The release of Lyzosyme from tablets coated with Poly (vinyl acetate). A study on the correlation between intrinsic viscosity and protein diffusion

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ABSTRACT

Studies have found a correlation between the intrinsic viscosity of a polymeric solution and the release of protein from the following created polymeric matrix. This study aims to investigate if the connection between intrinsic viscosity and release, also applies to Lysozyme-containing tablets coated with various Poly (vinyl acetate) solutions.

INTRODUCTION

Polymers have for many years been used as agents in pharmaceutical formulations, ranging from thickening agents in solutions to coating agents for prolonged release tablets.

This study aims to investigate how intrinsic viscosity of a specific polymer in various solvents can be used to control the release of model macromolecular drug from tablets coated with this polymer. The idea is that the release will be dependent on the polymer conformation in the solution used to coat the tablet cores.

The work of PJ Flory describes how the conformation of a polymer chain in a diluted solution ($c < c^*$) is dependent on the solvent[1], where if the solvent has higher affinity towards the polymer the polymer will appear more outstretched, compared to a solvent with lower affinity towards the polymer. To assess the relative conformation of a polymer in various solvent, the intrinsic viscosity, $[\eta]$, is very useful. Furthermore, the intrinsic viscosity can be directly linked to the conformation of the polymer through the Mark-Houwink-Sakurada (MHS) equation (Eq. 1)

$$[\eta] = K \cdot M^a \quad (1)$$

The MHS equation describes the relationship between the $[\eta]$ and the size of the polymer, M. The linear coefficient, K, and the shape dependent factor, a, are both solvent specific. For random polymers a = 0.5 at θ-conditions. In good solvents, with high affinity towards the polymer, the value of a is between 0.5 and 0.8. The higher the value of a, the more outstretched will the polymer appear[2].

This conformational difference of a specific polymer in various solutions has been suggested to be important for the network formation once the solvent evaporates and the individual polymer chains entangle into a solid matrix. The authors hypothesize that when the polymer chains are more outstretched in the feed solution, a more homogeneous network is obtained and thus decreasing the pore size in the microstructure of the network. If the pore size is smaller this should lead to a greater extent of retention by the assembled polymers and thereby a slower diffusion from/through the polymeric network. Wan *et al.*[3] showed a correlation between protein

release and solvent selection from spray-dried Poly (lactic-co-glycolic acid) (PLGA) particles containing Bovine Serum Albumin (BSA). The particles that originated from a feed solution of a relatively good solvent had a more prolonged release than particles originating from a relatively poor solvent. Madsen *et al*.[4] showed the same correlation for casted PLGA films containing BSA. Therefore, the aim of this study is to investigate if the same theory applies to tablet-coating using Poly (vinyl acetate) (PVAc) as the film forming polymer.

MATERIALS

Poly (vinyl acetate) (PVAc); M_W: 100.000 g/mol, Lysozyme from chicken egg white; ~70.000 U/mg; and acetone Chromasol® (ACE), for HPLC, ≥ 99.8 % were purchased from Sigma-Aldrich (Schnelldorg, DE). Calcium Hydrogen Phosphate Dihydrate (DC) USP/BP and Sepisperse™ Dry Jet 1011 Blue were purchased from Alsiano (Denmark). Magnesium Stearate, Ph.Eur; and Talc, Ph.Eur were obtained from Fagron Nordic, (Denmark). Ethanol, Ph.Eur. 96 % (v/v) and Dichloromethane (DCM) for IR spectroscopy, ≥ 99.5 % were purchased from CCS Healthcare (Sweden) and Fluka Analytical (Schnelldorg, DE), respectively.

METHODS

Intrinsic viscosity

Viscosity measurements of the polymer solutions were carried out using an Ubbelohde Semi-Micro dilution viscometer (No. 50, N212, Cannon instrument Company, USA) at 25 ± 0.2 °C. The time of flow (t) was measured at 5-8 different polymer concentrations, where all the solutions were in the dilute solution regime. All measurements were done in triplicates. The specific viscosity was calculated by Eq. 2:

$$\eta_{sp} = \frac{t - t_0}{t_0} \quad (2)$$

Subsequently, the reduced viscosity (η_{sp}/c) was calculated, where c is the polymer concentration in g/mL. The intrinsic viscosity ($[\eta]$) was obtained after extrapolation of η_{sp}/c as a function of c (Huggins plot), to a polymer concentration of zero[2].

Tableting

All Lysozyme tablet cores were produced in one batch to avoid batch-to-batch variations. Each core had an average mass of 150 mg, a diameter of 6 mm and consisted of 10 mg Lysozyme from chicken egg white, 7.5 mg Magnesium Stearate: Talc (1:9) and 132.5 mg Calcium Hydrogen Phosphate Dihydrate. The tablets were compressed on a DIAF excenter tabletting machine (TM20; DIAF, Denmark) to a crushing strength of 35 – 40 N.

Coating

All coatings were performed using a CC1/Lab Combi-Coater (GEA Niro Atomizer, Germany). Each coating was conducted with either 1000 or 2000 Lysozyme cores. Initial coatings were conducted using 300 g of tablets. However, later studies showed that less agglomeration of tablets occurred, when a smaller amount of tablets was used. Before each coating, the cores were run in the Combi-Coater for 10 min to de-dust, and round of the edges of the tablets. The Lysozyme tablets were weighed after this procedure.

Feed solution was applied at rates ranging from 1.5 g/min to 4.0 g/min using a Watson-Marlow liquid pumb (Model 503; Watson Marlow GmbH, Germany). Coatings with ACE-based feed solution could not be conducted at rates higher than 1.5 g/min due to tablet agglomeration. Coatings with DCM-based feed solutions could not be conducted at rates lower than 4.0 g/min, due to the equipment used. Atomization pressure was varied between

1.0 and 1.5 bar and the in-let temperature was kept at room temperature.

After each coating the mass of the tablets was measured in order to determine the amount of polymer applied to the tablet cores.

Release

For the studies of Lysozyme release, a method was developed which allowed for protein concentration determination by UV-absorption measured on the Nanodrop-2000C (Thermo Fischer Scientific, IL, US). A constant release volume of 20.0 mL was used for tablets containing 10 mg of Lysozyme. As the solubility of Lysozyme in H_2O is 10 mg/mL, the assumption of sink-condition was valid at all times. A new model was build based on the European Pharmacopeia monograph on "suppository disintegration rates measurements", Ph.Eur 2.9.2[5]. A basket for the sample tablet was printed on a 3D printer to match the size of a 40 mL beaker. The beaker was placed in a water bath at 37 °C and covered with a lid or parafilm to ensure minimal water evaporation. All release studies were conducted at 37 °C in MilliQ water. At each measurement a sample volume of 1.00 mL was replaced with fresh MilliQ water. When the study was terminated, each coated tablet was crushed in the solution and left for a few minutes to ensure complete Lysozyme dissolution. The solution was then filtered and measured. All concentration measurements were done in triplicate. Release studies were conducted minimum in triplicate and samples were taken at given intervals over 24 hours.

The amount of lysozyme released at a given time (Q_n) was calculated as the measured concentration (c_n) times the volume (V_{total}) plus the sum of the previously removed samples (Eq. 3). After 24 hours the tablet was crushed, the concentration measured and the total amount of Lysozyme in the tablet was determined (Q_{total}). The release was plotted as percentage released over time.

$$Q_n = \left(\sum_{i=0}^{n-1} c_{n-1} \cdot V_{sample}\right) + c_n \cdot V_{total} \quad (3)$$

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) images of the tablet films were obtained using a Hitachi TM3030 Tabletop scanning electron microscope. The tablets were cut in half and coated with gold. Pictures were taken at zoom 150x – 3000x.

RESULTS

Intrinsic viscosity

The intrinsic viscosity of PVAc in various solvents was determined using the Canon Ubbelohde Viscometer.

Table 1 - Schematic overview of the intrinsic viscosity of Poly (vinyl acetate) (PVAc) in various solvents at 25 °C. The work was conducted using a Canon Ubbelohde Viscometer.

Solvent	Intrinsic viscosity [η] (dL/g)
Dichloromethane	0.72
Acetone	0.34
Ethanol	0.28
Methanol	0.28
Pentanol	Insoluble
Water	Insoluble

In this study DCM was used as the *good solvent* and ACE as the *poor solvent* (Table 1).

Release profiles

The release of Lysozyme from tablets coated with a DCM-based feed solution was determined for three different amounts of coating (Fig. 1). The results indicate an inverse correlation between the amount of

coating and the release rate of Lysozyme, which is in line with existing literature[6, 7].

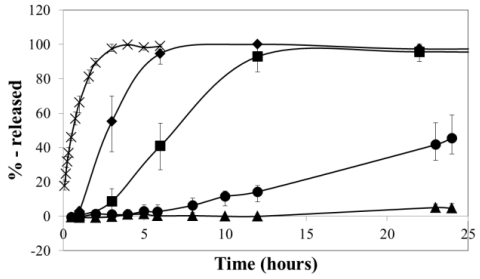

Figure 1 - Release of Lysozyme from tablets coated with various amounts of 4 % w/w PVAc feed solution in DCM. (♦): No coating, (▲): 1.9 mg/tablet, (●): 4.2 mg/tablet, (■): 7.6 mg/tablet

Coatings were conducted with ACE-based and DCM-based feed solutions and the release was measured over 24 hours (Fig. 2). Coatings with ACE-based feed solution were conducted at an atomization pressure of 1.5 bar, whereas coatings with DCM-based feed solutions were varied between 1.0 and 1.5 bar.

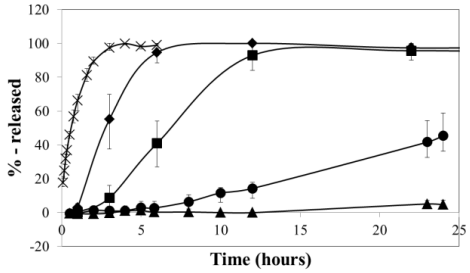

Figure 2 - Release of Lysozyme from tablets coated with: (×) No coating; (●) PVAc in DCM (4.2 mg/tablet) (1.0 bar); (▲) PVAc in Acetone (4.2 mg/tablet) (1.5 bar); (♦) PVAc in DCM (2.8 mg/tablet) (1.5 bar); (■) PVAc in Acetone (2.3 mg/tablet) (1.5 bar)

SEM images

SEM images were taken of the cross sections of the coated tablets to determine the structure and thickness of the film.

The coating originating from the DCM-based feed solution showed a thickness of ~70 µm and more porous characteristics (Fig. 3) than the film originating from the ACE-based feed solution. The ACE-originating coating had a thickness of ~40 µm despite similar amounts of applied PVAc (Fig. 4).

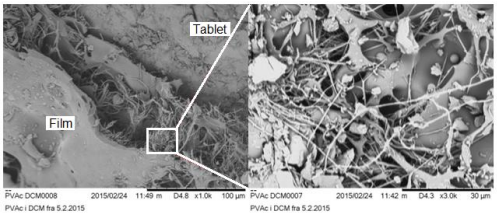

Figure 3 - Scanning electron microscopy images of films originating from 4 % w/w PVAc in DCM-based feed solution. Film applied: 2.8 mg/tablet. Atomization pressure: 1.5 bar. Left: Zoom 1000x, Right: Zoom 3000x.

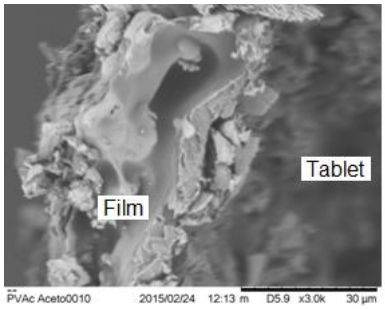

Figure 4 - Scanning electron microscopy image of a film originating from 4 % w/w PVAc in ACE-based feed solution. Film applied: 2.3 mg/tablet. Atomization pressure: 1.5 bar. Zoom: 3000x.

DISCUSSION

Atomization pressure

Tablets coated with an ACE-based feed solution at 1.5 bar showed a prolonged release compared to tablets coated with a

DCM-based feed solution at 1.0 bar, even though the amount of film pr. tablet was similar (Fig. 2, circles and triangles). This indicates that the atomization pressure might have an influence on the film formation, and as a consequence the release profiles of Lysozyme. The atomization pressure is a way to control the droplet size, with higher pressure leading to smaller droplet size. If a droplet becomes too large, an over wetting of the core surface will occur, potentially leading to agglomeration of the cores[6]. If the drops become too small, there is a risk of premature droplet evaporation[8]. Studies have described how an increased atomization pressure leads to an increased contact between droplet and core surface, ultimately leading to a more efficient and uniform film layer[8, 9]. Uneven film distribution due to variations in the atomization pressure, and subsequently the droplet size, could be an explanation to the difference between the release profiles of tablets coated with PVAc in DCM at 1.0 bar and PVAc in ACE at 1.5 bar (Fig. 2).

Viscosity of the feed solution

The difference in atomization pressure might explain the deviation from our hypothesis for the tablets coated under different conditions. However a difference in release is also observed, when the same atomization pressure (1.5 bar) is applied to the two feed solutions (Fig. 2, diamonds and squares). Tablets coated with the good solvent, DCM, show faster release than tablets coated with the poor solvent, ACE, which is against the hypothesis. This could, however, be explained by the differences in film porosity of the two films. The amount of film per tablet is almost the same in the two cases (2.8 vs. 2.3 mg/tablet), however the film from the DCM-based feed solution is almost twice as thick (Fig. 3 & 4). The DCM based film has a lower density, thereby being more porous, leading to a faster diffusion rate.

The more porous film created by the DCM-based feed solution could be due to differences in solution viscosity and subsequently the droplet size. The viscosity of pure DCM is higher than of pure ACE (Table 2), and as PVAc has a larger $[\eta]$ in DCM than in ACE (Table 1), the polymer is expected to display a larger hydrodynamic volume in the good solvent (DCM), which would lead to higher viscosity compared to the poor solvent (ACE). As such it must be expected that the viscosity of the DCM-based feed solution is higher than the viscosity of the ACE-based feed solution. Studies have shown that the viscosity of a solution determines the size of the droplet, with higher viscosity leading to a larger droplet size[10, 11]. The difference in droplet size between the two feed solutions could result in a more porous film layer obtained with the DCM-based feed solution and thereby explain the deviation from the existing literature.

Evaporation of solvent

Another potential explanation for the differences in both the film and the release observed through the spray-coated films is the differences in solvent volatility. Both coatings are conducted at room temperature, but the evaporation rate of DCM and ACE cannot be expected to be even at this temperature, as DCM is more volatile than ACE (Table 2).

Table 2 – Boiling point and viscosity and 25 °C of pure Acetone (ACE) and Dichloromethane (DCM)[12, 13]

Solvent	Viscosity mPa·s	Boiling point (B_p)
ACE	0.306	56 °C
DCM	0.413	40 °C

This means, that the evaporation of DCM may occur faster than ACE, at the same temperature, which increases the potential risk of premature droplet

evaporation[14]. This will lead to ineffective coating, as the polymer chains will not have time to form a strong network on the surface of the tablet core. Due to the faster evaporation of DCM, most of the solvent from the droplet will already have evaporated prior to contact with the tablet surface[15]. If most of the solvent has evaporated before contact with the tablet, the small polymer networks created in each droplet will not have sufficient time to coalescence to a uniform film layer. This uniforming step has been found to be the most important step in the film formation process and a shortening of this step would lead to a more porous, inconsistent film[16, 17].

Intrinsic viscosity and release

The studies conducted do not prove the expected correlation between $[\eta]$ and release. This is most likely due to the influence of the earlier explained process variables within the coating process and their influence on the created films. The influence that the solvent selection has on the pore size in the network's microstructure is simply not large enough to create a significant response when compared to the influence on the process variables of the coating process. To be able to make a justified comparison, it would be necessary to streamline the coating processes so that factors like droplet size, evaporation rate and liquid spray were alike.

From the experiments conducted, it is unclear whether the difference in the film is a consequence of a difference in droplet size or a difference in the solvent evaporation rate; however both variables have been previously found to be valid explanations for variations in films from spray-coating[8, 9, 15]. The porous characteristics of the DCM-films observed in the SEM images suggest that premature droplet evaporation is the primary explanation for the differences in the films. If true, it would be necessary to conduct the coatings with DCM-based feed solution at temperatures lower than 20 °C in order to slow the evaporation.

CONCLUSION

From the experiments conducted it has not been possible to verify that the results obtained by Wan *et al*. and Madsen *et al*. also apply to tablet coating. The studies do not show a correlation between the intrinsic viscosity and the release of Lysozyme from the coated tablets. This is most likely due to the process variables in the coating process; however the choice of polymer material and protein might also have an effect on the obtained results. Further studies are needed in order to clarify this matter.

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