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Research paper

Development of cationic nanocrystals for ocular delivery

Gregori B. Romero [a], Cornelia M. Keck [b], Rainer H. Müller [a], Nadia A. Bou-Chacra [c,*]

[a] Pharmaceutics, Pharmaceutical Nanotechnology & NutriCosmetics, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany
[b] Applied Pharmacy, University of Applied Sciences Kaiserslautern, Campus Pirmasens, Carl-Schurz-Str. 10-16, 66953 Pirmasens, Germany
[c] University of Sao Paulo, Faculdade de Ciências Farmacêuticas, Av. Lineu Prestes 580 – Bloco 15, Caixa-Postal 6608, CEP 05508-900 São Paulo, Brazil

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ABSTRACT

A cationic nanocrystal formulation containing dexamethasone acetate nanocrystals (0.05%) and polymyxin B (0.10%) for ophthalmic application was produced using a self-developed small scale method for wet bead milling. The formulation developed offers the advantage of increased saturation solubility of the drug (due to the nano-size of the crystals) and increased residence time in the eye (due to small size and increased mucoadhesion by the cationic charge) resulting ultimately in potential increased bioavailability. Characterization of the nanosuspensions by photon correlation spectroscopy (PCS) and transmission electron microscopy showed that the production method was successful in achieving dexamethasone crystals in the range of about 200–250 nm. The physical stabilization of the nanocrystals and generation of the positive charge were realized by using cetylpyridinium chloride (CPC) and benzalkonium chloride (BAC) at the concentration of 0.01%. In contrast to other cationic excipients, they are regulatorily accepted due to their use as preservatives. The drug polymyxin B also contributed to the positive charge. Positive zeta potentials in the range +20 to +30 mV were achieved. Isotonicity was adjusted using NaCl and non-ionic excipients (glycerol, sorbitol, dextrose). Physical and chemical stabilities were monitored for a period of 6 months at room temperature, 5 °C and 40 °C. Particle size of the bulk population assessed by PCS remained practically unchanged over 6 months of storage for the various formulations without isotonicity agents, and for the CPC-containing formulations with non-ionic isotonicity excipients. The chemical content also proved stable after 6 months for all 3 temperatures evaluated. *In vitro* investigation of mucoadhesion was tested using mucin solutions at different concentrations, and the generated negative zeta potential was used as a measure of the interaction. The zeta potential reversed to about −15 mV, indicating distinct interaction. The results show the potential of increased mucoadhesion of such cationic nanocrystals compared to standard eye drop formulations. The positively charged nanocrystal formulation also showed no *in vitro* cytotoxicity as assessed on fibroblast cell culture. In summary, 3 formulation candidates were identified being a promising alternative for ocular delivery with increased performance compared to what is presently available.

1. Introduction

Drugs formulated as conventional ophthalmic preparations, such as eye drops, are quickly removed from the ocular surface as a consequence of the protective mechanisms of the eye, i.e. reflex blinking, lacrimation and lacrimal fluid turnover. Therefore, the retention time of drugs on the eye is very limited and consequently, bioavailability is very low - normally less than 5%. After instillation, the excess volume of the instilled liquid is drained by the nasolacrimal duct. Additionally, the constant turnover of the lacrimal fluid (around 1 μL/min) associated with potential systemic absorption from the conjunctival sac capillaries contributes to the low concentration of drug on the eye surface [1]. Prolonged release dosage forms may discretely increase the bioavailability, but in clinical practice, these systems have not yet been widely accepted [2]. Permeation across corneal and conjunctival epithelial barriers is very limited (even for modern prolonged delivery); therefore, when the drug target is the posterior segment of the eye (retina, vitreous choroid), an alternative is to administer high doses of the drug by intravenous or intravitreal route [3].

Nevertheless, these invasive administration routes are not practical and only effective for a limited number of diseases (and drugs). Thus, currently, there are some formulation strategies are in development to increase the duration of action of the applied drug, e.g. gels, gelifying formulations, ointments, inserts [4]. The high viscous formulations can cause blurred vision after application, and user-

* Corresponding author.
E-mail addresses: gregoribahuromero@hotmail.com, romero.gregori@gmail.com (G.B. Romero), chacra@usp.br (N.A. Bou-Chacra).

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unfriendly are also inserts as the Ocusert® pilocarpine system (Ocusert Pilo) for the sustained topical ocular delivery of pilocarpine. It disappeared from the market. An optimum ocular drug delivery system should be one which can be delivered in the form of eye drops, causing no blurred vision or irritability and would need no more than one to two administrations per day [5]. This was the aim of the present study using nanocrystal suspensions.

Micro and nanoparticulate polymeric systems have been proposed as alternatives for ocular delivery [6]. However, one of the major problems related to these polymeric systems is that the drug release sometimes takes longer than the ocular residence time of the polymeric particles themselves. Liposomes have also been extensively investigated, but problems associated with their irritation potential and formulation stability are the main disadvantages [6]. Some other delivery systems currently being investigated for ocular delivery include dendrimers, cyclodextrins, nanoemulsions, niosomes and solid lipid nanoparticles (SLN) [7,8].

Problems associated with the previous delivery systems can be overcome by nanonization of the pure drug powder, resulting in drug nanocrystals (preferentially approx. 100–500 nm for maximum adhesiveness). Nanocrystals possess increased saturation solubility, dissolution velocity and additionally increased mucoadhesion [9]. After application to the eye fluid, they start immediately to dissolve fast (burst release), and the increased saturation solubility leads to an increased concentration gradient and thus increased diffusive flux into the eye surface. Not completely dissolved nanocrystals stay adhered to the eye surface for a longer time, thus acting as depot from which constantly new drug dissolves. From the regulatory point of view, in contrast to other nanoparticulate systems, nanocrystals have the distinct advantage that they are composed purely of drug; there is no matrix material such as polymer or lipid matrix. This is especially important for ocular delivery, since excipients legally authorized by regulatory agencies for ocular use are very limited [9]. In addition, because the drug loading of nanocrystals is 100% (i.e. they consist of pure drug), the instilled volume can be reduced/kept low, which contributes to a longer retention of the applied doses on the eye surface.

Mucin, the mucus layer that coats the corneal surface, is negatively charged. Therefore, the ideal carrier system for the eye would be a cationic particle with high adhesion to the mucosa. This principle was exploited before in other nanoparticulate systems by [10–12]. Therefore, cationic nanocrystals promoting an increase in the saturation solubility of the drug, together with increased mucoadhesiviness have the potential to distinctly improve the drug ocular bioavailability.

A problem is the selection of the electrostatic charge provider. Many cationic lipids do not possess a regulatorily accepted status for ocular administration and are often expensive. Similar problems exist for positively charged polymers such as chitosan or polyethylenimine (PEI).

In this study, positively charged dexamethasone acetate nanocrystals combined with polymyxin B sulfate in an ophthalmic formulation were developed. The problem of positive charge generation was solved by using a positively charged drug in combination with a positively charged preservative. The chemical and physical short-term stability of the ocular nanocrystal suspension (nanosuspensions) was assessed, as well as its *in vitro* mucoadhesion potential and its tolerability was confirmed by cytotoxicity investigations.

2. Materials and methods

2.1. Materials

Dexamethasone 21-acetate was purchased from TCI (Japan), polymyxin B sulfate from Biotika A.s. (Slovak Republic), benzalkonium chloride from Merck Schuchardt (Germany) and glycerol 85% from Fragon GmbH & Co. KG (Germany). Mucin type III, sodium chloride, dextrose, sorbitol and cetylpyridinium chloride were purchased from Sigma-Aldrich Chemie GmbH (Germany). Double distilled and ultrapurified water was obtained from a Milli-Q apparatus (Millipore GmbH, Germany). All other reagents were from analytical grade.

2.2. Nanosuspension production

The nanosuspension production was performed by a self-developed miniaturized wet bead milling method [13]. The coarse suspension was composed of 5% dexamethasone acetate, 1% stabilizer and optionally 1% polymyxin B sulfate (all w/w). The stabilizers tested were cetylpyridinium chloride and benzalkonium chloride.

Briefly, the coarse suspension was processed in a 2 mL glass vial containing yttria stabilized zirconium oxide beads with diameter of 0.05 mm as the grinding media. Stirring was performed on a magnetic stirring plate RCT basic (IKA-Werke GmbH & Co. KG, Germany). Milling efficiency was increased by a special arrangement of 3 stirrers located on top of each other.

2.3. Particle characterization

2.3.1. Photon correlation spectroscopy

The hydrodynamic diameter (z-average, z-ave) of the nanocrystals was determined by photon correlation spectroscopy (PCS), using a Zetasizer Nano ZS (Malvern Instruments, UK). The results are the z-average, which is the intensity weighted mean diameter of the bulk population, and the polydispersity index (PdI), which is a measure for the width of the size distribution. Samples were diluted in water to a suitable concentration and the average values were calculated from 10 single measurements.

2.3.2. Zeta potential

The zeta potential is a measure of the electrostatic charge on the surface of the particle and is a tool to predict the physical stability of colloidal suspensions. It was measured using a Zetasizer Nano ZS (Malvern Instruments, UK) in two different media: original dispersion medium of the nanosuspension (= solution with stabilizer and preservative) and Milli-Q water (adjusted to 50 μS/cm conductivity by addition of NaCl and at pH 5.5). The electrophoretic mobility was measured and converted into zeta potential by the Helmholtz-Smoluchowski equation.

2.3.3. Light microscopy (LM) and transmission electron microscopy (TEM)

To verify the presence of large particles or agglomerates, light microscopy using a microscope Orthoplan (Leitz, Germany) was performed at 160, 600 and 1000 fold magnifications. Additional particle characterization was performed by transmission electron microscopy (TEM) using a Tecnai G^2 20 S-TWIN (FEI company, USA).

2.4. Dilution and isotonicity adjustment

Nanosuspensions containing 5% (w/w) of dexamethasone acetate obtained from the milling process were further diluted to the desired concentration of the final ocular formulation and the tonicity was adjusted, resulting in a final formulation with 0.05% of dexamethasone, 0.1% of polymyxin B, 0.01% of the stabilizer and adequate concentrations of one of the tonicity agents (0.9% NaCl; 2.6% glycerol; 5.5% sorbitol; 5.0% dextrose) (all w/w).