Marinomed

Marinosolv® - A Novel Approach to Increase the Solubility and Improve the Bioavailability of Promising Low-Soluble Drugs

Whitepaper March 2024

Marinosolv®

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Advancing Pharmaceutical Development with Marinosolv®

80-90% of promising, highly potent drug candidates in the R&D pipeline fail due to their low solubility in aqueous formulations, and subsequently their low bioavailability. Therefore, a strong focus in pharmaceutical and drug delivery development is currently on novel and innovative solubility-enhancing techniques or new solubilizing agents, in order to face the challenge of low drug solubility. Furthermore, approximately 60% of new chemical entities (NCEs) have been reported to be classified in BCS (Biopharmaceutics Classification System, Figure 1) class II (low solubility, high permeability) or IV (low solubility, low permeability). 60-70% of them are classified as BCS class II.

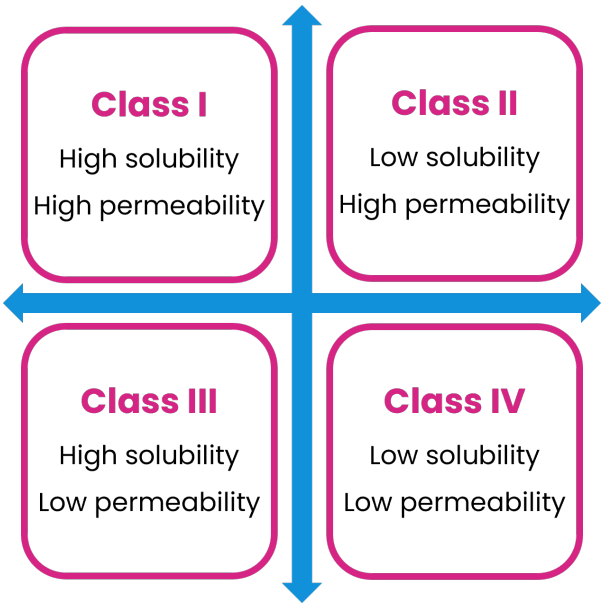


Figure 1: Biopharmaceutics Classification System (BCS) of drugs on the basis of their solubility and permeability

The solubility of a drug in aqueous formulations is a critical factor for the permeability and thereby bioavailability and systemic absorption into sensitive target tissues and/or at the site of action. Therefore, several different chemical and physical approaches to increase the solubility of a drug in aqueous formulations have become available in the last decade. One of them is Marinosolv®, a formulation technology that provides effective solubilization for a variety of highly hydrophobic small molecules and peptides, including APIs/NCEs.

Development of Marinosolv® as formulation technology

Marinosolv® enables a strongly increased solubilization and thereby enhanced bioavailability of hydrophobic small molecules and peptides and is applicable for substances classified as BCS class II and IV. The technology is particularly valuable during early-stage drug development, where careful consideration of each compound's unique physicochemical properties is essential. The key components of Marinosolv® are saponins (e.g., Escin, Glycyrrhizin) which create a solubility-enhancing environment, in combination with co-solvents and a stability enhancer (e.g. Dexpanthenol) (see Figure 2). Each drug product formulation is individually composed within the Marinosolv® matrix to optimize its effectiveness, considering the intended indication and application form.

This formulation technology is currently focusing on liquid and semi-solid formulation, e.g. providing aqueous solutions of API's that have been so far formulated as suspensions. Using the Marinosolv® formulation technology offers several new applications for highly insoluble substances in sensitive target tissues, including high local activity and low systemic side effects.

To investigate the potential of the Marinosolv® formulation technology, we have recently performed several solubility and stability studies, as well as permeability and efficacy studies in comparison to other solubility-enhancing techniques or new solubilizing agents. In this paper, we are summarizing the most and well-known approaches to increase the solubility, with a focus on approaches with a physical mode of action.

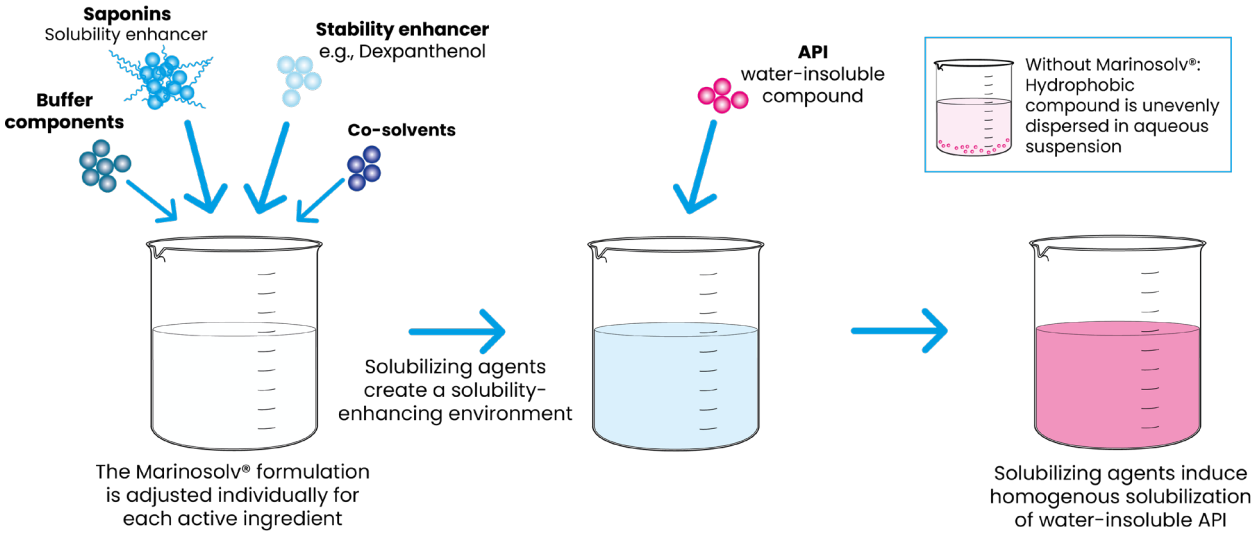


Figure 2: Marinosolv® formulation & solubilization of water-insoluble API

Approaches to increase the solubility of API's in drug products

There are two main approaches to improve the solubility of an API, especially in liquid formulations. Chemical approaches improve the characteristic of APIs to enhance their solubility, but also their physical and chemical stability. Physical approaches are focusing on optimizing the formulation development.

In this paper, both chemical and physical approaches were investigated and summarized. Using selected examples, similarities, differences, advantages, and disadvantages of Marinosolv® formulations were evaluated in comparison to other solubility-enhancing techniques or solubilizing agents, with a focus on the development of liquid formulations.

Chemical approaches

Salt formation

A very common method to increase the solubility of an API is the formation of a salt. However, it is not the only reason to employ a salt formation for an API, as this formation might also positively influence challenges in stability, toxicity, and absorption [1]. The selection is determined by the physical and chemical properties of the API, such as its pKa (acid dissociation constant, measured by the strength of an acid in solution), lipophilicity, hygroscopy, polymorphism, chemical stability, and its dissolution rate and solubility, respectively [1]. Salt formation is part of early drug development and should be finalized before formulation development.

Prodrug

A prodrug is a pharmacologically inactive compound which is metabolized into a pharmacologically active drug after intake [2]. This approach is preferred to improve the bioavailability of a compound, when a prodrug has improved absorption and/or distribution properties compared to the initial compound [3]. One of the best-known examples is Aspirin, which contains acetylsalicylic acid as a prodrug of salicylic acid. Until today, at least 30 prodrugs have been approved by the FDA [2]. The decision of using a prodrug or a drug should be made before formulation development.

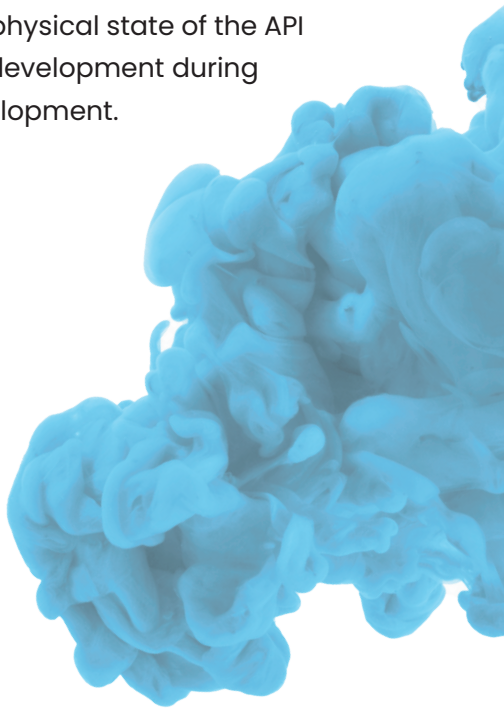
Particle size reduction

Particle size reduction is a method to increase the surface area of a compound in order to increase its dissolution rate and therefore bioavailability [4]. Well established, traditional methods are micronization (including milling and grinding), high-pressure homogenization, and spray drying [4]. Modern, non-conventional processes have been developed to overcome some challenges of these methods, such as thermal and chemical degradation, or the risk of a broader particle size distribution [4]. Such methods use supercritical fluids to induce a supersaturation, leading to precipitation of individual particles [5]. Until today, more than 30% of API's in the pharmaceutical development require micronization.

Use of amorphous drugs

Drug amorphization is defined as the transition from an organized crystalline structure to a disordered amorphous state. The process of drug amorphization increases the chemical potential of a drug compared to its crystalline form, resulting in increased solubilization [6]. This process leads to supersaturation and therefore improvements in bioavailability [6]. One of the first marketed

products using amorphization was a capsule formulation containing tacrolimus, a macrolide immunosuppressant [6]. The reported solubility of crystalline tacrolimus monohydrate and amorphous tacrolimus in phosphate buffer at pH 4.5 is 1.4 µg/ml and 45-50 µg/ml, respectively [6]. Therefore, we solubilized 100 µg/ml of an amorphous and a crystalline form of tacrolimus in a Marinosolv® formulation to compare their solubility. The powders were added to the same formulations and stirred for 24 hours, before being analyzed. The amorphous drug could be solubilized at a recovery of almost 100%, whereas the crystalline form showed a recovery of only about 25% (see Figure 3). Although the amorphous drug showed higher solubility, stability studies in a Marinosolv® formulation have not shown any improvements with the amorphous drug compared to the crystalline material. This highlights the importance of both the quality and physical state of the API and the formulation development during pharmaceutical development.



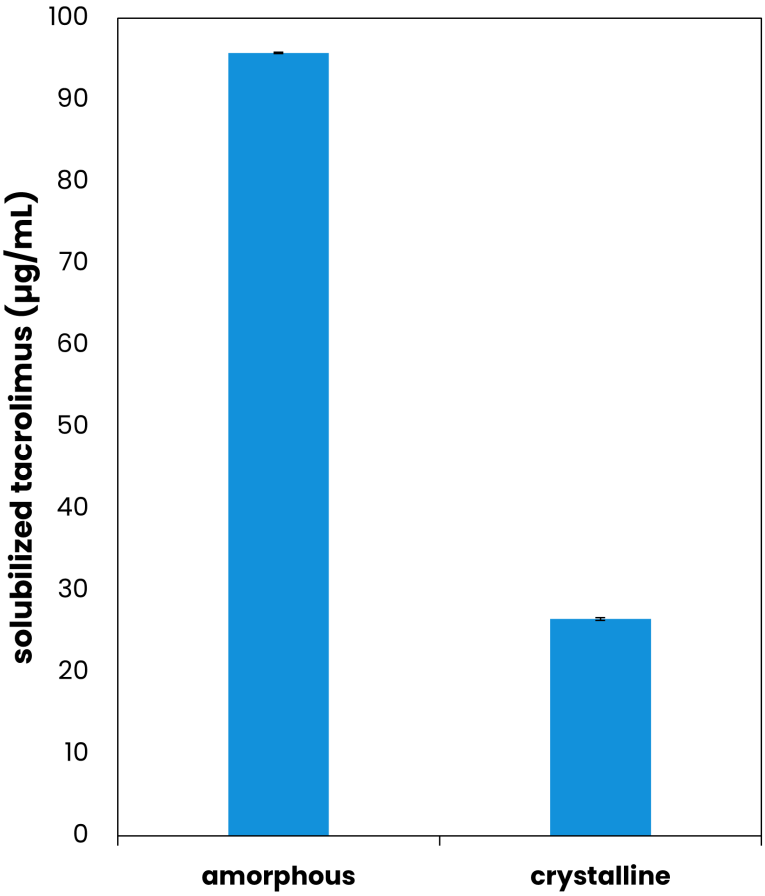


Figure 3: Amorphous versus crystalline tacrolimus solubilized in Marinosolv®

Physical approaches

Lipid formulations

Lipid-based drug delivery systems (LBDDS) are often used for oral administration of poorly water-soluble compounds [1]. They generally consist of a drug pre-solubilized in lipids, which increase the solubilization of a hydrophobic, low soluble drug, emulsified with an aqueous part. The combination of a lipid and an aqueous part are intended to form a stable lipid system [2]. Lipid-based formulations may be classified as solutions, emulsions, micellar systems, self-emulsifying drug delivery systems (SEDDS) and self-microemulsifying drug delivery system (SMEDDS), depending on their amount of oils, water-insoluble surfactants, water-soluble surfactants and hydrophilic cosolvents [1]. The *in-vivo* performance, and therefore bioavailability, is affected by the composition of the formulation [1]. Therefore, during pharmaceutical development, not only the physicochemical properties of the API but also the physiological and biochemical mechanisms related to resorption of the lipid-based formulation after oral intake should be considered.

Suspensions

Suspensions are aqueous systems in which insoluble, solid drugs are dispersed and are, depending on the particle size of the API, divided into colloidal (<1 µm) and coarse (>1 µm) suspensions [3]. There are several advantages for the use of suspensions; such as the avoidance of organic (co-)solvents, leading to a better tolerability of the formulation itself, or the generally higher stability of hydrophobic drugs in aqueous suspensions compared to solutions. On the other hand, there are also disadvantages, such as the low permeation of dispersed drugs or potentially causing irritation to sensitive tissues [4]. However, dissolved drugs permeate faster and in higher concentrations, leading to improved bioavailability compared to suspensions [4]. This is the reason why we have focused on the development of the Marinosolv® formulation technology.

Use of non-ionic surfactants

Surfactants are compounds with the ability to decrease the tension of a surface or the tension between two liquids, a liquid and a gas, or a liquid and a solid, and hence, are summarized as surface-active agents [5]. Non-ionic surfactants are water-soluble, temperature-sensitive compounds, containing covalently bonded oxygen-containing hydrophilic groups. The hydrophilic groups are bonded to hydrophobic parent structures. Since the number of hydrogen bonds decreases with increasing temperature, the water solubility is temperature-sensitive. Cremophor® EL (registered trademark of BASF Corp. and recently renamed to Kolliphor EL), and several poloxamers, such as poloxamer P124 and poloxamer P188 are examples of synthetic, non-ionic surfactants used as solubilizing agents, emulsifiers and stabilizers [6, 7].

During the pharmaceutical development of a preservative-free nasal spray containing dissolved fluticasone propionate, we investigated the potential to increase the solubility of this hydrophobic compound. Fluticasone propionate is a well-known and highly potent corticosteroid used in the treatment of allergic rhinitis and has a reported water solubility of 0.14 µg/ml [8]. Therefore, marketed nasal sprays are formulated as preserved suspensions. We developed a formulation in Marinosolv® containing at least 18 µg/ml dissolved fluticasone propionate (see Figure 4). Furthermore, we evaluated the solubility of fluticasone propionate using poloxamer P124, P188, and P407, showing the highest solubility at about 15 µg/ml for Poloxamer P407.

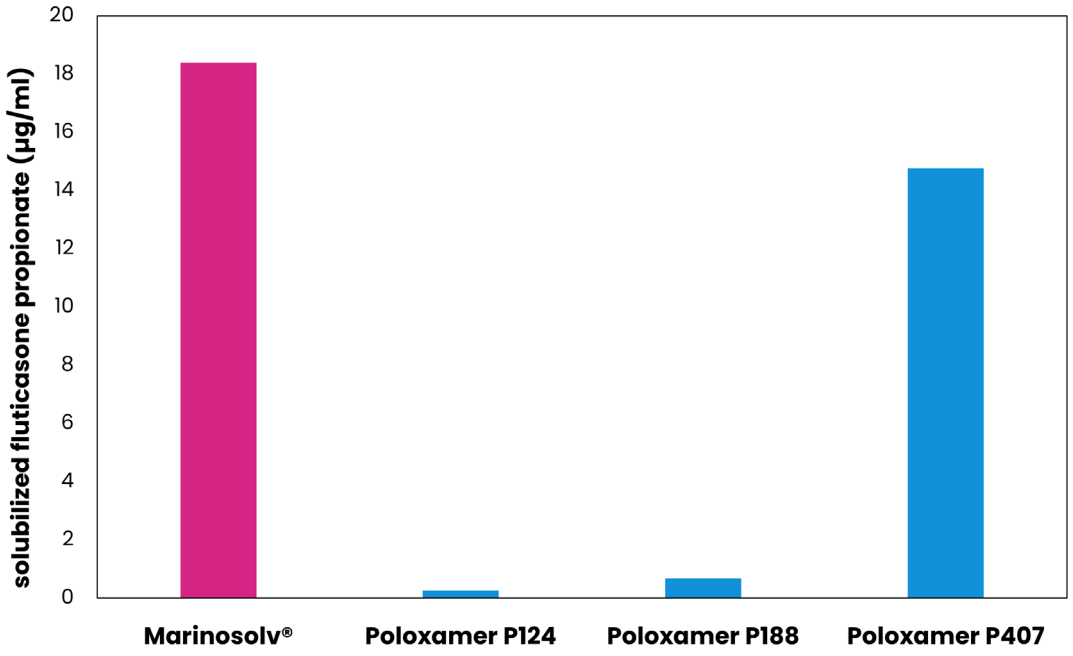


Figure 4: Solubility of fluticasone propionate in Marinosolv® compared to non-ionic surfactants

We have compared the drug permeation of fluticasone propionate *ex-vivo* into porcine nasal mucosa at a concentration of 15 µg/ml either formulated in Marinosolv® or with Kolliphor P407 at similar physico-chemical conditions (e.g. pH, osmolality and viscosity). Fresh porcine nasal mucosa was obtained from euthanized pigs. Uniform parts of the nasal mucosa were taken with a 10 mm biopsy punch. Nasal mucosa pieces were placed apical side up into a 48-well cell culture plate and 50 µL per 100 mg tissue of the respective formulation was applied onto the mucosal surface. Samples were incubated for up to 30 min in a humidity chamber. After the respective incubation period, treated mucosa pieces were extensively rinsed, frozen in liquid nitrogen, and stored at -80 °C until analysis. Quantification of permeated budesonide was done by HPLC-MS/MS after homogenization of the tissue.

The results shown in Figure 5 revealed a strongly reduced *ex-vivo* permeability for the formulation with Kolliphor P407. The Marinosolv® formulation showed a permeation of more than 2 µg fluticasone propionate per gram nasal mucosa after 30 minutes, whereas the Kolliphor P407 formulation reached its plateau after 10 minutes incubation time at 0.5 µg fluticasone propionate. Therefore, the Marinosolv® formulation is more beneficial for drug release compared to a Kolliphor P407 formulation.

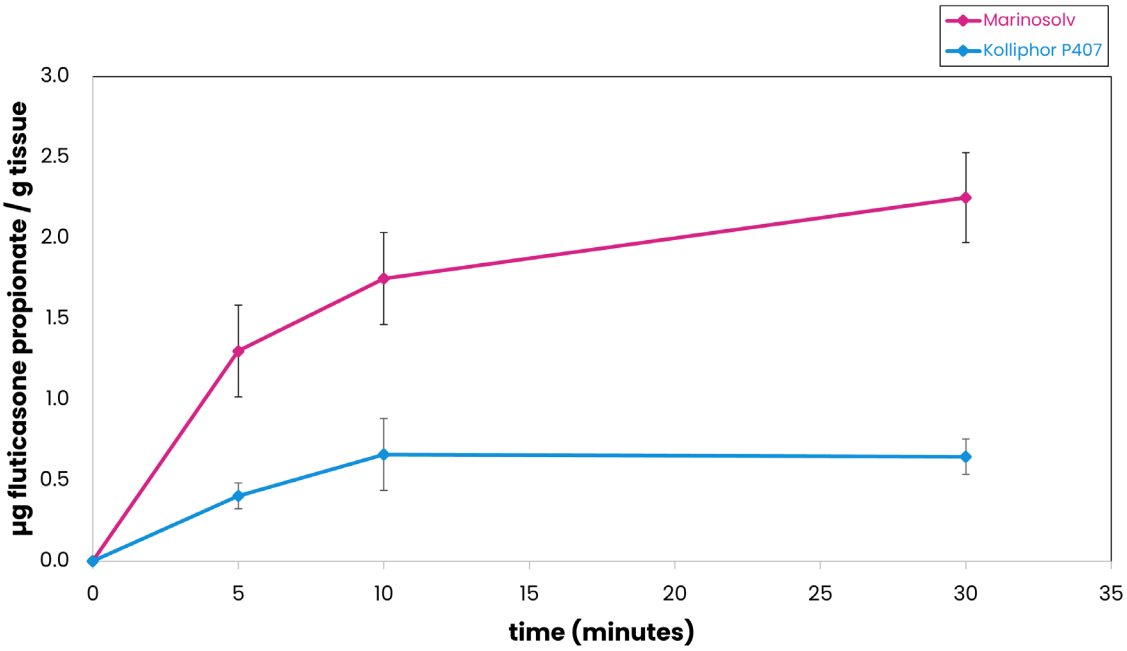


Figure 5: *Ex-vivo* permeation of fluticasone propionate into porcine nasal mucosa.

Another non-ionic surfactant commonly used in pharmaceutical development is the synthetic compound Polysorbate 80 (also known as Tween 80). It is a viscous, water-soluble yellow liquid and used in many oral and topical formulations as solubilizer. We evaluated its ability to solubilize budesonide, also a widely used corticosteroid in the treatment of allergic rhinitis and asthma. In a Marinosolv® formulation, the maximum of solubilized budesonide was about 480-490 µg/ml (see Figure 6), whereas when using Polysorbate 80, not more than 55 µg/ml could be dissolved.

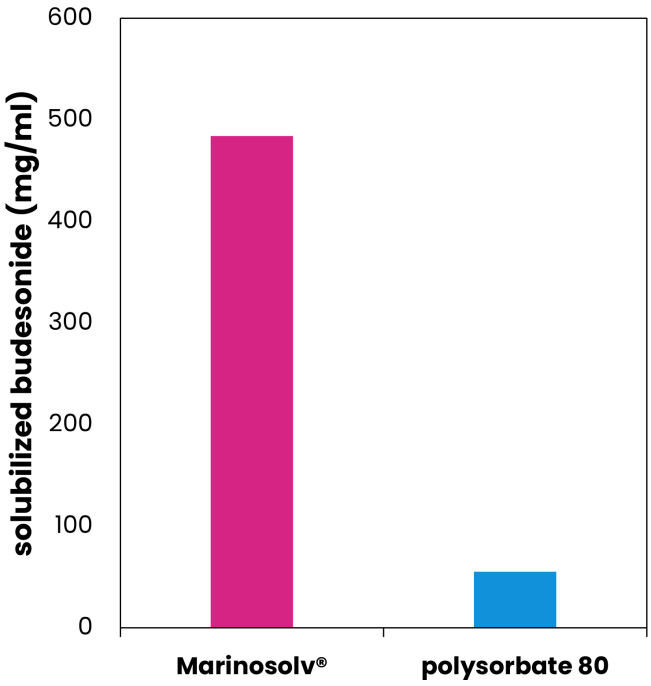


Figure 6: Solubility of budesonide in Marinosolv® compared to polysorbate 80

Complexations

Inclusion complexes are defined as a nonpolar molecule (or component of a molecule) incorporated into the cavity of a different molecule (called the "host"). Cyclodextrins are, based on their structure of cyclic oligosaccharides and their property of forming a toroid shape, the ideal host molecules for many hydrophobic, hardly soluble compounds [9]. Due to a specific arrangement of hydroxyl groups, the interior of the toroid shape is weakly hydrophobic, while the exterior is strongly hydrophilic. Thus, the interior can incorporate hydrophobic molecules, leading to enhanced solubility and stability of pharmaceutical products [9]. However, increasing the solubility of a drug does not automatically improve its bioavailability, since only a free form of the drug is able to penetrate lipophilic barriers [9]. Therefore, the bioavailability of a drug forming a complex with cyclodextrin depends on the ability of the complex to release the drug before permeation.

For budesonide, we have also investigated the drug permeation of budesonide *ex-vivo* into porcine nasal mucosa at a concentration of 300 µg/ml either formulated in Marinosolv® or with β-cyclodextrin at similar physicochemical conditions (e.g. pH, osmolarity, and viscosity). The experimental set up was similar to that for fluticasone propionate, only the incubation time was extended up to 60 minutes.

Results are shown in Figure 7, demonstrating that 300 µg/ml budesonide dissolved in Marinosolv® permeated faster and in higher amounts in the *ex-vivo* permeation experiment, reaching a plateau in drug permeation after 15 and a second even higher plateau after 45 minutes. At all time points, a significantly higher amount of solubilized budesonide could be detected with the Marinosolv® formulation, compared to the budesonide-cyclodextrin-complex. Hence, budesonide is released faster in a Marinosolv®-enabled formulation compared to a cyclodextrin-enabled budesonide formulation.

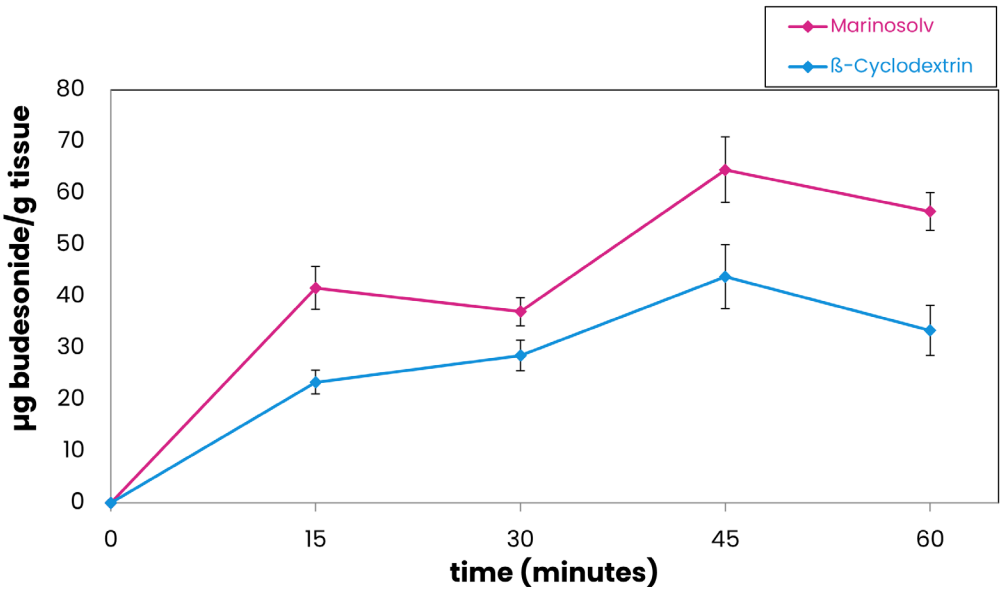


Figure 7: *Ex-vivo* permeation of budesonide into porcine nasal mucosa [10].

In another study, we investigated the efficacy of solubilized budesonide in Marinosolv® compared to cyclodextrin in an acute lung inflammation model. Lung inflammation was induced in female BALB/c mice by applying 3 mg/kg body weight E. coli lipopolysaccharide (LPS) intranasally. The mice were treated with 50 µl of 0.9% NaCl as placebo and 300 µg/ml budesonide in Marinosolv® or cyclodextin, respectively, in each nostril. Treatment was done 15 minutes after LPS challenge. Two hours after LPS challenge, mice were sacrificed and lungs were flushed to obtain bronchoalveolar lavage (BAL). BAL was analysed for their TNF-α concentration, a cytokine involved in systemic inflammation. The results, shown in Figure 8, revealed a significant TNF-α decrease for the Marinosolv® formulation, and a slightly, non-significant decrease for the cyclodextrin formulation. These results correlate with the results from the *ex-vivo* model, indicating an improved anti-inflammatory efficacy.

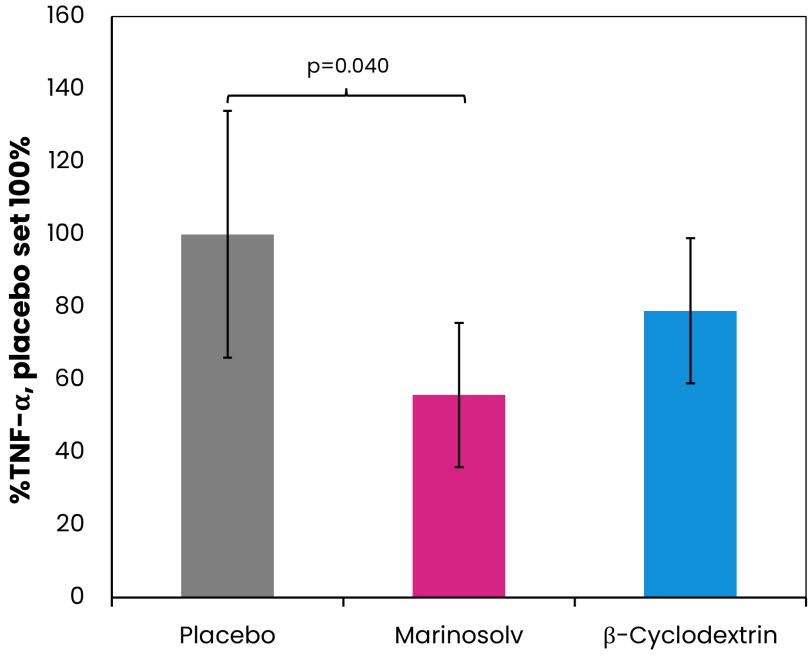


Figure 8: *In-vivo* TNF-α quantification after LPS-induced inflammation in a therapeutic set-up [10].

Drug carriers

Drug carriers are substrates such as liposomes, polymeric micelles, microspheres, and nanoparticles, used to control the release of drugs into the system, hence improving drug delivery. Semifluorinated alkanes (SFAs) have been recently used as new drug carriers in formulation development, since they are able to dissolve lipophilic drugs [11]. SFAs are colourless, non-aqueous liquids consisting of di-block molecules containing perfluorocarbon and hydrocarbon [11]. SFAs have been used in ophthalmology for vitreoretinal surgery for a long time and have recently also been used in the development of new liquid eye drops containing hydrophobic, hardly soluble drugs as solubility enhancer [11].

In our study, we compared the solubility and permeability of tacrolimus, an immunosuppressive drug, solubilized in Marinosolv®, in a cyclodextrin formulation, and in a formulation containing the semifluorinated alkane 1-(perfluorhexyl)octan, respectively. The water solubility of tacrolimus is only 1-2 µg/ml. Solubility studies of tacrolimus in Marinosolv® have shown a solubility of up to 1000 µg/ml. Hence, 200 µg/ml in Marinosolv® can be easily dissolved, just like in semifluorinated alkanes, whereas the solubility when using cyclodextrin is limited to about 150-160 µg/ml (see Figure 9).

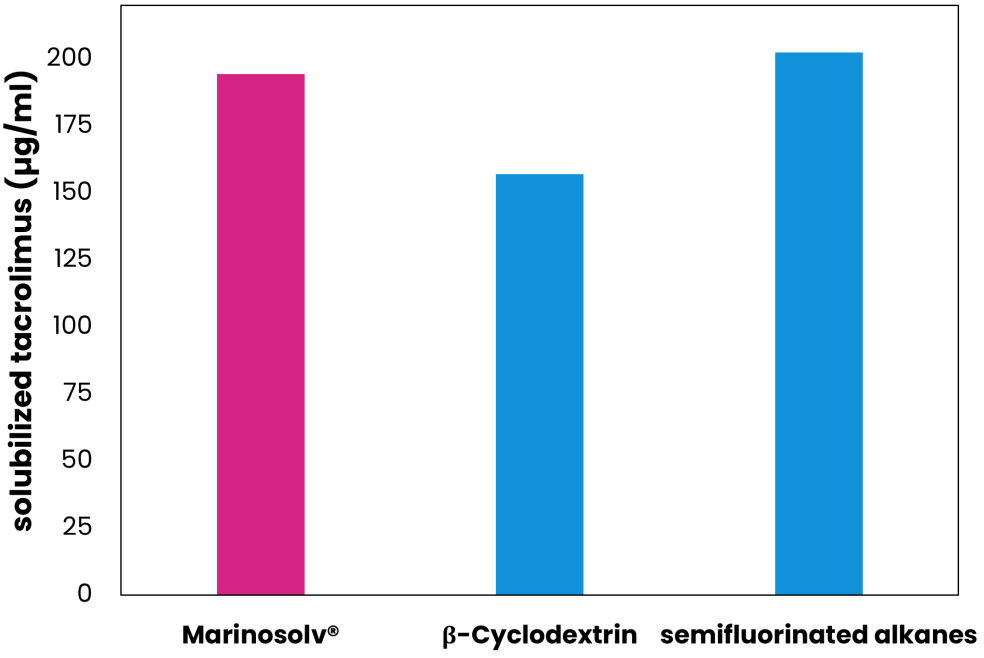


Figure 9: Solubility of tacrolimus in Marinosolv® compared to cyclodextrin and SFAs

Permeability studies have been performed *ex-vivo* on porcine eyes comparing 200 µg/ml tacrolimus dissolved in the three formulations mentioned above, and additionally using a marketed suspension of tacrolimus at a concentration of 1000 µg/ml. Explanted porcine eyes with surrounding eye sockets were instilled with 50 µl formulation, resulting in 10 µg tacrolimus for the Marinosolv®, the cyclodextrin and the SFA formulation and 50 µg for the suspension. After an incubation time of five minutes, the conjunctiva was harvested and frozen until analysis. The incubation time of five minutes was chosen, because a large portion of a topical drug is generally washed away from the corneal surface within the first 2-3 minutes [12]. Quantification of permeated tacrolimus was done by HPLC-MS/MS after homogenization of the tissue. The results, shown in Figure 10, reveal the highest permeability of 2 µg tacrolimus per gram conjunctiva when dissolved in Marinosolv®, compared to 1 µg when dissolved in cyclodextrin and only 0.5 µg when using SFAs. Using the suspension, similar amounts of tacrolimus compared to cyclodextrin permeated into the conjunctiva, but with a dose five times higher.

Comparing those four solubility-enhancing techniques, Marinosolv® showed the highest permeability, indicating an improved bioavailability.

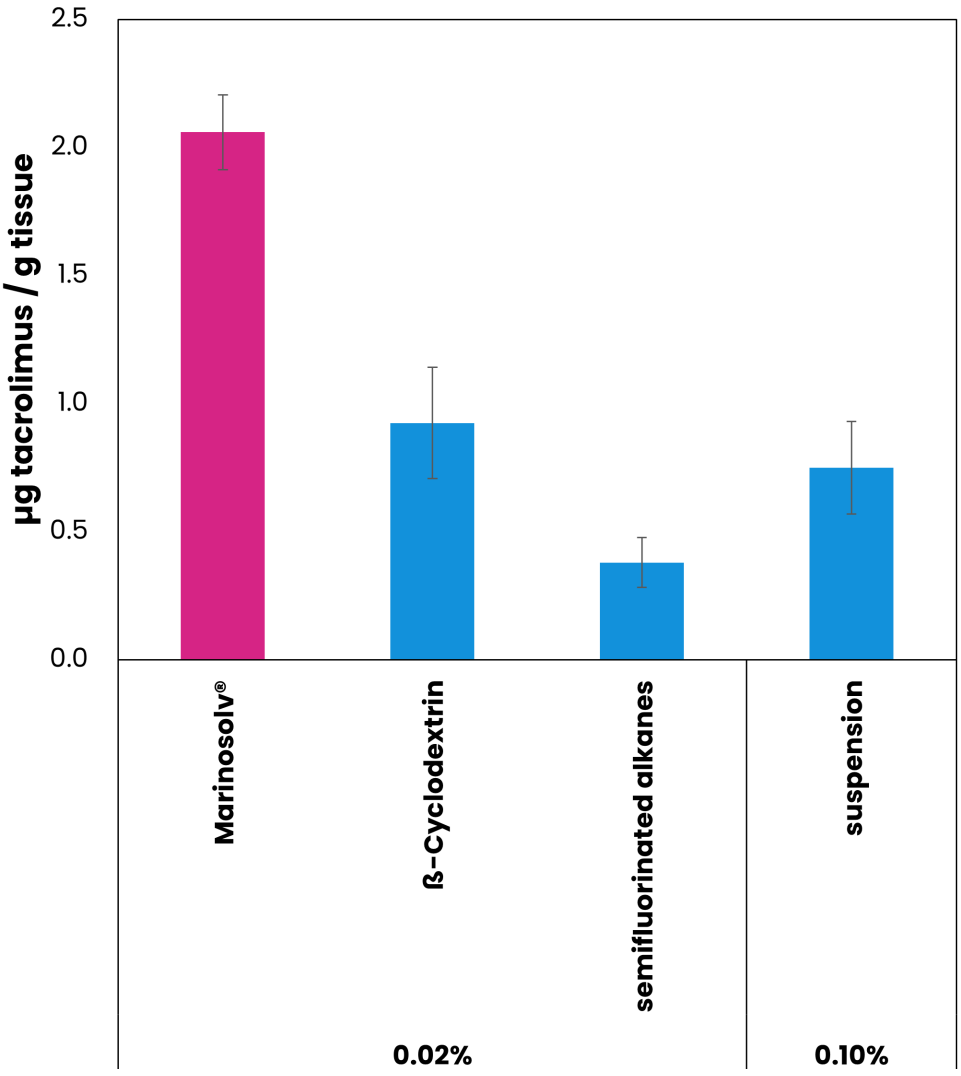


Figure 10: *Ex-vivo* permeation of tacrolimus into conjunctiva of porcine eyes after five minutes incubation time.

Summary

The data summarized here present a novel and promising approach for aqueous formulations containing hydrophobic, hardly soluble API's, called Marinosolv®. This IP-protected formulation technology not only increases the solubility of an API, but also enhances the permeability into several target tissues and thereby improves the bioavailability.

Based on our Marinosolv® formulation technology, several scientific papers have been published:

- **Siegl et al., European Journal of Pharmaceutics and Biopharmaceutics 134, 2019, 88-95.**
- **Nakowitsch et al., Pharmaceutics 2020, 12(9), 847.**
- **Zhang et al., Invest Ophthalmol Vis Sci. 2020; 61(1):4.**
- **Zieglmayer et al., Clin Exp Allergy. 2020; 00:1–13.**

Read here all Marinosolv®-related publications:
https://www.solv4u.com/en/news/publications

Furthermore, the benefits offered by a Marinosolv®-based formulation, such as safety and efficacy, have been validated in preclinical toxicological studies as well as in an ophthalmic phase II study and a pivotal phase III nasal spray study.

Benefits of Marinosolv®

- **Broadly applicable for small molecules and peptides**
- **Well-tolerated in systemic and local administration, including sensitive tissues**
- **Faster onset of action**
- **Clinically proven safety and efficacy**
- **Increased bioavailability in target tissue**
- **Significantly lower required dose compared to currently marketed products**
- **Improved local efficacy**
- **Lower systemic concentration of compound, reducing possible side-effects**
- **Lower environmental impact due lower possible dosing**
- **Sterile filtration followed by aseptic filling**
- **Preservative-free products**
- **Easily scalable process**
- **Increased sustainability of manufacturing**

Marinomed develops its own product candidates based on Marinosolv®, but also offers the formulation technology to external customers via its Solv4U technology partnerships, enabling a unique pharmaceutical development program, for both well-established API's and new chemical entities (NCE's).

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About Marinomed Biotech AG

Marinomed Biotech AG is an Austrian, science-based biotech company with globally marketed therapeutics and a growing development pipeline. The Company focuses on the development of innovative products based on two patent-protected technology platforms, Marinosolv® and Carragelose®. Marinomed is listed on the prime market segment of the Vienna Stock Exchange (VSE: MARI).

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