International Journal of Pharmaceutics 549 (2018) 466–475

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Novel approaches for improving stability of cysteamine formulations

Phillip Dixon, Kristin Powell, Anuj Chauhan*

Department of Chemical Engineering, University of Florida, Gainesville, FL 32611, United States

ARTICLE INFO

Keywords:
Cysteamine
Cystinosis
Eye drop formulations
Stability
Oxidation
Vitamin E
OMAC

ABSTRACT

Cystinosis is a genetic disorder that leads to the formation of cystine crystals in many organs in the body including cornea. Ocular manifestation of this disease is treated by eye drops of cysteamine which can easily oxidize into its disulfide cystamine. The rapid oxidation limits the shelf life as well the duration during which the drug can be used after opening the eye drop bottle. We evaluate two approaches of preventing the oxidation of cysteamine with the goal of increasing the time of use after opening the bottle to one month. The first approach integrates antioxidants such as catalase enzyme and vitamins C and E into the aqueous solution. Results show that catalase is the most effective additive as it decreases the oxidation rate by 58%, which on its own is not sufficient to reach targeted one month stability. The second approach focuses on incorporating diffusion barriers to prevent oxygen from reaching the cysteamine solution. This was accomplished by two methods: formulation of a hydrophobic layer which floats on the surface of the aqueous solution and integration of OMAC® oxygen-resistant material into the eye drop bottle. Both methods delay the onset of cysteamine degradation and decrease the rate of degradation. In particular, an eye drop bottle with three layers of OMAC® has less than 10% degradation after one month of opening the bottle and withdrawing a drop each day. By integrating all three methods, we designed a system where > 90% of cysteamine remains in the active form for 70 days after opening the bottle. In addition, we examine the use of OMAC® as heat-sealed pouches for storage of cysteamine eye drop bottles during packaging to eliminate the need for the current approach of freezing the formulation during shipping. The results show that such heat-sealed pouches would keep cysteamine stable for over one year at ambient conditions.

1. Introduction

Cystinosis is a genetic disorder affecting transport of the amino acid cystine across lysosomal membranes (Bishop, 2017; Gahl et al., 1982; Gahl et al., 2000), which leads to a build-up of cystine crystals and eventual cell damage and death. This crystal formation occurs in multiple tissues but it is most prevalent in the kidneys and corneas (Liang et al., 2015). If left untreated, cystinosis can lead to renal failure, stunted growth, and blindness (Nesterova and Gahl, 2008). The disease can be managed by using cysteamine which reacts with cystine through a thiol-disulfide interchange to form cysteine-cysteamine dimers that can then be transported out of the cell (Iwata et al., 1998; Jones et al., 1991). While cysteamine can be delivered via oral medication systemically, the avascular cornea cannot be treated by oral dosing. Instead, current ocular treatment relies on eye drop formulations of cysteamine instilled at a rate of four to twelve eye drops per day, with the exact dosing rate determined by the specific formulation and the severity of crystal formation.

Current commercial formulations are Cystaran™, a formulation of 4.4 mg mL^{-1} cysteamine available in the United States, and Cystadrops®, a gel formulation of 3.8 mg mL^{-1} cysteamine approved for marketing in Europe (Radojkovic, 2015). While Cystaran™ and Cystadrops® are effective at treating cystinosis, they both suffer from a short shelf life once the eye drop bottle is opened because exposure to air results in cysteamine oxidation (Reda et al., 2017). Specifically, cysteamine contains a thiol functional group which readily reacts with oxygen to produce a disulfide called cystamine (Bagiyan et al., 2003; Biaglow et al., 1984; Svensson, 1988) which is ineffective at treating the cystine crystals in the cornea (Iwata et al., 1998; Labbe et al., 2014). While acidic conditions have been shown to reduce the rate of cysteamine oxidation (Pescina et al., 2016), cysteamine oxidation can still occur over the time scale of several days. Thus, each commercial product attempts to reduce cysteamine oxidation to increase the duration of use after the bottle is opened.

Cystaran™ is packaged in low density polyethylene (LDPE) bottles, which allows oxygen to enter the solution at high rates by diffusing through the bottle. To counter the presence of oxygen and increase cysteamine stability, Cystaran™ bottles are frozen when shipped to

* Corresponding author.
E-mail addresses: pjdixon@ufl.edu (P. Dixon), kcpowell@ufl.edu (K. Powell), chauhan@che.ufl.edu (A. Chauhan).

https://doi.org/10.1016/j.ijpharm.2018.08.006
Received 15 March 2018; Received in revised form 26 July 2018; Accepted 6 August 2018
Available online 10 August 2018
0378-5173/

patients and the Cystaran™ solution is formulated with a pH between 4.1 and 4.5. At approximately −20 °C, Cystaran™ is stable for over one year with negligible oxidation rates (Reda et al., 2017; Biaglow et al., 1984). As mentioned above, the formulation's reduced pH also slows cysteamine degradation. However, the recommended shelf life of Cystaran™ is only one week after thawing and opening the bottle (Huynh et al., 2013). Additionally, freezing Cystaran™ bottles increases the cost of treatment for patients. When continuous temperature control is required, shipping costs are estimated to be approximately $8000 per year (Huynh et al., 2013). Frozen formulations also increase patient risk during power outages and cause logistical problems while traveling, potentially decreasing compliance. It should also be noted that the acidity of Cystaran™ may have negative side effects. Cysteamine solutions at or below a pH of 4 have been reported to cause a burning sensation upon application (Bozdag et al., 2008; Lim et al., 2014). While these sensations quickly dissipated and were not associated with any tissue damage, a neutral formulation with a pH close to the tear film pH would likely alleviate such side effects. Further, lowering the formulation pH decreases corneal permeability of cysteamine, necessitating addition of permeability enhancers to the formulation. For example, Cystaran™ includes benzalkonium chloride as both a permeability enhancer and a preservative. Unfortunately, while frequently used, benzalkonium chloride has several toxic side effects (Baudouin et al., 2010). Therefore, increasing corneal permeability by neutralizing the formulation may have the secondary benefit of reducing toxic side effects by allowing for preservative-free solutions.

In contrast, Cystadrops® is a gel formulation that does not need to be frozen and can instead be stored for six months in a refrigerated environment prior to opening. As a gel formulation, Cystadrops® uses carmellose sodium to enhance ocular residence time (Labbe et al., 2014). Such gelled formulations have been shown to have greater stability of cysteamine. For example, a gel formulation with hydroxypropylmethylcellulose (HPMC) was stable for one year when stored in a sealed glass flagon (Bozdag et al., 2008) and formulation containing sodium hyaluronate was stable for up to ten weeks (McKenzie et al., 2016). These formulations could be viable options for developing cysteamine eye drops, both for improved cysteamine stability and increased delivery to the cornea. This work focuses on improving the stability of neutral, aqueous cysteamine, but the approach developed here could directly be applied to the gel based formulations as well, potentially leading to even further improvements in stability.

For Cystadrops®, extended shelf life without freezing is accomplished by storage in a sealed amber vial with a bromobutyl stopper and an aluminum seal, which have a dramatically lower oxygen permeability than LDPE. Directly prior to use, the seal and stopper are removed and replaced with a polyvinyl chloride (PVC) and high density polyethylene (HDPE) dropper. While the amber vial does increase shelf life when sealed, its recommended shelf life is also only one week once the bottle is opened (Makuloluwa and Shams, 2018). This suggests that the plastic dropper does little to prevent oxygen from entering the bottle. Further, a recommendation by the European Union Committee for Medical Products for Human Use (CHMP) calls for a new storage technology since the glass vial droppers can be difficult for patients to assemble. Note, however, that Cystadrops® amber vials demonstrate how different packing materials can reduce temperature requirements for multiple month shelf life prior to first use. They also show that even with improved packaging, a major factor in cysteamine degradation is air which enters the eye drop bottle when it is opened. Thus, cysteamine degradation could be theoretically slowed either by reducing the intake of oxygen when the bottle is opened or by preventing oxygen's reaction with cysteamine.

Our main target is to design a system that can increase the duration of use after opening (defined per FDA guidelines as > 90% active cysteamine) from one week to one month while maintaining a neutral pH. Our second target is to design a system which maintains stability for one year before opening while stored at room temperature. Finally, we aim to design a system that does not require patients to assemble packaging components such as eye droppers. To achieve our objectives, we explore two different approaches:

1. Antioxidants – As mentioned above, oxygen which enters the system through the dropper aperture can have a significant effect on cysteamine oxidation. One possible solution is to add a chemical which scavenges oxygen or its generated radicals—commonly called an antioxidant. Cysteamine itself is considered an antioxidant, but a more potent scavenger could out-compete cysteamine for available oxygen or prevent the formation of an intermediary such as hydrogen peroxide (Luo et al., 2005; Quijano et al., 1997). While powerful iron-based antioxidants exist, pharmaceuticals require that antioxidants be bio-compatible. Further, iron ions in solution have been shown to catalyze the formation of free radicals, increasing the degradation rate of thiols (Kachur et al., 1998). Therefore, this paper examines the effects of vitamin C and vitamin E, two naturally occurring antioxidants found in the eye (Chen et al., 2009), which are suggested to have benefits to ocular and general health (Bursell et al., 1999; Christen et al., 2000; Christen et al., 1996; Padayatty et al., 2003; Zhang et al., 2015). Vitamin C is highly hydrophilic and can be added directly to the aqueous solution. This allows us to study how cysteamine behaves in the presence of a second antioxidant. In contrast, vitamin E has a very low solubility in aqueous formulations and can only be solubilized with the aid of a surfactant. Since emulsions have been shown to reduce oxygen transport and increase stability of other hydrophilic antioxidants (Coupland and McClements, 1996), emulsions using Tween 80 are also studied. Finally, the effect of the enzyme catalase, which can revert peroxide species back to diatomic oxygen, potentially starving the system of radicals required to oxidize cysteamine, is explored.
2. Barriers to oxygen – Oxygen can reach the eye drop bottle via diffusion through the bottle surface or with the air that is sucked into the bottle through the dropper aperture as pressure equalizes after dispensing a liquid drop. Oxygen penetration must be reduced for both modes of entry.
3. To reduce oxygen introduced through the dropper aperture, an insoluble oil layer is added at the water–air interface. An eye drop bottle always contains air on top of aqueous formulations. When a bottle is squeezed to dispense a liquid drop, an equal volume of air will enter the bottle to equalize the pressure. Clearly, oxygen that enters the bottle with this air can cause drug degradation. To slow this degradation, we propose to create an internal oxygen barrier in the bottle via a layer of oil that is insoluble with and less dense than the formulation. In addition to slowing oxygen transport into the aqueous formulation, the oil layer could also to store hydrophobic antioxidants to scavenge oxygen before it enters the aqueous cysteamine solution. Thus, we propose to design a two-component system containing the aqueous drug formulation and an additional oily phase which floats on top of the aqueous cysteamine solution, thereby providing a barrier to oxygen diffusion. It is critical that the presence of this barrier does not impede the drop dispensing dynamics and that the barrier oil is biocompatible.
4. To reduce oxygen introduced by diffusion through the bottle surface, one can either manufacture a thicker bottle using materials that are resistant to oxygen diffusion or coat an existing bottle with suitable materials. This method has already been shown to be effective with Cystadrops® amber vials. However, we propose to take this one step further and also cover the dropper and cap with oxygen resistant material. In this study, we opt for coating premade plastic eye drop bottles since this option does not require patients to assemble packaging components.
5. Since long-term shelf storage of one year at room temperature is desired prior to use, oxygen diffusion through packaging may be reduced further by a secondary sealed container. We investigate materials which are resistant to oxygen diffusion for this purpose.