Significance of UV-Vis spectroscopy in *in vitro* release studies

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1. Introduction

In recent years the interest in *in vitro* release studies from semisolid dosage forms has been rapidly growing. This results from the role of skin as an organ onto which active pharmaceutical ingredients are applied to get effective concentration in tissue compartment and even an equilibrium concentration in central compartment. The quality of semisolid forms depends on various factors such as properties of active compounds and other auxiliary substances, formulation type and even the preparation techniques. The release rate of the active ingredients is determined to a large extent by the appropriate selection of ointment components [1].

Dermatological products can influence the skin surface (disinfectants), deeper layers of the skin (antihistaminic) and organs, which are reached by the active substance thanks to blood circulation. Absorption of active compounds through the skin precedes differently than though the digestive system, because the skin acts as a protective barrier against harmful external factors [2, 3].

There are many different routes by which the active compound can reach deep into the skin such as direct permeation through the stratum corneum, penetration through sebaceous and sweat glands or permeation through hair follicle (Fig.1).

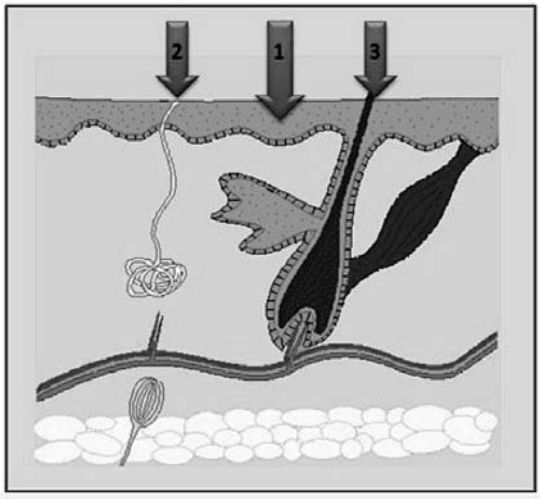

Fig. 1. Transdermal delivery of active compounds. The route through: 1 - epidermis, 2 - sweat glands, 3 - hair follicle

The controlled release of biologically active compounds from pharmaceutical dosage forms has significant meaning in estimating their efficiency. Therefore, the *in vitro* release studies of semisolid dosage forms have been carried out in the quality control laboratories. Especially, that the researches have to check the product activity after introduction of any changes in the composition and equipment or in the production process [4].

The general directives concerning the release studies can be found in Pharmacopoeia. It is essential to determine such parameters as: type, volume and temperature of the receiving medium, the stirring speed, sampling time and concentration analysis of the active compound (using UV-Vis spectroscopy or HPLC chromatography) [5].

The subject of investigation was the release studies of izotretinoin and heparin from semisolid dosage forms into a diffusion cell connected with UV-Vis spectrophotometer. The application of UV-Vis spectroscopy in release studies enables determination of the release kinetics of the bioactive substance from semisolid dosage forms to a receiving medium, process order and the properties and even then evaluation of mass transfer through the phase boundary.

2. Experimental part

2.1. Preparation of pharmaceutical formulations

- Gels

A portion of 21.25 mg of heparin sodium salt (Sigma-Aldrich) was weighted and 25 ml of water was added and stirred by a magnetic stirrer until the substance was completely dissolved. Then the solution was condensed to gel formulation by Xanthan gum (Evonic). Xanthan gum is a polysaccharide derived from bacteria. It is commonly used as a natural emulsifier and thickening agent soluble both in hot and cold water. A similar procedure was applied for the preparation of gel with izotretinoin (BASF).

- Oil-in-water emulsion

The ingredients of the oil phase: 6.5 g of peanut oil (Sunniva Med.), 1.5 g of cetyl alcohol (Sigma-Aldrich) and 2.0 g of glycerol monostearate (Sigma-Aldrich) were combined in a 25 ml beaker and heated upon gentle stirring on a magnetic stirrer hot plate until 60°C. 40 ml of distilled water was placed in the 100 ml beaker and then 21.25 mg of sodium heparin salt or 50 mg of izotretinoin were added in the usual manner. In both cases the components were subjected to magnetic stirring and heated to about 60°C. When oil and water phase reached the same temperature (60 °C), the ingredients of the oil phase were added to water phase. Then the mixture was homogenized on ULTRA-TURRAX® Tube Drive Workstation (IKA).

- Lanolin ointment

45 g of lanolin (Fluka) was weighted on a Petri dish. 5 ml of distilled water was placed in the 10 ml beaker. Then 21.25 mg of sodium heparin salt or 50 mg izotretinoin were added and all ingredients were subjected to magnetic stirring until complete dissolution of the active compound. Finally the aqueous phase was mixed with the oil phase using a mortar and a pestle.

2.2. The in vitro release studies

In order to estimate the release kinetics of izotretinon and heparin from various semisolid dosage forms, the VanKel 7010 diffusion cell connected with a spectrophotometer UV-Vis Cary 50 Bio (Varian) was used (Fig. 2).

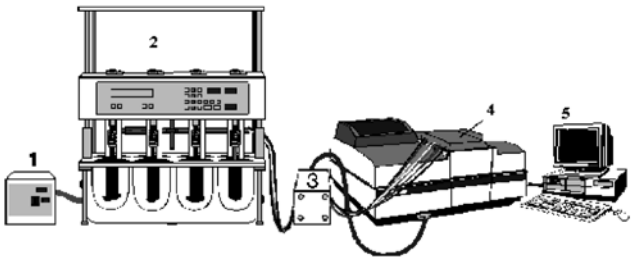

Fig. 2. Vankel VK-7010 Apparatus:1-heated bath, 2-module where the active substance is released, 3- peristaltic pump, 4- UV-Vis spectrophotometer UV-Vis, 5-computer [6]

The temperature of the process was maintained at 32°C. The enhancer cell was filled with 1 g of the tested product containing the active substance, then the Cuprophan membrane, which imitates the skin barrier, was placed over the sample surface. The release studies of izotretinoin were carried out in phosphate buffer pH 5.5 with the addition of ethanol (ratio 65:35), while in the experiment with heparin the aqueous azure II solution (the equilibrium mixture of azure B N,N,N-trimethylthionine and methylene blue pH 6) was applied as a receiving medium. The amount of the released active substance was calculated using the following equation:

$$\%\ \text{released} = \left(\frac{A_p}{A_w}\right)\left(\frac{m_w\,[mg] \times C_w}{V_w\,[ml]}\right)\left(\frac{1}{D_w}\right)\left(\frac{V_p\,[ml]}{m_p\,[mg]}\right) \times 100$$

where: A_p – sample absorbance, A_w – standard absorbance, m_w – standard weight, m_p – label content, C_w – standard purity, D_w – dilution factor, V_w – standard volume, Vp – medium volume (phosphate buffer).

3. Results and discussion

Quantitative assessment of heparin and izotretinoin released with the use of UV-Vis spectrophotometry, likewise most instrumental analysis, is a comparative technique. Therefore the sample analysed is compared with an appropriate standard (calibration curves). Within the framework of this study the absorbance of the sample and standard solutions (of a known concentration) was measured (in cuvette of the same thickness) at predetermined intervals (30 min) for 20 hours to investigate pharmaceutical bioavailability.

Reliability of quantitative assessment was achieved by the selection of a proper wavelength characteristic only of the substances analyzed and not of the receiving medium applied (such as phosphate buffer). In the experiment with izotretinoin, the UV spectra were recorded at 345 nm, while for heparin the UV absorption maximum was detected at 190 nm. As many other pharmaceutical substances absorb in the wavelength range 190÷210 nm, the measurement of the active compound would not be reliable. Therefore, an appropriate coordination complex (azure II) was added to heparin to shift the absorption band to longer wavelength, bearing in mind that the reaction of coordination complexes should be selective, fast and reproducible.

According to literature data [7,8] the absorption maximum of heparin-azure II complex was detected at 500÷530 nm. The complex spectrum recorded in laboratory showed the absorbance increase in the same range of wavelength (Fig. 3). Additionally, it is worth mentioning that after introducing heparin into the aqueous solution of azure II the change in colour from blue to violet (characteristic of azure II-heparin complex) was observed.

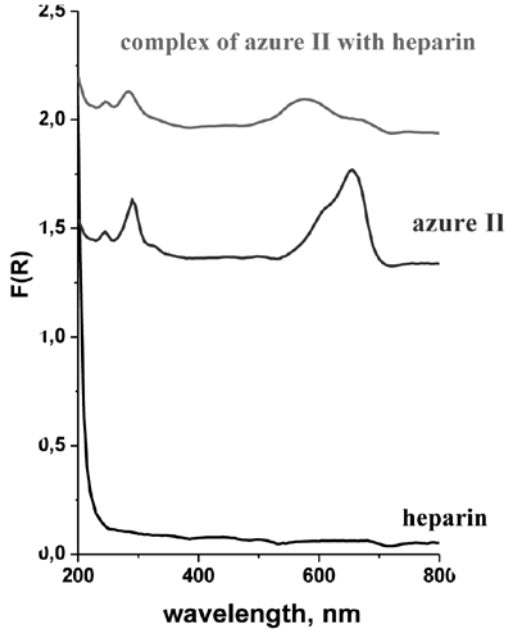

Fig. 3. UV-Vis spectrum of heparin (λ_{max} 190 nm), aqueous solution of azure II (λ_{max} 658 nm) and azure II-heparin complex (λ_{max} 530nm)

The *in vitro* release of the active compound is highly connected with diffusion process from the formulation to the receiving medium. The results obtained by UV-Vis spectrometry proved that in both cases (heparin and izotretinoin) the release rates depended on the pharmaceutical formulation.

The heparin release profiles from gel and o/w emulsion (Fig. 4) demonstrate that in the preparation there are not any substances which can inhibit the diffusion of the active substance. The release process occurred very fast when the membrane Cuprophan (Cellulose acetate) was used. During the first two hours of the analysis, 60% of the active substance was released, after that the released amount slightly increased to reach a constant level 65 % for o/w emulsion and 81% for gel. In the case of heparin release from lanolin ointment, the active substance appeared in the receiving medium after 6.5 hours. These results are connected with the high hydrophobicity and high viscosity of this type of formulation. The maximum of heparin released was very low (6%).

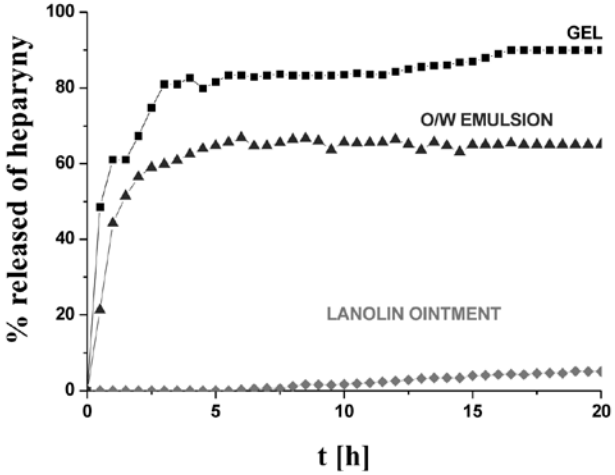

Fig. 4. Release profiles of heparin from various formulations

The effect of the formulation type was also noted for izotretinoin. The release profiles presented in figure 5 indicate that izotretinoin diffuses gradually both from gels and o/w emulsion. The regular increase was observed during the first 7 hours and then the equilibrium state was reached. The pharmaceutical availability of izotretinoin introduced into gel was 52%, while in o/w emulsion it was 19%. The lanolin ointment caused the inhibition of izotretinoin diffusion. Only after 10 hours the active substance was detected (1%) in the receiving medium.

The maximum of izotretinoin release was 3%. The high amount of oil phase was responsible for high viscosity of the ointment and as a result the diffusion from this formulation was inhibited.

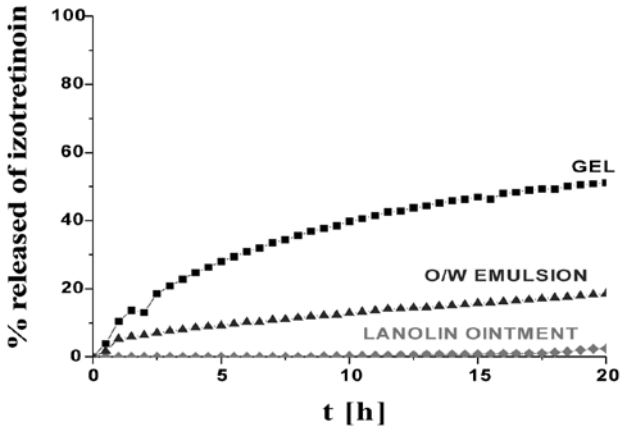

Fig. 5. Release profiles of izotretinoin from various formulations

Conclusion

The application of the spectroscopic methods (especially UV-Vis spectrometry) in *in vitro* release studies is very important for the

development of new, effective semisolid dosage forms applied on the skin. The obtained results show that both heparin and izotretinoin added to the semisolids of high viscosity (lanolin) permeate slower through the dialysis membrane imitating skin barrier than when added to products of low viscosity (Xantan gum). A low amount of the oil ingredients in the pharmaceutical formulation favours the bioavailability of its active substance.

Translation into English by the Author

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