel network [21. When the drug was depleted from the surface, the silica gel degradation became a factor involved in the heparin release rate.

The results from the *in vitro* release of heparin show that addition of ormosils decreased the release rate of heparin from the microparticles. This is probably a result of an increased hydrophobicity when METES is introduced in the silica network, or it is due to the already discussed decrease of pore volume in the system with TEOS/α,ω-silane-terminated PEG.

Silica gel degradation of the microparticles is shown in Fig 4. After 21 days the amount of the silica gel left was 91.10 % for TEOS/α,ω-silane-terminated PEG system, and even 95.89 % for TEOS based and 96.17 % for TEOS/METES based xerogels.

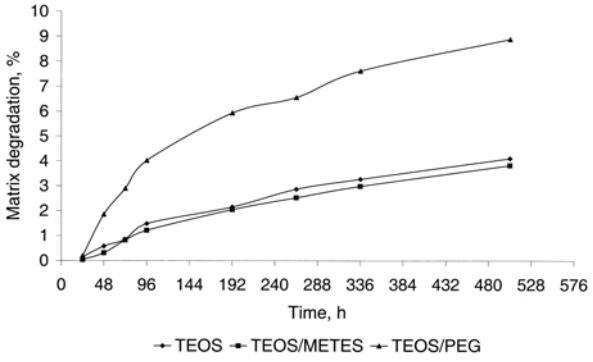

Fig 4. Degradation of different xerogel formulations

Degradation plots were fitted to first order kinetics model and the following values for R were obtained: (0.977; slope = $3.65 \cdot 10^{-5}$) for TEOS microparticles, (0.978; slope = $3.49 \cdot 10^{-5}$) for TEOS/METES microparticles, and (0.945; slope = $7.73 \cdot 10^{-5}$) for TEOS/α,ω-silane-terminated PEG microparticles.

The cumulative percent of degraded silica gel network was similar when TEOS and TEOS/METES microparticle were compared. Almost two times higher amount of degraded silica gel was obtained in the case of TEOS/α,ω-silane-terminated PEG microparticles. This is probably due to the increased hydrophilicity of the silica network and enhanced intake of water into the system, which favors hydrolysis as a mechanism of degradation of the silica xerogel network.

Heparin was released faster than the silica gel microparticles were degraded (Fig 5). Literature data suggests that for highly water soluble drugs, the release rate is controlled mainly by diffusion through the matrix, whereas the erosion process is the main factor for release of low water-soluble drugs. Thus, the release rate is probably a combination of diffusion of the dissolved heparin through the xerogel network and degradation-generated pores in the xerogel, and surface erosion over time.

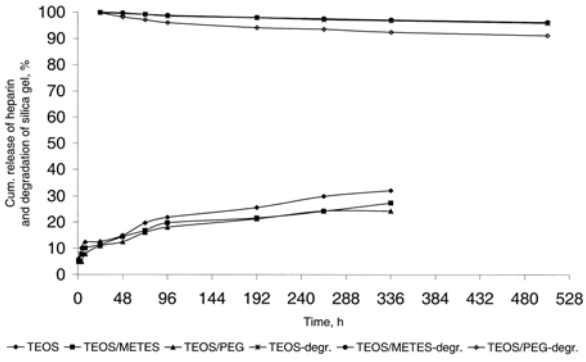

Fig 5. In vitro release of heparin and degradation of silica gel microparticles

Compared with silica xerogel monoliths, spray dried silica gels degrade more slowly [2]. During spray-drying at an increased temperature (135 ˚C), the hydrolyzed silica sol is very rapidly dried into small particles which most likely results in a more condensed structure.

CONCLUSIONS

Spray drying is a promising method for preparation of sol-gel derived silica gel microparticles for controlled release of heparin. From a manufacturing point of view, spray drying offers

the advantage of being single-step process which can be readily scaled up.

It has been shown that heparin release from the microparticles may be controlled by addition of organically modified precursors that cause changes in the microstructure of the silica network.

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