

## Increased uptake of lipophilic immunosuppressive compounds in cornea and retina based on solubilization in an aqueous formulation

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### Abstract

**Purpose:** Second generation corticosteroids and many macrolide immunosuppressants are hardly soluble in aqueous solutions. Hence, these substances are formulated as dispersions for application to ocular tissues. However, dissolved drugs permeate faster into different ocular compartments and are less likely washed out before reaching therapeutic levels than solid dispersed drug particles. Here, we show that the novel biocompatible aqueous formulation Marinolv allows solubilizing of lipophilic drugs like Fluticasone propionate and Tacrolimus. For visualization of the increased uptake into cornea and retina of solubilized lipophilic compounds in comparison to dispersions, fluorescently labeled estradiol was used in porcine ex-vivo models.

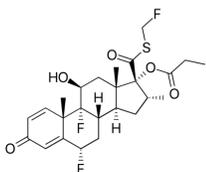
**Methods:** Fluticasone propionate<sup>1</sup>, Tacrolimus<sup>2</sup>, and fluorescently labeled estradiol were solubilized in the Marinolv formulation. The concentrations of dissolved compounds were determined by standard HPLC methods. For visualization of the permeation, fluorescently labeled estradiol or Tacrolimus was applied as Marinolv solution or dispersion onto porcine eyes ex-vivo and the amount of compound was determined by light scattering microscopy (LSM). Tacrolimus concentration in porcine cornea was determined by HPLC-MS/MS.

**Results:** Fluticasone propionate, Tacrolimus and fluorescently labeled estradiol can be dissolved in Marinolv, a formulation suitable for ocular application. An increase of solubility of approximately 200-fold was observed for all three compounds<sup>3</sup>. The ex-vivo visualization experiment with fluorescently labeled estradiol showed that dispersed estradiol hardly penetrates into eye compartments<sup>5,6</sup>. In contrast, Marinolv solubilized estradiol was detected within the cornea and the sclera/retina tissues in remarkable amounts. Application of dissolved Tacrolimus versus a marketed product resulted in a markedly increased uptake into porcine cornea as demonstrated in an ex-vivo model<sup>4</sup>.

**Conclusion:** Utilization of Marinolv enables the solubilization of otherwise insoluble compounds for ocular application. The increased penetration of these compounds into ocular tissues, visualized with solubilized labeled estradiol and demonstrated with Tacrolimus, suggests an enhanced tissue availability of dissolved drugs in comparison to dispersions. The application of Marinolv may enable the development of otherwise insoluble drugs for the treatment of ocular diseases.

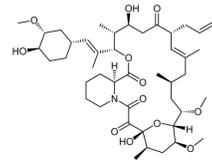
### Corticosteroids

**Figure 1. Chemical structure of Fluticasone propionate**  
Belongs to the class of glucocorticosteroids with experience for intranasal application to treat asthma and allergic rhinitis.

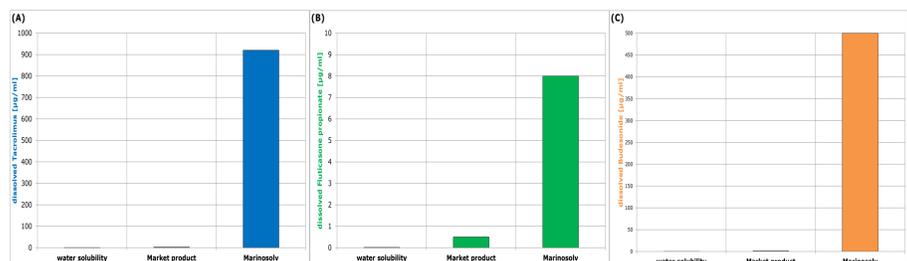


### Immunosuppressants

**Figure 2. Chemical structure of Tacrolimus**  
A neutral macrolide calcineurin inhibitor indicated after organ transplantations, ulcerative colitis, as an ointment in atopic dermatitis and as dispersions against ocular diseases such as dry eye.

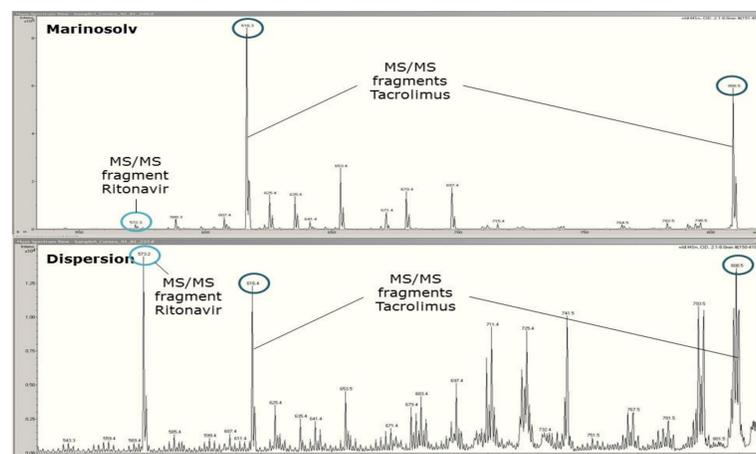


### Solubility of Tacrolimus & Fluticasone propionate in Marinolv solutions compared to marketed products formulated as dispersions



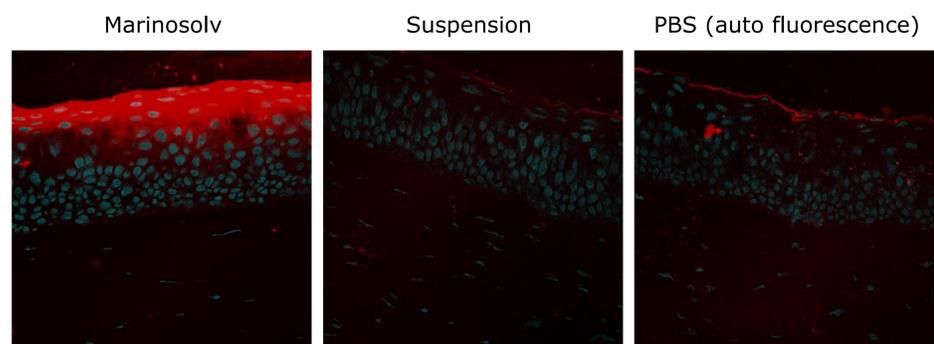
**Figure 3.** Corticosteroids & Immunosuppressants are hardly soluble in water and thus formulated as dispersions in marketed products. An increase of the solubility in ocular application improves the availability and thus permeability into anterior and posterior eye compartments, such as Cornea, Retina & Sclera. Improved solubility of (A) Tacrolimus (B) Fluticasone propionate (C) Budesonide in **Marinolv** formulations compared to marketed product and the solubility in water.

### Quantification of Tacrolimus in porcine cornea treated with Marinolv compared to a marketed product formulated as dispersion by HPLC-MS/MS

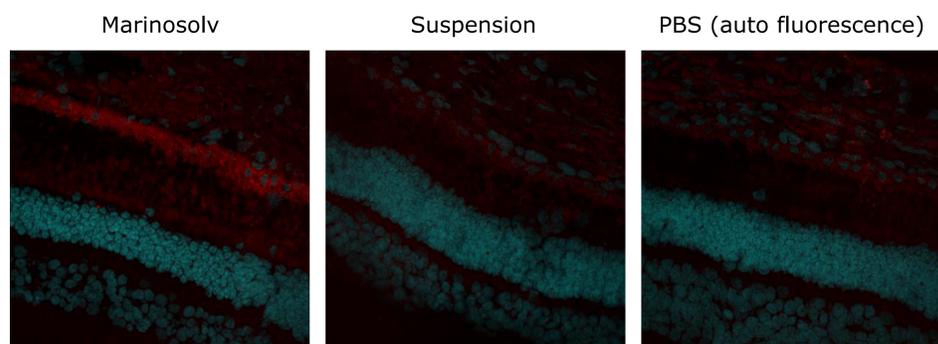


**Figure 4.** Quantitative analysis was performed using Ritonavir as internal standard, spiked into treated tissue samples. Compounds are separated on a heated reversed phase column and specific fragment ions are detected with an ion trap mass spectrometer. Eyes treated with Tacrolimus in **Marinolv** showed a 40-fold higher permeation into porcine **cornea**, than eyes treated with the dispersion.

### Increased tissue permeation of dissolved estradiol compared to a dispersion (ex-vivo porcine eyes)



**Figure 5.** Fluorescently labeled estradiol either dissolved using **Marinolv** or as **dispersion** was applied onto the porcine eyes (60 min), PBS was used as control for auto fluorescence of the tissue. Penetration into the **cornea** is shown.



**Figure 6.** Fluorescently labeled estradiol either dissolved using **Marinolv** or as **dispersion** was applied onto the porcine eyes (60 min), PBS was used as control for auto fluorescence of the tissue. Penetration into the **sclera/retina** is shown.