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Characterization of the Properties of Powder Excipients Commonly Used in Pharmaceutical Compounding

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Characterization of the properties of powder excipients commonly used in pharmaceutical compounding

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ABSTRACT

Capsules are the most popular and versatile dosage form produced in pharmaceutical compounding. In the preparations of capsules, powder excipients are nonactive ingredients that can be used for the purpose of increasing bulk, enhancing flow, and improving stability of a powder formulation. Flow properties of powder excipients can impact not only the compounding procedure, but also the uniformity and quality of the final product. In this study, an FT4 Powder Rheometer was used to compare dynamic, bulk, and shear properties of the following four powder excipients: Loxoral, Loxasperse, a commercial powder excipient, and lactose monohydrate. Changes in flow energy, measured as the powders are exposed to external conditions such as air, compression, consolidation, and shear forces, were used to compare flow properties of the four excipients. Results show Loxasperse to have superior dynamic properties, indicating more efficient packing potentials. Though Loxoral did not have the most favorable flow in terms of dynamic properties, Loxoral outranked the other excipients when comparing bulk and shear properties. Taking into account processing equipment and the external environment, compounding pharmacists can reevaluate current formulas to include excipients with more favorable flow properties, resulting in more uniform and higher quality products.

KEYWORDS: Capsules, flow properties, flowability, pharmaceutical compounding, powder excipients, powder rheometer

Technical Report:

Characterization of the Physical and Microbiological Properties of LoxaSperse®

Abstract: LoxaSperse is a proprietary blend of micronized xylitol and poloxamers, designed to be mixed with active substances in order to improve water solubility, dispersibility, and to prevent microbial growth. The physical and microbiological properties of LoxaSperse were characterized by three laboratory tests performed with LoxaSperse and LoxaSperse with itraconazole.

Introduction:

LoxaSperse is a proprietary blend of micronized xylitol and micronized poloxamers, designed to be mixed with active substances in order to improve their water solubility and dispersibility. The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is ample evidence for their safety and efficacy (Durairaj *et al*., 2006; Plataki *et al*., 2011). Xylitol and poloxamers exhibit antimicrobial activity and, therefore, LoxaSperse is also expected to prevent microbial growth (Veyries *et al*., 2000; Zabner *et al*., 2000). LoxaSperse mixtures are dry powders, packaged as non-sterile capsules or sachets for dispersion or dissolution in sterile water prior to the administration of compounded medicines for nebulization and irrigation.

Methodology:

The physical and microbiological properties of LoxaSperse were characterized by three types of laboratory tests performed on LoxaSperse and LoxaSperse with itraconazole, a triazole antifungal that is active against a wide spectrum of microorganisms (*Martindale 35*, 2007).

Physical Properties: To determine the particle size distribution of LoxaSperse and LoxaSperse with itraconazole, two different tests were performed respectively: Static Laser Light Scattering and Optical Microscopy.

Microbiological Properties: To characterize the antimicrobial activity of LoxaSperse and LoxaSperse with itraconazole, two different Minimum Inhibitory Concentration (MIC) methods were performed against fungal and bacterial strains by the Broth Microdilution Method and Agar Dilution Method. All strains were obtained from the American Type Culture Collection (ATCC). To estimate microbiological growth in LoxaSperse, water activity of the powder excipient base was determined using the AquaLab Water Activity Meter (AquaLab, 2008; 2013).

Results and Discussion:

The physical and microbiological properties of LoxaSperse are discussed separately below.

Physical Properties of LoxaSperse

Particle Size Distribution: The particles in a sample are not perfectly mono-disperse (i.e., every single particle with exactly the same dimensions) but, instead, they commonly consist of a statistical distribution with particles of differing dimensions. Several tests may be performed in order to characterize this physical property (Malvern, 2012).

Static Laser Light Scattering: This test provides a volume weighed distribution, in which the contribution of each particle in the distribution relates to the volume of that particle (Malvern, 2012). LoxaSperse 6.4% in sterile water exhibits a narrow distribution of particles (**Figure 1**), which demonstrates the optimal physical characteristics and performance of the powder excipient base.

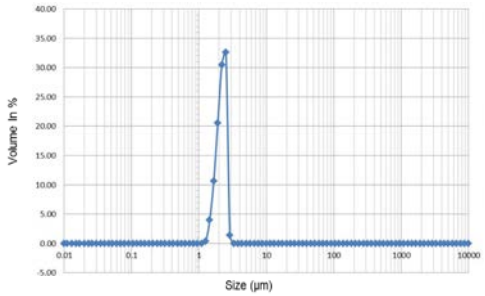

Figure 1. *Particle size distribution of LoxaSperse in sterile water.*

Optical Microscopy: Microscopic examination is suitable to determine the distribution of particles of inhalable size (European Commission JRC, 2002) and, therefore, optical microscopy was performed to characterize the effect of LoxaSperse in the particle size distribution of itraconazole. An AmScope Microscope Digital Camera was used for photographic characterization of itraconazole (1%) in sterile water, with and without LoxaSperse, at 200x magnification (AmScope, 2013). This test was performed in accordance with the respective 'Physical Test' of the US Pharmacopeia (The United States Pharmacopeial Convention, 2013). It was observed that, following the addition of LoxaSperse, large aggregates of itraconazole were converted into small aggregates and single particles (Figure 2). It is therefore concluded that LoxaSperse optimizes the particle size distribution of itraconazole in sterile water.

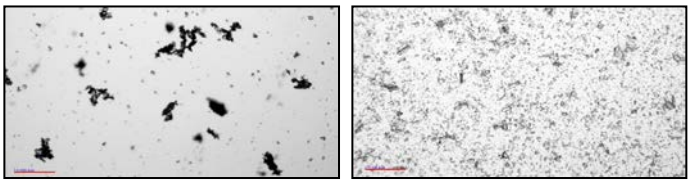

Figure 2. *(Left) Itraconazole 1% in sterile water. (Right) Itraconazole 1% and LoxaSperse in sterile water. Both at 200x magnification.*

Microbiological Properties of LoxaSperse

Minimum Inhibitory Concentration: MIC is the lowest concentration of an antimicrobial that will inhibit visible growth of a microorganism after overnight incubation. MIC is the gold standard research tool to determine *in vitro* activity of antimicrobials (Andrews, 2001). A lower MIC is indicative of a better antimicrobial agent.

Broth Microdilution Method: The *in vitro* antifungal activity of itraconazole and LoxaSperse with itraconazole (9:1) was determined against four fungal strains using the National Committee for Clinical Laboratory Standards (NCCLS) reference methods for yeast and filamentous fungi (Espinel-Ingroff, 2002; NCCLS, 2002a; 2002b). A lower MIC was found

in LoxaSperse with itraconazole than in itraconazole itself (**Table 1**). It is concluded that the LoxaSperse mixture has improved antifungal activity against all fungal strains tested.

Table 1. *MIC (µg/mL) of itraconazole and itraconazole with LoxaSperse (9:1) against four fungal strains (filamentous and yeast).*

Fungal strains	***A.fumigatus*** ATCC 204305	***A.niger*** ATCC 16404	***C.albicans*** ATCC 90028	***R.oryzae*** ATCC 9363
Itraconazole	0.5	0.5	≤0.125	0.25
Itraconazole +LoxaSperse	0.2	0.2	0.025	0.20

Agar Dilution Method: The *in vitro* antimicrobial activity of LoxaSperse was determined against eight microbial strains. An MIC of 17% LoxaSperse was achieved for the majority of the microbial strains tested (**Table 2**). No antimicrobials were added to this test.

Table 2. *MIC (%) of LoxaSperse against eight microbial strains.*

Microbial strains	Concentration of LoxaSperse			
	15%	**16%**	**17%**	**18%**
E.coli *ATCC 8739*	Growth	Growth	**No growth**	**No growth**
E.coli *ATCC 8739*	Growth	Growth	**No growth**	**No growth**
S.aureus *ATCC 6538*	Growth	Growth	**No growth**	**No growth**
P.aeruginosa *ATCC 9027*	Growth	Growth	**No growth**	**No growth**
C.albicans *ATCC 10231*	Growth	Growth	**No growth**	**No growth**
A.niger *ATCC 16404*	Growth	Growth	**No growth**	**No growth**
S.typhimurium *ATCC 14028*	Growth	**No growth**	**No growth**	**No growth**
S.aureus MRSA *ATCC 33591*	Growth	**No growth**	**No growth**	**No growth**

Water Activity (a_w): is defined as the amount of available, or free, water in a system and is a measure of how efficiently water can take part in a chemical reaction. Reducing the a_w minimizes undesirable chemical reactions and microbiological growth. Most bacteria do not grow at a_w<0.91 and no microbiological growth is possible at a_w<0.60. The a_w is a better index of microbial growth than total water content (Blandamer *et al*., 2005; AquaLab, 2008; 2013). The a_w of LoxaSperse was measured after 90 days storage at three different temperatures. An average a_w of 0.321 (with desiccant) and a_w of 0.456 (without desiccant) was measured (Table 3).

Table 3. *Water activity of LoxaSperse, with and without desiccant, after 90 days of storage at three different temperatures.*

Temperature	Water Activity (a_w) (with desiccant)	Water Activity (a_w) (without desiccant)
T=4°C (±1°C)	0.297	0.409
T=25°C (±1°C)	0.321	0.471
T=45°C (±1°C)	0.344	0.489

It is concluded that no microbiological growth is possible in LoxaSperse, after 90 days storage at T<45˚C, due to its low a_w (<0.60).

Conclusions:

LoxaSperse with itraconazole has improved particle size distribution in sterile water and also improved antifungal activity compared to itraconazole alone. Considering the MIC and a_w of LoxaSperse, it is also concluded that LoxaSperse prevents microbial growth as expected.

References:

Andrews, J. (2001) 'Determination of minimum inhibitory concentrations', *Journal of Antimicrobial Chemotherapy*, 48 (S1), p.5-16.

AquaLab (2008) *Water Activity Meter Operator's Manual.* Washington: Decagon Devices, Inc.

AquaLab (2013) *Microbial Growth*. Available at: http://www.aqualab.com/applications/microbial-growth (Accessed: 10 May 2013).

AmScope (2013) *Microscope Cameras*. Available at: http://www.amscope.com/Camera.html (Accessed: 8 June 2013).

Blandamer, M., Engberts, J., Gleeson, P. and Reis, J. (2005) 'Activity of water in aqueous systems: A frequently neglected property', *Chemical Society Reviews*, 34, p.440-458.

Durairaj, L., Launspach, J., Watt, J.L., Mohamad, Z., Kline, J. and Zabner, J. (2007) 'Safety assessment of inhaled xylitol in subjects with cystic fibrosis', *Journal of Cystic Fibrosis*, 6 (1), p.31-34.

Espinel-Ingroff, A. (2002) 'Antifungal susceptibility methods and their potential clinical relevance', *LabMedicine*, 8 (33), p.626-31.

European Commission JRC (2002) *Guidance Document on the Determination of Particle Size Distribution, Fibre Length and Diameter Distribution of Chemical Substances* [Online]. Available at: http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/5555/1/EUR%2020268%20EN.pdf (Accessed: 8 June 2013).

Malvern (2012) 'A Basic Guide to Particle Characterization', *Inform White Paper*. Worcestershire: Malvern Instruments Limited.

Martindale 35 (2007) [CD-ROM]. RPSGB and Sweetman, S. (ed.). Available: Pharmaceutical Press.

NCCLS (2002a) *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard*. 2nd edn. Pennsylvania: NCCLS.

NCCLS (2002b) *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard*. Pennsylvania: NCCLS.

Plataki, M., Lee, Y., Rasmussen, D. and Hubmayr, R. (2011) 'Poloxamer 188 facilitates the repair of alveolus resident cells in ventilator-injured lungs', *American Journal of Respiratory and Critical Care Medicine*, 184, p.939-947.

The United States Pharmacopeial Convention (2013) 'Physical Tests / <776> Optical Microscopy'. *USP 36 -NF 31.* Rockville: USP, p.343-344.

Veyries, M., Faurisson, F., Joly-Guillou, M. and Rouveix, B. (2000) 'Control of staphylococcal adhesion to polymethylmethacrylate and enhancement of susceptibility to antibiotics by Poloxamer 407', *Antimicrobial Agents and Chemotherapy*, 44 (4), p.1093-1096.

Zabner, J., Seiler, M., Launspach, J., Karp, P., Kearney, W., Look, D., Smith, J. and Welsh, M. (2000) 'The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing', *Proceedings of the National Academy of Sciences of the United States of America*, 97 (21), p.11614-11619.

Technical Report:

The Antimicrobial Activity of Itraconazole and LoxaSperse® Against Biofilms of *C. albicans*

Abstract: Itraconazole is a broad-spectrum, triazole antifungal agent, class II drug molecule (low solubility–high permeability) according to the Biopharmaceutical Classification System (BCS). LoxaSperse is an excipient manufactured by PCCA and can be used as a chemical dispersing or solubilizing agent in irrigation or nebulization formulations, improving the solubility and dispersibility of poorly water soluble Active Pharmaceutical Ingredients (APIs). The *in vitro* antimicrobial activity of itraconazole in a LoxaSperse formulation was evaluated against *Candida albicans* biofilms and compared to the same activity of reference antifungal drugs (itraconazole, fluconazole and amphotericin B), in order to verify the benefits of the LoxaSperse formulation. The LoxaSperse formulation reduced Minimum Biofilm Inhibitory Concentration (MBIC) 10-fold compared to the value of itraconazole alone. Improvement in antimicrobial activity of the LoxaSperse/itraconazole formulation could be attributed to the improved dissolution rate and solubility enhancement caused by the base over the poorly water-soluble itraconazole.

Purpose:

To evaluate the *in vitro* antimicrobial activity of itraconazole in a LoxaSperse formulation, Loxasperse alone, and Itraconazole EP Micronized, fluconazole and amphotericin B (reference antifungal drugs) against *Candida albicans* biofilms.

Introduction:

Local delivery of medication to the sinuses and lungs is highly desirable, especially in patients with specific sinus and pulmonary diseases such as cystic fibrosis, asthma, chronic sinus and pulmonary infections, and lung cancer. The principal advantages include reduced systemic side effects and higher doses of the applicable medication at the site of drug action (Harvey and Schlosser, 2009; Pilcer and Amighi, 2010).

Many existing APIs and an increasing number of new drugs are often poorly water-soluble drugs (Zhang *et al.*, 2011). Drug insolubility, regardless of the administration route, commonly generates bioavailability or efficacy problems. Different techniques exist to increase drug dissolution and/or solubility, which often require the use of specific excipients. In the ear, nose and throat (ENT) injuries and illness field, excipients should be chemically and physically stable, inert to the API and exhibit no side effects (Duret *et al*., 2012).

LoxaSperse is a proprietary excipient manufactured by PCCA for use as a chemical dispersing or solubilizing agent in oral, sinus, inhalation, rectal and topical formulations. It consists of a blend of micronized xylitol and micronized poloxamers, designed to be mixed with APIs in order to improve their water solubility and dispersability (PCCA, 2013). Xylitol is a 5-carbon sugar with low transepithelial permeability which is poorly metabolized by bacteria (Durairaj *et al*., 2007). Poloxamers are a series of synthetic block copolymers of poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) (PEO–PPO–PEO) with varying molecular weights and block ratios. They are non-ionic amphiphilic surfactants possessing excellent wetting, antifoaming and solubilizing properties (Moebus *et al*., 2009). The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is evidence of their safety. (Durairaj *et al*., 2007; Jagannath *et al*., 1995; Plataki *et al*., 2011; Zabner *et al*., 2000). LoxaSperse is a base that allows for the preparation of non-sterile capsules and powder sachets that are added to sterile water or normal saline by the patient at the moment of administration (PCCA, 2013).

Candida infections have increased dramatically over the past years, being reported as the fourth most common nosocomial bloodstream pathogen. Candidemia represents 10% of all nosocomial blood-stream infections (Burgess *et al*., 2000).
The traditional treatment uses amphotericin B, but it has changed to relatively less toxic alternatives, such as the triazole antifungals itraconazole and fluconazole (Wroblewska *et al*., 2002).

Itraconazole has a broader spectrum of activity than other azole antifungals (De Beule, 1996). However, poor oral bioavailability, variable absorption and gastrointestinal toxicity due to the hydroxypropyl-β-cyclodextrin component of the oral solution limit itraconazole to a second or third line treatment option for invasive fungal infections (Vaughn *et al*., 2007). Itraconazole is a typical Biopharmaceutical Classification System (BCS) Class II drug with low solubility–high permeability (Amidon *et al*., 1995). An inhaled itraconazole delivery system has shown an interesting potential for treating pulmonary invasive fungal infections with improvement of its efficacy (Duret *et al*., 2012).

Methodology:

Materials: Itraconazole EP Micronized (lot number C149307) and PCCA Formula #10342 (4 g of Itraconazole EP Micronized + 37.574 g of LoxaSperse) were provided by PCCA (Houston, TX, USA) as powders. Itraconazole and PCCA Formula #10342 were prepared on the day of the assay. Fluconazole and amphotericin B (Sigma Aldrich®) were obtained as powders and stored at 4°C. Stock solutions (10.24 mg/mL) of these two reference actives were prepared in sterile water.

Strain: *Candida albicans* isolate ATCC 90028 was obtained from American Type Culture Collection (Manassas, VA) and used in the course of this study.

Methods: A Minimum Biofilm Inhibitory Concentration (MBIC) of itraconazole in a LoxaSperse formulation, LoxaSperse excipient, itraconazole, fluconazole and amphotericin B was measured for the *C. albicans* biofilm according to the NCCLS M27-A broth microdilution method (NCCLS, 1997). The testing medium used for growing was RPMI 1640 (American Biorganics, Inc., Niagara Falls, NY) supplemented with L-glutamine (Sigma Aldrich®). Yeast inocula (100 µL of 1 x 10^6 cells/mL) were added to each well of 96-well microtiter plates (Corning) and incubated at 37°C for 48h. After biofilm formation, medium was aspirated and non-adherent cells were

removed by thoroughly washing the biofilms three times in sterile phosphate-buffered saline (PBS, Sigma Aldrich®). The antifungal drug and LoxaSperse solutions (samples) were then added to the biofilms in serially diluted concentrations (1,024 to 0.5 µg/ml, from stock [concentrated] solutions of each sample prepared in RPMI medium directly) and incubated for a further 48h at 35°C. A series of sample-free wells and biofilm-free wells were also included to serve as positive and negative controls, respectively. The MBIC was defined as the lowest concentration of sample that produced a 50% reduction of fungal growth compared with the growth control. Cell viability was determined by using CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega, 2013).

Results and Discussion:

All biofilms formed on the microtiter plates over 48h displayed consistent CellTiter 96® dye solution readings when the intensity of the colorimetric product was measured in a microtiter plate reader at 570 nm. The MBIC value of itraconazole in a LoxaSperse formulation (expressed as concentration of itraconazole) showed efficient result in comparison with the MBIC values for raw itraconazole, fluconazole and amphotericin B tested against *C. albicans* ATCC 90028, as reported in Table 1. The LoxaSperse formulation improved the antimicrobial potential of itraconazole approximately 10-fold. Biofilm from *C. albicans* strain tested was intrinsically resistant to fluconazole (MBIC > 1024 µg/mL). The polyene antifungal amphotericin B was highly active (MBIC = 0.5 µg/mL) against *C. albicans* ATCC 90028. The findings for fluconazole and amphotericin B are in accordance with the literature (Ramage *et al*., 2001).

Table 1. *Minimum Biofilm Inhibitory Concentrations against C. albicans ATCC 90028.*

Sample	Minimum Biofilm Inhibitory Concentration (MBIC) (ug/mL)
Amphotericin B	0.5
Fluconazole	>1,024
Itraconazole	1024
LoxaSperse	>10,240
Itraconazole/LoxaSperse	98.5

Conclusions:

Itraconazole has an increased *in vitro* antimicrobial activity against *Candida* biofilms when associated with the LoxaSperse excipient. It may be due to the benefits caused by the base in terms of the dissolution rate and saturation solubility of the poorly water-soluble itraconazole, providing a higher *in vitro* dissolved drug concentration that induced an enhanced inhibition of microbial growth.

Financial Disclosure:

For this study, PCCA contracted a third party laboratory with no proprietary or financial interests in the test products, or equity interest in PCCA.

References:

Adjei A. L., Gupta P. K. (1997) Inhalation Delivery of Therapeutic Peptides and Proteins. Marcel Dekker Inc., New York, Basel, Hong Kong.

Amidon G. L., Lennernas H., Shah V. P., Crison J. R. (1995) A theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability, *Pharmaceutical Research*, 12: 413-420.

Burgess D. S., Hastings R. W., Summers K. K., Hardin T. C., Rinaldi M. G. (2000) Pharmacodynamics of fluconazole, itraconazole, and amphotericin B against *Candida albicans*, *Diagnostic Microbiology and Infectious Disease*, 36: 13-18.

De Beule K. (1996) Itraconazole: pharmacology, clinical experience and future development, *International Journal of Antimicrobial Agents*, 6: 175-181.

Durairaj L., Launspach J., Watt J. L., Mohamad Z., Kline J., Zabner J. (2007) Safety assessment of inhaled xylitol in subjects with cystic fibrosis, *Journal of Cystic Fibrosis*, 6: 31-34.

Harvey R. J., Schlosser R. J. (2009) Local Drug Delivery, Otolaryngologic Clinics of North America, 42: 829-845.

Duret C., Wauthoz N., Sebti T., Vanderbist F., Amighi K. (2012) Solid dispersions of itraconazole for inhalation with enhanced dissolution, solubility and dispersion properties, *International Journal of Pharmaceutics*, 428: 103-113.

Jagannath C., Allaudeen H. S., Hunter R. L. (1995) Activities of Poloxamer CRL8131 against *Mycobacterium tuberculosi*s In Vitro and In Vivo, *Antimicrobial Agents and Chemotherapy*, 39: 1349-1354.

Moebus K., Siepmann J., Bodmeier R. (2009) Alginate–poloxamer microparticles for controlled drug delivery to mucosal tissue, *European Journal of Pharmaceutics and Biopharmaceutics*, 72: 42-53.

National Committee for Clinical Laboratory Standards. (1997) Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard. NCCLS document M27-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.

PCCA (2013). LoxaSperse. Available at: http://www.pccarx.com/bases/LoxaSperse (Accessed: October/ 2013).

Pilcer G., Amighi K. (2010) Formulation strategy and use of excipients in pulmonary drug delivery, *International Journal of Pharmaceutic*s, 392: 1-19

Plataki M., Lee Y. D., Rasmussen D. L. Hubmayr R. D. (2011) Poloxamer 188 Facilitates the Repair of Alveolus Resident Cells in Ventilator-injured Lungs, *American Journal of Respiratory and Critical Care Medicine*, 184: 939-947.

.Promega (2013) CellTiter 96® Non-Radioactive Cell Proliferation Assay, Technical Bulletin, Available at: http://www.promega.com/~/media/Files/Resources/Protocols/... (Accessed: 14 October 2013).

Ramage G., Walle K. V., Wickes B. L. López-Ribot J. L. (2001) Standardized Method for In Vitro Antifungal Susceptibility Testing of *Candida albicans* Biofilms, *Antimicrobial Agents and Chemotherapy*, 45: 2475-2479.

Vaughn J. M., Wiederhold N. P., McConville J. T., Coalson J. J., Talbert R. L., Burgess D. S., Johnston K. P., Williams III R. O., Peters J. I. (2007) Murine airway histology and intracellular uptake of inhaled amorphous itraconazole, *International Journal of Pharmaceutics*, 338: 219-224.

Wroblewska M. M., Swoboda-Kopec E., Rokosz A., Krawczyk E., Marchel H., Luczak M. (2002) Epidemiology of clinical isolates of *Candida albicans* and their susceptibility to triazoles, *International Journal of Antimicrobial Agents*, 20: 472-475.

Zabner J., Seiler M. P., Launspach J. L., Karp P. H., Kearney W. R., Look D. C., Smith J. J., Welsh M. J. (2000) The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing, *Proceedings of the National Academy of Sciences*, 97: 11614-11619.

Zhang J., Wu L. B., Chan H. K., Watanabe W. (2011) Formation, characterization, and fate of inhaled drug nanoparticles, *Advanced Drug Delivery Reviews*, 63: 441–455.

Technical Report:

Antimicrobial Effectiveness Testing of a Budesonide LoxaSperse® Dispersion

Abstract: LoxaSperse is a powder excipient base used for nebulization and irrigation designed to improve dispersability and solubility of Active Pharmaceutical Ingredients (APIs). PCCA tested the performance of PCCA Formula #10341 (budesonide 0.5 mg in a LoxaSperse mixture) and measured its efficacy against microbial activity when mixed with sterile water. The intent is not to determine efficacy of budesonide as an antimicrobial. The Antimicrobial Effectiveness Test (AET) was conducted at 0.5h, 6h, 28h and 168h – serially diluted, and plated for colony counts. Budesonide LoxaSperse dispersions required 0.5h to 28h to significantly reduce the number of viable bacterial cells (*E. coli, S. aureus* and *P. aeruginosa*). The results of this study demonstrate that accidental or intentional contamination of the finished or reconstituted preparation did not result in microbial growth.

Purpose:

The intent of this study was to evaluate results of purposeful inoculation of the formulation with microorganisms specified in USP <51> (The United States Pharmacopeial Convention, 2013a), for nasal and inhalation use with modified Antimicrobial Effectiveness Test (AET) methodology and to determine the *in vitro* efficacy of formulas containing LoxaSperse to reduce microbial counts or inhibit viable cell growth.

Introduction:

LoxaSperse is a powder excipient base used for nebulization and irrigation. LoxaSperse is a blend of specially micronized xylitol with an optimized ratio of micronized poloxamers, designed to improve the dispersability and solubility of APIs (PCCA, 2013). The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is ample evidence of their safety and efficacy (Durairaj *et al.*, 2006; Jagannath *et al.*, 1995; Plataki *et al.*, 2011; Zabner *et al.*, 2000). Budesonide is a corticoid with mainly glucocorticoid activity (*Martindale 35*, 2007). PCCA tested the performance of Formula #10341, budesonide 0.5 mg in a LoxaSperse mixture, and measured its efficacy against microbial activity when mixed with sterile water.

Methodology:

The efficacy of budesonide LoxaSperse dilutions were evaluated by serially diluting the formula in sterile water and plating for colony counts with *S. aureus, P. aeruginosa, E. coli*, *C. albicans* and *A. niger* at intervals of 0.5h, 6h, 28h and 168h.

Materials and Methods:

A Budesonide Micronized USP (lot number C158080) capsule was prepared by PCCA (Houston, TX, USA) following the instructions on PCCA Formula #10341 (budesonide 0.5 mg in a LoxaSperse mixture). The final solutions were subsequently prepared by an outside laboratory at time of testing by adding one budesonide capsule (PCCA Formula #10341) to 10 mL of sterile water.

Bacterial Strains:

The strains were from the American Type Culture Collection (ATCC, Manassas, VA). All strains were maintained as glycerol stock solutions at -80°C. Working stocks were grown on tryptic soy or Sabouraud agar media at 35°C.

Antimicrobial Effectiveness Test (AET):

Growth, harvesting, and enumeration of *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans* and *A. niger* were performed according to universal AET procedures (Moser and Meyer, 2011). 1 mL aliquots of the test articles were prepared in 15 mL polycarbonate test tubes. 10 µL of cell culture (diluted in phosphate buffered saline, (PBS, Sigma Aldrich®) was added to each 1 mL aliquot to initiate the AET assay. 10 µL of cell culture was also added to 1 mL PBS for initial colony counts at the start of the AET assay. During the AET assay, 100 µL of the mixture was removed at intervals of 0.5h, 6h, 28h, and 7d (168h), serially diluted, and plated for colony counts. Final colony counts, reported in CFU/mL and Log_{10} reductions in viable cell numbers, are discussed in this report.

Results and Discussion:

Initial colony counts of *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans* indicated that a 10^2 to 10^4 CFU/mL product challenge was performed for these organisms (**Table 1**). *A. niger* colonies were not obtained from these initial plates (≤10 CFU/mL, **Table 1**), but counts from subsequent plates indicated that 10^1 to 10^2 spores were present at the start of the AET (**Table 2**).

Over the course of the AET, viable cell/spore counts varied depending upon the test article, where it was prepared, and the test organism.

E. coli: a 1-Log reduction after 6h and no viable cells after 28h.
S. aureus: little change in cell viability was observed over 168h, when no viable cells were recovered.

C. albicans: viable cells were recovered and continued to increase in number over the course of the AET.

A. niger: little change in the number of viable cells was observed.

P. aeruginosa: a 2-Log reduction after 0.5h. No viable cells were recovered after 6h.

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Table 1. Initial colony counts from adjusted cultures.

Organism	CFU/mL
Control	≤10*
E. coli	9.7 x 10^3
A. niger	≤10*
C. albicans	3.2 x 10^2
P. aeruginosa	5.9 x 10^3
S. aureus	1.0 x 10^4

Table 2. Recovered cell counts from AET (CFU/mL).

CFU/mL at time (h):				
Organism	**0.5**	**6**	**28**	**168**
Control	≤10*	≤10*	≤10*	≤10*
E. coli	2.6 x 10^3	4.6 x 10^2	≤10*	≤10*
A. niger	1.0 x 10^1	1.0 x 10^1	1.0 x 10^1	≤10*
C. albicans	2.5 x 10^2	6.7 x 10^2	≤10*	3.0 x 10^3
P. aeruginosa	4.0 x 10^1	≤10*	≤10*	≤10*
S. aureus	7.98 x 10^3	6.36 x 10^3	8.50 x10^3	≤10*

*≤10 denotes below detection limits USP <1227> (The United States Pharmacopeial Convention, 2013b).

Conclusions:

The test article containing budesonide and LoxaSperse required 0.5h to 28h to significantly reduce the number of viable bacteria (*E. coli, S. Aureus* and *P. aeruginosa*). *A. niger* showed a decrease in the number of viable cells up to 168h. The chosen formula when intentionally contaminated with microorganisms specified in USP 51 resisted microbial growth. Further, this study demonstrated this formulation after reconstituted was not at risk or did not support microbial growth.

Financial Disclosure:

For this study, PCCA contracted a third party laboratory with no proprietary or financial interests in the test products, or equity interest in PCCA.

References:

Durairaj, L., Launspach, J., Watt, J.L., Mohamad, Z., Kline, J. and Zabner, J. (2007) 'Safety assessment of inhaled xylitol in subjects with cystic fibrosis', *Journal of Cystic Fibrosis*, 6 (1), p.31-34.

Jagannath, C., Allaudeen, H. and Hunter, R. (1995) 'Activities of Poloxamer CRL8131 against *Mycobacterium tuberculosis in vitro* and *in vivo*', *Antimicrobial agents and chemotherapy*, 39 (6), p.1349-1354.

Martindale 35 (2007) [CD-ROM]. RPSGB and Sweetman, S. (ed.). Available: Pharmaceutical Press.

Moser, C. and Meyer, B. (2011) 'Comparison of compendial antimicrobial effectiveness tests: a review', *AAPS PharmSciTech*, 12 (1), p.222-226.

PCCA (2013) *Loxasperse*. Available at: http://www.pccarx.com/pcca-products/pcca-exclusives/bases/loxasperse (Accessed: 10 October 2013).

Plataki, M., Lee, Y., Rasmussen, D. and Hubmayr, R. (2011) 'Poloxamer 188 facilitates the repair of alveolus resident cells in ventilator-injured lungs', *American Journal of Respiratory and Critical Care Medicine*, 184, p.939-947.

The United States Pharmacopeial Convention (2013a) 'Microbiological Tests / <51> Antimicrobial Effectiveness Testing'. *USP 36 -NF 31*. Rockville: USP, p.54-55.

The United States Pharmacopeial Convention (2013b) 'General Information / <1227> Validation of Microbial Recovery from Pharmacopeial articles. *USP 36 -NF 31*. Rockville: USP, p.989-991.

Zabner, J., Seiler, M., Launspach, J., Karp, P., Kearney, W., Look, D., Smith, J. and Welsh, M. (2000) 'The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing', *Proceedings of the National Academy of Sciences of the United States of America*, 97 (21), p.11614-11619.

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Technical Report:

Antimicrobial Effectiveness Testing of a Gentamicin LoxaSperse® Dispersion

Abstract: LoxaSperse is a powder excipient base used for nebulization and irrigation designed to improve dispersibility and solubility of Active Pharmaceutical Ingredients (APIs). PCCA tested the performance of PCCA Formula #10337 (gentamicin 80 mg in a LoxaSperse mixture), and measured its efficacy against microbial activity when mixed with sterile water. The intent is not to determine efficacy of gentamicin as an antimicrobial. The Antimicrobial Effectiveness Test (AET) was conducted at 0.5h, 6h, 28h and 168h – serially diluted, and plated for colony counts. Gentamicin LoxaSperse dispersions reduced the number of viable bacteria (*E. coli, S. aureus* and *P. aeruginosa*) within 0.5h of exposure and no bacterial growth was observed in the test article up to 128h after exposure. A 3-Log to 4-Log reduction in viable bacterial cells was observed within 0.5h. The results of this study demonstrate that accidental or intentional contamination of the finished or reconstituted preparation did not result in microbial growth.

Purpose:

The intent of this study was to evaluate results of purposeful inoculation of the formulation with microorganisms specified in USP <51> (The United States Pharmacopeial Convention, 2013a) for nasal and inhalation use with modified Antimicrobial Effectiveness Test (AET) methodology and to determine the *in vitro* efficacy of formulas containing LoxaSperse to reduce microbial counts or inhibit viable cell growth.

Introduction:

LoxaSperse is a powder excipient base used for nebulization and irrigation. LoxaSperse is a blend of specially micronized xylitol with an optimized ratio of micronized poloxamers, designed to improve the dispersability and solubility of APIs (PCCA, 2013). The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is ample evidence of their safety and efficacy (Durairaj *et al.*, 2006; Jagannath *et al.*, 1995; Plataki *et al.*, 2011; Zabner *et al.*, 2000). Gentamicin is an aminoglycoside antibiotic and has bactericidal action against many gram-negative aerobes and against strains of staphylococci (*Martindale 35*, 2007). PCCA tested the performance of Formula #10337 which is gentamicin (80 mg) in a LoxaSperse mixture and measured efficacy against microbial activity when mixed with sterile water.

Methodology:

The efficacy of gentamicin LoxaSperse dilutions were evaluated by serially diluting the formula in sterile water and plating for colony counts with *S. aureus, P. aeruginosa, E. coli, C. albicans* and *A. niger* in the intervals of 0.5h, 6h, 28h and 168h.

Materials and Methods:

A Gentamicin Sulfate USP (lot number C150822) capsule was prepared by PCCA (Houston, TX, USA) following the instructions on PCCA Formula #10337 (gentamicin 80 mg/LoxaSperse). The final solutions were prepared by an outside lab at time of testing by adding one capsule of Gentamicin Sulfate USP (PCCA Formula #10337) to 10 mL of sterile water.

Bacterial Strains:

The strains were from the American Type Culture Collection (ATCC, Manassas, VA). All strains were maintained as glycerol stock solutions at -80°C. Working stocks were grown on tryptic soy or Sabouraud agar media at 35°C.

Antimicrobial Effectiveness Test (AET):

Growth, harvesting, and enumeration of *S. aureus, P. aeruginosa, E. coli, C. albicans* and *A. niger* were performed according to universal AET procedures (Moser and Meyer, 2011). 1 mL aliquots of the test articles were prepared in 15 mL polycarbonate test tubes. 10 µL of cell culture diluted in Phosphate Buffered Saline (PBS, Sigma-Aldrich®) was added to each 1 mL aliquot to initiate the AET assay. 10 µL of cell culture was also added to 1 mL PBS for initial colony counts at the start of the AET assay.

During the AET assay, 100 µL of the mixture was removed at intervals of 0.5h, 6h, 28h, and 168h, serially diluted, and plated for colony counts. Final colony counts, reported in CFU/mL and Log_{10} reductions in viable cell numbers are discussed in this report.

Results and Discussion:

Initial colony counts of *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans* indicated that a 10^2 to 10^4 CFU/mL product challenge was performed for these organisms (**Table 1**). *A. niger* colonies were not obtained from these initial plates (≤10 CFU/mL, **Table 1**), but counts from subsequent plates indicated that 10^1 to 10^2 spores were present at the start of the AET (**Table 2**).

Over the course of the AET, viable cell/spore counts varied depending upon the test article, where it was prepared, and the test organism.

No viable cells of *E. coli, S. aureus* or *P. aeruginosa* were recovered after 0.5h exposure.

C. albicans: a 1-Log reduction observed after 0.5h and no viable cells were observed after 24h.

A. niger: colony forming spores were recovered up to 128h in solutions.

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Antimicrobial Effectiveness Testing of a Gentamicin LoxaSperse® Dispersion

Table 1. Initial colony counts from adjusted cultures.

Organism	**CFU/mL**
Control	≤10*
E. coli	9.7 x 10^3
A. niger	≤10*
C. albicans	3.2 x 10^2
P. aeruginosa	5.9 x 10^3
S. aureus	1.0 x 10^4

Table 2. Recovered cell counts from AET (CFU/mL).

	CFU/mL at time (h):			
Organism	0.5	6	28	168
Control	≤10*	≤10*	≤10*	≤10*
E. coli	≤10*	≤10*	≤10*	≤10*
A. niger	5.0 x 10^1	4.0 x 10^1	2.0 x 10^1	1.0 x 10^1
C. albicans	2 x 10^1	2 x 10^1	≤10*	≤10*
P. aeruginosa	≤10*	≤10*	≤10*	≤10*
S. aureus	≤10*	≤10*	≤10*	≤10*

*≤10 denotes below detection limits USP <1227> (The United States Pharmacopeial Convention, 2013b).

Conclusions:

The Test Article containing Gentamicin Sulfate USP and LoxaSperse reduced the number of viable bacteria (*E. coli, S. aureus* and *P. aeruginosa*) within 0.5h of exposure and no bacterial growth was observed up to 168 h after exposure. A 3-Log to 4-Log reduction in viable bacteria was observed within 0.5h (**Tables 1-2**). A 1-Log reduction in the number of viable *C. albicans* cells was observed within 6h and no *C. albicans* cells were recovered after 24h (a 2-Log reduction). The gentamicin and LoxaSperse formulation continued to reduce the number of viable *A. niger* spores throughout testing. Additionally, the low number of spores introduced at the initiation of the AET and the subsequent low limit of detection prevented the observation of a significant 1-Log reduction of viable spores. The chosen formula when intentionally contaminated with microorganisms specified in USP 51 resisted microbial growth. Further, this study demonstrated this formulation after reconstituted was not at risk or did not support microbial growth.

Financial Disclosure: For this study, PCCA contracted a third party laboratory with no proprietary or financial interests in the test products, or equity interest in PCCA.

References:

Durairaj, L., Launspach, J., Watt, J.L., Mohamad, Z., Kline, J. and Zabner, J. (2007) 'Safety assessment of inhaled xylitol in subjects with cystic fibrosis', *Journal of Cystic Fibrosis*, 6 (1), p.31-34.

Jagannath, C., Allaudeen, H. and Hunter, R. (1995) 'Activities of Poloxamer CRL8131 against *Mycobacterium tuberculosis in vitro* and *in vivo*', *Antimicrobial agents and chemotherapy*, 39 (6), p.1349-1354.

Martindale 35 (2007) [CD-ROM]. RPSGB and Sweetman, S. (ed.). Available: Pharmaceutical Press.

Moser, C. and Meyer, B. (2011) 'Comparison of compendial antimicrobial effectiveness tests: a review', *AAPS PharmSciTech*, 12 (1), p.222-226.

PCCA (2013) *Loxasperse*. Available at: http://www.pccarx.com/pcca-products/pcca-exclusives/bases/loxasperse (Accessed: 10 October 2013).

Plataki, M., Lee, Y., Rasmussen, D. and Hubmayr, R. (2011) 'Poloxamer 188 facilitates the repair of alveolus resident cells in ventilator-injured lungs', *American Journal of Respiratory and Cri tical Care Medicine*, 184, p.939-947.

The United States Pharmacopeial Convention (2013a) 'Microbiological Tests / <51> Antimicrobial Effectiveness Testing'. *USP 36-NF 31*. Rockville: USP, p.54-55.

The United States Pharmacopeial Convention (2013b) 'General Information / <1227> Validation of Microbial Recovery from Pharmacopeial articles. *USP 36 -NF 31*. Rockville: USP, p.989-991.

Zabner, J., Seiler, M., Launspach, J., Karp, P., Kearney, W., Look, D., Smith, J. and Welsh, M. (2000) 'The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing', *Proceedings of the National Academy of Sciences of the United States of America*, 97 (21), p.11614-11619.

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Technical Report:

Antimicrobial Effectiveness Testing of Antihistamine and Corticosteroid in LoxaSperse® Dispersion

Abstract: LoxaSperse™ is a powder excipient base used for nebulization and irrigation designed to improve dispersibility and solubility of Active Pharmaceutical Ingredients (APIs). PCCA tested the performance of LoxaSperse formulations containing fluticasone propionate alone and in combination with levocetirizine dihydrochloride, and measured its efficacy against microbial activity when mixed with sterile water. The intent was not to determine clinical efficacy of the API(s) used as antimicrobials but to determine the ability of the dry powder preparation to resist microbial growth. The Antimicrobial Effectiveness Test (AET) was performed at 0.5h, 6h, 28h and 168h – serially diluted, and plated for colony counts. LoxaSperse formulations required 0.5h to significantly reduce and completely eliminate viable *S. aureus* and *P. aeruginosa. The same effect against* viable *E. coli* and *C. albi*cans required 168h. LoxaSperse formulations prevented *A. niger* proliferation over 7 days of testing. The results of this study demonstrate that accidental or intentional contamination of the finished or reconstituted preparation did not result in microbial growth.

Purpose:

The intent of this study was to evaluate results of purposeful inoculation of the formulations with microorganisms specified in USP <51> (The United States Pharmacopeial Convention, 2013a), for nasal and inhalation use with modified Antimicrobial Effectiveness Test (AET) methodology and to quantitatively determine the *in vitro* effectiveness of formulations containing LoxaSperse to prevent microbial proliferation and/or kill the organisms.

Introduction:

LoxaSperse is a powder excipient base used for nebulization and irrigation. LoxaSperse is a blend of specially micronized xylitol with an optimized ratio of micronized poloxamers, designed to improve the dispersibility and solubility of active pharmaceutical ingredients (APIs) (PCCA, 2013). The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is ample evidence of their safety and efficacy (Durairaj *et al.*, 2006; Jagannath *et al.*, 1995; Plataki *et al.*, 2011; Zabner *et al.*, 2000).

Fluticasone propionate is one of the most prescribed inhaled corticosteroids in the United States, being the preferred therapy for persistent asthma by acting directly on the pulmonary airways through topical anti-inflammatory effects (Colice *et al*., 2013). Levocetirizine dihydrochloride is a second-generation antihistamine for the relief of symptoms associated with allergic rhinitis and uncomplicated skin manifestations of chronic idiopathic urticaria. It is known that current treatment options for allergic rhinitis include antihistamines and corticosteroids (Singh-Franco *et al*., 2009).

In order to verify the effectiveness of LoxaSperse formulations against microbial activity, capsules containing LoxaSperse with fluticasone propionate alone and in combination with levocetirizine dihydrochloride were mixed with sterile water. The final suspensions designed for nasal administration and local effect were assayed by AET methodology for 7 days.

Methodology:

Materials: Fluticasone Propionate USP Micronized (lot number C145638), and Levocetirizine Dihydrochloride (lot number C150499) were obtained from PCCA (Houston, TX, USA) as well as the excipient LoxaSperse (lot number 5994620). Capsules size #1 were filled with the following formulations and stored at 4°C: *formulation 1*, fluticasone propionate (180 mg) in LoxaSperse (448 mg); *formulation 2*, fluticasone propionate (180 mg) and levocetirizine dihydrochloride (265 mg) in LoxaSperse (448 mg). The test solutions were prepared by EPS by adding the contents of 1 capsule of each formulation to 10 mL of sterile water.

Microorganisms Strains: *E. coli* ATCC 8739, *A. niger* ATCC 16404, *C. albicans* ATCC 13231, *P. aeruginosa* ATCC 9027 and *S. aureus* ATCC 6538 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). All strains were maintained as glycerol stock solutions at -80°C. Working stocks were grown on tryptic soy (bacteria growth) or Sabouraud dextrose (fungi growth) agar media at 35°C.

Antimicrobial Effectiveness Test (AET):
Growth, harvesting, and enumeration of *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans* and *A. niger* were performed according to universal AET methodology (Moser and Meyer, 2011) with minor modifications. 1 mL aliquots of the test solutions (formulations) were prepared in 15 mL polycarbonate test tubes. 10 µL of cell culture (from 10^4 to 10^5 CFU/mL stock, diluted in phosphate buffered saline, PBS, Sigma Aldrich®) was added to each 1 mL aliquot to initiate the AET assay. 10 µL of cell culture was also added to 1 mL PBS for initial colony counts at the start of the AET assay. During the AET assay carried out at 20-25°C (room temperature), 100 µL of each challenged contaminated test solution was removed at intervals of 0.5h, 6h, 24h, and 7d (168h), serially diluted, and plated for colony counts on specific growth media. The results are presented as final colony counts, reported in CFU/mL and Log_{10} reductions in viable cell numbers at defined time intervals, being compared to the time zero performed on the PBS control inoculum levels.

Results and Discussion:

Initial colony counts of *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans* and *A. niger* indicated that a 10^2 to 10^3 CFU/mL product challenge was performed for these organisms (Table 1).

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Over the course of the AET, viable cell/spore counts changed according to the test solution (formulation) and organism tested. Formulation 1 eliminated the viable cells of *S. aureus* and *P. aeruginosa* in 0.5h and kept the solution free of bacteria for 7 days. *E. coli* was progressively (1-Log reduction/time interval from 0.5h) eliminated in 7 days, while *C. albicans* had the cell counts reduced only after 24h of incubation, being killed at 7 days. Formulation 2 induced the death of *S. aureus* in 0.5h, maintaining the solution free of bacteria for 7 days. A 2-Log reduction was achieved for *P. aeruginosa* in 0.5h exposure, with the solution completely cleared of bacteria by 6h and lasting through 7 days. This formulation showed a similar profile as formulation 1 against *E. coli* and *C. albicans*. The cell counts of *A. niger* did not change significantly over time for both formulations.

Table 1. Initial colony counts from adjusted cultures.

Organism	CFU/mL
Control	≤10*
E. coli	2.04 x 10^3
A. niger	2.4 x 10^2
C. albicans	1.5 x 10^2
P. aeruginosa	1.07 x 10^3
S. aureus	4.7 x 10^2

Table 2. Recovered colony counts from AET (CFU/mL).

CFU/mL at time (h):					
Organism	**F**	**0.5**	**6**	**24**	**168**
Control		≤10*	≤10*	≤10*	≤10*
E. coli	*1* *2*	2.6 x 10^3 2.3 x 10^3	7.9 x 10^2 5.9 x 10^2	50 60	≤10* ≤10*
A. niger	*1* *2*	6.0 x 10^2 2.6 x 10^2	5.4 x 10^2 3.2 x 10^2	50 7.8 x 10^2	2.4 x 10^2 1.7 x 10^2
C. albicans	*1* *2*	1.5 x 10^2 2.1 x 10^2	4.4 x 10^2 1.5 x 10^2	2.7 x 10^2 1.3 x 10^2	≤10* ≤10*
P. aeruginosa	*1* *2*	≤10* 10	≤10* ≤10*	≤10* ≤10*	≤10* ≤10*
S. aureus	*1* *2*	≤10* ≤10*	≤10* ≤10*	≤10* ≤10*	≤10* ≤10*

*≤10 denotes below detection limits USP <1227> (The United States Pharmacopeia Convention, 2013b); F = formulation

Conclusions:

Both formulations containing LoxaSperse required 0.5h to significantly reduce and completely eliminate viable *S. aureus* and *P. aeruginosa* and no bacterial growth was observed in the solutions up to 7 days. This behavior characterizes a 2-Log to 3-Log reduction in viable bacterial cells. *E. coli* counts were reduced over time and completely killed in 7 days while *C. albicans* was killed at 7 days. *A. niger* remained viable throughout the test. The chosen formulas when intentionally contaminated with microorganisms specified in USP <51> resisted microbial growth. Further, this study demonstrated these formulations after reconstitution were not at risk or did not support microbial growth.

Financial Disclosure:

PCCA contracted Emeryville Pharmaceutical Services (EPS, Emeryville, CA) to conduct this study. EPS has no proprietary or financial interests in the test products, or equity interest in PCCA, the sponsor of the study.

References:

Colice, G., Martin, R.J, Israel, L., Roche, N., Barnes, N., Burden, A., Polos, P., Dorinsky, P., Hillyer, E.V., Lee, A.J., Chisholm, A., von Ziegenweidt, J., barion, F., Price, D. (2013) 'Asthma outcomes and costs of therapy with extrafine beclomethasone and fluticasone', *Journal of Allergy and Clinical Immunology*, 132(1):45-54.

Durairaj, L., Launspach, J., Watt, J.L., Mohamad, Z., Kline, J. and Zabner, J. (2007) 'Safety assessment of inhaled xylitol in subjects with cystic fibrosis', *Journal of Cystic Fibrosis*, 6 (1), p.31-34.

Jagannath, C., Allaudeen, H. and Hunter, R. (1995) 'Activities of Poloxamer CRL8131 against *Mycobacterium tuberculosis in vitro* and *in vivo*', *Antimicrobial agents and chemotherapy*, 39 (6), p.1349-1354.

Moser, C. and Meyer, B. (2011) 'Comparison of compendial antimicrobial effectiveness tests: a review', *AAPS PharmSciTech*, 12 (1), p.222-226.

PCCA (2013) *Loxasperse*. Available at: http://www.pccarx.com/pcca-products/pcca-exclusives/bases/loxasperse (Accessed: 10 October 2013).

Plataki, M., Lee, Y., Rasmussen, D. and Hubmayr, R. (2011) 'Poloxamer 188 facilitates the repair of alveolus resident cells in ventilator-injured lungs', *American Journal of Respiratory and Critical Care Medicine*, 184, p.939-947.

Singh-Franco, D., Ghin, H.L., Robles, G.I., Borja-Hart, N., Perez, A. (2009) 'Levocetirizine for the Treatment of Allergic Rhinitis and Chronic Idiopathic Urticaria in Adults and Children', *Clinical Therapeutics*, 31(8): 1664-1687.

The United States Pharmacopeial Convention (2013a) 'Microbiological Tests / <51> Antimicrobial Effectiveness Testing'. *USP 36 -NF 31*. Rockville: USP, p.54-55.

The United States Pharmacopeial Convention (2013b) 'General Information / <1227> Validation of Microbial Recovery from Pharmacopeial articles. *USP 36 -NF 31*. Rockville: USP, p.989-991.

Zabner, J., Seiler, M., Launspach, J., Karp, P., Kearney, W., Look, D., Smith, J. and Welsh, M. (2000) 'The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing', *Proceedings of the National Academy of Sciences of the United States of America*, 97 (21), p.11614-11619.

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Technical Report:

Microbiological Quality of LoxaSperse® Dispersion Evaluated by Mikrocount® Combi

Abstract: LoxaSperse™ is a powder excipient base used for nasal nebulization and irrigation designed to improve dispersibility and solubility of Active Pharmaceutical Ingredients (APIs). PCCA evaluated the incidence of microbial contamination of a LoxaSperse formulation containing fluticasone propionate for a period of 24 h after the powder was mixed with sterile water under realistic conditions for use by the patient. The resultant LoxaSperse dispersion was subjected to total viable microbial count examination using a Mikrocount® Combi kit. Dip-slide tubes coated on both sides with specific media that allow distinguished bacterial and fungal growth were used in determined time intervals in order to assess the microbiological quality of the preparation. Neither microbial contamination nor physical characteristic changes were detected in the LoxaSperse dispersion containing fluticasone propionate throughout the 24 h study time period. The results showed that this non-sterile pharmaceutical preparation in sterile water avoided microbiological contamination in the absence of a preservative for at least 24 h after formulation reconstitution.

Introduction:

LoxaSperse is a powder excipient base used for nasal nebulization and irrigation. It is a blend of specially micronized xylitol with an optimized ratio of micronized poloxamers, designed to improve the dispersability and solubility of active pharmaceutical ingredients (APIs) (PCCA, 2013). The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is ample evidence of their safety and efficacy (Durairaj *et al.*, 2006; Jagannath *et al.*, 1995; Plataki *et al.*, 2011; Zabner *et al.*, 2000).

LoxaSperse formulations are dispensed as powders in capsules or sachets without preservatives. Due to an extremely low water activity of less than 0.5, microbiological growth cannot occur in this powder (PCCA, 2013). At the time of use, the patient must add a predetermined amount of sterile normal saline or sterile water to an appropriate cup, empty the powder contents into the liquid and mix gently to form a dispersion that will be administered via irrigation or nasal nebulization. From the moment of adding water to the powder, the non-sterile LoxaSperse dispersion could possibly become a good environment to support microbial contamination and proliferation.

The aim of this study was to evaluate the capability of the LoxaSperse formulation to remain exempt of microorganisms for 24 h after the non-sterile preparation was mixed with water under realistic conditions for use by the patient, and to draw conclusions about its microbial risk for use in nasal therapy. Fluticasone propionate was chosen as the API in the LoxaSperse formulation tested due to its ample use as a corticosteroid with topical anti-inflammatory effects against asthma (Colice *et al*., 2013).

Mikrocount® Combi was the microbial monitoring system used in the microbiological assay. It is a simple tool applicable to determine the total number of microorganisms present in any pharmaceutical and cosmetic sample, which provides rapid and reliable microbial control. The simple sampling and evaluation of results through different agar medium for bacteria and fungi confers practicability to a wide scope.

Methodology:

Materials: Fluticasone Propionate USP Micronized (lot number C145638) and the excipient LoxaSperse (lot number 5994620) were obtained from PCCA (Houston, TX, USA). A capsule size #1 was filled with 3 mg of fluticasone propionate in LoxaSperse and stored at room temperature. The test dispersion was prepared in a sterile plastic cup by adding the contents of one capsule to 10 mL of sterile water under non-sterile conditions. Mikrocount® Combi tubes were supplied in a 10-unit box by Schülke Inc. Each tube contains a dip-slide coated on one side with TCC-agar medium (light pink medium – bacterial growth), while on the other side with Rose-bengal-agar (red medium – fungal growth).

Total Viable Microbial Count Assay:

The microbiological quality of the LoxaSperse formulation after reconstitution with sterile water was evaluated using the Mikrocount® Combi kit. The assay was undertaken at room temperature for 24 h after the test dispersion was placed in a plastic cup open to environmental exposure (absent of any protective cover), according to the kit's instructions. A single dip-slide tube was used for microbial measurement of the test dispersion at each of the following time-points of exposure: 1, 2, 3, 4, 5, 6, 7, 8 and 24 h. The lid of each tube was loosened to remove the slide without touching the agar surfaces. Each slide was dipped into the test dispersion for a few seconds in a way in which both sides stayed completely wet. The excess liquid was drained off the slide, the slide was re-inserted into the tube, and the lid was tightly fixed to close the tube. The bacterial or fungal growth was visually analyzed by colony counting on the respective agar surface, after 48 h (bacteria) or 96 h (fungi) of incubation at 30°C, with the tube remaining sealed. The results can be compared with an evaluation chart in order to characterize in CFU/mL, which represents the degree of microbial contamination of the preparation.

Results and Discussion:

The microbiological assay using a microbial test kit (Mikrocount® Combi) revealed no microbial contamination in the LoxaSperse

dispersion containing fluticasone propionate for at least 24 h after it was mixed with sterile water and placed in an adequate container specified for patient use. No changes in its physical characteristics were observed throughout the study (Figures 1 to 3).

Figure 1. Fluticasone Propionate in LoxaSperse dispersion after the formulation was reconstituted in a sterile plastic cup.

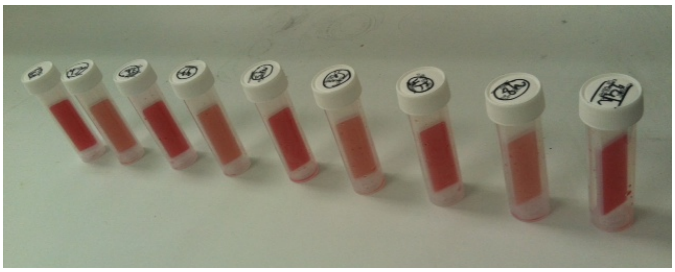

Figure 2. Dip-slide tubes used for testing LoxaSperse dispersion at 1, 2, 3, 4, 5, 6, 7, 8 and 24 h after the powder contents were mixed with sterile water.

Figure 3. Representative dip-slide with double agar sides free of microbial contamination. This dip-slide was used to test the LoxaSperse dispersion for up to 24 h of environmental exposure.

Conclusions:

The LoxaSperse formulation containing Fluticasone Propionate 3 mg, as a non-sterile pharmaceutical preparation in sterile water used for nasal nebulization/irrigation, is able to prevent microbial contamination for up to 24 h, making it appropriate for immediate use in nasal nebulization/irrigation. The LoxaSperse dispersion, even without a preservative in its formulation, was free from microbial contamination during the first 24 h after reconstitution under realistic conditions for use by the patient.

Financial Disclosure: PCCA is the full sponsor of the study.

References:

Colice, G., Martin, R.J, Israel, L., Roche, N., Barnes, N., Burden, A., Polos, P., Dorinsky, P., Hillyer, E.V., Lee, A.J., Chisholm, A., von Ziegenweidt, J., barion, F., Price, D. (2013) 'Asthma outcomes and costs of therapy with extrafine beclomethasone and fluticasone', *Journal of Allergy and Clinical Immunology*, 132(1):45-54.

Durairaj, L., Launspach, J., Watt, J.L., Mohamad, Z., Kline, J. and Zabner, J. (2007) 'Safety assessment of inhaled xylitol in subjects with cystic fibrosis', *Journal of Cystic Fibrosis*, 6 (1), p.31-34.

Jagannath, C., Allaudeen, H. and Hunter, R. (1995) 'Activities of Poloxamer CRL8131 against *Mycobacterium tuberculosis in vitro* and *in vivo*', *Antimicrobial agents and chemotherapy*, 39 (6), p.1349-1354.

PCCA (2013) *LoxaSperse*. Available at: http://www.pccarx.com/pcca-products/pcca-exclusives/bases/loxasperse (Accessed: 10 October 2013).

Plataki, M., Lee, Y., Rasmussen, D. and Hubmayr, R. (2011) 'Poloxamer 188 facilitates the repair of alveolus resident cells in ventilator-injured lungs', *American Journal of Respiratory and Critical Care Medicine*, 184, p.939-947.

Schülke (2014) *Mikrocount® combi*. Available at: http://www.schuelke.com/int/en/default/48159.php (Accessed: 17 March 2014)

Zabner, J., Seiler, M., Launspach, J., Karp, P., Kearney, W., Look, D., Smith, J. and Welsh, M. (2000) 'The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing', *Proceedings of the National Academy of Sciences of the United States of America*, 97 (21), p.11614-11619.

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Technical Report:

Evaluating the Effects of LoxaSperse® Using an *In Vitro* Model of Human Respiratory Tract Tissue

Abstract: LoxaSperse™ is an excipient manufactured by PCCA that can be used in compounding as a dispersing or solubilizing agent for active pharmaceutical ingredients (APIs) in nasal nebulizations or irrigations. It can help improve the solubility and therefore potential bioavailability of poorly water soluble drugs or combinations of drugs used in the treatment of respiratory and pulmonary diseases. The *in vitro* toxicity profile of LoxaSperse was evaluated using normal tracheal/bronchial epithelial cells in an assay which closely resembles the epithelial tissue of the respiratory tract. At concentrations of 0.01 µg/µL, 0.1 µg/µL, and 1 µg/µL, LoxaSperse was shown to be substantially less toxic than the positive control, Polysorbate 20 NF, with mean percent cell viabilities of 96%, 98%, and 69% for LoxaSperse compared to 65%, 28%, and 7% for the toxicant Polysorbate 20 NF, respectively. In addition, LoxaSperse exhibited a low toxicological profile similar to that of a known nontoxic respiratory agent, Monohydrate Lactose inhalation grade (negative control). The results of this study suggest a positive safety profile for the use of LoxaSperse as an excipient for compounding with APIs used in the treatment of respiratory and pulmonary diseases.

Purpose:

The objective of this study is to compare the toxicity profile of LoxaSperse against a known toxic (Polysorbate 20 NF) and commercial nontoxic (Monohydrate Lactose inhalation grade) agent in the pulmonary system using an *in vitro* model of the human respiratory tract tissue.

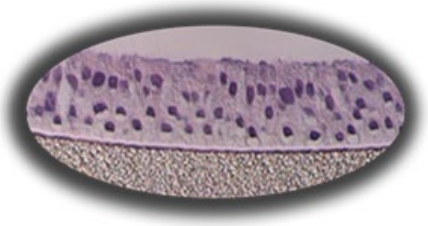
Introduction:

Local delivery of medication to the sinuses and lungs is highly desirable, especially in patients with specific sinus and pulmonary diseases such as cystic fibrosis, asthma, chronic sinus and pulmonary infections, and lung cancer. The principal advantages include reduced systemic side effects and higher doses of the applicable medication at the site of drug action (Harvey & Schlosser, 2009; Pilcer & Amighi, 2010).

Many existing APIs and an increasing number of new drugs are often poorly water soluble (Zhang et al., 2011). Drug insolubility, regardless of the administration route, commonly generates bioavailability or efficacy problems. Different techniques exist to increase drug dissolution and/or solubility, which often require the use of specific excipients. The excipients used in nasal nebulizations and irrigations should be chemically and physically stable, inert to the API used with them, and exhibit no side effects (Duret *et al*., 2012).

LoxaSperse is a proprietary excipient manufactured by PCCA for use in compounding as a dispersing or solubilizing agent for APIs in nasal nebulization and irrigation formulations. It consists of a blend of micronized xylitol and poloxamers. This combination is designed to be mixed with APIs in order to improve the drugs' water solubility and dispersability (PCCA, 2013). Xylitol is a 5-carbon sugar with low transepithelial permeability and is poorly metabolized by bacteria (Durairaj *et al*., 2007). Poloxamers are a series of synthetic block copolymers of poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) or PEO–PPO–PEO with varying molecular weights and block ratios. They are nonionic amphiphilic surfactants possessing excellent wetting, antifoaming, and solubilizing properties (Moebus *et al*., 2009). The use of xylitol and poloxamers in nasal nebulizations and irrigations is thoroughly referenced in the literature, and there is evidence of their safety (Durairaj *et al*., 2007; Jagannath *et al*., 1995; Plataki *et al*., 2011; Zabner *et al*., 2000). LoxaSperse is an excipient base used in compounding that allows for the preparation of non-sterile capsules and powder sachets that are added to sterile water or water for injection by the patient at the time of administration (PCCA, 2013).

This study was conducted to determine the toxicological profile of LoxaSperse in human-derived tracheal/bronchial epithelial cells (TBE) which have been cultured to form a multilayered, highly differentiated model which closely resembles the epithelial tissue of the respiratory tract (Figure 1). Histological cross-sections of both the *in vitro* tissue and a normal human bronchiole reveal a pseudostratified epithelial structure. The LC50 (the concentration at which the survival of the exposed cells is 50% of the maximum value) was determined for LoxaSperse (test compound), Polysorbate 20 NF (positive control), and a commercial Monohydrate Lactose inhalation grade (negative control) as a comparative measure of toxicity among the compounds.



In vitro human respiratory tract tissue

Normal human bronchiole

Figure 1. Histology – *in vitro* human respiratory tract tissue and normal human bronchiole. Formalin fixed, paraffin embedded and H&E stained (100x).

Methodology:

Materials: Polysorbate 20 NF and a commercial Monohydrate Lactose inhalation grade were selected due to their known toxic and nontoxic effects in the respiratory tract tissue, respectively. Given that LoxaSperse is designed to be an excipient for respiratory drugs, low doses of LoxaSperse were used in this study. LoxaSperse (Lot: 6212182), Polysorbate 20 NF (Lot: C162820), and a commercial Monohydrate Lactose inhalation grade (Lot: 10765667) were provided by PCCA (Houston, TX, USA) as powders and were dissolved in sterile water to make a 1 µg/µL stock solution for each compound. The stock solution was then diluted to make the 0.1 µg/µL (1:9 dilution) and 0.01 µg/µL (1:99 dilution) solutions. All compounds were prepared on the day of the assay.

Methods: In the *in vitro* model, the human tracheal/bronchial epithelial cells were cultured on specially prepared cell culture inserts using serum free media to form ciliated, bronchiole-like structures, which resemble the human epithelial tissue of the respiratory tract. This multilayered, highly differentiated 3D model was used to determine the toxicological profile of LoxaSperse in the respiratory tract tissue.

The human tracheal/bronchial tissue inserts were incubated on a 6-well plate with each compound at concentrations of 0.01 µg/µL, 0.1 µg/µL, and 1 µg/µL for 3 hours with 5% CO_2 at 37°C and ≥ 90% humidity. After the 3-hour incubation period, each tissue insert was individually removed from its plate, decanted into a waste beaker, and rinsed 3 times with phosphate buffered saline (PBS). After the final rinse, excess liquid was shaken off and each treated tissue insert was dosed with 300 µL of MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide). MTT is a water soluble, yellow tetrazolium salt which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Toxic substances that damage the mitochondrial enzyme, succinate dehydrogenase, inhibit the reduction of MTT; therefore, the amount of reduced MTT is proportional to the number of viable cells. Following a 3-hour incubation with MTT, each insert was removed and gently rinsed with PBS. Once residual MTT and PBS have been removed, the inserts were placed into one 24-well extraction plate, which was immersed into 2 mL of extraction solution. The plate was then sealed in a plastic bag and stored overnight at room temperature.

After the extraction period, the liquid within each insert was decanted back into the 6-well plate. The remaining extractant solution was then agitated, and a 200 µL aliquot of each extract was removed for evaluation. A Molecular Device SpectraMax M5 Microplate Reader was used to determine the absorbance of each extract at 570 nm – the wavelength that corresponds to the amount of reduced MTT or cell viability.

Data Analysis: The percent cell viability for each well was measured from the absorbance or optical density (OD) reading while using the mean defined absorbance of the diluent (sterile water) as control. Toxicological profiles as a function of log concentration were generated using GraphPad Prism 5 (La Jolla, CA, USA). The LC50s were interpolated from a sigmoidal dose response curve fitting analysis. All results were expressed as mean ± standard error of the mean.

Results and Discussion:

Using a nonlinear regression model, the toxicological profiles of LoxaSperse, Polysorbate 20 NF (positive control), and a commercial Monohydrate Lactose inhalation grade (negative control) were measured as shown in Figure 2.

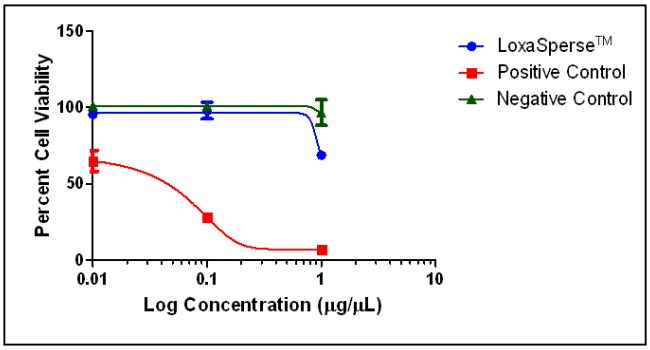


Figure 2: Graph shows the toxicological profiles of LoxaSperse, Polysorbate 20 NF (positive control), and a commercial Monohydrate Lactose inhalation grade (negative control).

At concentrations of 0.01 µg/µL, 0.1 µg/µL, and 1 µg/µL, LoxaSperse was shown to be substantially less toxic than the positive control, Polysorbate 20 NF, with mean percent cell viabilities of 96%, 98%, and 69% for LoxaSperse compared to 65%, 28%, and 7% for the toxicant Polysorbate 20 NF, respectively. In addition, no clinically significant differences in toxicological profiles were observed between LoxaSperse and the nontoxic agent, Monohydrate Lactose inhalation grade, indicating very minimal toxicity of LoxaSperse in the human respiratory tract tissue. Unlike the toxic agent, Polysorbate 20 NF, percent cell viabilities for LoxaSperse and Monohydrate Lactose inhalation grade did not decrease to less than 50%; thus, the predicted LC50s for LoxaSperse and Monohydrate Lactose inhalation grade were at least 7-fold higher than that of Polysorbate 20 NF (predicted LC50 for LoxaSperse = 7.95 µg/µL; predicted LC50 for Monohydrate Lactose inhalation grade = 7.64 µg/µL; LC50 for Polysorbate 20 NF = 1.11 µg/µL). Altogether, these results indicate that exposure of human tracheal/bronchial epithelial cells to LoxaSperse produced very minimal toxicity, with LoxaSperse exhibiting low toxicological profile similar to that of a known nontoxic respiratory agent.

Conclusions:

LoxaSperse is an excipient used to enhance the water solubility and dispersibility of APIs, leading to increased bioavailability, which can potentially improve drug efficacy and therapeutic outcome. Using LoxaSperse as an excipient in compounded preparations allows for APIs with low water solubility and combinations of APIs to be delivered locally to the area of treatment. LoxaSperse improves the dissolution of poorly water soluble drugs into hydrophilic and permeable tissue compartments such as the oral and nasal cavities. This study showed that LoxaSperse as an excipient produced very minimal toxicity in the human respiratory tract tissue. The results of this study suggest a positive safety profile for the use of LoxaSperse as an excipient for compounding with APIs used in the treatment of respiratory and pulmonary diseases.

Financial Disclosure: PCCA contracted Consumer Product Testing Company, Inc. (Fairfield, NJ) to conduct this study. Consumer Product Testing Company, Inc. has no proprietary or financial interests in the test products or equity interest in PCCA, the sponsor of the study.

References:

Durairaj L., Launspach J., Watt J. L., Mohamad Z., Kline J., Zabner J. (2007) Safety assessment of inhaled xylitol in subjects with cystic fibrosis, *Journal of Cystic Fibrosis*, 6: 31-34.

Harvey R. J., Schlosser R. J. (2009) Local Drug Delivery, Otolaryngologic Clinics of North America, 42: 829-845.

Duret C., Wauthoz N., Sebti T., Vanderbist F., Amighi K. (2012) Solid dispersions of itraconazole for inhalation with enhanced dissolution, solubility and dispersion properties, *International Journal of Pharmaceutics*, 428: 103-113.

Jagannath C., Allaudeen H. S., Hunter R. L. (1995) Activities of Poloxamer CRL8131 against *Mycobacterium tuberculosis* In Vitro and In Vivo, *Antimicrobial Agents and Chemotherapy*, 39: 1349-1354.

Moebus K., Siepmann J., Bodmeier R. (2009) Alginate–poloxamer microparticles for controlled drug delivery to mucosal tissue, *European Journal of Pharmaceutics and Biopharmaceutics*, 72: 42-53.

PCCA (2013). LoxaSperse. Available at: http://www.pccarx.com/bases/LoxaSperse (Accessed: October/ 2013).

Pilcer G., Amighi K. (2010) Formulation strategy and use of excipients in pulmonary drug delivery, *International Journal of Pharmaceutics*, 392: 1-19.

Plataki M., Lee Y. D., Rasmussen D. L. Hubmayr R. D. (2011) Poloxamer 188 Facilitates the Repair of Alveolus Resident Cells in Ventilator-injured Lungs, *American Journal of Respiratory and Critical Care Medicine*, 184: 939-947.

Zabner J., Seiler M. P., Launspach J. L., Karp P. H., Kearney W. R., Look D. C., Smith J. J., Welsh M. J. (2000) The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing, *Proceedings of the National Academy of Sciences*, 97: 11614-11619.

Zhang J., Wu L. B., Chan H. K., Watanabe W. (2011) Formation, characterization, and fate of inhaled drug nanoparticles, *Advanced Drug Delivery Reviews*, 63: 441–455.

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Respiratory System

SCIENTIFIC CASE STUDY

PCCA LoxaSperse®

Chronic Rhinosinusitis

SUMMARY: An adult female suffering from chronic rhinosinusitis was prescribed a compounded medicine post endoscopic sinus surgery. According to the patient's self-reported assessment, the treatment with the compounded medicine contributed to a 100% improvement (full recovery) of the patient's chronic rhinosinusitis symptoms (e.g. nasal congestion, facial pain and headache).

Submitted by: Ashley Berthelot, CAO, Sales and Marketing Director at Professional Arts Pharmacy, Lafayette, LA.

Introduction:

Chronic rhinosinusitis (also known as chronic sinusitis) is a complex inflammatory disease of the nose and paranasal sinuses characterized by at least 8-12 weeks of recurrent symptoms, such as nasal congestion and discharge (anterior/posterior nasal drip), facial pressure and/or reduction of smell, which commonly co-exists with asthma. Chronic rhinosinusitis is the second most prevalent respiratory disease among adults, women in particular, affecting 12.1% of the U.S. population and approximately 28.5 million patients. The current overall expenditure with chronic rhinosinusitis has been recognized as a socioeconomic burden, considering the number of work days missed, office visits, surgical interventions (e.g. endoscopic sinus surgery) and medicines prescribed [1-3].

The purpose of this case study is to discuss the management of chronic rhinosinusitis using a LoxaSperse compounded medicine. LoxaSperse is a proprietary powder excipient used in compounding for nasal nebulization or nasal and wound irrigation (Figure 1). Multiple active ingredients are often combined with LoxaSperse in the form of capsules or sachets to be mixed with saline or sterile water prior to use [4].

Case Report:

A 59 year-old Caucasian female has been suffering from chronic rhinosinusitis for 20 years and was subjected to 3 endoscopic sinus surgeries in the years of 2001, 2009 and 2013. The patient is asthmatic and her condition deteriorates with the asthma exacerbations. Several commercial medicines (e.g. Claritin-D®) were used throughout this long period without success. Approximately 2 weeks following the third surgery, the patient was prescribed a compounded medicine containing levofloxacin 125 mg, mupirocin 100 mg and fluticasone propionate 3 mg in LoxaSperse (Figure 2) – capsules to be opened prior to dosing, mixed with sterile saline and administered using a NasoNeb® Nasal Nebulizer, an intranasal drug delivery system which delivers aerosols to the nasal and paranasal sinus cavities (Figure 1) [5]. The patient was instructed to administer the compounded medicine 3 times daily, for a period of 8 months (post-surgery). The patient observed that her chronic rhinosinusitis symptoms, such as headache, congestion and posterior nasal drips, improved considerably following treatment with the compounded medicine (Figure 2).

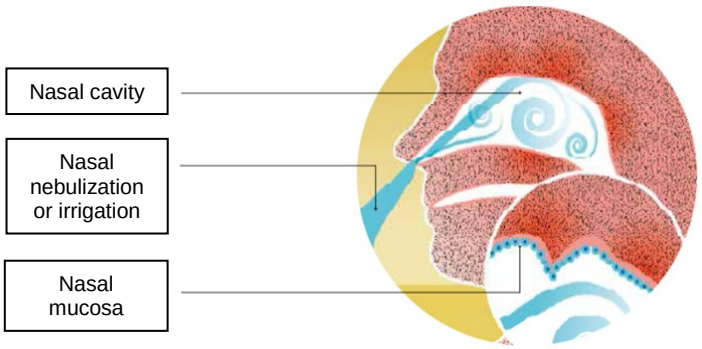

Figure 1. *Schematic representation of the nasal cavity, nasal nebulization or irrigation and the nasal mucosa.*

Levofloxacin hemihydrate USP	12.800 g
Mupirocin USP	10.000 g
Fluticasone propionate USP micronized	0.300 g
LoxaSperse®	22.994 g
Capsules size #0	100 units

Figure 2. *Compounded medicine prescribed post-surgery (PCCA Formula #11657).*

Methodology:

Valid and reliable assessment of outcomes is essential in scientific case studies and, therefore, 2 validated research instruments were selected, as follows:

1. *Numeric Rating Scale (NRS)*: a generic, unidimensional, self-reported questionnaire that consists of a segmented, 11-point intensity scale (from 0 to 10). The raw change and percent change are calculated taking into account the baseline and endpoint scores selected by the patient. The NRS is commonly used to assess pain [7] and it was adapted in this case study to measure the overall severity of the chronic rhinosinusitis symptoms, before and after treatment with the compounded medicine.

2. *Sino Nasal Assessment Questionnaire (SNAQ) 11*: a patient focused, rhinosinusitis specific outcome measure that consists of a multidimensional 11-point assessment.

This self-reported questionnaire covers a list of symptoms and social/emotional consequences often found

Chronic Rhinosinusitis

in chronic rhinosinusitis. Patients are invited to classify their level of problem as follows: no problem (n=0), very mild problem (n=1), mild problem (n=2), moderate problem (n=3), severe problem (n=4) and problem as bad as it can be (n=5). The first three questions (i.e. nasal blockage, congestion and facial pain) are depicted as the most relevant and, therefore, the individual scores are multiplied by 3 or 2. All other questions (e.g. sneezing, cough, headache) have a maximum score of 5. As a result, the SNAQ-11 total score range from 0 (completely asymptomatic) to 80 (worst possible symptoms) [8]. Written permission was obtained to use the SNAQ-11 for scientific purposes, copyright by F.F. Fahmy (United Kingdom). Written informed consent was obtained from the patient to publish this case study.

Results and Discussion:

The patient reported a NRS baseline score of 10, which corresponds to the worst possible chronic rhinosinusitis symptoms (before treatment); and an endpoint score of 0, which corresponds to no chronic rhinosinusitis symptoms (after treatment). The NRS raw change (baseline to endpoint) of 10 points indicates a 100% improvement of the chronic rhinosinusitis symptoms, according to the patient's self-reported assessment. Considering that 30% is the minimum level of change that represents a clinically important outcome [7], this study results demonstrate that the compounding treatment contributed to a significant clinical improvement of the patient's chronic rhinosinusitis symptoms.

The patient completed all questions of the SNAQ-11 questionnaire (adapted), before and after treatment with the compounded medicine, as displayed in Table 1.

Parameters	*Score (n) Before*	*Score (n) After*
Blocked nose	3 (x3)	0
Nasal congestion	5 (x3)	0
Facial pain	5 (x2)	0
Nasal discharge	5	0
Phlegm	5	0
Sneezing	5	0
Cough	5	0
Altered smell	5	0
Headache	5	0
Earache	4	0
Fatigue	4	0
Total	72	0

Table 1. *SNAQ-11 scores, individual and total, before and after treatment with the compounded medicine.*

Before treatment with the compounded medicine, the patient classified her symptoms as 'bad as can be' (n=5) for the majority of the questions, with the exception of blocked nose, earache and fatigue (Table 1). The total score of 72 corresponds to 90% of the maximum score of 80, which indicates a very severe problem, according to the patient's self-reported assessment of her condition.

After treatment with the compounded medicine, the patient classified all her symptoms as 'no problem' (n=0), as displayed in Table 1. The total score decreased from 72 pre-treatment to 0 post-treatment, which corresponds to an improvement of 100% and suggests a full recovery of the chronic rhinosinusitis symptoms, according to the patient's self-reported assessment of her condition.

Conclusions:

A valid and reliable assessment of outcomes is essential in scientific case studies and requires the use of validated research instruments for a meaningful estimate of treatment outcomes. To demonstrate the effectiveness of a compounded medicine in chronic rhinosinusitis, both NRS and SNAQ-11 validated questionnaires were used. According to the patient's self-reported assessment, the treatment with the compounded medicine contributed to a 100% improvement (full recovery) of the patient's chronic rhinosinusitis symptoms.

References:

1. Centers for Disease Control and Prevention (2014) Summary Health Statistics for U.S. Adults: National Health Interview Survey, 2012 [Online]. Available at:

http://www.cdc.gov/nchs/fastats/sinuses.htm (Accessed: 01 August 2015).

2. Hamilos, D.L. (2011) 'Chronic rhinosinusitis: epidemiology and medical management', *Journal of Allergy and Clinical Immunology*, 128 (4), p. 693-707, doi: 10.1016/j.jaci.2011.08.004.

3. Bachert, C., Pawankar, R., Zhang, L. *et al.* (2014) 'Consensus document, ICON: chronic rhinosinusitis', *World Allergy Organization Journal*, 7 (25), doi:10.1186/1939-4551-7-25.

4. Phan, H., Banov, D., Delancy, M. and Brockbank, K. (*in press*) 'Characterization of the properties of powder excipients commonly used in pharmaceutical compounding', *Particulate Science and Technology*, doi: 10.1080/02726351.2015.1071298.

5. MedInvent (2015) NasoNeb Nasal Nebulizer. Available at:

http://nasoneb.com/index.php?page=nebulizer (Accessed: 29 July 2015).

6. Fahmy, F.F., McCombe, A. and Mckiernan, D.C. (2002) 'Sino nasal assessment questionnaire, a patient focused, rhinosinusitis specific outcome measure', *Rhinology*, 40 (4), p. 195-197.

7. Farrar, J.T., Young Jr., J.P., LaMoreaux, L. *et al.* (2001) 'Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale', *Pain*, 94 (2), p. 149-158.

8. Fahmy, F.F., McCombe, A. and Mckiernan, D.C. (2002) 'Sino nasal assessment questionnaire, a patient focused, rhinosinusitis specific outcome measure', *Rhinology*, 40 (4), p. 195-197.

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