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In vitro simulation of distribution processes following intramuscular injection

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Abstract: There is an urgent need for *in vitro* dissolution test setups for intramuscularly applied dosage forms. Especially biorelevant methods are needed to predict the *in vivo* behavior of newly developed dosage forms in a realistic way. There is a lack of knowledge regarding critical *in vivo* parameters influencing the release and absorption behavior of an intramuscularly applied drug. In the presented work the focus was set on the simulation of blood perfusion and muscle tissue. A solid agarose gel, being incorporated in an open-pored foam, was used to mimic the gel phase of muscle tissue and implemented in a flow through cell. An aqueous solution of fluorescein sodium was injected. Compared to recently obtained *in vivo* results the distribution of the model substance was very slow. Furthermore an agarose gel of lower viscosity an open-pored foam and phosphate buffer saline pH 7.4 were implemented in a multi-channel-ceramic membrane serving as a holder for the muscle imitating material. Blood simulating release medium was perfused through the ceramic membrane including filling materials. Transport of the dissolved fluorescein sodium was, in case of the gel, not only determined by diffusion but also by convective transport processes. The more realistic the muscle simulating materials were constituted the less reproducible results were obtained with the designed test setups.

Keywords: biorelevant; flow-through-cell; intramuscular injection; *in vitro* dissolution.

1 Introduction

Long acting depot formulations for subcutaneous or intramuscular application are getting into the focus of pharmaceutical industry as drug delivery systems. They offer the advantage of a constant drug release over a long period of time and therewith less fluctuating plasma levels compared to a daily application of other dosage forms. Furthermore, the gastrointestinal pathway can be circumvented which is very useful for drugs with stability problems in the gastrointestinal tract or a small absorption window in the small intestine. Nevertheless, there is a lack of *in vitro* dissolution methods for parenterally administered dosage forms [1]. This might be caused by the great variability of parenterally applied formulations but also by the lack of data describing most critical parameters influencing drug absorption *in vivo*. In the following work the focus was set on intramuscularly applied dosage forms. It is expected that, next to other parameters, blood flow within the muscle tissue, the formation of a depot and the exact location of the injection within the muscle tissue pose critical variables on drug absorption into the systemic circulation [2]. Within the presented *in vitro* study these parameters were taken into consideration. Thus it was the aim of the study to simulate the process of injection into the muscle tissue, the blood flow through the muscle tissue (and injected depot) and the muscle tissue itself.

2 Material and methods

For the imitation of the gel phase of the muscle tissue being embedded between the muscle fibres an open-pored foam (Carpenter®, Germany) in combination with an agarose gel 2% was used. The thermoreversible agarose gel was heated to 90°C and consequently incorporated in the foam before cooling and solidification. The foam comprised four radial arranged cavities. Before solidification of the gel phase the foam was mounted on metal rods that were attached over a thread to a stainless steel plate. This plate was fitted into a flow through cell (FTC) which

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possessed a conical part that was shortened by 10 mm in favor of the cylindrical part, compared to the compendial FTC for tablets (see Figure 1).

After the gel became solid the metal rods were removed and 30 µl of an aqueous solution of fluorescein sodium were injected centrally, using an injection stencil, into the gel-foam-block. Consequently, the block was perfused (35 ml/min) within the FTC by the release medium (phosphate buffer saline pH 7.4, PBS, 37°C) operating in a closed system. At predetermined time points samples were taken from the reservoir and replaced by fresh buffer. The fluorescein contents of samples were quantified using a fluorescence reader (Varioskan Flash Multimode Reader, Thermo Fisher Scientific, USA) with calibration samples on each well-plate. Furthermore, the shape of the depot was observed at certain time points after the injection of an aqueous solution of methylene blue by cutting the block in half.

Besides the gel-foam-block the same foam saturated with PBS solution and agarose gels with lower viscosities were tested as muscle tissue imitating materials reflecting *in vivo* conditions in a potentially more realistic way. The release test setup had to allow for the implementation of the non-solid agarose gel. For this purpose a porous multi-channel-ceramic membrane (inopor®, Germany) saturated with the release medium was used as a holder. Afterwards 30 µl of an aqueous solution of fluorescein sodium were injected centrally into the filled inner cavity. Besides the foam and agarose gel 0.05%, PBS was used as filling material. A plug was set on the cavity which was provided with a tube (see Figure 2). The prepared membrane was then placed in a beaker filled with 500 ml PBS. A peristaltic pump led the medium out of the beaker via the tube through the inner cavity of the ceramic membrane. The flow rate was set to 2 ml/min. Due to its porous structure the medium passed the membrane and was recirculated into the reservoir. At certain time points samples were taken from the reservoir and replaced with fresh buffer. The quantification of the substance in the samples was performed using fluorescence spectroscopy, as stated above.

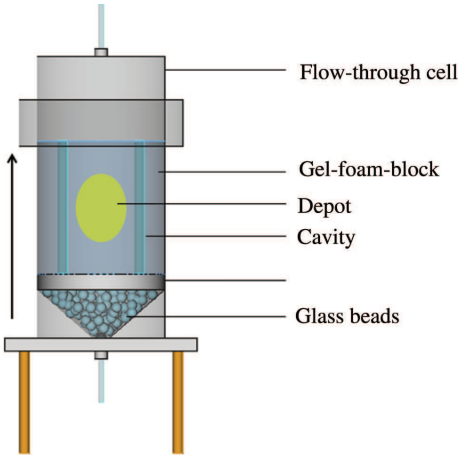

Figure 1: Prepared gel-foam-block with injected depot in FTC. (Arrow indicates the flow direction of the release medium).

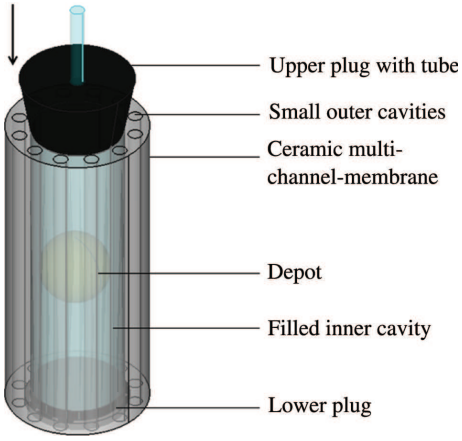

Figure 2: Prepared ceramic membrane with injected depot. (Arrow indicates the flow direction of the release medium).

3 Results

The gel-foam-blocks allowed the injection of an aqueous depot. The depot spread over the time and was removed slowly after perfusion with PBS (see Figure 3).

About 30 h after the injection of an aqueous solution of fluorescein sodium 100% of the injected amount were found in the release medium. The standard deviations within the first 12 h were remarkable (see Figure 4).

Also the used open-pored foam, which was saturated in PBS allowed the injection of an aqueous solution and the formation of a depot. Nevertheless, the depot was only

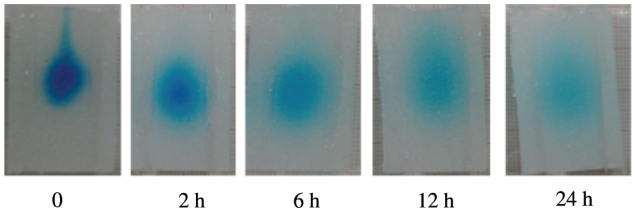

Figure 3: Spreading of an aqueous depot of methylene blue centrally injected into an agarose-foam-block with four radial arranged cavities and perfused at 35 ml/min in a modified FTC.

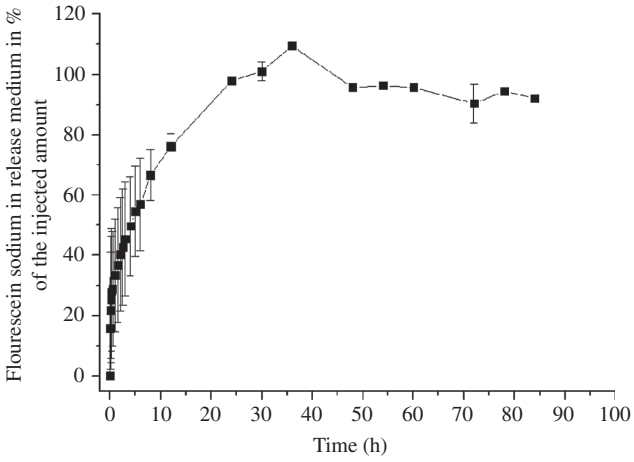

Figure 4: Distribution of fluorescein sodium into the release medium after the injection of an aqueous solution into the agarose-foam-block located in the FTC perfused at 35 ml/min over the time. (n = 3; MW ± SD).

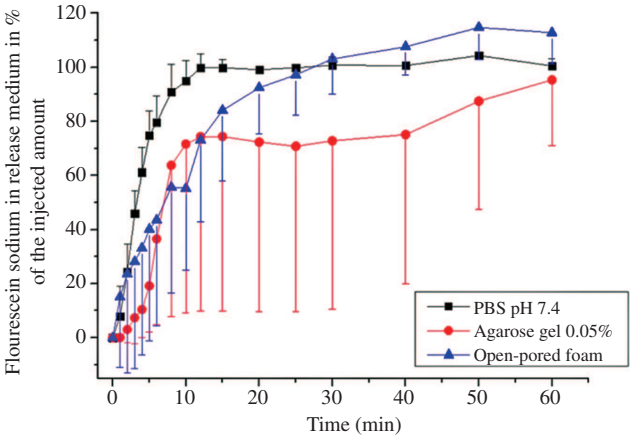

Figure 6: Distribution of fluorescein sodium into the release medium after the injection of an aqueous solution into the inner cavity of the ceramic membrane filled with PBS pH 7.4, Agarose gel 0.05% or an open-pored foam being perfused at 2 ml/min over the time. (n = 3; MW ± SD).

stable for a few minutes before spreading throughout the whole foam. It was found that an agarose gel at a concentration of 0.05% without a foam structure yielded the formation of a depot. The spreading process was slower and more controlled than in the PBS soaked foam (results not shown) but faster than in the gel-foam-block (Figure 5).

Figure 6 shows the curves describing the distribution of the injected flourescein sodium from PBS, agarose gel 0.05%, and open-pored foam all located within the ceramic membrane into the release medium over the time. The fastest distribution occurred after the injection into PBS reaching a plateau after about 15 min. A slower distribution process, taking about 60 min, was observed using the foam as well as the agarose gel. The distribution of the substance from the agarose gel was not completed within that time and showed a plateau between 10 and 40 min. The standard deviations in case of the foam and especially in case of the agarose gel were remarkable. Filling the inner cavity of the ceramic membrane with PBS led to more reproducible results.

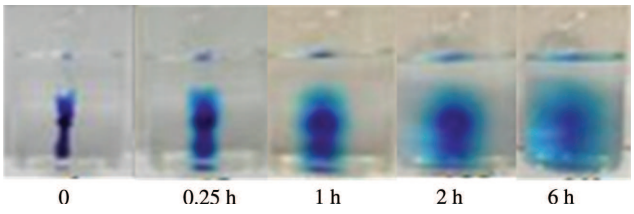

Figure 5: Spreading of an aqueous depot (methylene blue) being injected in an agarose gel 0.05% over the time.

4 Discussion

It is expected, that the extent of the influence of the different physiological parameters that are affecting drug release and absorption after intramuscular application is not constant but depends on the formulation. In the presented study the focus was set on simulating blood perfusion, which is thought to be relevant especially in fast releasing dosage forms [3], and the simulation of the muscle tissue, having an impact on the formation of a depot and on the time for removal of the depot from the muscle tissue [2]. The application of a FTC-system allows the simulation of blood perfusion through the muscle tissue. The flow rate can be easily adapted. Volumes of the release medium were chosen with respect to the maintenance of sink-conditions. Though *in vivo* no sink-conditions are present it is crucial to maintain them *in vitro* in order to achieve reproducible results. In general, muscle tissue consists of muscle fibres surrounded by connective tissue. The connective tissue consists of a framework of collagen fibres in which a gel phase is embedded. Since an injection into the surrounding connective tissue is very likely a simulation of this tissue for the imitation of the muscle tissue seems to be a promising approach. *In vitro* an agarose gel was used as a hydrogel. Since it was the aim to implement and perfuse the imitated muscle tissue in a FTC it had to be of solid consistency. Injecting into a solid gel led to a backflow along the puncture channel. Consequently the gel was incorporated in an open-pored foam interrupting the tight framework of the gel and enabling the forming of a depot. Since a diffusion

of the solved model substance through the framework had to be possible an open-pored foam had to be used. A recently described *in vivo* study in rats [2] showed, that an aqueous depot is removed from the muscle tissue within 2 – 6 h whilst maximal plasma levels were already achieved within about 30 min after the injection. Thus, absorption processes were finished at the latest after 2 h, when no drug was quantified in the blood anymore [2]. The spreading and the removal of the depot in the gel-foam-block took much longer time. The time that was needed until the whole amount of the injected model substance was found in the release medium was about 30 h. Thus, the diffusion process through the solid gel was slower than the diffusion process of the drug molecules in the muscle tissue. Furthermore the standard deviations between the single trials were very high. One reason for that can be an uneven texture of the gel-foam-block. Another reason might be a non reproducible injection technique although a standardization of the injection depth and angle was assured by using an injection stencil.

In vivo the transport of a dissolved substance is thought not only to be determined by its diffusion but also by convective transport processes. Furthermore, the viscosity of the gel phase of the muscle tissue is considerably lower than of a solid hydrogel. For this reason another test setup was applied. An agarose gel of low viscosity, allowing the injection and forming of a depot without incorporating the gel in a foam, and the foam without the use of a gel were tested as muscle imitating materials. Since the agarose gel 0.05% is in a liquid state an implementation into the FTC was not possible. A porous ceramic membrane was used as a holder for this material. In order to perfuse the imitated muscle tissue, under consideration of convective transport mechanisms, the release medium was pumped through the membrane filled with either the foam, the agarose gel 0.05% or PBS, after the injection of the aqueous solution of fluorescein sodium. PBS was used as filling material in order to investigate the influence of the ceramic membrane as a barrier. The new test setup accelerated the time needed for a complete distribution of the injected model substance into the release medium to 10–60 min. This reflects the time needed for complete absorption *in vivo* in a better way. In the case of the foam or the low viscosity gel were filled in the inner cavity of the membrane, the times to reach a plateau were increased compared to a filling with PBS. Thus the filling materials have a greater effect on the distribution of flourescein than the ceramic itself. Furthermore, the standard deviations increased greatly using muscle imitating materials. The results were not reproducible anymore. In case of the agarose gel the reason for that may be different dilution processes of the gel due to the perfusion of the ceramic membrane. It cannot be excluded that liquid nests were formed influencing the transport time of the dissolved model substance. In the case of PBS and the foam only convective transport processes may play a role. The use of an agarose gel requires transport via diffusion and convective processes, but in a non reproducible way.

In summary, the presented test setups reflect *in vivo* conditions in some parts. The more biorelevant the method is designed the more problems emerge in creating reproducible results. Nevertheless, it may give a hint that the observed high variability in plasma levels *in vivo* after intramuscular injection may result, from the constitution of the muscle tissue and therefore be dependent on the injection site.

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Author's Statement

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