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SHORT COMMUNICATION

Monitoring model drug microencapsulation in PLGA scaffolds using X-ray powder diffraction

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KEYWORDS

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Abstract The microencapsulation of three model drugs; metronidazole, paracetamol and sulphapyridine into Poly (DL-Lactide-Co-Glycolide) (PLGA) scaffolds were probed using X-ray Powder Diffraction (XRPD). Changes in the diffraction patterns of the PLGA scaffolds after encapsulation was suggestive of a chemical interaction between the pure drugs and the scaffolds and not a physical intermixture.

1. Introduction

Following tremendous advancement in pharmaceutical and medical research/technology, the use of biodegradable materials/polymers such as Poly (DL-Lactide-Co-Glycolide) (PLGA) nanoparticles/scaffolds/microparticles has become increasingly essential as a drug delivery system.

Examples include, inclusion of pharmaceutical excipient into polymeric matrices e.g. polymeric films, microsphere and nanospheres (Bibby et al., 2000), the use of nanoparticles containing therapeutic agents for site-targeted drug delivery and optimisation of drug treatment effects (Barzegar-Jalali et al., 2012, 2008; Hornig et al., 2009; Lee, 2004) and use of an electrospun Active Pharmaceutical Ingredient (API)-loaded resorbable Poly Lactide (PLA) fibre for local periodontitis treatment (Markus et al., 2012; Jandt and Sigusch, 2009; Balamurugan et al., 2008), etc.

PLGA has added advantages over other natural and synthetic polymers; it is biocompatible, it can be used to synthesise nano/micro-particles with tuneable physico-chemical properties and is an FDA (United States Food and Drug Administration) approved polymer (Imbuluzqueta et al., 2011; Ravi et al., 2008; Anderson and Shive, 1997.).

It not only increases the sustained release but also guards against enzymatic degradation of drugs. (Sabzevari et al., 2013; Tahara et al., 2011; Javadzadeh et al., 2010).

The relevance of the characterisation of the interaction between polymer and drug(s) cannot be over emphasised

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because of its denotations on formulation outcomes, product integrity, drug-release kinetics as well as probable polymorphic transitions. (Singh and Kim, 2007; Hsiue et al., 1998; Jansen et al., 1998; Katzhendler et al., 1998; Lorenzo-Lamosa et al., 1998; Puttipipatkhachorn et al., 2001; Wong et al., 2002; Takka, 2003).

This is particularly important in the pharmaceutical drug development process where a drug product with optimal characteristics must be identified in the early stages in order to prevent unwanted 'surprises' in the later stages of development or after the drug has been approved for usage. (Hilfiker, 2006; Preface, 2004).

The type of formulations used during the various stages of drug development also determines the final properties of the drug product: for example when suspensions are used, particle size must be controlled as this can affect bioavailability. (Pirttimäki et al., 1993; Jain and Banga, 2010).

The purpose of this study is to analyse the nature of the interaction (during microencapsulation) between three model drugs; metronidazole, paracetamol, sulphapyridine (shown in Fig. 1a–c respectively overleaf) and PLGA scaffolds using X-ray Powder Diffraction (XRPD).

XRPD was particularly chosen to monitor the encapsulation because of the superiority of the technique in differentiating between the structural properties of different materials. (Justin, 1999; Cullity, 1995; Karjalainen et al., 2005).

More so all three drugs used in the study have reported crystal structures (Ramukutty and Ramachandran, 2012; Burley et al., 2007; Gharaibeh and Al-Ard, 2011; Basak et al., 1848; Bar and Bernstein, 1985; Bernstein, 1988; Kirby et al., 2011) while the PLGA scaffold is amorphous thus making it easier to compare any changes that may arise in the X-ray patterns after encapsulation with already published data.

Scanning Electron Microscopy (SEM) was also used to probe the physical morphologies of the PLGA scaffolds before and after encapsulations in order to corroborate data from the XRPD studies.

2. Materials and methods

PVA (fully hydrolysed) and sulphapyridine were purchased from Sigma Aldrich Co. 3050 Spruce Street, St. Louis, MO 63103 USA. PLGA was supplied by Evonik Degussa Corporation, 750 Lakeshore parkway, Birmingham, AL. 35211 USA. Metronidazole was procured from Nacalai Tesque Inc. Kyoto Japan; Paracetamol from R & M Marketing Essex, UK. HPLC grade Dichloromethane (DCM) was obtained from Fisher Scientific, Bishop Meadow road, Loughborough, UK, LE11 5RG. Distilled water was produced for this study using ELGA Purelab flex (Chemopharm Selangor, Malaysia), while Phosphate Buffered Saline (PBS, (pH 7.4)) tablets were supplied by Oxoid limited, Basingstoke, Hampshire, UK.

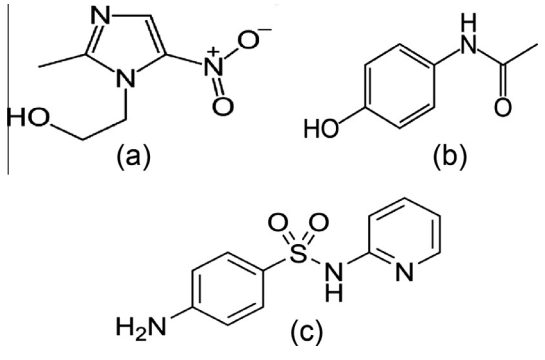

Figure 1 Molecular structures of metronidazole (a), paracetamol (b) and sulphapyridine (c).

2.1. PLGA scaffolds preparation with drug encapsulation

The PLGA scaffolds incorporated with the model drugs were prepared using the water in oil in water (w/o/w) emulsion technique (Kirby et al., 2011). 1 g of PLGA was weigh and dissolved in 5 ml of DCM, to this was added 250 µl of PBS solution (the model drugs were dissolved in separate 100 ml solutions of PBS at the following concentrations; metronidazole 0.5007% w/v, paracetamol 0.1409% w/v and sulphapyridine 0.09425 w/v according to their reported aqueous solubilities) (Gouda et al., 1977; Jensen et al., 1990; Ribeiro et al., 2012). The resulting mixture was homogenised (Silverson L5M-A) at 9000 rpm for 2 min. The new PLGA/DCM/PBS (plus model drug) mixture was then added to 200 ml of PVA solution which was then homogenised at 3000 rpm for 2 min. The double emulsion was then stirred for 2 min at 300 rpm and the microparticles formed were washed under a continuous flow of water in a sieve (Fisher test sieve number 230) and freeze dried.

2.2. XRPD analysis

XRPD patterns were collected on a Bruker D8 Advance diffractometer system (Bruker AXS, Madison, WI, USA), operating in a Bragg–Brentano geometry, at a wavelength (λ) of 1.54059 Å, 40 kV voltage, and a current of 40 mA (with a Cu $K\alpha_1$ radiation source), using a LynxEye detector, samples were contained in flat Poly (methyl methacrylate) (PMMA) sample holder. Data acquisition was done at 0.025°/0.1 s step size over a total period of 5 min.

2.3. Scanning Electron Microscopy (SEM)

SEM images were obtained using the LEO 1450 (LEO Company LTD UK) variable pressure scanning electron microscope, at an accelerated voltage of 10 kV.

3. Results and discussion

Figs. 2a–2c show overlay plots of the XRPD patterns obtained before and after PLGA scaffold-drug encapsulation with metronidazole, paracetamol and sulphapyridine respectively.

Under ordinary visual inspection of Figs. 2a–2c, the changes in the XRPD patterns of the PLGA scaffolds are not obvious; but on closer inspection, weak peaks (*) are evident on the X-ray patterns of the scaffolds after encapsulation even at the extremely low drug concentration used as shown in Figs. 3a–3c (black = PLGA scaffolds before encapsulation, green = PLGA scaffolds after encapsulation, in all cases); indicative of an interaction between the drugs and scaffolds.

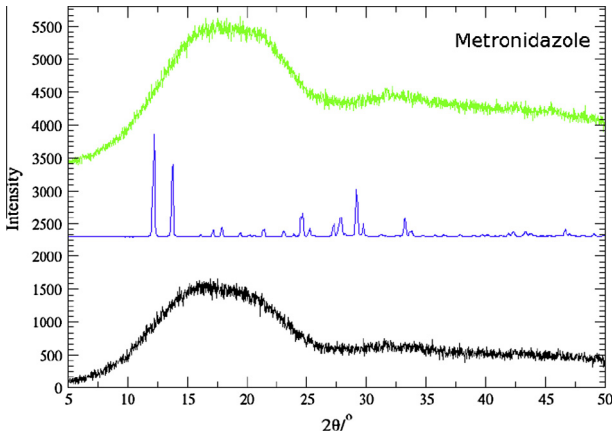

Figure 2a XRPD patterns of PLGA scaffolds before (black), after encapsulation (green) and pure metronidazole (blue).

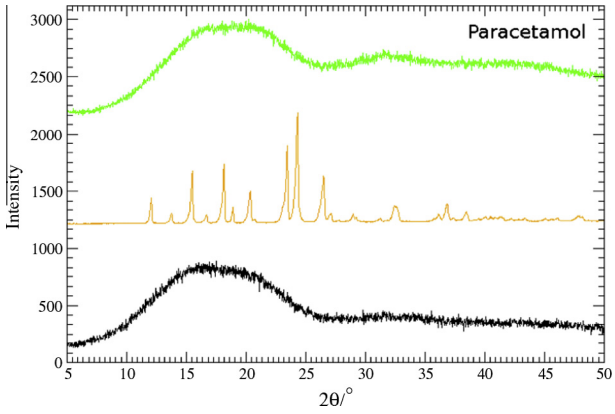

Figure 2b XRPD patterns of PLGA scaffolds before (black), after (green) encapsulation and pure paracetamol (orange).

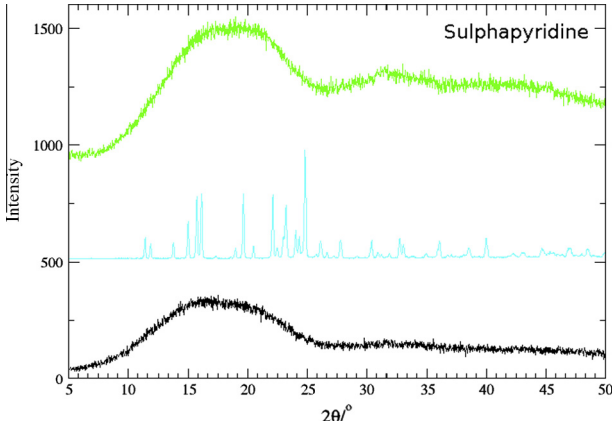

Figure 2c XRPD patterns of PLGA scaffolds before (black), after (green) encapsulation and pure sulphapyridine (cyan).

While the appearance of new (weak) peaks is apparent in the encapsulated scaffolds as shown in Figs. 3a–3c, these peaks do not occur in regions where the pure drugs exhibit characteristic peaks i.e. 12.15°, 13.80° (metronidazole), 23.40°, 24.34° (paracetamol) and 19.59°, 24.78° (sulphapyridine).

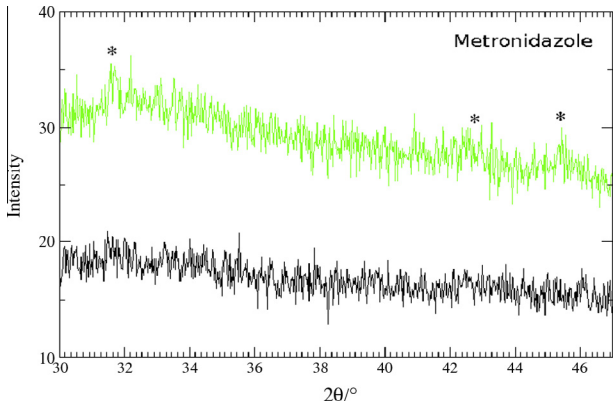

Figure 3a XRPD offset patterns of metronidazole encapsulation.

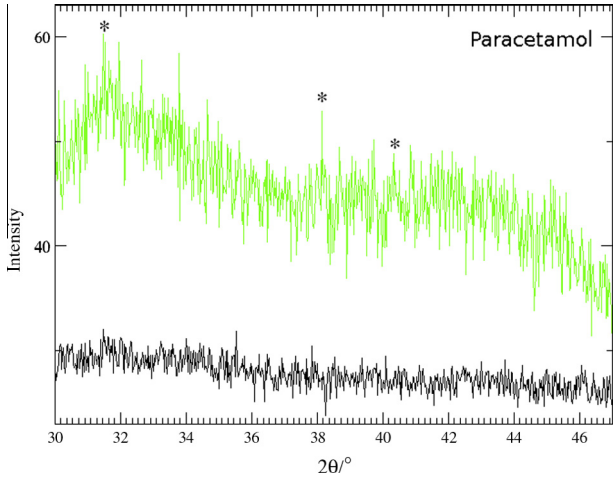

Figure 3b XRPD offset patterns for paracetamol encapsulation.

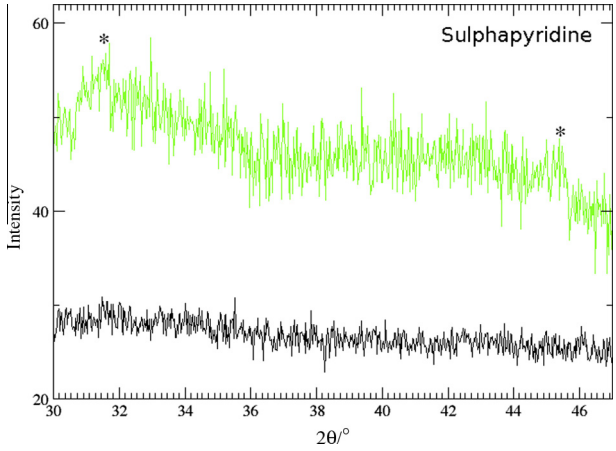

Figure 3c XRPD offset patterns for sulphapyridine encapsulation.

As such, these said peaks cannot be attributed to a physical interaction between the drugs and the scaffolds. (Gouda et al., 1977; Jensen et al., 1990; Ribeiro et al., 2012; Guns et al., 2010; Lehto et al., 2006; Tita et al., 2011).

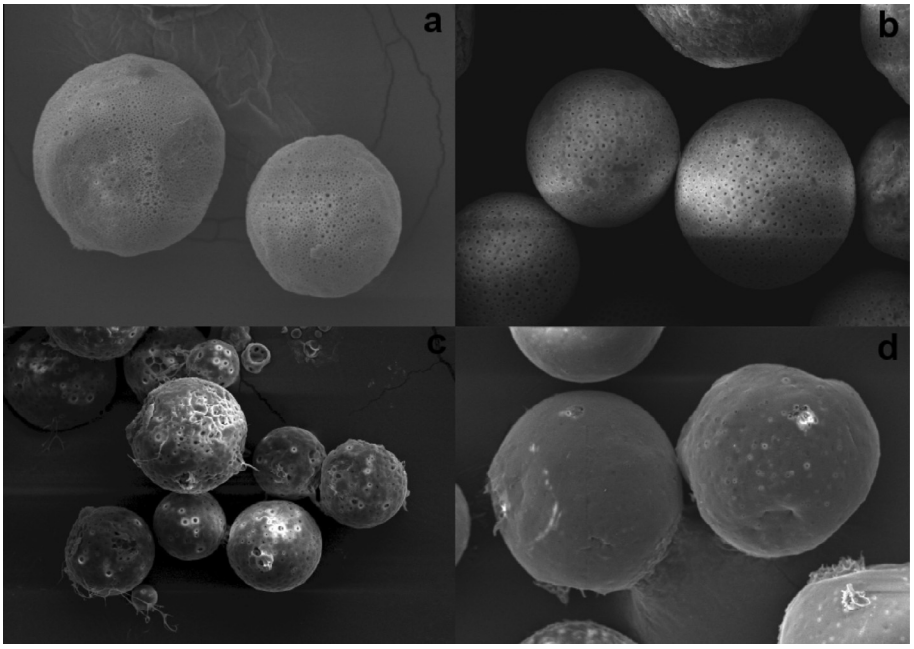

Figure 4 SEM images of PLGA scaffold before encapsulation (a) and after encapsulation with (b) metronidazole (c) paracetamol and (d) sulphapyridine (scale and magnification were 10 μm and 500× in all cases respectively).

Similarly, Fig. 4a–d below show the SEM images of the PLGA scaffolds before and after encapsulation with the model drugs used in this study.

Fig. 4 above clearly shows that there is an interaction between the PLGA scaffold and the model drugs as the scaffolds are markedly different before and after encapsulation.

In conclusion, it has been shown that metronidazole, paracetamol and sulphapyridine can be encapsulated into PLGA scaffolds even at low concentration as permitted by their aqueous solubilities. XRPD has proven to be a veritable tool for monitoring the observed encapsulation, although the appearance of new/weaker peaks in the X-ray patterns of the encapsulated scaffolds cannot be adduced to a physical interaction between the drugs and scaffold rather it is suggestive of a stronger chemical interplay. SEM images also support the deductions from the study, with the physical appearances of the PLGA scaffolds significantly different after encapsulation.

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