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Research paper

Formulation and antibacterial profiles of clay–ciprofloxacin composites

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ABSTRACT

The ability of clay minerals to adsorb and desorb drugs, including antibacterial molecules, is an attractive and exciting prospect for healthcare applications. The antibacterial ciprofloxacin was adsorbed onto kaolin, montmorillonite K-10, and Laponite® RD then the subsequent antibacterial activity of the composites formed was confirmed. The effects of time, pH, and CIP concentration were investigated. X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy were used to confirm the mechanism of ciprofloxacin adsorption onto the clay minerals. Dispersion pH was the most important variable influencing the adsorption of ciprofloxacin onto the clay minerals and the mechanism of adsorption was confirmed as cation exchange. Adsorption isotherms and application of Langmuir and Freundlich models showed that a ciprofloxacin monolayer was formed at different concentrations on each of the clay minerals tested. Kinetic studies showed that maximal CIP adsorption was achieved within the first hour of adsorption. Antibacterial activity of clay–ciprofloxacin composites against the common skin bacteria *Staphylococcus epidermidis* and *Propionibacterium acnes* was demonstrated. This work showed that clay–ciprofloxacin composites are potential delivery systems for ciprofloxacin molecules. As a result, this could make them ideal candidates to take forward for healthcare applications, including the development of novel wound dressings.

1. Introduction

Clays and clay minerals have recently seen a resurgence in interest for healthcare applications and have been utilised in the formulation of medicines for a number of years as both excipients and active ingredients (Aguzzi et al., 2007; Lopez-Galindo et al., 2007; Viseras et al., 2007). For generations mankind has been using clays to self-medicate, possibly in mimicry of other animals, and such use has been indicated in texts throughout history and across the world (Carretero, 2002; Choy et al., 2007; Del Hoyo, 2007; Williams and Haydel, 2010). Clay minerals are well documented adsorbents for bacteria (Rong et al., 2008; Zhao et al., 2012) and viruses (Lipson and Stotzky, 1983; Schiffenbauer and Stotzky, 1982), but also for the adsorption of cations such as toxins and other molecules that may be released by such pathogens and waste from the healing process (Deng et al., 2010; Swartzen-Allen and Matijevic, 1974; Tateo and Summa, 2007).

In modern medicine complicated skin and soft tissue infections are regularly treated with the use of antimicrobial agents. The inability of current medicines to deliver antibiotics across the skin in sufficient concentrations means patients are often required to take antibiotics orally or be admitted to hospital for intravenous therapy (Bennett-Marsden, 2010; Seaton, 2009). Twenty-six in every 100,000 hospital admissions in the UK are due to skin and soft tissue infections (Seaton, 2009) creating an increased burden on the healthcare system. The use of systemic antibacterial agents can result in a number of problems for patients such as gastrointestinal side effects and the destruction of gut flora — resulting in an increased risk of fatal *Clostridium difficile* infection (Healy and Freedman, 2006; Kluytmans and Struelens, 2009; Schweiger and Weinberg, 2004).

The ability of clay minerals to adsorb cations onto their negatively charged surfaces also extends to antibacterial molecules. A number of researchers have published work investigating the adsorption of antibacterial molecules onto clay minerals and their subsequent release (Chang et al., 2009; Jung et al., 2008; Li et al., 2010; Parolo et al., 2008, 2010; Porubcan et al., 1978; Wang et al., 2010, 2011; Wu et al., 2010). However, few have considered the activity of these antibacterial agents when adsorbed or desorbed from clay minerals, with even fewer applying the work to clinical problems. Clay–antibacterial composites with the ability to deliver antibacterial agents in a sustained manner and adsorb excess water and waste (Williams and Haydel, 2010) thus benefiting the healing of skin and soft tissue infections in a multitude of ways is an attractive prospect.

The antibiotic ciprofloxacin (CIP) is frequently used in the UK for the treatment of a number of infections including skin and soft tissue infections (Healy and Freedman, 2006; Sandwell and West Birmingham Hospitals NHS Trust, 2011; The Royal Liverpool and Broadgreen University Hospitals NHS Trust, 2012). CIP is a concentration dependent broad spectrum antibacterial with greater effect on Gram-negative bacteria and some effect on Gram-positive bacteria (Eboka and Okeri, 2005; Phillips et al., 1990; Wispelwey, 2005). The pharmacokinetics of CIP allow it to reach the skin in significant amounts even when administered orally or parenterally (Vance-Bryan et al., 1990), making it an attractive choice for clinicians when other first-line therapies (such as

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beta-lactams) have failed or are inappropriate for the patient (Sandwell and West Birmingham Hospitals NHS Trust, 2011; The Royal Liverpool and Broadgreen University Hospitals NHS Trust, 2012).

The overall purpose of this work is to investigate the possibility of using clay–antibiotic composites in the treatment of wounds, which would form the basis of developing appropriate pharmaceutical formulations. In this study, the effects of pH, time and CIP concentration on the mechanism of CIP adsorption onto kaolin, montmorillonite K10, and the synthetic clay mineral Laponite® RD were investigated. The antibacterial effects of clay-CIP composites formed were tested against two common skin bacteria *Staphylococcus epidermidis* and *Propionibacterium acnes*.

2. Materials and methods

2.1. Materials

Montmorillonite K-10 (MMTK10, cation exchange capacity (CEC) = 119 meq/100 g) was obtained from Acros Organics (UK), kaolin washed and sieved (KN, CEC = 8 meq/100 g) was obtained from Fischer Scientific (UK), and Laponite® RD (LRD, CEC = 53 meq/100 g) was obtained from Rockwood Additives Ltd. (UK). The ciprofloxacin (CIP) was obtained from Sigma Aldrich (UK).

2.2. Adsorption of CIP onto clay minerals

To test the effects of dispersion pH on CIP adsorption 1 g of KN, MMTK10, and LRD was dispersed in 80 mL deionised water at 7000 rpm for 2 h. A 250 mg in 20 mL solution of CIP was added to the clay mineral dispersions and the pH adjusted to between 1 and 12 with dilute NaOH and HCl, and stirred for 24 h at 7000 rpm. The dispersions were filtered over vacuum and washed twice with deionised water. The amount of CIP remaining in the filtrates and washes was measured by UV–vis spectrophotometry scanning between 200 and 400 nm, in turn determining the amount of CIP adsorbed by the clay minerals.

For the adsorption kinetics KN (500 mg), MMTK10 (100 mg), and LRD (10 mg) were dispersed in 100 mL deionised water and mixed for 2 h as described above. The dispersion pH was adjusted to 7.4 (optimum pH for CIP adsorption) before adding the 2 mg in 10 mL CIP solution. Samples were taken at regular time intervals between 0 and 120 min then less regularly until 48 h. Samples were filtered through a 0.2 μm Minisart filter. CIP adsorption was determined by UV–vis spectrophotometry and the data used to determine the kinetics of CIP adsorption onto each clay mineral.

The effect of CIP concentration on adsorption was investigated on 250 mg in 25 mL dispersions of each clay mineral. The concentration of CIP ranged from 5 mg/L to 200 mg/L and the dispersion pH set to 7.4 to optimise CIP adsorption. Dispersions were stirred at 7000 rpm for 24 h before 1 mL samples were removed and centrifuged (3000 rpm for 10 min). CIP adsorption was determined through UV–vis spectrophotometry. Adsorption isotherms were plotted and adsorption isotherm models were applied from the data obtained.

Table 1
Clay–CIP composites further analysed along with their designated codes.

Clay	Concentration CIP adsorbed	Composite code
Kaolin	4.79 mg/g	KN-A
	27.54 mg/g	KN-B
	81.53 mg/g	KN-C
Montomorillonite K-10	49.76 mg/g	MMTK10-A
	90.70 mg/g	MMTK10-B
	117.02 mg/g	MMTK10-C
Laponite® RD	15.85 mg/g	LRD-A
	104.31 mg/g	LRD-B
	128.09 mg/g	LRD-C

Clay–CIP composites were freeze dried for FTIR and XRD analyses and microbiological assays. Table 1 gives details of the clay–CIP composite codes referenced within this paper and their associated CIP concentrations.

2.3. Mechanism of adsorption

To determine the interaction of functional groups on the CIP molecules with the clay mineral surface, and to detect clay-structure changes, FTIR analysis was undertaken on a Perkin Elmer Spectrum BX FTIR spectrometer with GladiATR attachment. Sixteen scans were undertaken between 4000 and 400 cm^{-1} with an interval of 1.0 cm^{-1} and a resolution of 2.0. Spectra for the antibiotic prepared at varying pH were used to confirm peak shift(s) as a result of charge variation.

Powder XRD was used to detect structural changes to the clay minerals and determine the location of the antibiotic molecules within the clay–CIP composites. A Rigaku Miniflex X-ray diffractometer was utilised with a CuKα1 radiation source and measurements were taken at 2θ/min between 3 and 60° 2θ. Interlayer spacing was determined through the d_{001} value of the *001* peak.

The clay minerals, CIP, and simple physical solid blends of the clay minerals and CIP were analysed to identify if any spectra or diffractogram changes arose. The clay–CIP composites prepared were analysed in the same way and compared to all the standards listed above.

2.4. Microbiological testing

Liquid cultures of *S. epidermidis* were grown in nutrient broth under aerobic conditions at 37 °C whilst cultures of *P. acnes* were grown in brain–heart infusion broth under anaerobic conditions. McFarland standards were made up as per the standard methodology (McFarland, 1907) and the resultant turbidity was measured with UV–vis at 600 nm. Bacterial cultures were diluted in their respective liquid media until the UV–vis turbidity measurements were equivalent to McFarland 0.5 (approximately 1.5×10^8 CFU/mL). The prepared McFarland 0.5 cultures were further serially diluted by a factor of ten with the respective growth medium before use.

To determine the number of colony forming units (CFUs) 100 μl of McFarland 0.5 culture and the serial dilutions of it were spread onto pre-poured agar plates. *S. epidermidis* was grown on nutrient agar, which was incubated in aerobic conditions whereas *P. acnes* was grown on brain–heart agar under anaerobic conditions. Both bacteria were grown at 37 °C for 24 h and 48 h, respectively. Individual colonies were counted and back calculated to derive the number of CFUs per millilitre in the McFarland 0.5 cultures.

The well-diffusion method was deployed to examine the antibacterial effects of the clay–CIP composites formed. One millilitre of a 1 in 10 dilution of the McFarland 0.5 culture was added to 19 mL of molten agar (appropriate for each bacterium, as above) and poured into plates and cooled. Wells, 5 mm in diameter, were made in the solid agar. Aliquots of CIP solutions (50 μl) in the range of 5 μg/mL to 250 μg/mL, were added into wells to act as standards. Clays and selected clay–CIP composites were dispersed in sterile deionised water to a concentration of 40 mg/mL, of which 50 μL aliquots were added into the wells. The plates were incubated in the same way as described above. Inhibition of growth was observed as clear zones of no bacterial growth around the wells and the radii were measured using digital callipers.

3. Results and discussion

3.1. Effect of dispersion pH

Changes in dispersion pH produced profound differences in the amount of CIP adsorbed onto MMTK10 and KN (Fig. 1). CIP adsorption onto MMTK10 was observed to increase between pH 1.0 and 7.4. This can be explained as CIP is positive below pH 6.1 and predominantly