International Journal of Pharmaceutics 379 (2009) 1–8

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Drug distribution in enteric microparticles

Suchada Nilkumhang, Mohamed A. Alhnan, Emma L. McConnell, Abdul W. Basit*

Department of Pharmaceutics, The School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, UK

ARTICLE INFO

Article history:
Received 27 January 2009
Received in revised form 28 May 2009
Accepted 28 May 2009
Available online 6 June 2009

Keywords:
Eudragit
Arlacel 83
Acrylic
Enteric polymers
Emulsion solvent evaporation
Microspheres
Confocal laser scanning microscopy

ABSTRACT

The aim of this study was to assess the distribution of three fluorescent drug or drug-like molecules in enteric microparticles. Microparticles were prepared using the pH-responsive methylmethacrylate polymer Eudragit L by an emulsion solvent evaporation process. In the process drug and polymer are dissolved in ethanol, and dispersed in a liquid paraffin external phase using sorbitan sesquioleate as stabiliser. The incorporation and distribution of riboflavin, dipyridamole and acridine orange into these microparticles were investigated using confocal laser scanning microscopy (CLSM). The influence of the physicochemical properties of the molecules (solubility in the inner phase, partition coefficient [ethanol/paraffin]) on the distribution, encapsulation efficiency and pH-responsive dissolution behaviour of the microparticles were examined. The drug that tended to partition in ethanol rather than liquid paraffin (riboflavin) was efficiently encapsulated and evenly distributed. In contrast, compounds which partitioned in favour of the liquid paraffin localised towards the surface of the microparticles and exhibited lower encapsulation efficiency (dipyridamole and acridine orange). All three sets of drug-loaded microparticles showed a limited release in acid (<10% release); drug distribution appeared to have a minimum effect on drug release. This microparticle technology has the potential to provide effective enteric drug release with a wide variety of molecules.

1. Introduction

Emulsion solvent evaporation for the preparation of drug-loaded microparticles is a conceptually simple technique, but achieving appropriate release characteristics from such particles can prove an elusive goal. The apparent simplicity of the three stages (1) emulsification of polymer/drug solution and external phase (2) solvent diffusion to the external phase and then evaporation through the emulsion/air interface resulting in particle hardening, and (3) filtration/washing belies a multi-factorial process, dependent on the interplay between drug, formulation and process parameters (Arshady, 1991; Shukla and Price, 1991; Watts et al., 1992; Perumal, 2001).

The physicochemical properties of encapsulated drugs are thought to be key variables influencing microparticle quality (Arshady, 1991) having influence on parameters like encapsulation efficiency and on "burst release" (Bodmeier and McGinity, 1987; Alex and Bodmeier, 1990; Matsumoto et al., 1997). Burst release can be attributed to fast drug diffusion through the polymer matrix or drug being present on the surface of the microparticle and so drug distribution within the microparticle is important; the localisation of drug in the inner core or on the surface of the particle affects the diffusion path length and the release profile (Yang et al., 2001). Moreover, drug concentration at a particular location in the microparticles can result in partial or complete drug crystallization within the microparticle. This prevents drug being in the amorphous form and inherently affects drug release from the microparticles. To date, little is known on the effect of the partition behaviour and solubility of drugs on their distribution behaviour within modified release microparticles, in particular microparticles used for oral delivery.

In order to study the drug distribution within microparticles, appropriate visualisation techniques are necessary. Light microscopy suffers from poor resolution and scanning electron microscopy cannot identify and localise compounds within the same sample. Confocal laser scanning microscopy (CLSM) has several advantages: it eliminates out of focus fluorescent light from the focal plane; it produces non-destructive three-dimensional optical sections without prior sample preparation and improves the axial (z: along the optical axis) and lateral (x and y: in the specimen plane) optical resolution and imaging contrast (Yan et al., 1994); and it is possible to obtain thin (0.5–1.5 μm) or thick (up to 100 μm) optical sections of a specimen along the z axis which can be compiled to reconstruct its three-dimensional (3D) structure (Prasad et al., 2007). By using sufficient fluorescent markers, CLSM also allows one to visualize and identify different compounds and structures within the same specimen. One drawback is that CLSM can only visualize fluorescent materials and to visualize non-

* Corresponding author. Tel.: +44 20 7753 5865; fax: +44 20 7753 5865.
E-mail address: abdul.basit@pharmacy.ac.uk (A.W. Basit).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.05.066

Table 1
Summary of physicochemical properties of dipyridamole, acridine orange and riboflavin (molecular weight, log Po/w and pKa values obtained from literature).

Drug	Molecular weight (Da)	$\log P_{o/w}$	pKa	Solubility in 0.1N HCl (pH 1.2) (mg/ml)	Solubility in phosphate buffer (pH 6.8) (mg/ml)	Solubility in ethanol (mg/ml)	$\log K_{ethanol/liquid\ paraffin}$
Dipyridamole	504.6	2.7	6.4	20.41	0.0045	26.22	1.9
Acridine orange	265.4	3.4	9.8	22.99	2.54	154.13	1.1
Riboflavin	376.4	−1.5	10.2	0.11	0.14	0.107	2.5

fluorescent materials, fluorescent labelling is required which may alter the physicochemical properties of the compound.

CLSM has been extensively used in biological and medical studies and is being increasingly employed in pharmaceutical science for evaluation and characterisation of solid dosage forms. It has been used to evaluate the film coating properties of tablets and pellets (Guo et al., 2002; Ruotsalainen et al., 2003; Missaghi and Fassihi, 2004) to investigate drug release mechanisms from modified release dosage forms (Cutts et al., 1996; Lamprecht et al., 2000b; Guo et al., 2002; Liu et al., 2009) and to analyze the polymer distribution in microcapsules (Lamprecht et al., 2000a,c, 2003). Protein encapsulation into microparticles has been investigated (Lamprecht et al., 2000b; Yang et al., 2001; Wischke et al., 2006), but only one study has looked into the distribution of a small molecule in biodegradable PLGA microspheres (Berkland et al., 2003). In this latter study, although different drug distributions were demonstrated, the long degradation time of PLGA (~2 weeks) means that the effects of these were difficult to elucidate. However, no previous work has been reported on the distribution of small molecules within microspheres designed for oral drug delivery. Previous research by our group has resulted in a novel method for the preparation of uniform and robust enteric microparticles (Kendall et al., 2009; Nilkumhang and Basit, 2009) using acrylic (methacrylate) polymers. These particles showed an excellent controlled release of prednisolone in acidic conditions, and rapid drug release when the pH was raised above the pH dissolution thresholds of the respective polymers. They also demonstrated excellent morphology and narrow size distribution. Here we fabricate these uniform polymeric enteric microparticles from Eudragit L polymer with three different fluorescent compounds (as model drugs). The effect of the differing physicochemical properties of the fluorescent compounds on their distribution patterns within the microparticles was studied using CLSM and the distribution was correlated with encapsulation efficiency and *in vitro* drug release.

2. Materials and methods

2.1. Materials

Eudragit L was obtained from Evonik (Darmstadt, Germany). Dipyridamole, riboflavin and acridine orange were purchased from Sigma–Aldrich (Poole, UK). All other chemicals were of analytical grade.

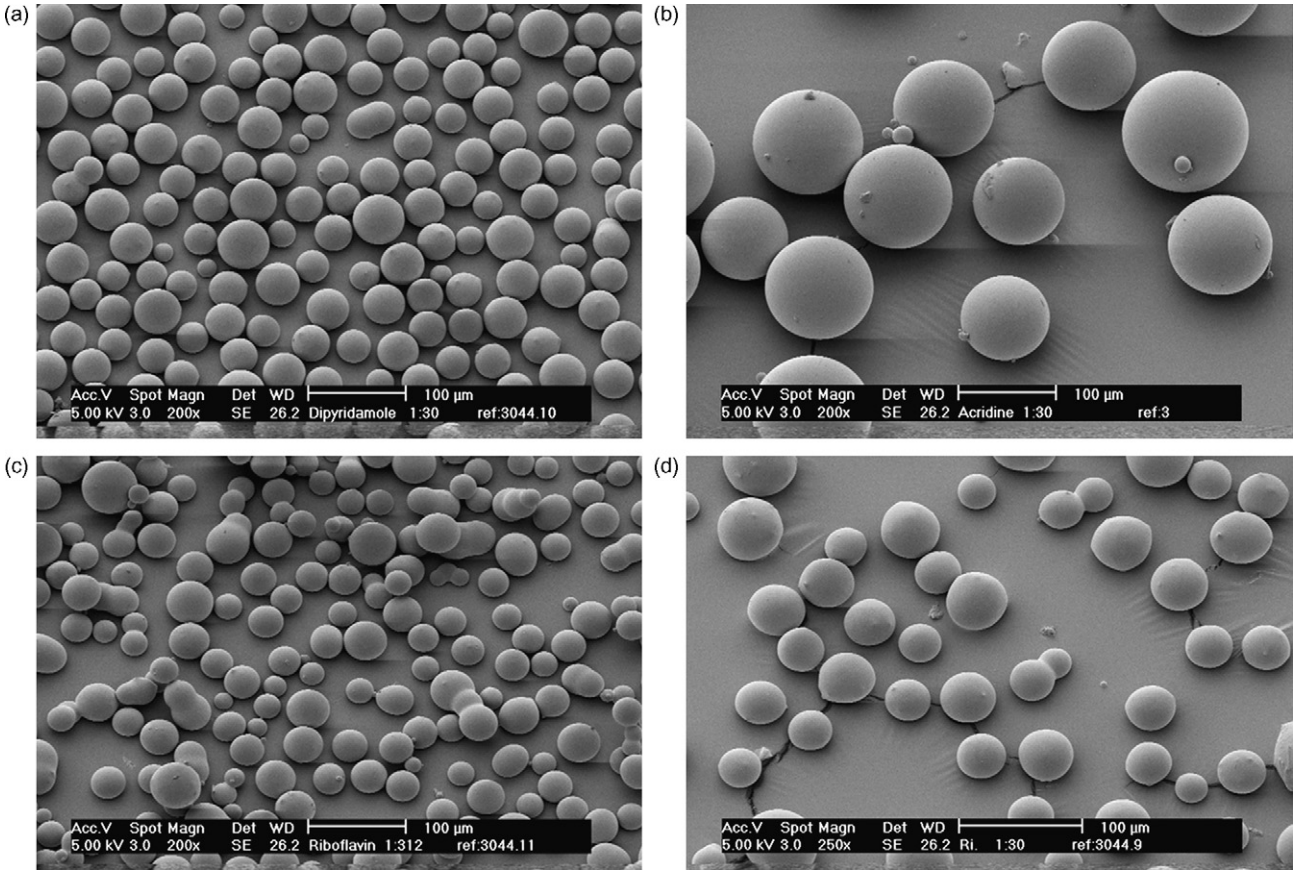

Fig. 1. SEM images of (a) dipyridamole, (b) acridine orange, (c) riboflavin (solution) and (d) riboflavin (suspension) Eudragit L microparticles.

Table 2
Summary of characteristics of the Eudragit L microparticles.

Drug	Polymer to drug ratio	Encapsulation efficiency (%)	Size		Batch yield (%)
			Dv50 (μm)	Span	
Dipyridamole	30:1	77.5 ± 0.3	47.8 ± 0.8	0.6	89.5 ± 1.1
Acridine orange	30:1	50.2 ± 1.5	82.8 ± 3.6	1.1	89.8 ± 1.7
Riboflavin (suspension)	30:1	79.6 ± 0.5	31.8 ± 0.3	1.1	90.3 ± 0.9
Riboflavin (solution)	312:1	85.6 ± 2.6	50.9 ± 2.3	1.4	91.3 ± 1.5

2.2. Determination of saturation solubility and the apparent partition coefficient (ethanol/liquid paraffin)

In a darkroom, excess quantities of the fluorescent drugs were added to 10 ml of different solvents (ethanol, hydrochloric acid (HCl) 0.1N and phosphate buffer pH 6.8). The vials were agitated in a horizontal shaker at room temperature for 24 h. The contents of the vials were filtered through 0.22 μm disposable filter and then the filtrate was diluted with the appropriate solvent. The UV absorbance of dipyridamole, riboflavin and acridine orange filtrate were read at wavelengths of 260, 270 and 270 nm respectively in ethanol and 282, 266, and 268 nm respectively in aqueous media and the saturation concentration was calculated with reference to a standard curve.

The apparent partition coefficient (K) of the ethanol/liquid paraffin system was studied by the shake-flask method. Prior to the start of the experiment, both phases (ethanol and liquid paraffin) were pre-saturated with each other; ethanol was equilibrated with an excess of liquid paraffin and left overnight at room temperature and vice versa. In a darkroom, the drug stock ethanolic solution (3 ml) was added to 33 ml of liquid paraffin and left shaken at room temperature for 18 h, simulating the duration that the drug would be in the emulsification process when making microparticles. The experiment was repeated with three different drug concentrations. The mixture was then centrifuged at 2500 rpm for 30 min to separate the two phases. The ethanol phase was then analyzed by UV spectrophotometry for drug concentration. The ethanol/liquid paraffin apparent partition coefficient ($K_{\text{ethanol/liquid paraffin}}$) was calculated using Eq. (1).

$$K_{\text{ethanol/liquid paraffin}} = \frac{\text{Concentration of solute in ethanol}}{\text{Concentration of solute in liquid paraffin}} = \frac{W_{\text{ethanol}}/V_{\text{ethanol}}}{(W - W_{\text{ethanol}})/V_{\text{liquid paraffin}}} \quad (1)$$

where W is the weight of the initial solute in stock solution (3 ml), W_{ethanol} is the weight of the solute remaining in ethanol at equilibrium, V_{ethanol} and $V_{\text{liquid paraffin}}$ are volumes of ethanol and

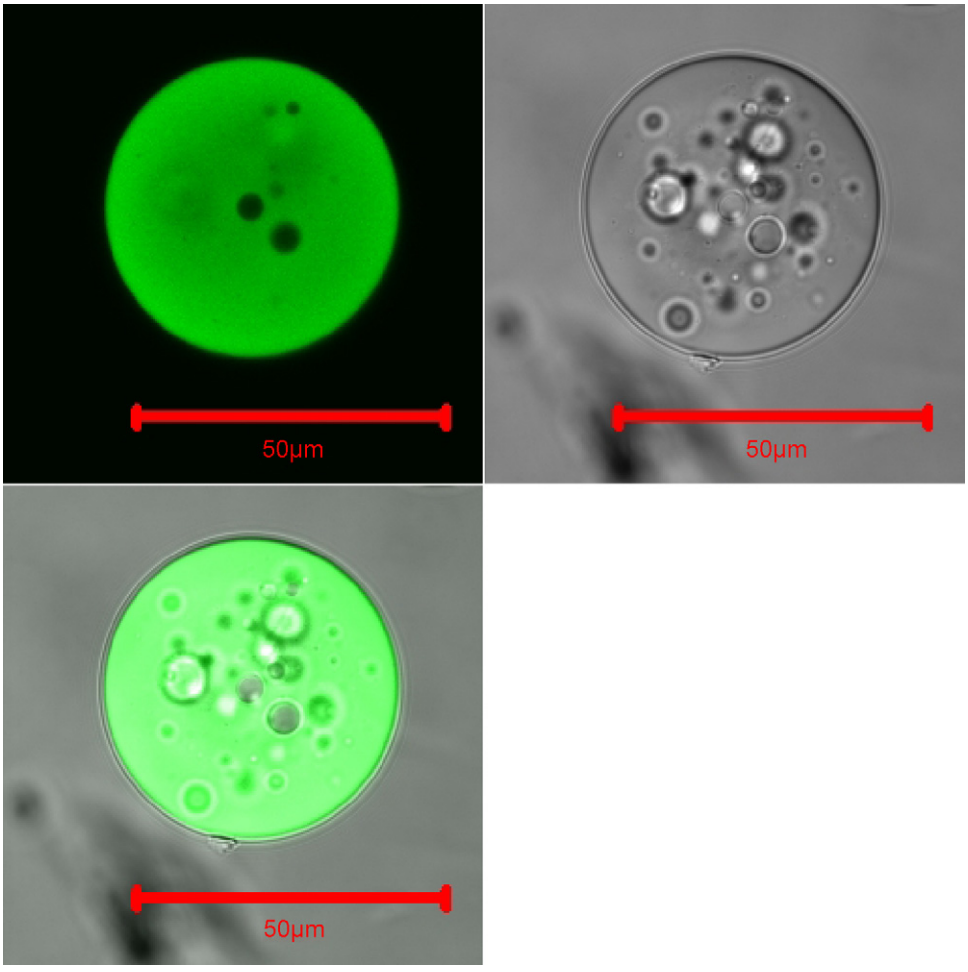

Fig. 2. Dipyridamole loaded microparticles: CLSM image (top left), transmitted light image (top right), overlaid CLSM and transmitted light image (bottom left).

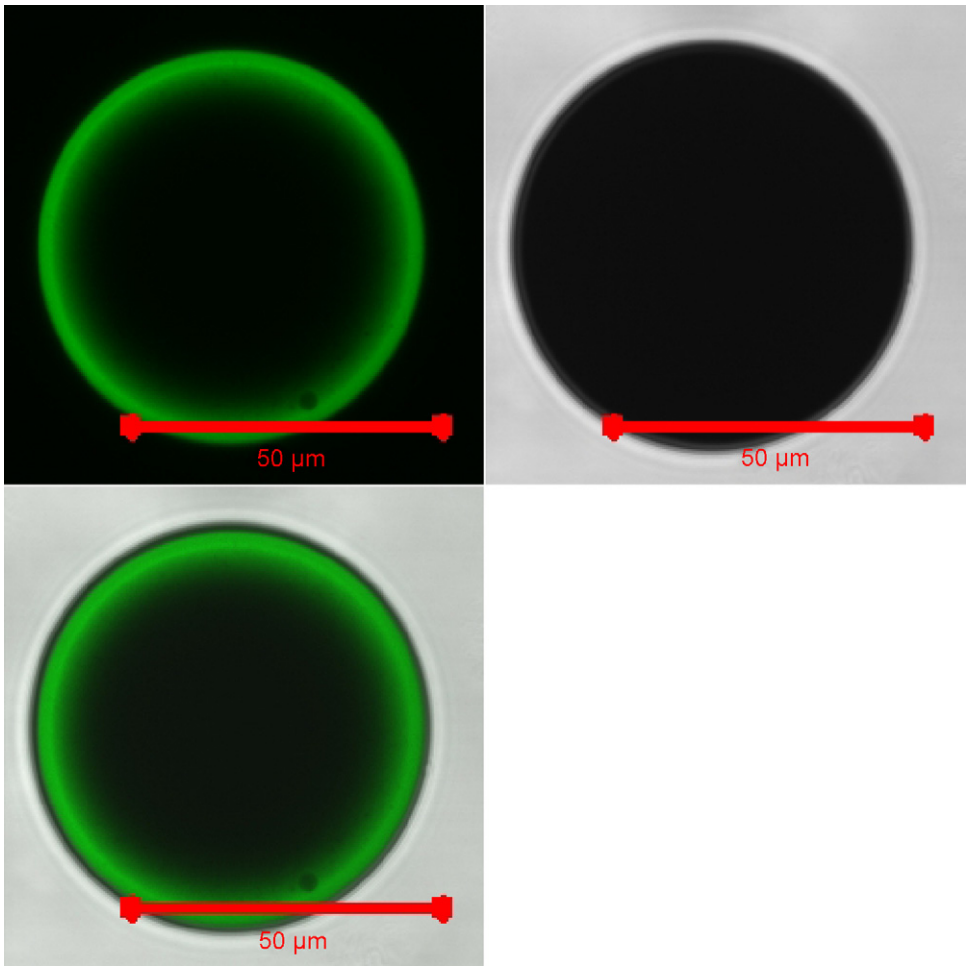

Fig. 3. Acridine orange loaded microparticles: CLSM image (top left), transmitted light image (top right), overlaid CLSM and transmitted light image (bottom left).

liquid paraffin, respectively. The logarithm of $K_{\text{ethanol/liquid paraffin}}$ was used as an indicator of drug partition in the emulsion system.

2.3. Preparation of microparticles

Microparticles were prepared using a novel emulsion solvent evaporation method (Kendall et al., 2009). Three grams of Eudragit L and 100 mg of dipyridamole, riboflavin or acridine orange were added to ethanol (30 ml) at polymer to drug ratios of 30:1 (polymer solution concentration 10%, w/v). At this drug loading dipyridamole and acridine orange were soluble in the ethanol and riboflavin was in suspension. Therefore riboflavin was additionally investigated at a polymer to drug ratio of 312:1 at which level riboflavin was solubilised in ethanol (polymer solution concentration of 3%, w/v). It was established through previous work that reducing the initial polymer concentration did not impact on microparticle encapsulation efficiency and release properties (Nilkumhang and Basit, 2009). The drug/polymer solutions/suspension was emulsified into liquid paraffin (165 g) containing 1% (w/w) sorbitan sesquioleate (Arlacel 83) as emulsifier under agitation. The emulsion was stirred for 18 h. 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 (room temperature, 1000 mBar). All the batches of microparticles were prepared in triplicate. Microparticle preparation was undertaken in a darkroom to protect the fluorescent compounds from light, and the microparticles were stored in light proof containers.

2.4. Characterization of microparticle size, morphology, encapsulation efficiency and batch yield

The microparticle size was measured by laser diffraction using a Mastersizer S (Malvern Instruments Ltd., Worcestershire, UK) after suspending the microparticles in 0.1 M HCl. The measurements were conducted in triplicate. The average size of microparticles was expressed as a median diameter (Dv50), which is a particle diameter at 50% cumulative volume. The morphology and surface topography of the microparticles were examined by scanning electron microscopy (SEM). Samples were adhered to SEM stubs using carbon discs, and were gold coated, using an EMITEC K550 sputter coater for 3 min at 40 mA. After coating, the samples were transferred to a Philips XL20 Scanning Electron Microscope for imaging.

The encapsulation efficiency was determined by dissolving 40 mg of microparticles in 100 ml methanol in a darkroom; followed by the addition of 0.1 M HCl to 10 ml of the methanolic solution to precipitate the pH sensitive polymer and made up to 100 ml. Samples were filtered through 0.22 μm disposable filters and the drug content was analysed by UV spectrophotometry as previously stated. The experiment was carried out in triplicate for each sample. Drug encapsulation efficiency is calculated from Eq. (2).

$$\text{Encapsulation efficiency} = \frac{\text{Calculated mass of drug compound in microparticles}}{\text{Theoretical mass of drug compound in microparticles}} \times 100 \quad (2)$$

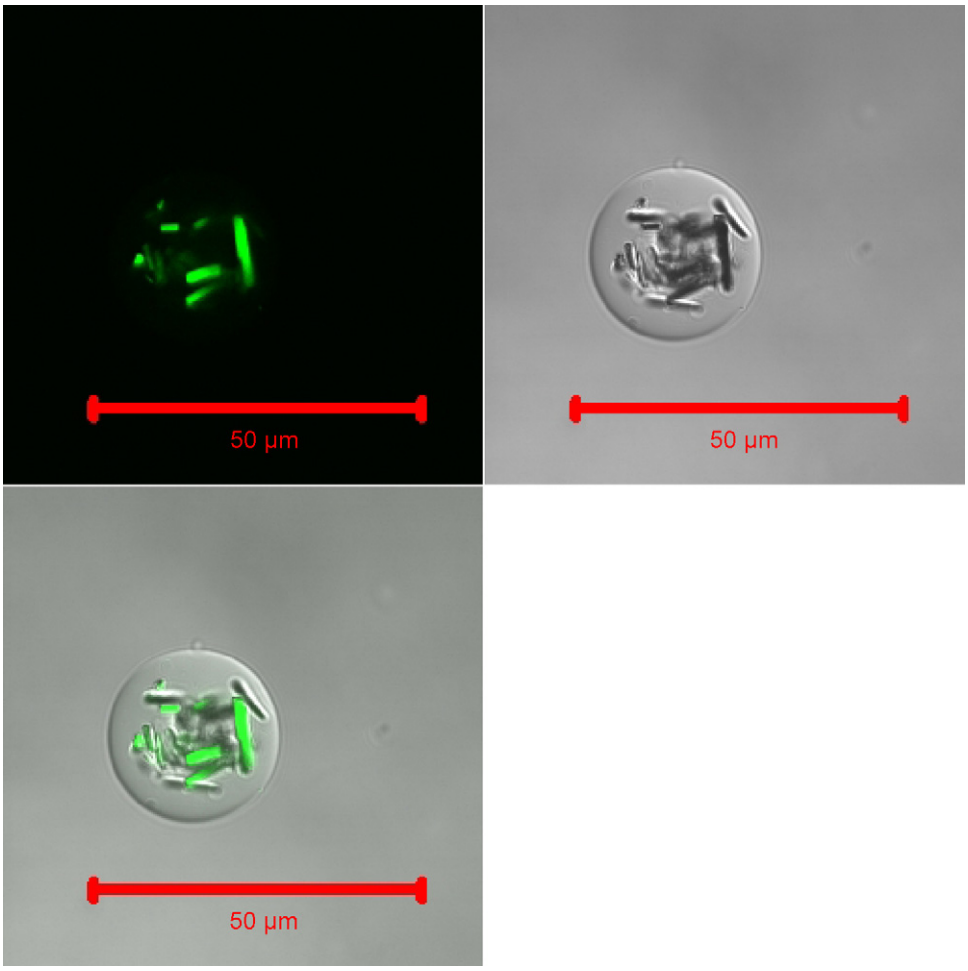

Fig. 4. Riboflavin suspension loaded microparticles: CLSM image (top left), transmitted light image (top right), overlaid CLSM and transmitted light image (bottom left).

The yield of microparticles for each batch was calculated using Eq. (3).

$$\%\,\text{Batch yield} = \frac{\text{Mass of harvested microparticles}}{\text{Original mass of polymer} + \text{original mass of drug}} \times 100 \quad (3)$$

2.5. In vitro drug release studies

Drug release studies were carried out under sink conditions using a pH change method and USP II (paddle) apparatus (Pharmatest PTWS3C Dissolution Bath, Hainburg, Germany) with Icalis dissolution software to evaluate release at gastric and intestinal pH. The dissolution baths were covered in aluminium foil to protect the fluorescent compounds from light degradation during the experiment. Microparticles were filled into a size 0 gelatin capsule. 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 ± 0.5 °C, was added to each vessel and the pH was adjusted to 6.8 ± 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 the UV absorbance was read as previously stated. The experiments were carried out in triplicate for each formulation.

2.6. Confocal laser scanning microscopy

The fluorescent compound distribution within the microparticles was investigated using a Zeiss LSM 510 Meta laser scanning confocal microscope, equipped with an argon laser. An exciting wavelength of 488 nm was employed and confocal images were taken with a 63× objective. The iris, amplifier offset, detector gain control and all other settings were kept constant during all experiments. The microparticles were placed onto a glass slide, and the images were captured. Combining the green fluorescence channel with the transmitted light channel allows observation of the internal structure of the microparticles.

3. Results

The parameters of solubility and partition coefficient (in ethanol/paraffin) for dipyridamole, acridine orange and riboflavin were calculated, and are shown in Table 1, along with literature values for molecular weight, pKa and partition coefficient in octanol/water.

Uniform, spherical microparticles were successfully produced from all three compounds using the emulsion solvent evaporation process (Fig. 1). A summary of their characteristics is found in Table 2. It can be seen that the microparticles generally had a high encapsulation efficiency (>77%) apart from acridine orange which had only 50% of the theoretical drug loading encapsulated in the microparticles. The particle sizes varied according to which drug was encapsulated; acridine orange

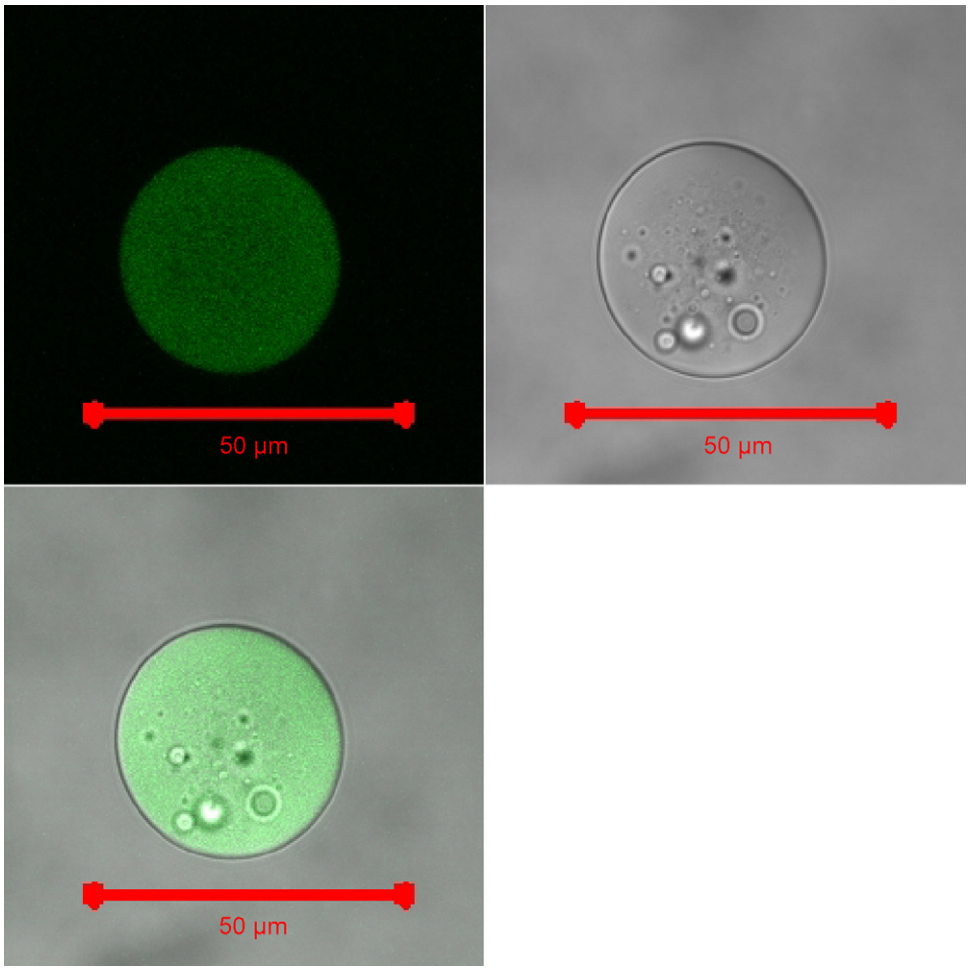

Fig. 5. Riboflavin solution loaded microparticles: CLSM image (top left), transmitted light image (top right), overlaid CLSM and transmitted light image (bottom left).

particles having the largest median diameter and riboflavin having the smallest. The yields were generally high at around 90%.

The distribution of the fluorescent compounds in the Eudragit L microparticles is shown in Figs. 2–5. Dipyridamole is evenly distributed throughout the polymer matrix and no drug crystals are evident (Fig. 2). Acridine orange shows a distribution towards the surface of the polymeric microparticle, with the microparticle core being devoid of any of the fluorescent compound (Fig. 3). Microparticles loaded with riboflavin suspension (polymer:drug 30:1) show obvious crystals of the fluorescent molecule which are located at the centre of the microparticle (Fig. 4), while microparticles loaded with riboflavin solution (polymer:drug 312:1) show an even distribution throughout the polymer matrix (Fig. 5).

The release of the fluorescent compounds in a pH-change dissolution test is shown in Fig. 6. Under acidic conditions the release of dipyridamole, acridine orange, riboflavin (suspension) and riboflavin (solution) are 3.6%, 9.9%, 3.3% and 5.5% of drug content respectively. When the pH is increased to pH 6.8 rapid release of the compounds was seen. In the case of dipyridamole and acridine orange most of the compound is released in the first minute after the pH change. The release of riboflavin from the Eudragit L microparticles prepared with the suspension is slower, with around 50% being released in the first minute, and thereafter being released gradually over the next 3 h. Interestingly, no apparent relationship between drug distribution within the microparticle and *in vitro* release pattern was found.

4. Discussion

The drug-loaded microparticles are similar to those reported previously with prednisolone (Kendall et al., 2009), confirming the universal nature of this emulsion solvent evaporation method.

The encapsulation efficiency of riboflavin and dipyridamole were approaching 80%. Acridine orange had poor encapsulation efficiency. There is a link between the encapsulation efficiency and the partition of the drug [acridine orange, dipyridamole

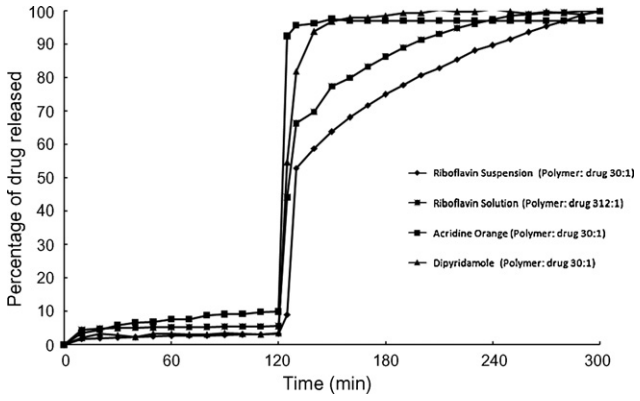

Fig. 6. *In vitro* release of riboflavin (suspension), riboflavin (solution), acridine orange, and dipyridamole from Eudragit L microparticles at pH 1.2 for 2 h followed by pH 6.8.

and riboflavin (solution)] between ethanol and liquid paraffin ($R^2 = 0.952$) or ethanol solubility ($R^2 = 0.996$). It is interesting to highlight that a poor correlation ($R^2 = 0.591$) was observed between encapsulation efficiency and octanol water partition coefficient ($P_{o/w}$), a commonly used indicator of drug lipophilicity. Consequently, the partition coefficient of the drug between the internal and external phase can be considered as a more reliable indicator of drug encapsulation efficiency in the microencapsulation process. Upon emulsification the drug and polymer solution form discrete droplets through which the ethanol diffuses to liquid paraffin (external phase) allowing the microparticles to harden. As this occurs, the drug will partition to its preferred phase. If the preferred phase is liquid paraffin then the encapsulation efficiencies are expected to be low (Bodmeier and McGinity, 1987). Acridine orange had the lowest affinity for the ethanolic phase and as such demonstrated the lowest encapsulation efficiency. The riboflavin (solution) is efficiently encapsulated inside the microparticles; this compound had both low partition into the external phase and had poor solubility in ethanol which meant it could not diffuse across into the external phase.

The effects of partition and solubility are also apparent in the drug distribution throughout the polymer matrix. When microparticles were loaded with dipyridamole there was a distribution throughout the polymeric matrix, with a higher intensity towards the surface, and the fairly homogeneous distribution is a reflection of the good solubility in ethanol. Riboflavin, in contrast to dipyridamole, was preferentially located in the inner core of the particles in the form of large crystals (Fig. 4). As the solubility of riboflavin in ethanol was very low (0.107 mg/ml), during the preparation only a small amount of riboflavin was dissolved in ethanol and a large amount of non-dissolved riboflavin was suspended in the ethanol and was encapsulated as such. Using a polymer to drug ratio of 312:1 the distribution of dissolved riboflavin can be seen. There is an absence of crystals confirming that those crystals seen at the higher drug concentration (30:1) were due to un-dissolved riboflavin, and not recrystallization from solution.

The distribution of acridine orange was distinct from the other molecules. Acridine orange was found to be preferentially distributed on the surface (Fig. 3) which was due to the migration of the lipophilic drug towards the liquid paraffin during the solvent evaporation process.

It was anticipated that the distribution of the fluorescent compounds within the microparticle would have an influence on the release profile in acid and buffer. Interestingly, release from all microparticles was still within the USP specifications for a delayed release product in acid (<10% of drug content). Drug release can also be affected by the size of the microparticles and solubility of the compound in the dissolution media. For example, despite high dipyridamole and acridine orange solubility in acid (Table 1), the release of both drugs from the microparticles were limited after 2 h in the gastric medium (Fig. 6). The larger size of acridine orange loaded microparticles compared to the other molecules' microparticles seems to have little influence on restricting drug release from the microspheres. This can be partially attributed to the peripheral distribution of the acridine orange within the microparticles. Riboflavin microparticles prepared from suspension had a slower drug release rate than those prepared from a solution (Fig. 6). It is thought that this is due to the extra dissolution step; following the dissolution of the polymer there needs solubilisation of the un-dissolved riboflavin crystals.

In summary, although drug solubility in the inner phase and partition between the internal and external oil phases in the emulsion are important factors influencing drug encapsulation and distribution within the microparticles, they do not adversely affect the ability of the microparticles to provide pH-responsive drug release.

5. Conclusion

Confocal laser scanning microscopy was instrumental in investigating the internal structures of the microparticles and the distribution of different fluorescent compounds. The physicochemical properties of the model drugs had an influence on the encapsulation efficiency and distribution within the microparticles. The compounds that are preferentially localised in the ethanol phase rather than the liquid paraffin were efficiently encapsulated inside the microparticle (riboflavin). In contrast, drugs that partitioned in favour of the liquid paraffin phase were more likely to be localised towards the surface of the microparticles and exhibited lower encapsulation efficiency (dipyridamole and acridine orange). Despite their different distribution patterns within the microparticles and solubilities in the acidic medium, the release of the three molecules was well-controlled in the acidic medium; drug distribution appeared to have a minimum effect on drug release. This highlighted the efficiency of this micron-size system of controlling drug release and requires further investigations about the determining factors that directly influence drug release.

References

Alex, R., Bodmeier, R., 1990. Encapsulation of water-soluble drugs by a modified solvent evaporation method. I. Effect of process and formulation variables on drug entrapment. J. Microencapsul. 7, 347–355.

Arshady, R., 1991. Preparation of biodegradable microspheres and microcapsules. 2. Polyactides and related polyesters. J. Control. Release 17, 1–22.

Berkland, C., Kim, K., Pack, D.W., 2003. PLG microsphere size controls drug release rate through several competing factors. Pharm. Res. 20, 1055–1062.

Bodmeier, R., McGinity, J.W., 1987. Poly(lactic acid) microcapsules containing quinidine base and quinidine sulfate prepared by solvent evaporation technique. J. Microencapsul. 4, 279–288.

Cutts, L.S., Hibberd, S., Adler, J., Davies, M.C., Melia, C.D., 1996. Characterising drug release processes within controlled release dosage forms using the confocal laser scanning microscope. J. Control. Release 42, 115–124.

Guo, H.X., Heinamaki, J., Yliruusi, J., 2002. Diffusion of a freely water-soluble drug in aqueous enteric-coated pellets. AAPS PharmSciTech 3, E16.

Kendall, R., Alhnan, M.A., Nilkumhang, S., Murdan, S., Basit, A.W., 2009. Fabrication and in vivo evaluation of highly pH-responsive acrylic microparticles for targeted gastrointestinal delivery. Eur. J. Pharm. Sci. 37, 284–290.

Lamprecht, A., Schafer, U.F., Lehr, C., 2000a. Characterization of microcapsules by confocal laser scanning microscopy: structure, capsule wall composition and encapsulation rate. Eur. J. Pharm. Biopharm. 49, 1–9.

Lamprecht, A., Schafer, U.F., Lehr, C.M., 2000b. Structural analysis of microparticles by confocal laser scanning microscopy. AAPS PharmSciTech 1, E17.

Lamprecht, A., Schafer, U.F., Lehr, C.M., 2000c. Visualization and quantification of polymer distribution in microcapsules by confocal laser scanning microscopy (CLSM). Int. J. Pharm. 196, 223–226.

Lamprecht, A., Yamamoto, H., Takeuchi, H., Kawashima, Y., 2003. Microsphere design for the colonic delivery of 5-fluorouracil. J. Control. Release 90, 313–322.

Liu, F., Lizio, R., Schneider, U.J., Petereit, H.U., Blakey, P., Basit, A.W., 2009. SEM/EDX and confocal microscopy analysis of novel and conventional enteric-coated systems. Int. J. Pharm. 369, 72–78.

Matsumoto, A., Matsukawa, Y., Suzuki, T., Yoshino, H., Kobayashi, M., 1997. The polymer-alloys method as a new preparation method of biodegradable microspheres: principle and application to cisplatin-loaded microspheres. J. Control. Release 48, 19–27.

Missaghi, S., Fassihi, R., 2004. A novel approach in the assessment of polymeric film formation and film adhesion on different pharmaceutical solid substrates. AAPS PharmSciTech 5, 1–8.

Nilkumhang, S., Basit, A.W., 2009. The robustness and flexibility of an emulsion solvent evaporation method to prepare pH-responsive microparticles. Int. J. Pharm. 337, 135–141.

Perumal, D., 2001. Microencapsulation of ibuprofen and Eudragit RS 100 by the emulsion solvent diffusion technique. Int. J. Pharm. 218, 1–11.

Prasad, V., Semwogerere, D., Weeks, E.R., 2007. Confocal microscopy of colloids. J. Phys.:Condensed Matter 19, 1–25.

Ruotsalainen, M., Heinamaki, J., Guo, H., Laitinen, N., Yliruusi, J., 2003. A novel technique for imaging film coating defects in the film-core interface and surface of coated tablets. Eur. J. Pharm. Biopharm. 56, 381–388.

Shukla, A.J., Price, J.C., 1991. Effect of drug loading and molecular weight of cellulose acetate propionate on the release characteristics of theophylline microspheres. Pharm. Res. 8, 1396–1400.

Watts, P.J., Barrow, L., Steed, K.P., Wilson, C.G., Spiller, R.C., Melia, C.D., Davies, M.C., 1992. The transit rate of different-sized model dosage forms through the human colon and the effects of a lactulose-induced catharsis. Int. J. Pharm. 87, 215–221.

Wischke, C., Lorenzen, D., Zimmermann, J., Borchert, H.H., 2006. Preparation of protein loaded poly(D,L-lactide-co-glycolide) microparticles for the antigen delivery to dendritic cells using a static micromixer. Eur. J. Pharm. Biopharm. 62, 247–253.

Yan, C., Resau, J.H., Hewetson, J., West, M., Rill, W.L., Kende, M., 1994. Characterization and morphological analysis of protein-loaded poly(lactide-co-glycolide) microparticles prepared by water-in-oil-in-water emulsion technique. J. Control. Release 32, 231–241.

Yang, Y.Y., Chung, T.S., Ng, N.P., 2001. Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. Biomaterials 22, 231–241.