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Influence of Enzymes and Surfactants on the Disintegration Behavior of Cross-Linked Hard Gelatin Capsules During Dissolution

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ABSTRACT Gelatin exhibits cross-linking upon storage at stress conditions. Capsules stored at these conditions fail to show appropriate in vitro dissolution. The aim of this study is to show the effect of surfactants in the medium on the disintegration of the gelatin capsule. This is demonstrated in the presence and absence of the enzymes pancreatin and pepsin, the function of which is to improve the dissolution. Sodium lauryl sulfate (SLS) and Tween 80 are tested as surfactants. When SLS is used in the medium, dissolution is significantly hampered due to the formation of a less soluble precipitate of gelatin. Compared to SLS, Tween 80 shows far better disintegration and solubility results in dissolution media with neutral or low pH. Therefore, it is concluded in this study that Tween 80 is preferred when a surfactant is necessary to comply with sink condition requirements.

KEYWORDS In vitro dissolution, gelatin capsules, cross-linking, enzymes, surfactants

INTRODUCTION

Gelatin is a mixture of water soluble proteins derived primarily from collagen. It is extensively used in the pharmaceutical capsule industry due to its favorable properties, which include solubility in aqueous solutions, ease of swallowing, and strong flexible backbone (Gold et al., 1997).

When storing hard gelatin capsules under light stress conditions or elevated temperature and high humidity conditions, gelatin cross-linking occurs. This can cause considerable changes in the in vitro dissolution profiles of drugs. Cross-linking reveals from the formation of a swollen, rubbery, water-insoluble membrane during dissolution testing. This water-insoluble gelatin film acts as a barrier restricting drug release. The current literature tends to indicate that these effects primarily impact the in vitro testing methodology rather than the in vivo bioavailability of drugs formulated in hard gelatin capsules (Digenis et al., 1994). Since protease enzymes are present in the gastrointestinal fluids, the inclusion of enzymes in a dissolution medium may simulate the

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physiological conditions that an ingested dosage form encounters during transit through the gastrointestinal tract (Dahl et al., 1991). Gastric juice, at pH 1.2, contains the enzyme pepsin. Pancreatin is present in the duodenum and operates therefore at a neutral pH. Both enzymes have an effect on scission of certain peptide bonds within the gelatin molecule, thus liberating the drug which it encapsulates.

The objective of this study was to evaluate if surfactants, added to the dissolution medium as a solubility enhancer, negatively affect the disintegration of cross-linked hard gelatin capsules, independent of the absence or presence of enzymes (Dey et al., 1993; Murthy et al., 1989). The tested surfactants were sodium lauryl sulfate (SLS) and Tween 80.

MATERIALS AND METHODS

Materials

The enzymes used were pepsin and pancreatin (both from Sigma, Zwijndrecht, The Netherlands). Milli-Q water used in the dissolution procedure was of analytical quality. The tested formulation contained a hydrophilic drug (Org 12962) with a solubility of 0.1 mg/mL in both water and aqueous fluids with a pH varying from 1.0 to 8.0 (see Fig. 1).

The formulation was filled into green opaque hard gelatin capsules (L730 A, no. 2 from Capsugel) and contained 2.84 mg Org 12962 (salt), 20.0 mg maize starch, 4.0 mg hydroxypropylcellulose, 1.0 mg magnesium stearate, 3.0 mg colloidal anhydrous silica, and lactose 200M for a total weight of 200.0 mg per capsule. These hard gelatin capsules were stored in open containers at room temperature/ambient humidity or at a constant condition of 40°C/75% RH. The latter condition was chosen to accelerate physical changes. Samples were tested for their in vitro dissolution performance at 1, 3, 4, 5, 6, and 14 months. Graphs shown have been selected as most typical examples to illustrate the phenomena occurring. Because SLS is preferably used to improve the solubility of the drug, SLS (purity approx. 99%, from Sigma) was tested for its influence on the in vitro performance. Also Tween 80 (Ph. Eur. quality) was tested, being a non-ionogenic surfactant.

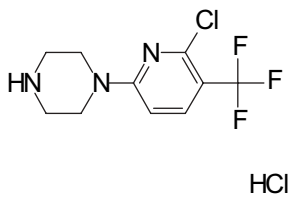

FIGURE 1 **Structural Formula of Org 12962.**

Methods

All dissolution tests were conducted with a USP apparatus 2 (rotating paddle method) at 37°C (dissolution tester CD6, IKA Laboratory Technology from Janke & Kunkel, Landsmeer, The Netherlands, and dissolution tester AT7 from Sotax, Besel, Switzerland). USP paddles rotating at a speed of 50 rpm were used. The dissolution medium was 500 mL of deionized water or 0.1 N HCl. All dissolution media were used with and without pepsin and pancreatin. Pepsin was used with an activity level of 1330 units/mg protein (1:10000) and concentrations of 0.42 – 4.98 mg/mL. Pancreatin activity was at least equivalent to the USP specification and was used at concentrations of 0.05 – 0.50 mg/mL.

Sampling was performed (8452A Diode Array from Hewlett Packard, Amstelveen, The Netherlands, and Lambda 25 UV/VIS spectrometer from Perkin Elmer, Groningen, The Netherlands) at 5, 10, 15, 30, 45, and 60 min by an automated system. Samples were analyzed at a wavelength of 260 nm. The absorbance values were then compared to a standard calibration curve. Because the enzymes pancreatin and pepsin interfere at the used wavelength, an absorbance correction was performed for the present enzyme concentration.

Automatically taken samples were filtered through a 45 µm solvent resistant filter (Distec polyethylene). The dissolution data reported in the various figures are the average of three individual capsule determinations.

RESULTS AND DISCUSSION

Figure 2 shows the cross-linking effect for the capsules on the dissolution in water. Storage at 40°C/75% RH for 6 months results in a much slower disintegration compared to a storage time of 1 month at 25°C/60% RH. Addition of pancreatin (0.05 mg/mL) to the dissolution medium stimulates the disintegration behavior of the capsules, but does not render an equal dissolution profile as obtained for the capsules stored at 25°C/60% RH for 1 month. Similar results were obtained after dissolution in 0.1 N hydrochloric acid (HCl) in the absence or presence of pepsin (data not shown).

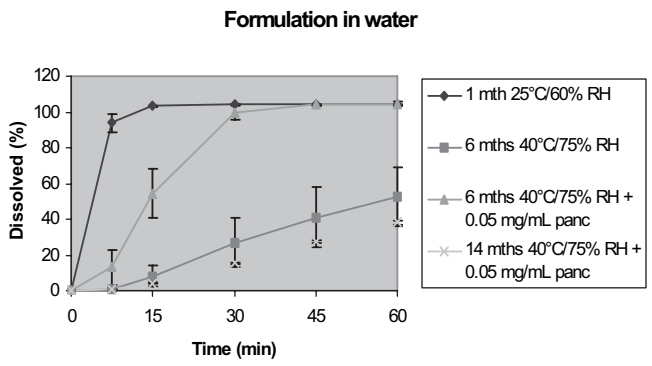

FIGURE 2 **Effect of Storage Conditions and Storage Times on the In Vitro Release.**

Prolonged storage further deteriorates the dissolution characteristics. In fact, addition of pancreatin does not have any value anymore.

Often surfactants are added to the dissolution media to improve the solubility of the drug in order to comply with sink condition requirements. The effect of SLS on the activity of enzyme is shown in Fig. 3. As can be seen, 0.3 m/V% SLS worsens the disintegration (Fig. 3, upper graphics) compared to a dissolution medium of water with pancreatin. Addition of SLS exhibits similar adverse effects on the disintegration of the capsules in 0.1 N HCl/pepsin (Fig. 3, graphics below).

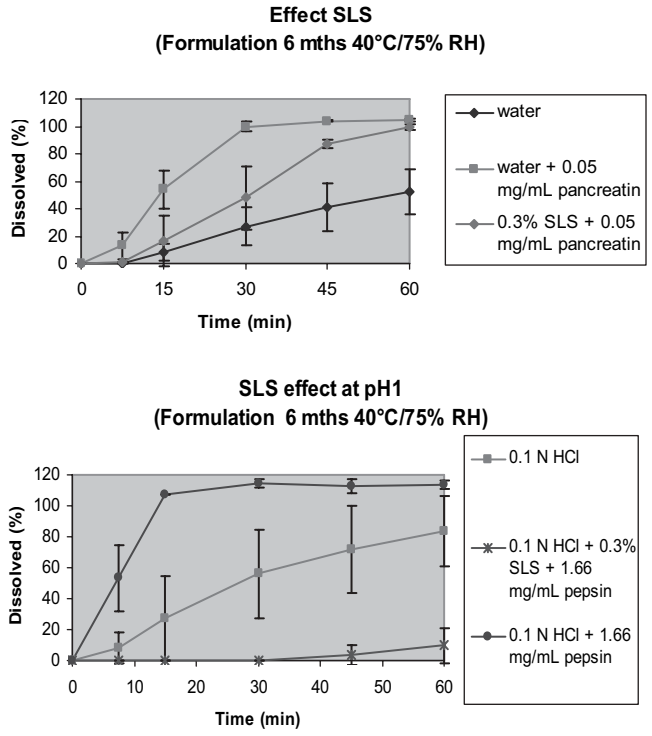

FIGURE 3 **Effect of 0.3 m/V% SLS on the Disintegration of Hard Gelatin Capsules.**

The huge effect of SLS, as shown in Fig. 3 for pepsin, is not found to such an extent for pancreatin. Obviously, the low pH appears to be important for this. It should be mentioned that in the absence of SLS, the dissolution rate of gelatin is known to decrease slightly as pH increases from 1 to 7. This is due to the inherent pH solubility behavior of gelatin, which has an isoelectric point around pH 5–8 (Podezeck R. Jones, 2004; Zhao et al., 2004). In the presence of 0.3% SLS, the dissolution of gelatin shells slowed down significantly in the low pH medium as shown in Fig. 3. Visually, the undissolved gelatin shell was observed to become like "stringy cheese," which took a much longer time to dissolve. Earlier literature study (Zhao et al., 2004) reports that this stringy cheese precipitate is formed through some interactions between SLS and gelatin. Elemental analysis showed that there was an increase in the sulfur content for the unknown precipitate obtained from the gelatin, which suggests that the unknown precipitate contained bound SLS. Apparently SLS delays the disintegration behavior of the capsules, which is not necessarily a neutralization of the enzymatic activity. However, there possibly may exist an interaction between SLS and the enzymes not allowing capsule disintegration. To our knowledge there is a lack of previous studies using both surfactant and enzymes in the dissolution media (Marchais et al., 2003).

Figure 4 illustrates the effect of SLS concentration on the dissolution profiles of cross-linked capsules. Water was chosen as the dissolution medium. The critical micelle concentration (CMC) of SLS in water is 0.023% (The American Pharmacutical Association, 1984). Therefore, the tested concentrations of 0.3% and 1.75% SLS in water are well above the CMC of SLS. The 1.75 m/V%

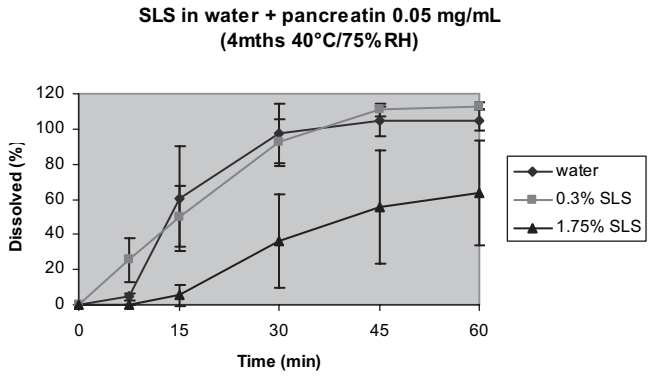

FIGURE 4 **Effect of SLS Concentration on the In Vitro Release of the Formulation in the Presence of Pancreatin.**

SLS concentration gives worse disintegration than the dissolution media with lower SLS concentration (Fig. 4).

Because a dissolution medium of 1.75 m/V% SLS containing 0.05 mg/mL pancreatin gives poor disintegration of the hard gelatin capsules, higher pancreatin concentrations were used to determine the compensation of this SLS effect. Figure 5 shows no evident effect in pancreatin concentration towards disintegration behavior.

Also, higher pepsin concentrations were used to determine the influence of enzyme concentration on the SLS effect. Figure 6 shows no effect of pepsin concentration also probably due to the dominating effect of SLS. When comparing Fig. 3 (lower graph) and Fig. 6, it must be noticed that the storage period of the gelatin capsules at stressed conditions is evident also for the negative impact on disintegration behavior.

As stated, SLS exhibits an interaction with gelatin which yields a poorly soluble precipitate. Sodium lauryl sulfate (SLS) is an ionic surfactant. Tween 80 was also tested as a commonly used non-ionic surfactant. Figure 7 shows a sort of lag-time; it takes a certain time before release from the capsules starts. On the other hand, the surfactant seems to have limited effect on the gelatin, which reveals most pronounced from the pepsin data. This is obvious when comparing Fig. 3 (lower graph) with Fig. 7. Also, the effect of enzyme concentration in 0.5 V/V% Tween 80 was observed.

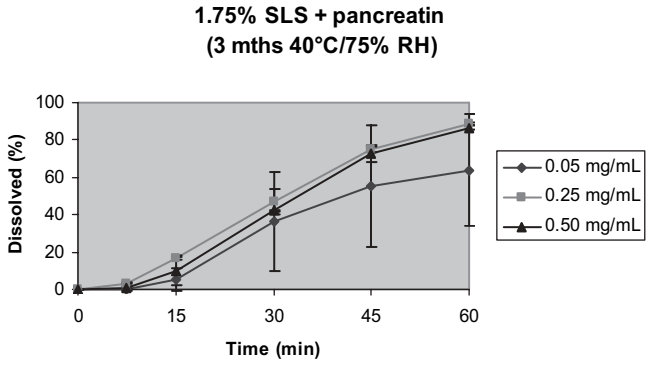

FIGURE 5 **Effect of Pancreatin Concentration on the Disintegration Behavior of Hard Gelatin Capsules.**

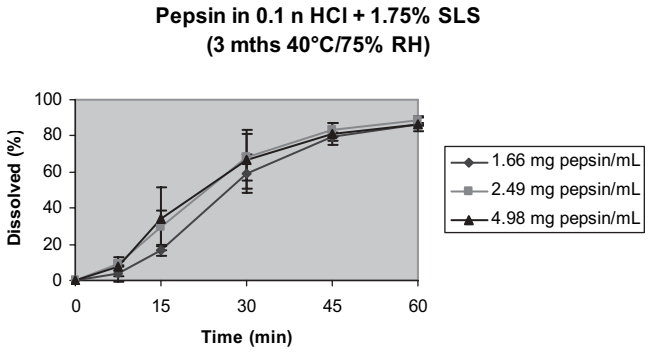

FIGURE 6 **Effect of Pepsin Concentration on the Disintegration Behavior of Hard Gelatin Capsules.**

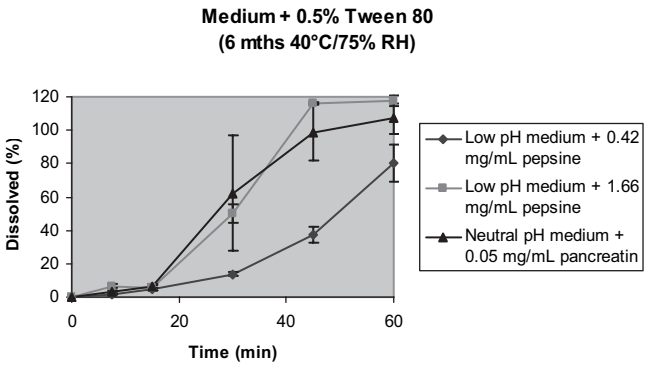

FIGURE 7 **Effect of Tween 80 and Enzyme Concentration on the In Vitro Release of Cross-Linked Hard Gelatin Capsules.**

CONCLUSION

This work demonstrates the effect of incorporating the surfactants SLS or Tween 80 and the enzymes pepsin or pancreatin into the dissolution medium to evaluate in vitro drug release profiles from hard gelatin capsules exposed to a condition of 40°C/75% RH. Medium containing pancreatin or pepsin improved the dissolution performance of the stressed hard gelatin capsules, compared to dosage forms tested in deionized water or 0.1 N HCl. There does not seem to be an optimal working concentration for pancreatin and pepsin.

Sodium lauryl sulfate (SLS) slows down the disintegration of gelatin shells significantly, especially in low pH media, due to the formation of a less soluble precipitate of gelatin. Because SLS is typically added to dissolution media to improve the dissolution of poorly water soluble drugs, the slowdown of gelatin capsule shell disintegration can potentially counteract this effect. Compared to SLS, Tween 80 shows better results. Knowing that storage for a longer period at 40°C/75% RH has its influence on the disintegration behavior of the hard gelatin capsule, it is conceivable that after a certain storage period the capsule may not dissolve satisfactorily even in the presence of enzymes. Nevertheless, when using a dissolution method for hard gelatin capsules, stressed at 40°C/75% RH, the addition of enzymes to the dissolution medium is recommended.

When adding surfactants to the dissolution medium, it should be kept in mind that this might affect the disintegration behavior of the hard gelatin capsules. The addition of SLS to the dissolution medium negatively influenced the disintegration behavior, in the presence and absence of enzymes, while addition of Tween 80 did not have such a negative effect. When a surfactant is necessary to comply with sink condition requirements, Tween 80 is preferred.

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