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Short communication

Critical aspects of *in vitro* Release (IVRT) method development for PEG based ointments

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Introduction

The advantages of hydrophilic PEG based ointments in treatment of skin conditions where dry skin is preferable has led to a significant increase of such formulations on the market. In addition to the aforementioned fact, formulations of this matrix type leave no grease like appearance, are easily washable and most importantly hinder drug penetration due to the effect of stratum corneum dehydration shown by Rowe et al (2009). With this on one side and the new regulative issued by the Food and Drug Administration and European Medicines Agency on the other, the need of a suitable *in vitro* release test is vast and of the outmost importance. The following text will dive into a discussion of the specifics in the process of analytical method development of an IVRT utilizing Franz Diffusion cell apparatus for PEG based ointments.

Materials and methods

Materials

The materials that were used are as following: membranes of polycarbonate, regenerated cellulose, polyethersulphone cellulose acetate, PTFE, Strat M and silicone composition obtained from various providers, Phosphate buffer Saline obtained from Sigma Aldrich, Absolute Ethanol(100%) supplied by Alkaloid AD, HPLC-DAD Agilent 1260 Quaternary series system, ACE C8, 250 mm x 4.6 mm i.d; 5µm or alternative HPLC column, Acetonitrile Isocratic Grade supplied by Supelco, Ammonium acetate, Glacial acetic acid and Potassium hydroxide (KOH) supplied by Merck and Automated Vertical Diffusion Cell Apparatus with 6 cells supplied by Hanson.

IVRT condition

The Franz Diffusion Cell apparatus that was utilized consisted from an automated sampling station, water bath with heater, magnetic stirrer and 6 glass cells with an effective volume of ~9.5 mL. The magnetic stirrer speed was set at 400 rpm as this speed allowed for suitable homogenization of the sample but with no back diffusion. The temperature of the water bath was set at 32.5°C in order to obtain comparable temperature to that of the skin. The acceptor medium was defined through sink analysis of the API and consisted of 10% phosphate buffer saline set at pH 7.4 : absolute ethanol = 80:20%. In the study various membranes were tested and will be discussed later. Sampling volume with appropriate washing step of the tubing was defined at 0.5 mL. The test was conducted for a time of 6h as per the guidance of the EMA's Draft guideline on quality and equivalence of topical products with sampling points at 10, 20, 30, 60, 120, 180, 240, 300 and 360 minutes.

Results and discussion

The development of the IVRT method began with the choice of polycarbonate membrane impregnated with polyvinyl pyrrolidone in order to obtain adequate wetting in water based media (Nuclepore Track-Etched membrane). The pore size of this particular membrane was about 0.05 µm. This membrane showed no biding capacity of the API and was the first one tested. When observing the formulation applied on the cells a reoccurring phenomena was observed where due to the osmotic effect of the PEG excipient the acceptor media was pulled inside of the donor compartment. This is quite significant as PEGs are very soluble in water based media leading to the solubilization not only of the API but the matrix as well. This effect had serious implications on the

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obtained results as the recovery of the released amount of API versus the applied was ~100%. This is not acceptable as some of the profiles may be with a low value for the coefficient of correlation < 0.92 or low degree of linearity when plotted according to the Higutchi plot. This is due to the fact that Higutchi release plots according to Siepmann 2011 expect lower degrees of API release in the later time points as the release in this time is due to the diffusion of the API from the layers distant to the membrane (longer diffusion path). But this is not the case as water dissolves the matrix and allows complete release of the API. The development continued with regenerated cellulose membrane with pore size of 0.2µm. This membrane was significantly thicker which somewhat inhibited the aforementioned problem and the recovery was significantly lower ~50%. The coefficient of correlation values were also satisfactory > 0.97. A different challenge was observed with this membrane as it could not discriminate between the test formulation and the formulation with altered characteristics. The formulation with altered composition had drastically higher viscosity which should have resulted in slower release rate of the API but the profiles from the two formulations seemed identical when 90% confidence intervals for the cumulative amount and flux were generated. This is not acceptable as the utility of an IVR method lies as quality control test which should possess sufficient discriminatory power. Cellulose acetate membrane was also used but similar results to the regenerated cellulose membrane were obtained. According to Theo Kapanadze et al. for most fast-release matrix types as are PEG based ointments earlier sampling times (between zero to four hours) could be more discriminative and be of higher quality indicating value than later time points. This approach was taken into consideration and the later time points (after 3 hours) were omitted in the process of constructing the 90% confidence interval. The acceptance limits proposed by EMA's guideline were adopted (90-111%.) The calculated 90% confidence interval for the cumulative amount was 108-121% which showed significant difference between the formulations. On the other hand, the 90% confidence interval for the slopes lied within the predetermined acceptance criteria showing no significant difference between the test and discriminatory formulation. Although this methodology can be useful for some PEG based ointments adopting only this method did not solve the discriminatory problem.

Further in development polyethersulphone membrane (PES)was tested. This membrane showed similar release profile to the Nuclepore Track-Etch membrane and wasn't conducted to any further analysis. Next membrane considered was Strat M. The acceptor medium used for this experiment was modified as only 10% ethanol was compatible with this membrane. This membrane is a synthetic membrane that imitates the outermost layer of the skin or the stratum corneum. The Strat M membrane inhibited the process of dissolving the matrix but due to inconsistencies in the process of its synthesis the results were extremely variable. The results gathered also had significant lag period and low recovery ~2% after 6 hours. This due to the lipophilic nature of the membrane which acts as a rate limiting step in the diffusion process.

As Strat M is incompatible for IVRT, other synthetic membranes like PTFE and silicone were tested. The PTFE experiments had quite large variability with very low linearity. The silicone membrane on the other hand was quite promising as it inhibited the osmotic effect of the PEGs due to high lipophilic character and small to non-existent pores. As a low release membrane the recovery was significantly lower but was acceptable. The profile was satisfactory for the tested formulation and as the release was drastically slower satisfactory discrimination was observed. Literature search revealed that a different paper by Bhagukar et al. (2016) also commented on the advantages of using such low release membranes in fast releasing matrices as in PEG based formulations.

Conclusion

Even though IVR tests with Franz Diffusion cell cannot be used for quality control of finished drug products yet, the regulative is changing fast and it might be incorporated as such. With this the need of a suitable IVRT for problematic, fast releasing matrices is growing. From the obtained results presented above the silicone membrane showed most promising characteristics in testing for most PEG based formulations. As in this study only one formulation of this type was tested and only one other paper was found on this particular problem further testing is needed to prove or disprove the current findings.

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