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The robustness and flexibility of an emulsion solvent evaporation method to prepare pH-responsive microparticles

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ABSTRACT

A microparticle preparation method based on an emulsion of ethanol in liquid paraffin stabilised using sorbitan sesquioleate which produces enteric microparticles of excellent morphology, size and pH-sensitive drug release was assessed for its robustness to changes in formulation and processing parameters. Prednisolone and methacrylic acid and methyl methacrylate copolymer (Eudragit S) were the drug and polymer of choice. Emulsion solvent evaporation procedures are notoriously sensitive to changes in methodology and so emulsion stirring speed, drug loading, polymer concentration and surfactant (emulsifier) concentration were varied; microparticle size, encapsulation efficiency, yield and *in vitro* dissolution behaviour were assessed. The yield and encapsulation efficiency remained high under all stirring speeds, drug loadings and polymer concentrations. This suggests that the process is flexible and efficiency can be maintained. Surfactant concentration was an important parameter; above an optimum concentration resulted in poorly formed particles. All processing parameters affected particle size but this did not alter the acid resistance of the microparticles. At high pH values the smaller microparticles had the most rapid drug release. In conclusion, the microparticle preparation method was resistant to many changes in processing, although surfactant concentration was critical. Manipulation of particle size can be used to modify the drug release profiles without adversely affecting the gastro-resistant properties of these pH-responsive microparticles.

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1. Introduction

Oral drug delivery offers great potential benefits for a patient in terms of convenience and compliance, but delivering drugs to different regions in the gastrointestinal (GI) tract is challenging. The physiology of the GI tract is complex and has a great impact on the effectiveness of modified release systems (McConnell et al., 2008a). Single unit dosage forms (tablets or capsules) for modified release present problems such as erratic gastric emptying (Coupe et al., 1991; Fadda et al., 2009; Ibekwe et al., 2008a) or incomplete drug delivery in the GI tract (Schroeder et al., 1987; Ibekwe et al., 2006). Multiple unit dosage forms such as granules, pellets or microparticles have been used to counter these issues. Microparticles in particular may have benefits because of their small particle size which is anticipated to provide more rapid emptying from the stomach and more reproducible transit through the small intestine and colon. The larger surface area of the microparticles should facilitate drug dissolution resulting in rapid drug release, and more reproducible absorption and bioavailability.

One of the most common approaches for the preparation of microparticles for site-specific drug delivery is based on emulsification solvent evaporation. The emulsification solvent evaporation technique involves a three step processes. In the first step, a combination of polymer and drug solution (internal phase) is emulsified into the continuous phase. Then the solvent evaporates through the emulsion/air interface resulting in polymer precipitation and particle hardening. In the last step, microparticles are separated from the continuous phase by filtration and then they are washed by an appropriate solvent. Although emulsification solvent evaporation is a conceptually simple technique, it is normally difficult to achieve the appropriate drug release characteristics from this process. This is because the actual particle formation depends on many variables; type of drug, type of polymer and drug, polymer and emulsifier concentration, volume ratio of the internal and external phase, stirring speed and solvent evaporating temperature (Watts et al., 1990; Arshady, 1991; Shukla and Price, 1991; Perumal, 2001). A novel microparticle preparation procedure was developed by Kendall et al. (2009). Here we investigate the effect of formulation (drug content, polymer concentration and emulsifier concentration) and process parameters (stirring speed) on the characteristics of microparticles to determine how robust the preparation procedure is to changes in these parameters.

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Poly (methacrylic acid, methyl methacrylate) (Eudragit S) was selected as a model polymer, as it is used extensively in modified release coatings for drug delivery to the distal intestine. Tablets coated with Eudragit S are used clinically for the treatment of ulcerative colitis, but have been shown to pass through the gut intact in some instances (Safdi, 2005; Sinha et al., 2003; Ibekwe et al., 2008b). The same has been shown for pellet formulations (McConnell et al., 2008b). Eudragit S microparticles may be a solution to this problem. Prednisolone was selected as a model drug, and drug-loaded microparticles were prepared. The aims of this study were to identify the key process and formulation variables affecting the properties of microparticles prepared by emulsion solvent evaporation and to understand how they interact. Ultimately, this knowledge should allow the microparticles to be "tailor-made" for specific drug delivery in the gastrointestinal tract.

2. Materials and methods

2.1. Materials

Eudragit S was obtained from Evonik (Darmstadt, Germany) and prednisolone was purchased from Sanofi Aventis (Romainville, France). All other chemicals were of analytical grade and purchased from Sigma–Aldrich (Poole, UK).

2.2. Preparation of microparticles

Prednisolone loaded Eudragit S microparticles were prepared using a novel emulsion solvent evaporation method (Kendall et al., 2009). Eudragit S and prednisolone were dissolved in ethanol (30 ml) and emulsified into liquid paraffin (165 g) containing sorbitan sesquioleate (Arlacel 83) under agitation. Microparticles were collected by vacuum filtration through a glass filter (pore size 4) and then washed three times with 50 ml of *n*-hexane and dried in a vacuum oven. All the batches of microparticles were prepared in triplicate. To investigate the impact of stirring speed, the drug content, polymer concentration (in ethanol) and emulsifier concentration (in liquid paraffin) were kept constant at 9.1% (w/w), 5% (w/v) and 1% (w/w) while the stirring was set at either 300, 500, 1000 or 1500 rpm. To investigate the effect of drug content, the polymer concentration and emulsifier concentration were kept constant at 5% (w/w) and 1% (w/w), respectively and the stirring was kept at 1000 rpm while the drug concentration was varied (9.1%, 16.7%, 50.0% and 66.7% (w/w)). To investigate the polymer concentration, drug content and emulsifier concentration

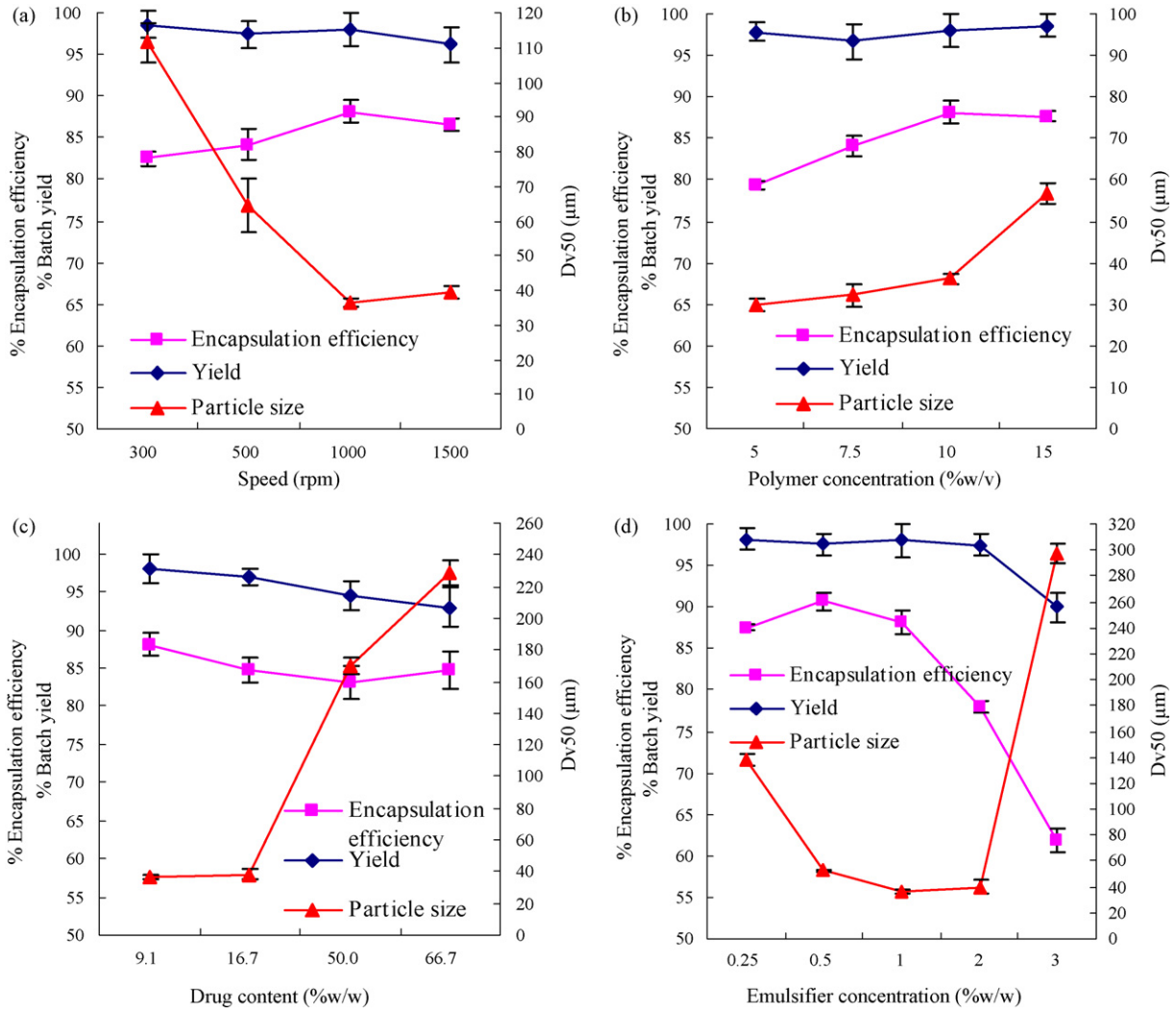

Fig. 1. Effect of (a) stirring speed (b) polymer concentration (c) drug content and (d) emulsifier concentration on the particle size (*Dv*50), batch yield and encapsulation efficiency of Eudragit S/prednisolone microparticles.

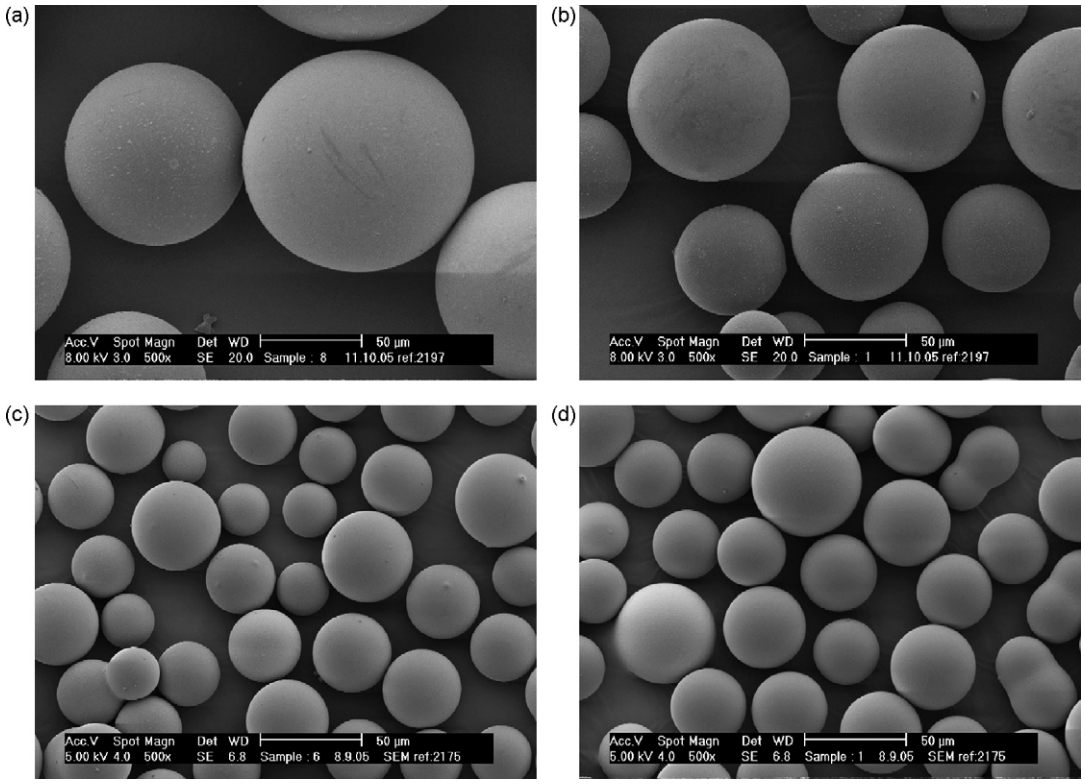

Fig. 2. SEM of microparticles formed from different stirring rates: (a) 300 rpm, (b) 500 rpm, (c) 1000 rpm, and (d) 1500 rpm.

were kept constant at 9.1% (w/w) and 1% (v/v), respectively and the stirring was kept at 1000 rpm while the polymer concentration was increased from 5% to 7.5%, 10.0% and 15.0% (w/v). To investigate the emulsifier concentration the drug content and polymer concentration were kept constant at 9.1% (w/w) and 5% (w/v) respectively and the stirring was kept at 1000 rpm and the emulsifier was used at 0.25%, 0.5%, 1.0%, 2.0% and 3.0% (w/w).

2.3. Microparticle characterisation

The yield of microparticles for each batch was calculated using the following equation:

$$\%\,\text{batch yield} = \frac{\text{weight of harvested microparticles}}{\text{total amount of polymer and drug used}} \times 100$$

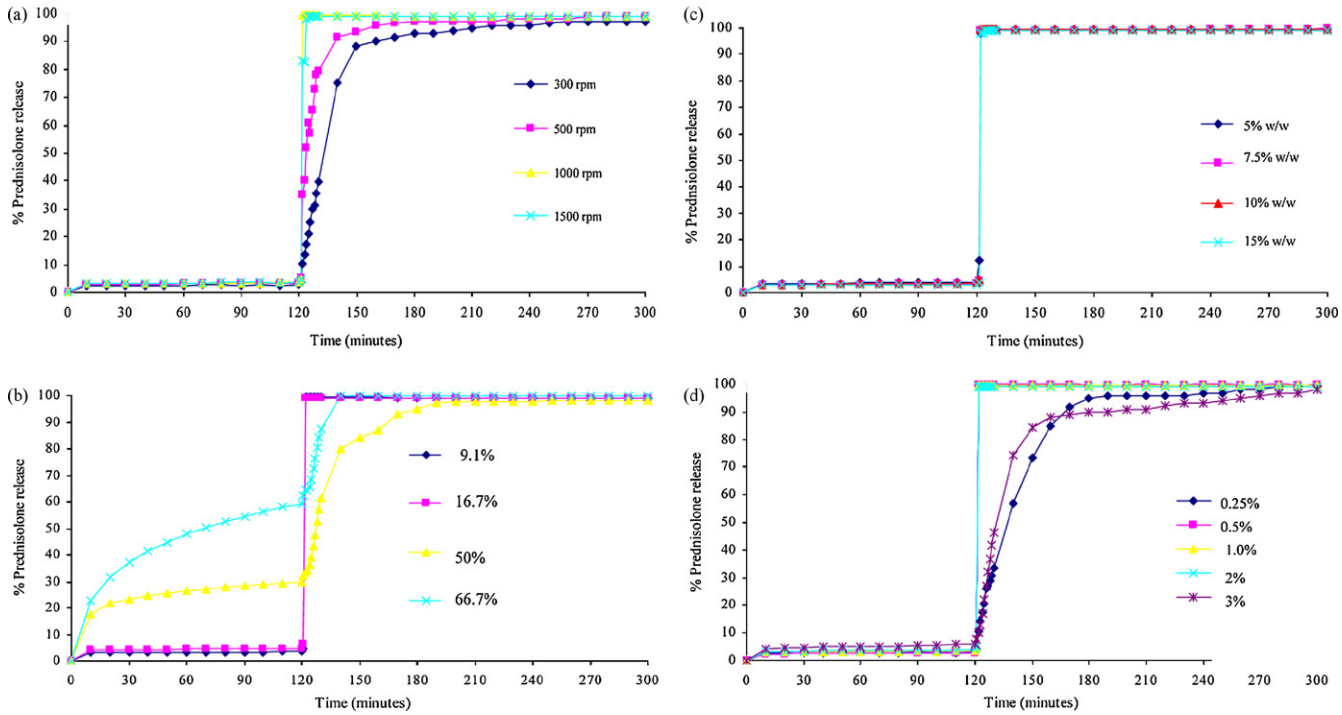

Fig. 3. *In vitro* drug release of prednisolone loaded Eudragit S microparticles fabricated using various (a) stirring speeds (b) drug contents (c) polymer concentrations and (d) emulsifier concentrations at pH 1.2 for 2 h followed by an adjustment to pH 7.4 (Error bars have been omitted for clarity).

After suspending the microparticles in 0.1 M HCl, the particle size was measured by laser diffraction using a Mastersizer S (Malvern Instruments Ltd., Worcestershire, UK). The measurements were conducted in triplicate. The average size of microparticles was expressed as a median diameter, $D(v,50)$, which is a particle diameter at 50% cumulative volume.

The morphology and surface topography of microparticles were examined by scanning electron microscopy (SEM). The samples were fastened to a holder using double sided carbon adhesive tape and then coated with gold using a gold sputter module in a high vacuum evaporator (Emitech K550, Ashford, Kent, England) and images were taken using the scanning electron microscope (Phillips XL30, Eindhoven, Holland).

Determination of prednisolone encapsulation efficiency was calculated using the following method. Forty milligrams of microparticles were dissolved in 100 ml methanol; 10 ml of this methanolic solution was added to 0.1 M HCl to precipitate the pH-sensitive polymer and made up to 100 ml. Samples were filtered through 0.22 μm disposable filters and assayed for prednisolone spectrophotometrically at 245 nm. The measured absorbance was converted to drug concentration using a standard curve for the known concentration of the drug in 10% methanol in 0.1 M HCl. The experiment was carried out in triplicate for each sample.

$$\text{encapsulation efficiency}\,(\%) = \frac{\text{calculated amount of prednisolone in microparticle}}{\text{theoretical amount of prednisolone in microparticles}} \times 100$$

Release studies were carried out under sink conditions. Three hundred milligrams of microparticles were accurately weighed and filled into a size 0 hard gelatin capsule. To evaluate the *in vitro* drug release at gastric and intestinal pH, the dissolution test was carried out using a pH change method and USP II dissolution apparatus. The microparticle filled capsule, secured inside a stainless steel sinker was introduced into 750 ml of 0.1 M HCl. After 120 min, 250 ml of 0.2 M tri-sodium phosphate, which had been equilibrated to $37 \pm 0.5\,^{\circ}C$, was added to each vessel and the pH was adjusted to 7.4 ± 0.05 with 2 M NaOH. The experiment was then run for a further 180 min. Throughout the experiment, the speed of the paddle was 100 rpm and the temperature of the medium was maintained at 37 ± 0.5. During the dissolution test, the samples were taken and filtered through 0.2 μm filters and prednisolone content was mea-

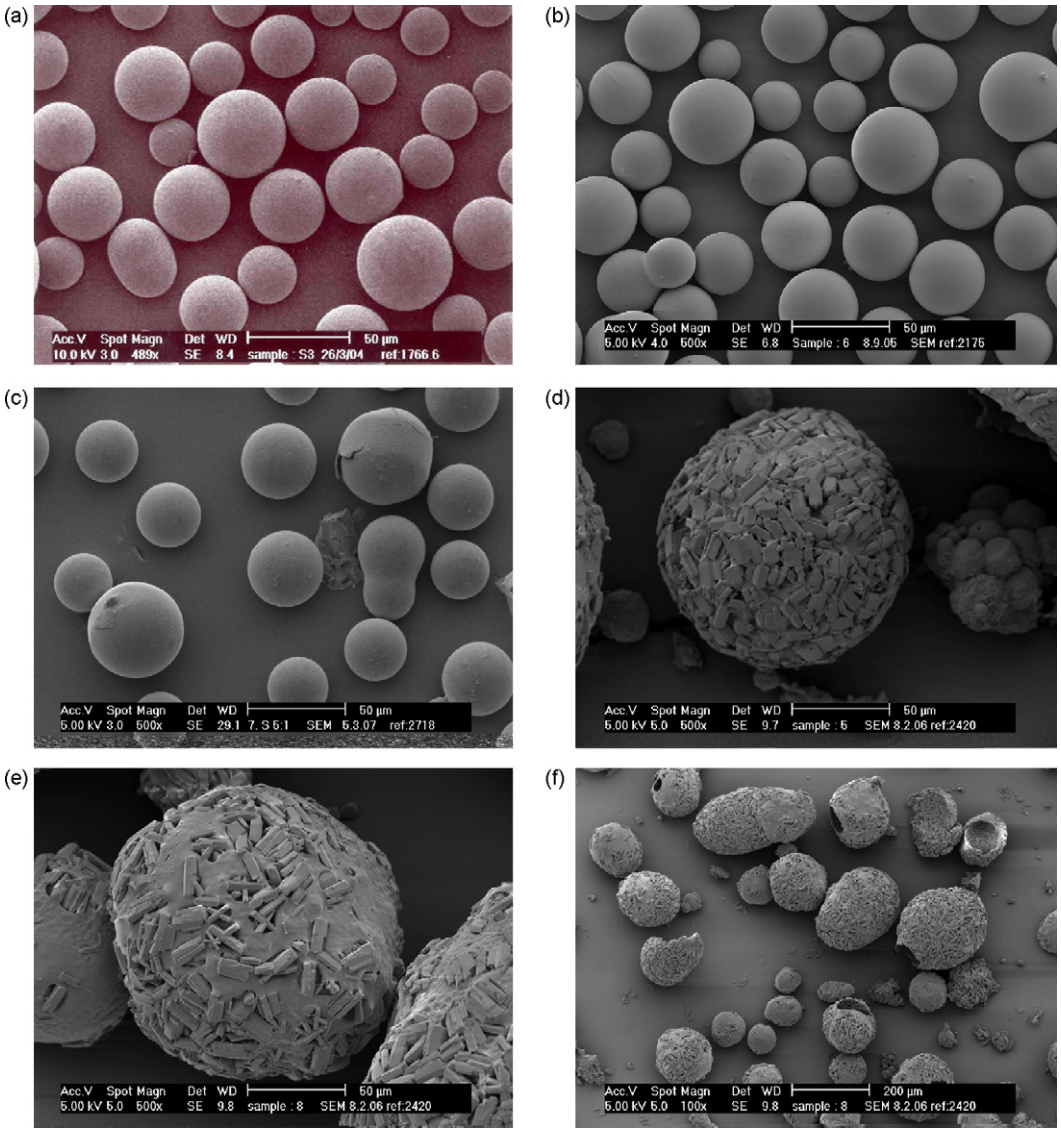

Fig. 4. SEM of microparticles loaded with different levels of drug: (a) drug free, (b) 9.1% (w/w), (c) 16.7% (w/w), (d) 50% (w/w), (e) 66.7% (w/w) and (f) 66.7% (w/w) (lower magnification).

sured using a UV spectrophotometer at 247 nm. The amount of prednisolone in the dissolution medium was calculated with reference to a standard curve of prednisolone. The experiments were carried out in triplicate for each formulation.

3. Results and discussion

The solvent evaporation method was used to successfully produce a range of microparticles, prepared under various process parameters.

3.1. Stirring speed

Particle size was reduced when stirrer speed was increased (Figs. 1a and 2). Stirring provides energy to disperse the organic/polymeric phase in the liquid paraffin phase, and a high shear provided by the propellers produces a smaller droplet size, and consequently, smaller particles are formed after solvent evaporation (Benita et al., 1984; Huang and Ghebre-Sellassie, 1989; Jeffery et al., 1991; O'Donnell and McGinity, 1997; Gabor et al., 1999). Interestingly, increasing the stirring speed from 1000 to 1500 rpm did not further reduce the particle size. The encapsulation efficiency increased slightly with the increase in the stirring rate (Fig. 1a). Stirring speed has no discernable effects on yield; the productivity of the preparation methodology is maintained.

The *in vitro* drug release profiles of the Eudragit S microparticles at pH 1.2 for 2 h following by pH 7.4 are shown in Fig. 3a. After 2 h in acid, the release of prednisolone from microparticles at the entire range of stirring rates was restricted to less than 4% which complies with USP specification for delayed release products. Importantly, the particle size (which was altered by stirring speed) did not affect the ability of the particle to control release in acidic conditions. Even more than halving the size of microparticles from ~100 μm (at 300 rpm) to ~40 μm (at 1000–1500 rpm) did not negatively affect this control of release. When the pH is increased to 7.4 (above the pH threshold of Eudragit S, pH 7), drug release is seen with all microparticles. However, those produced by more rapid stirring (1000 and 1500 rpm) had faster release. This is likely to be due to the smaller size of these particles and their larger surface area to volume ratio.

3.2. Drug loading

Yield and encapsulation efficiency remained high at all drug loadings. Particle size was increased when drug content was increased (Figs. 1b and 4). This may be because of the increase in drug concentration in the inner phase leading to an increase in inner phase viscosity and droplet size and the formation of larger sized particles (Krishnamachari et al., 2007). As drug content increased, the encapsulation efficiency was slightly decreased. Yang et al. (2001) suggested that an increase in drug content led to an enhancement of the drug concentration in the emulsion droplets and consequently increased the concentration gradient between the emulsion droplets and the continuous phase; as a result increasing the amount of drug partitioning into the continuous phase.

At high drug loadings there was poor microparticle formation demonstrated by changes in morphology (Fig. 4e–f). Microparticles with high drug content (50% and 66.7%, w/w) were hollow and had an extensive amount of crystalline prednisolone on the surface whereas the morphology of microparticles loaded with lower levels of drug showed a smooth surface and dense structure (Fig. 4a–d). It can be assumed that the high proportion of drug in the droplets is able to precipitate when solvent removal occurs, leaving crystalline deposits on the microparticle wall aided by the fact that lipophilic crystals tend to accumulate at the interface with liquid paraffin. This high proportion of drug on the surface accounts for the initial burst release in drug release studies (Fig. 3b) after 2 h in acid; consequently these high drug loading microparticles fail the USP test for enteric coated products. In contrast, prednisolone release from microparticles with low drug content (9.1% and 16.6%,

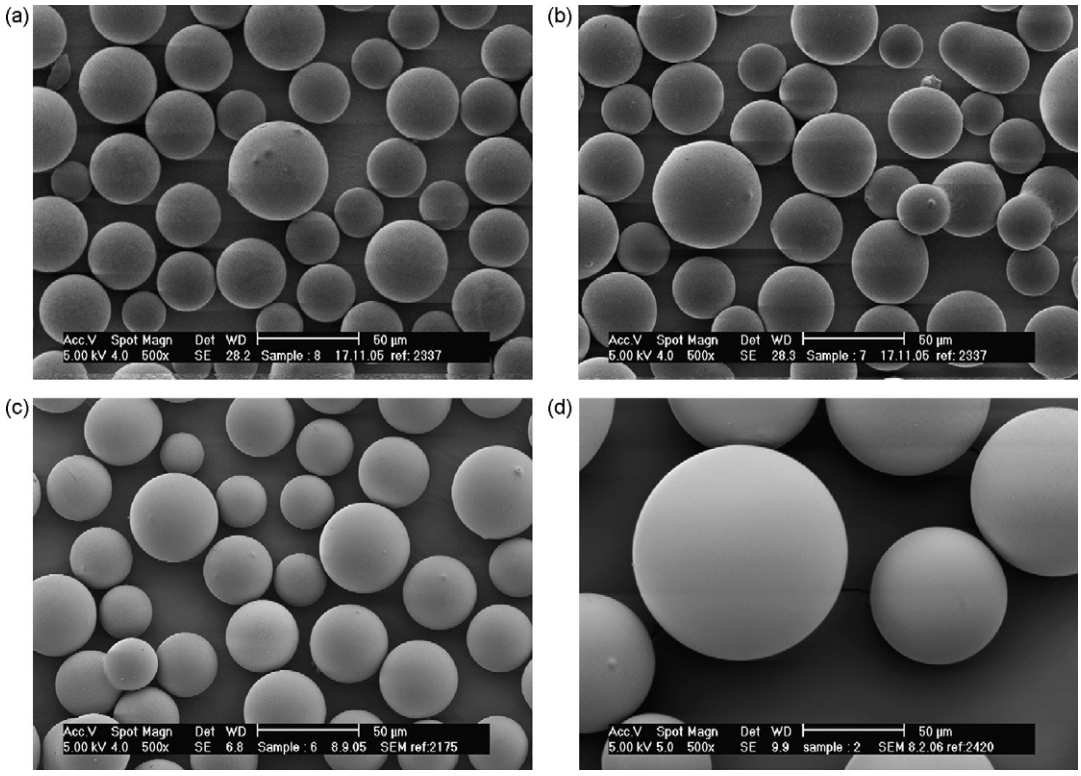

Fig. 5. SEM of microparticles formed from different polymer concentrations: (a) 5% (w/v), (b) 7.5% (w/v), (c) 10% (w/v), and (d) 15% (w/v).

w/w) was restricted to less than 5% after 2 h in acid. When the pH was increased, a rapid release was observed in these microparticles due to the large surface area of small microparticles. In contrast, drug release from microparticles with higher drug contents (50% and 66.7%, w/w) at pH 7.4 was found to be slower, taking approximately 20 and 70 min, respectively for the complete release of prednisolone. The slow dissolution of prednisolone at pH 7.4 could be due to the large size of the microparticles and the presence of crystalline drug deposits.

3.3. Polymer concentration

The particle size as well as drug encapsulation efficiency tended to increase when polymer concentrations were increased (Fig. 1c). The size distribution of microparticles was slightly narrower when polymer concentration decreased. There are two possible explanations for these effects. An increase in the polymer concentration can lead to an increase in the viscosity of the internal phase. When the viscosity of the internal phase is increased, the efficiency of the stirring is reduced and large sized microparticles result. The increased viscosity as polymer levels increase can impede drug mobility in the droplets, and this was observed as an increase in encapsulation efficiency at high polymer levels (Fig. 1c). These high polymer levels may also lead to rapid polymer precipitation on the droplet surface and rapid microparticle solidification, resulting in the hindering of drug diffusion, effectively trapping it in the particle (Bodmeier and McGinity, 1988). The polymer levels did not affect the morphology (Fig. 5) or the batch yield (Fig. 1c).

The effect of polymer concentrations on the release profiles of the Eudragit S particles in 0.1 M HCl and pH 7.4 phosphate buffer is shown in Fig. 3c. After 2 h in 0.1 M HCL, the release of prednisolone was restricted to less than 4% for all formulations. When the pH increased, a rapid prednisolone release was observed from the microparticles prepared at the entire range of polymer concentrations. The polymer concentrations used in this study have minimal impact on the release profile of prednisolone from Eudragit S microparticles.

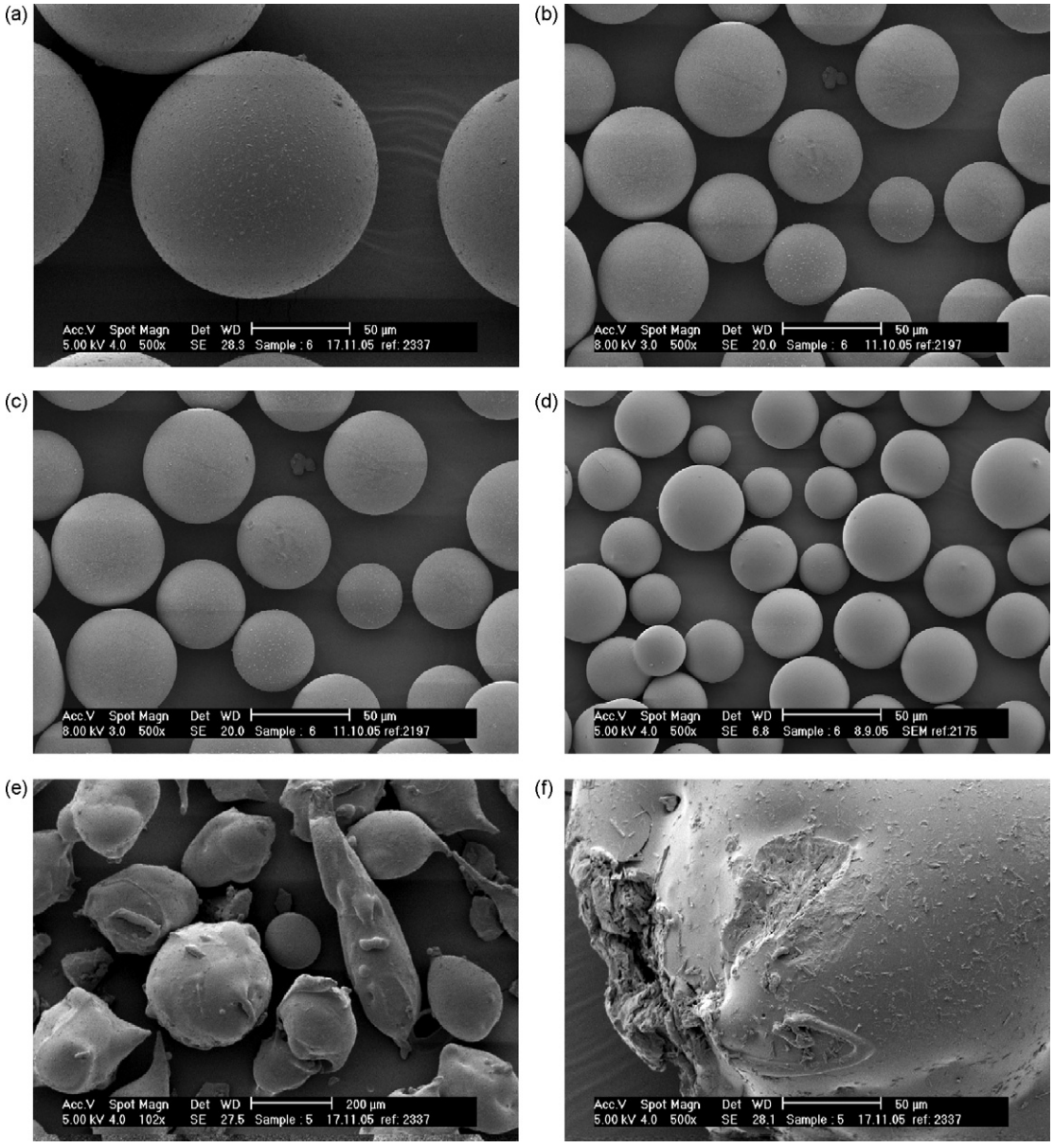

Fig. 6. SEM of microparticles formed from different emulsifier concentrations: (a) 0.25% (w/w), (b) 0.5% (w/w), (c) 1% (w/w), (d) 2% (w/w), (e) 3% (w/w): high magnification, and (f) 3% (w/w): low magnification.

3.4. Emulsifier concentration

The particle size and size distribution were increased when the surfactant concentration was reduced from 1% to 0.25% (w/w) (Figs. 1d and 6). The function of the surfactant is to decrease the interfacial tension between the dispersed droplets and the continuous phase and to protect the droplets from collision and coalescence (Arshady, 1990). Low emulsifier concentrations may be insufficient to shield the entire droplet surface; droplets are more susceptible to collision and fusion (Jeffery et al., 1993; Sansdrap and Moes, 1993; Dinarvand et al., 2002). There was little change in the size of the microparticles prepared by using emulsifier at 1% and 2% (w/w), suggesting that 1% (w/w) may be an optimum value for this formulation. When the concentration of emulsifier was increased from 2% to 3% (w/w), a dramatic increase in particle size and size distribution was observed. The lower encapsulation efficiency and larger particle size at 3% suggest that the critical micelle concentration has been exceeded which directly affected emulsion stability. The presence of micelles might induce collision or bridge discrete droplets leading to the formation of irregular shaped microparticles. The decrease in encapsulation efficiency may be due to an increase in the solubility of the drug in the oil phase. The drug is more likely to partition into the oil phase; decreasing microparticle encapsulation efficiency (Kristmundsdottir and Ingvarsdottir, 1994). An emulsifier concentration of 1% (w/w) was considered to be an optimum concentration as it provided small particle size with high drug encapsulation efficiency.

The effect of the emulsifier concentration on the release profiles of the Eudragit S microparticles in 0.1 M HCl and pH 7.4 phosphate buffer is shown in Fig. 3d. After 2 h of exposure to 0.1 M HCL, the release of prednisolone from all formulations was found to be less than 6% in spite of the size differences of the microparticles. When the pH was increased, rapid prednisolone release was observed from the microparticles prepared by using an emulsifier concentration of 0.5–2% (w/w) due to the small size of these microparticles. In contrast, the rate of drug release from microparticles with an emulsifier concentration of 0.25% and 3% (w/w) was slower due to the larger size of these microparticles.

4. Conclusion

Stirring speed, drug loading and polymer concentration all had very minimal effects on microparticle yield and encapsulation efficiencies. This suggests that the microparticle preparation methodology is robust, and is not sensitive to the studied range of changes in these process parameters. This imparts an economic advantage since the yields remain high and is promising for scale-up. The microparticle characteristic which is most susceptible to changes in processing parameters in microparticle size but importantly large changes in size did not adversely affect the gastroresistant properties of the microparticles. As the pH was increased, the size did have some effect on the rate of drug release, and so size could be used to tailor drug release without sacrificing yield or encapsulation efficiency. The most important formulation parameter proved to be emulsifier concentration. This work suggests that an optimum concentration of sorbitan sesquioleate of 1% (w/w) is required to maintain emulsion stability for microparticle formation.

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