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Hyaluronan based materials with catanionic sugar-derived surfactants as drug delivery systems

F. Roig[a], M. Blanzat[b], C. Solans[c,d], J. Esquena[c,d], M.J. García-Celma[a,d,*]

[a] Departament de Farmàcia i Tecnologia Farmacèutica i Fisicoquímica, IN2UB, Universitat de Barcelona, Joan XXIII s/n, 08028 Barcelona, Spain
[b] Laboratoire des IMRCP, Université de Toulouse, CNRS UMR 5623, Université Toulouse III - Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex 9, France
[c] Institut de Química Avançada de Catalunya (IQAC), Consejo Superior de Investigaciones Científicas (CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
[d] CIBER-BBN (Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina), Jordi Girona 18-26, 08034 Barcelona, Spain

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ABSTRACT

In the present work novel drug delivery systems consisting in highly porous Hyaluronan foams for the administration of a non-steroidal anti-inflammatory drug (NSAID), ketoprofen, have been obtained. A sugar-derived surfactant associated with ketoprofen was prepared and incorporated into the porous hyaluronan materials. The association between a lactose derived surfactant, $Lhyd_{12}$, and ketoprofen was obtained by acid-base reaction and its physicochemical properties were studied. Tensiometric and dynamic light scattering (DLS) determinations showed the formation of catanionic surfactant aggregates, $Lhyd_{12}$/ketoprofen, in aqueous solution. Furthermore, the catanionic surfactants allowed greater solubilisation of ketoprofen. Hyaluronan porous materials were developed using butanediol diglycidyl ether as crosslinking agent. The profile release of $Lhyd_{12}$/ketoprofen from hyaluronan based materials shows differences as a function of the aggregation state of catanionic surfactant.

1. Introduction

Soft matter drug delivery systems have recently received substantial attention, in particular from the nanomedicine field. In fact, these carriers increase drug bioavailability and activity, while decreasing its toxicity, because drug efficiency is often altered by its non-controlled biodistribution. The design of the appropriate drug delivery system is then important to optimize drug efficiency [1]. In order to meet the needs, a wide range of drug delivery systems have already been developed. These systems include matrix and vesicular carriers, with a large range of size and structures [2]. The use of a biocompatible polymer matrix is an interesting approach because it allows controlling the release of low molecular weight drugs for various routes of administration such as: oral, parenteral, ocular, etc. [3]. Furthermore, these materials have the following advantages: reduction of side effects and improvement of drug bioavailability, solubilization of lipophilic drugs, and lower treatment costs [4]. Hyaluronan (HA), belongs to the family of glycosaminoglycans and consists on N-acetyl-D-glucosamine and D-glucuronic acid [5]. This polymer is an important component of the extracellular matrix of connective tissue and is found in various parts of the human body such as: skin, cartilage, vitreous humour and intra-articular joint fluid [6]. It also plays an important role in cartilage matrix stabilization, cell proliferation, control of morphogenesis, cancer metastases, inflammation processes and wound healing [7–12]. HA is degradable *in vivo* by enzymes such as hyaluronidase present in human tissues [13]. A suitable approach to avoid fast elimination from the human body could be the preparation of HA materials chemically crosslinked that show an increase in their resistance against hyaluronidase [14,15].

Polymeric materials can be obtained by crosslinking hydrophilic polymers in bulk [16] (hydrogels) or by the use of colloidal systems as templates for the preparation of materials with controlled porosity (solid foams). The incorporation of a polymer in the continuous phase of a highly concentrated emulsion, allows the preparation of porous materials with very high pore volume [17]. These materials have found a number of applications, including biomaterial engineered devices and drug delivery systems [18].

In addition, vesicular drug delivery systems made of catanionic surfactants have also proved their great contribution to drug solu-

* Corresponding author at: Departament de Farmàcia i Tecnologia Farmacèutica i Fisicoquímica, IN2UB, Universitat de Barcelona, Joan XXIII s/n, 08028, Barcelona, Spain.
E-mail address: mjgarcia@ub.edu (M.J. García-Celma).

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bility in water and drug delivery by means of spontaneous vesicles formation [19–21]. These vesicles also showed an increase of the therapeutic effect of the drug together with a sustained diffusion through the skin [19,22]. The strategy adopted in this work was to evaluate the impact of the combination of both drug delivery systems by preparing a novel complex drug delivery system. These advanced materials consist in the association between a drug, ketoprofen (KP), and a sugar-derived surfactant to form an ion-pair, "catanionic" surfactant, which would later be incorporated into a crosslinked polymer matrix based on HA. To ensure the formation of a KP catanionic surfactant, the selected surfactant has to be capable of forming an ionic acid-base pair with the carboxylic acid of KP. This feature means that the surfactant requires the presence of a basic group that is able to react with the acid group of the drug. In addition, the surfactant has to be biocompatible and biodegradable without inducing toxicity to ensure biocompatibility of the ion pair within the body. Furthermore, the physicochemical characteristics of the surfactant should lead to drug solubilization. In this context, sugar-derived surfactants represent a suitable choice for this purpose. Apart from their biocompatibility and biodegradability, these surfactants can be obtained from sugars that are natural raw materials available in large quantities.

The aim of this work was to develop a novel drug delivery system based on HA materials (hydrogels and solid foams) loaded with KP catanionic surfactants and determine the differences in the release behavior when the aggregates of catanionic surfactants are formed.

2. Experimental

2.1. Materials

Hyaluronic acid sodium salt from *Streptococcus equi.* of molecular weight around 2 million Daltons with 97% purity was obtained from *Sigma-Aldrich*. The chemical crosslinker butanediol diglycidyl ether (BDDE) with a molecular weight 202.25 g mol^{-1} with 95% purity was obtained from *Sigma-Aldrich*. Nonionic surfactant Cremophor RH455 (CRH 455) with an HLB between 14 and 16 was obtained from *BASF*. Miglyol 812, medium chain triglycerides, was obtained from *Fagron*. Ketoprofen ($C_{16}H_{14}O_3$), (KP), non-steroidal anti-inflammatory drug (NSAIDs) used as anionic precursor surfactant, was from *Fagron* with 99.8% purity. Phosphate buffer solution pH 7.4, (PBS) was prepared from: KH_2PO_4 from *Fagron Iberica S.A.V*, Na_2HPO_4 from *Probus S.A*, NaCl from *Acofarma*®, and *Milli-Q*® deionized water. Cellulose tubular membrane was purchased from *Orange Scientific*. Its properties are a 12.000-14.000 Da nominal MWCO, and 20 μm wall thickness. Mobile phase for HPLC (pH 3.0) was prepared from 45% of aqueous phase comprising: Citric acid from *Acofarma*® with 99.5% purity, NaCl from *Acofarma*®, NaOH from *Acofarma*®, and *Milli-Q*® deionized water; and 55% of organic phase acetonitrile obtained from *Carlo Erba Reagents*, with 99.9% purity.

2.2. Methods

2.2.1. Synthesis of KP catanionic surfactant

The catanionic sugar-derived surfactant was obtained by an acid–base reaction between equimolar amounts (0.66 mmol) of cationic and anionic precursor surfactants in 30 mL of water. N-dodecylamino-1-deoxylactitol, designated as $Lhyd_{12}$, was used as cationic precursor surfactant. $Lhyd_{12}$ was obtained as previously described from a reductive amination of dodecylamine with lactose [23,24]. An NSAID, KP was used as the anionic precursor surfactant. The two components were added in ultrapure water and stirred for 24 h. The resulting homogeneous solution was lyophilized. After lyophilization a white powder corresponding to the catanionic sugar-derived surfactant was obtained with a quantitative yield (Fig. 1).

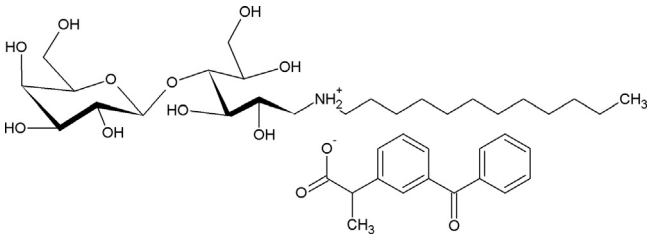

Fig. 1. Molecular structure of KP catanionic surfactant as a result of ionic association between $Lhyd_{12}$ and ketoprofen.

2.2.2. Physicochemical characterization of KP catanionic surfactant

2.2.2.1. Fourier transform – infrared spectroscopy (FT-IR). Infrared spectra were obtained with a Perkin-Elmer IR FT 1760X. KBr discs with a concentration of 0.5 w/w% were prepared of KP catanionic surfactant.

2.2.2.2. Surface tension measurements. The values of surface tension as a function of catanionic surfactant concentration were measured by the Wilhelmy plate method using a Kruss Tensiometer Easy Dyne at 25.0 °C ± 0.1 °C. The catanionic solutions were prepared by dissolving weighted amounts of dry catanionic associations in ultrapure water. Solutions were stirred at room temperature during a few minutes.

2.2.2.3. Dynamic light scattering (DLS). Dynamic light scattering was performed using a Malvern Zetasizer Nano-ZS, ZEN3600, with a measuring range of 0.5 nm to 10 μm. The light source used was a He-Ne laser with a wavelength of 633 nm. The temperature was regulated at 25.0 °C with a Peltier with an accuracy of ± 0.1 °C. The measuring angle was 173°. Samples of aqueous solutions of KP catanionic surfactant (3.5×10^{-3} M) were introduced into cells (pathway, 10 mm). The deconvolution of the measured intensity autocorrelation function of the samples was realized with the multiple narrow modes program that uses a non-negatively constrained least squares (NNLS) fitting algorithm to obtain the distribution of diffusion coefficients (D) of the solutes. The apparent equivalent hydrodynamic diameter (d_h) was determined using the Stokes-Einstein equation. Hydrodynamic diameter values were obtained from three different runs.

2.2.3. Preparation of chemically crosslinked hydrogels

For the preparation of HA hydrogels, 50 mg of sodium hyaluronate were introduced into test tubes of 12 × 75 mm, to which 500 μL of crosslinking solution, consisting of BDDE (5% v/v) in alkaline media (0.2 M NaOH), were added. Then, the HA and the crosslinking solution were stirred with a vortex till a homogeneous mixing. The resulting mixture was incubated at 25 °C for 24 h and the HA crosslinked hydrogel was obtained. The epoxy groups of BDDE react with the hydroxyls present in the HA polymer [25].

2.2.4. In vitro *cell viability analysis*

Cell viability in the presence free BDDE crosslinker, BDDE crosslinked hyaluronan hydrogels and KP catanionic surfactant was assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay [26]. For each assay, HeLa cells were seeded in Dulbecco's modified Eagle's medium supplemented with FBS and antibiotics. Then, the culture medium was replaced with samples at the required concentrations. 100 μL of BDDE solution at the same concentration that in the hydrogel and the corresponding dilutions 1:5 and 1:10 v/v, 100 μL