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Spray-dried mucoadhesives for intravesical drug delivery using *N*-acetylcysteine- and glutathione-glycol chitosan conjugates

Nunzio Denora [a,*,1], Angela Lopedota [a,1], Mara Perrone [a], Valentino Laquintana [a], Rosa M. Iacobazzi [a,b], Antonella Milella [c], Elisabetta Fanizza [c,d], Nicoletta Depalo [d], Annalisa Cutrignelli [a], Antonio Lopalco [e], Massimo Franco [a]

[a] Department of Pharmacy – Drug Sciences, University of Bari "A. Moro", Orabona, St. 4, 70125 Bari, Italy
[b] Istituto Tumori IRCCS "Giovanni Paolo II", Flacco, St. 65, 70124 Bari, Italy
[c] Department of Chemistry, University of Bari "A. Moro", Orabona, St. 4, 70125 Bari, Italy
[d] CNR-Institute for Physical and Chemical Processes UOS, Bari, Italy
[e] Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66047, USA

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ABSTRACT

This work describes *N*-acetylcysteine (NAC)- and glutathione (GSH)-glycol chitosan (GC) polymer conjugates engineered as potential platform useful to formulate micro-(MP) and nano-(NP) particles via spray-drying techniques. These conjugates are mucoadhesive over the range of urine pH, 5.0–7.0, which makes them advantageous for intravesical drug delivery and treatment of local bladder diseases. NAC- and GSH-GC conjugates were generated with a synthetic approach optimizing reaction times and purification in order to minimize the oxidation of thiol groups. In this way, the resulting amount of free thiol groups immobilized per gram of NAC- and GSH-GC conjugates was 6.3 and 3.6 mmol, respectively. These polymers were completely characterized by molecular weight, surface sulfur content, solubility at different pH values, substitution and swelling degree. Mucoadhesion properties were evaluated in artificial urine by turbidimetric and zeta (ζ)-potential measurements demonstrating good mucoadhesion properties, in particular for NAC-GC at pH 5.0. Starting from the thiolated polymers, MP and NP were prepared using both the Büchi B-191 and Nano Büchi B-90 spray dryers, respectively. The resulting two formulations were evaluated for yield, size, oxidation of thiol groups and *ex-vivo* mucoadhesion. The new spray drying technique provided NP of suitable size (<1 μm) for catheter administration, low degree of oxidation, and sufficient mucoadhesion property with 9% and 18% of GSH- and NAC-GC based NP retained on pig mucosa bladder after 3 h of exposure, respectively.

Statement of Significance

The aim of the present study was first to optimize the synthesis of NAC-GC and GSH-GC, and preserve the oxidation state of the thiol moieties by introducing several optimizations of the already reported synthetic procedures that increase the mucoadhesive properties and avoid pH-dependent aggregation.
Second, starting from these optimized thiomers, we studied the feasibility of manufacturing MP and NP by spray-drying techniques. The aim of this second step was to produce mucoadhesive drug delivery systems of adequate size for vesical administration by catheter, and comparable mucoadhesive properties with respect to the processed polymers, avoiding thiolic oxidation during the formulation. MP with acceptable size produced by spray-dryer Büchi B-191 were compared with NP made with the apparatus Nano Büchi B-90.

1. Introduction

Mucoadhesion is most commonly defined as the ability of natural or synthetic polymers to adhere to the surface of mucosal tissues. Mucoadhesive drug delivery systems (MDDS) offer the

* Corresponding author.
E-mail address: nunzio.denora@uniba.it (N. Denora).
[1] These authors contributed equally to this study as first authors.

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advantages of extending the residence time at the site of interest, protecting the drug from enzymatic degradation and increasing its bioavailability, making less frequent dosing possible [1–3]. Hence, MDDS have become a useful approach to target and release the drug cargo to particular sites or tissues such as the bladder mucosa [3,4,5]. Although bladder diseases have a considerable impact on society, their treatment remains an important challenge for research. Intravesical instillation therapy using MDDS has become a promising approach for the treatment of bladder diseases, such as interstitial cystitis and bladder cancer [6,7]. Such approach has the advantage to selectively deliver high concentrations of drugs to the target site, limiting the undesirable side effects, compared to oral or intravenous therapies. However, local administrations into the bladder of drugs via solutions is often ineffective due to the low permeability of urothelium, the short residence time, and the pH of urine, typically 5.0–7.0, may limit solubility or permeability at some drugs. Moreover, the instilled drug solutions become diluted with urine and are washed out of the bladder during voiding, necessitating repeated infusions of the drug.

Several natural polymers have been already used as MDDS. Among them, chitosans (CS) are an interesting class of mucoadhesive cationic polymers composed of D-glucosamine and *N*-acetyl-D-glucosamine units. However, there are some limitations in the use of these polymers as bladder MDDS, such as the tendency to form aggregates and loss of surface charge at pH values greater than 5.5 [8]. Hence, the use of a chemically modified CS characterized by an ethylene glycol portion, and known as glycol chitosan (GC), is more suitable when a pH independent water solubility and a positive charge retention are desirable at physiological or urine pH values. Recently, it has been shown that chemical modification of the structure of some polymers, such as CS, GC, polyacrylic acid, cellulose derivatives, pectin, and alginates, can improve the mucoadhesive properties by the introduction of thiol groups, displaying potential utility for intravesical drug delivery [3,5,7]. However, besides this advantage, thiomers show reasonably low stability in aqueous solutions, as they are subject to thiol oxidation at pH $\geqslant$ 6 unless sealed under inert conditions [9]. In fact, it has been reported that *N*-acetylcysteine- and glutathione-GC polymer conjugates, with some mucoadhesive properties, are characterized by high amounts of oxidized thiols, namely disulfide groups. Most likely this can be ascribed both an oxidizing synthetic procedure and an excessive purification of raw materials detrimental for mucoadhesion properties [10]. Hence, the optimization of synthetic approach and purification of thiomers and their suitable formulations would be highly advantageous in developing MDDS. In a study conducted by Barthelmes et al., thioglycolic CS microparticles (MP) and nanoparticles (NP) were generated by spray-drying and ionic gelation [3]. *In vitro* and *in vivo* mucoadhesion tests revealed that thiolated MP and NP were more adherent to the mucosa than the unmodified materials. Furthermore, a direct comparison between thiolated MP and NP indicated a longer retention of NP in the rat bladder.

Inspired from these findings, the aim of the present study was first the synthesis of *N*-acetylcysteine- and glutathione-GC conjugates (NAC-GC and GSH-GC), preserving the oxidation state of the thiol moieties, and second, the feasibility to formulate the above mentioned thiomers as MP and NP by using spray-drying techniques. Namely, GSH- and NAC-GC polymer conjugates were here synthesized by developing appropriate synthetic processes and purification methods which offer some significant advantages over those already reported in literature [10], such as high control on their molecular weight, retention of their water solubility at pH of at least 5.0, as well as, lower amount of surface disulfide groups, thus ensuring improved mucoadhesive properties of the two novel thiolated GC derivatives.

Furthermore, an innovative approach was here designed and implemented to clearly discriminate the thiol groups from the disulfide ones by means XPS-ESCA analysis and mostly by exploiting a selective derivatization of the two polymer conjugates carrying the thiol groups with a molecule close in structure to the Ellman's reagent [10,11].

Finally, for the first time these new polymers were formulated as MP and NP by spray drying technique. In particular, NP were prepared by Buchi Spray Dryer B-90 that recently was advised as a valid tool in the pharmaceutical field [12], thus providing opening and promising instructions to process glycol chitosan and their derivate polymers in this new apparatus, never studied up to now.

The MP and NP based on the two novel thiolated GC-polymer derivatives and characterized by good bio-adhesively on bladder mucosa represent promising carriers, which can be potentially able to improve the therapeutic efficiency of specific drug by increasing its bioavailability, solubility, and retention time and thus finally resulting useful for local intravesical treatment of bladder diseases.

2. Materials and methods

Glycol chitosan (degree of polymerization $\geqslant$ 400; GC), glutathione (GSH), *N*-acetylcysteine (NAC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC), 5,5′-dithiobis(2-nitrobenzoic acid) (Ellmann's reagent), 4,4′-bis(trifluoromethyl)-2,2′-dinitrodiphenyldisulphide (BTDDS), sodium-borohydride, *N*-hydroxysuccinimide (NHS), porcine stomach mucin (type II, bound sialic acid ~1%), fluorescein diacetate (FDA) and the component for artificial urine were purchased from Sigma-Aldrich, Italy.

2.1. Synthesis of glutathione-glycol chitosan polymer conjugate (GSH-GC)

The synthesis of GSH-GC polymer conjugate was accomplished using a modified procedure previously reported [10]. In particular, we adapted this synthetic procedure from that developed for GSH-CS polymer conjugate [9]. Briefly, GSH (1.5 g, 4.8 mmol), NHS (0.6 g, 5.8 mmol), and EDAC (1.1 g, 5.8 mmol), were dissolved in 10 mL of a hydrochloric acid solution (pH 5.0). After 30 min, GC (0.5 g) dissolved in the same acid solution (10 mL), was added dropwise over the course of 5 min. To optimize the synthetic procedure, three different batches were planned differing on the reaction time of 1, 3 and 6 h. After 1, 3 and 6 h of incubation under continuous stirring at room temperature, the corresponding polymer conjugates were purified using an Amicon Centrifugal Filtration Device (100 kDa MWCO regenerated cellulose membrane). The loaded device was centrifuged at 4000 rpm for 20 min, concentrating the GSH-GC polymer conjugate solution to approximately 1 mL. This concentrated material was diluted in an additional 10 mL of fresh Milli-Q water and centrifuged again under identical conditions. Each sample was washed three times. The final concentrated GSH-GC dispersions were frozen at −20 °C and lyophilized (Christ Alpha 1-4 LSC) for 24 h under reduced pressure (0.016 mbar). The recovered solid polymers were sealed under nitrogen and stored in desiccator at 4 °C until further use.

2.2. Synthesis of N-acetylcysteine-glycol chitosan polymer conjugate (NAC-GC)

For the synthesis of NAC-GC polymer conjugate, we used the same procedure adopted for GSH-GC thiomer above described. In particular, NAC (0.8 g, 4.8 mmol), NHS (0.6 g, 5.8 mmol), and EDAC (1.1 g, 5.8 mmol) were dissolved in hydrochloric acid solution (pH 5.0), and after 30 min a solution of GC (0.5 g) in 10 mL of hydrochloric acid solution (pH 5.0), was added dropwise to the first