# Laponite clay as a carrier for *in situ* delivery of tetracycline

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Although smectite clays have gained much interest in recent years as potential sustained local drug delivery vehicles, they have not been yet utilized or assessed as a local drug delivery device for treatment of periodontal disease. In this paper we showed that unique nanostructure and gel-formation ability of clay holds great promise as an effective drug carrier. In particular, we demonstrated the feasibility of using laponite XLG, a smectite clay, as a carrier for *in situ* delivery of tetracycline that can potentially be used in the treatment and prevention of periodontal disease. The intercalation of tetracycline between the layers of laponite at different acidic pHs and different concentrations of tetracycline was examined. The laponite–tetracycline composite was characterized using X-ray diffraction (XRD), Fourier transformed infrared spectroscopy (FT-IR) and differential thermal analysis (DTA). The results showed that, in a pH and concentration dependent manner, tetracycline can be intercalated between the layers of laponite nanoparticles. The release study in a simulated saliva solution demonstrated a sustained release of tetracycline from laponite over a 72 hour period. The antimicrobial activity of the released tetracycline was maintained and was not affected by the intercalation of tetracycline into the carrier (laponite) and its release from the carrier.

## Introduction

Periodontal disease is a "*collective term ascribed to several pathological conditions characterized by degeneration and inflammation of gums, periodontal ligaments, alveolar bone and dental cementum*".[1] Some of these diseases such as gingivitis and periodontitis are caused by dental plaque[2] that contains pathogenic bacteria, which are in part responsible for the inflammation seen in these diseases. Therefore, by controlling these pathogenic microflora, the development of periodontal disease can be prevented. Systemic administration of antibiotics has thus been advocated for the treatment of these diseases.[3] The antimicrobial agents that have been and continue to be used in the treatment of periodontal disease include amoxicillin with clavulanic acid or metronidazole, clindamycin, doxycycline, minocycline and tetracycline.[3] Tetracycline is a broad spectrum antimicrobial agent that has been used in various applications over the last 60 years[4] and since the early 1980s, has been applied in adjunctive therapy to scaling and root planing (deep cleaning of the root surface), yielding successful clinical outcomes.[3] It has been shown to be effective against both Gram positive and negative organisms, including beta-lactamase

producing strains, against which penicillins are ineffective.[4] However, concerns emerged with respect to gastrointestinal intolerance and development of bacterial resistance with systemic use of tetracycline.[4] For these reasons, intra-periodontal pocket delivery systems have been developed to combat periodontal disease, eliminating the side effects of systemic administration of antibiotics and improving the treatment efficacy and timeframe.[4–6] Periodontal pockets form when pathogenic microorganisms colonize the sub-gingival areas of teeth, causing degeneration of collagen, resorption of alveolar bone and migration of gingival epithelium along the tooth surface.[5] As periodontal disease is often localized to the immediate vicinity of the periodontal pocket, it makes the pocket a suitable site in which to place a drug carrier for local delivery of an antimicrobial agent.[4–6] Local drug delivery in the periodontal pocket not only causes negligible impact on microflora of other body sites but also provides greater concentrations of the drug at the relevant site than is possible with systemic delivery.[3] Numerous forms of local drug delivery systems have been developed in pursuit of the optimal formulation to combat periodontal disease,[7–10] but one that has attracted great interest in recent years is the use of clay-based biomaterials as drug delivery vehicles.[11]

Clay biomaterial has long been known to reduce the oral bioavailability of some medications.[11] One of the earliest examples of this was reported in 1965 when Sorby *et al.* observed that the absorption of promazine decreased when co-administered

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with an anti-diarrheal agent containing attapulgite.[12] This soon led to the realization that such interactions could be used to achieve biopharmaceutical benefits[11] and so in recent decades, this phenomenon has been utilized to produce sustained drug delivery systems, particularly using smectite clays such as montmorillonite. In the montmorillonite structure, the imperfection of crystals induces a negative charge between the clay's silica and alumina sheets.[13] This repulsive electrostatic force between silica and alumina sheets creates layers, leading to a large total surface area and thereby good adsorbability and drug carrying capability. Due to these favourable properties, montmorillonite with intercalated tetracycline,[14,15] timolol,[13] ibuprofen[16] and donepezil[17] has been investigated. Another less commonly studied smectite clay, laponite, is observed to have smaller particle size, lower polydispersity of dimension and charge, and better stability and dispersability in an aqueous suspension when compared to montmorillonite.[18] This supports the potential for laponite to be used as an effective drug carrier. However, with regard to its use as a modified-release drug carrier, laponite XLG (a gel forming grade of laponite) has only been combined and studied with itraconazole.[19] Furthermore, the potential use of clay biomaterials for periodontal applications has not been studied and very limited data are available on the behaviour of clay in saliva or gingival crevicular fluid.

The present study investigated the feasibility of using laponite XLG as a drug carrier for *in situ* delivery of tetracycline. The effect of pH on the intercalation kinetics of tetracycline between the laponite layers was studied to determine the optimal pH that maximizes intercalation of tetracycline. The release of tetracycline from the laponite–tetracycline composite (L–T) was investigated in a simulated saliva solution over 72 hours at 37 °C. The changes in layer spacing (*d*-spacing) of laponite was determined using X-ray diffraction (XRD) to provide evidence for the intercalation of tetracycline between the laponite layers. Fourier transformed infrared spectroscopy (FT-IR) was conducted to provide information on the nature of the chemical interaction between tetracycline and laponite and the thermo-behavior of L–T was assessed using differential thermal analysis (DTA). Zeta potential measurements were performed to determine the charge of the clay particles, as this was hypothesized to be a key factor in both extent of intercalation and release of tetracycline.

## Materials and methods

### Materials

All materials were of analytical grade and used as purchased from the manufacturer, including tetracycline hydrochloride (Sigma-Aldrich, Australia) and laponite XLG, $Na_{0.7}Si_8(Mg_{3.5}Li_{0.3})O_{20}(OH)_4$, (Rockwood, Austin, USA).

### Preparation of L–T under different tetracycline concentrations and pHs

To prepare the L–T samples, 1% laponite suspension was prepared by dispersing laponite powder in deionised water. Solutions of tetracycline hydrochloride were added to the laponite suspension producing final concentrations of 0.1%, 0.2% and 0.3% tetracycline in 1% laponite suspension. The pH of each solution was adjusted to 3, 4 or 6 using HCl and NaOH. The mixtures were mixed vigorously with magnetic stirring for 1 h to allow full intercalation of tetracycline into the clay. The suspensions were centrifuged at 5000 rpm for 5 minutes to precipitate the L–T composite. The non-intercalated tetracycline ($T_{ni}$) in the supernatant was measured using spectrophotometry at 270 nm (Shimadzu UV-1800 UV). The prepared L–T samples were washed twice with deionized water and the amount of tetracycline was measured in the washout solutions ($T_w$) and the resultant powder was freeze-dried. The theoretical maximum percentage of tetracycline that was intercalated into each L–T samples ($T_i$) was calculated using the following equation:

$$T_i = T_t - T_{ni} - T_w \qquad (1)$$

where, $T_t$ is the total amount of tetracycline used in preparing each L–T composite, $T_{ni}$ is the amount of non-intercalated tetracycline left in the supernatant, and $T_w$ is the amount of tetracycline measured in the washout solutions.

### De-intercalation study

To determine how much tetracycline was incorporated into laponite and the amount of tetracycline available for release, a de-intercalation study was carried out using 5% potassium carbonate ($K_2CO_3$).[20] L–T composites with different concentrations of tetracycline were prepared at pH 3, 4, and 6. The L–T powder was added into 5% $K_2CO_3$ solution and mixed with magnetic stirring for 3 h at room temperature to allow full de-intercalation of tetracycline from the laponite. The amount of de-intercalated tetracycline ($T_{di}$) in the supernatant was determined using UV spectrophotometry at 270 nm. The percentage of de-intercalated tetracycline $T_{di}\%$ was calculated using the following equation:

$$T_{di}\% = (T_{di}/T_i) \times 100 \qquad (2)$$

where, $T_{di}$ is the amount of de-intercalated tetracycline in the supernatant and $T_i$ is the theoretical maximum percentage of tetracycline intercalated in laponite, calculated using eqn (1).

### Release study

To study the effect of background constituents present *in vivo*, on the release of tetracycline from L–T composites, an *in vitro* simulated saliva solution was prepared containing 1.2 mM $CaCl_2$, 0.72 mM $KH_2PO_4$, 30 mM KCl and 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), at pH 7.2 (ref. 21). Ten mg of L–T powder was added to 10 mL of simulated saliva-like solution, sealed, and shaken at 50 rpm, at 37 °C. Saliva-like solution (SLS) was used as a release medium due to the potential application of L–T composite for treatment of periodontal diseases. At pre-determined time intervals, 500 μL aliquots were removed and replaced with fresh medium. The aliquots were centrifuged at 5000 rpm for 5 minutes and the concentration of tetracycline was determined using UV spectrophotometry at 270 nm. The release study was performed over 72 h in triplicate. The release profile of tetracycline from L–T

samples was also carried in acidic SLS (pH 5.2), mimicking the acidic condition of bacterial metabolic activities in periodontal diseases. Tetracycline release in acidic condition was only performed for L–T samples prepared at pH 4, as this group demonstrated the highest tetracycline release in SLS at pH 7.2.

### Powder diffraction

To determine whether the intercalation of tetracycline into laponite increased the distance between the laponite layers, XRD was performed on laponite and 0.3% L–Ts samples prepared at pH 3, 4 and 6. XRD patterns were obtained by Cu Kα radiation ($\lambda = 0.154$ nm) using a XRD-6000 (Shimadzu) spectrometer. The diffraction patterns were recorded at 40 kV and 30 mA with a scanning angle range from 2 to 70° at a scan speed of $1.2°$ min$^{-1}$ and increments of 0.02°. The $d$-spacing of basal (001) reflection (the distance between the clay's layers) was then calculated using Bragg's eqn (3).[22]

$$\lambda = 2d \sin \theta \qquad (3)$$

where $\lambda$ is the wavelength of Cu Kα radiation (1.5406 Å), $d$ is the spacing between the scattering clay's layers ($d$-spacing), and $\theta$ is the diffraction angle, which is the angle between the incident X-ray and the scattering clay's layers (or lattice planes).

### FT-IR spectroscopy

To determine whether tetracycline interacts with laponite structure, the FT-IR spectrum was obtained using Varian 660-IR in the range of 600–4000 cm$^{-1}$ with 2 cm$^{-1}$ spectral resolution on the 0.3% L–T samples produced at pH 4.

### Thermal properties

DTA analysis was performed on laponite alone, tetracycline alone and 0.3% L–T samples prepared at pH 4, using TGA-2950 (TA instruments) under dynamic N$_2$ flow. Samples were loaded onto a ceramic pan and heated from room temperature to 800 °C at a rate of 10 °C min$^{-1}$.

### Particle charge

Zeta potential measurements were carried out in suspensions of 0.025% w/v L–T powder in phosphate–citric buffer made of 0.2 M dibasic sodium phosphate and 0.1 M citric. To determine the charge of the laponite clay during intercalation of tetracycline, the pH of suspensions for the zeta potential measurements was adjusted to the pH at which the L–T samples were prepared. Similarly, to determine the charge of the laponite clay in the release study, 0.025% w/v L–T suspensions were prepared in phosphate–citric buffer at pH 7.2. The zeta potential of the samples was measured using a Zetasizer Nano ZS with a disposable folded capillary cell.

### *In vitro* antimicrobial activities of released tetracycline

The antimicrobial property of released tetracycline from L–T samples (prepared using 0.3% tetracycline at pH 4) was determined using an agar well diffusion assay. Mueller–Hinton (MH)

agar plates were inoculated by swabbing for confluence with a standard inoculum of either a Gram negative, *Escherichia coli* (*E. coli*), NCTC 9001[ATCC 11775], or a Gram positive, *Staphylococcus aureus* (*S. aureus*), Oxford NCTC 6571. These two microorganisms have been widely used as standard strains to determine the antimicrobial activities of materials.[23] Three 7 mm diameter wells were aseptically cut into the inoculated plate using a sterile cork borer. Carefully one well was filled with 100 μL of L–T sample, as negative control the second well was filled with 100 μL pure laponite gel and as positive control the third well was filled with 100 μL of tetracycline formulated in agar gel. The concentration of tetracycline in control group (third well) was the same concentration of tetracycline that was measured in the extract obtained from L–T sample (described below) using UV. The gels were incubated at 37 °C overnight and the inhibition zone of each sample was measured in mm using a ruler.

The minimum inhibitory concentration (MIC) of eluted tetracycline from the L–T samples was determined using standard serial two-fold dilutions in MH broth according to NCCLS guidelines.[24] The amount of tetracycline released (extracted) from the L–T samples was obtained according to the ISO standard 10993-12. Briefly, 1 g of L–T sample was incubated in 2 mL of saliva-like solution (SLS) at 37 °C for 24 hours in an incubator under shaking. The extracts were centrifuged at 5000 rpm for 5 minutes to remove insoluble particles. The clear supernatant was aliquoted and filter sterilized using a 0.2 μm polyethersulfone membrane (Millex, Millipore Ireland Ltd). Serial two-fold dilutions of the extract in MH broth were prepared and a standard inoculum of an overnight culture of either *E. coli* (NCTC 9001[ATCC 11775]) or *S. aureus* (Oxford NCTC 6571) was added and the tubes were incubated at 37 °C overnight. The minimum concentration of the extract, which inhibited visible growth of bacteria was reported as the minimum inhibitory concentration (MIC). The negative control tubes contained MH broth with the extract obtained from laponite gel and the positive control tubes contained MH broth with tetracycline.

## Results and discussion

### Intercalation of tetracycline between laponite layers

Results of the intercalation study (Table 1) demonstrated that regardless of the pH used in sample preparation, the percentage of intercalated tetracycline into laponite steadily decreased as the concentration of tetracycline used in sample preparation increased. As the concentration of tetracycline increased, this reflects an increase in the number of tetracycline molecules that are competing for intercalation between the clay's layers: the higher the number of available tetracycline molecules to intercalate, the lower percentage of them will be intercalated.

One of the main mechanisms of intercalation into smectite clay is cation exchange.[15] This process occurs when cations within the clay (Na$^+$) are exchanged for cations in solution, which in this case, is the cationic form of tetracycline (Fig. 1). This process is dependent upon the charge of both the clay and the tetracycline. According to the manufacturer, the cation exchange capacity (CEC) of used laponite XLG is about 60 meq. per 100 g. Given the milliequivalent weight of Na$^+$ is 23 mg

**Table 1** The weight percentage of intercalated tetracycline (from total available tetracycline in L–T preparation) under different pHs and tetracycline concentrations (w/v%) used in L–T preparation[a]

| Tetracycline (w/v%) used in L–T sample preparation | 0.1% | 0.2% | 0.3% |
|---|---|---|---|
| pH 3 | $98.9 \pm 0.6\%^{a}$ | $98.0 \pm 0.4\%^{a}$ | $95.6 \pm 0.7\%^{a}$ |
| pH 4 | $99.2 \pm 0.1\%^{a}$ | $97.7 \pm 0.2\%^{a}$ | $95.5 \pm 0.2\%^{a}$ |
| pH 6 | $94.3 \pm 5.4\%^{b}$ | $81.6 \pm 2.8\%^{b}$ | $68.3 \pm 7.2\%^{b}$ |

[a] Values (mean $\pm$ SD of three replicates) in the same column and not having the same superscript are significantly different ($p < 0.05$) *post hoc* (Tukey).

meq.$^{-1}$, 100 g laponite can maximum hold 1.38 g of Na$^+$ and if all Na ions are replaced by cationic tetracycline, that means 100 g of laponite can maximum hold 26.67 g or 26.67% tetracycline (the molecular weight of tetracycline and sodium are respectively 444.435 and 23 g mol$^{-1}$). Therefore for 0.1% (0.1% tetracycline in 1% laponite) and 0.2% L–T samples the amount of added tetracycline was less than the maximum holding capacity of laponite for tetracycline and for 0.3% L–T sample the amount of added tetracycline was slightly higher than laponite holding capacity for tetracycline. Based of the above calculation, the % of Na ions that can be replaced by tetracycline if all of tetracycline was intercalated are approximately 38%, 75% and 100% for 0.1, 0.2, and 0.3% L–T samples respectively.

Laponite structure contains both a non-pH-dependent (a constant negative charge area) and a pH-dependent charge area, which later becomes positively charged in acidic environments.[18] On the other hand, tetracycline has three p$K_a$'s that affect its total charge.[25] At pH < 3.3, it exists predominantly as a monocationic species. Between pH 3.3 and 7.7, a deprotonation step occurs causing the tetracycline molecule to acquire a negative charge and making it zwitterionic. Then at pH 7.7 to 9.5, a second negative charge gives the molecule a net charge of −1 and above pH 9.5, it becomes dianionic. Varying pH during L–T preparation therefore causes the tetracycline molecules to adopt a cationic or zwitterionic state, which can impact the amount of tetracycline that is intercalated into the laponite structure. The results showed that regardless of the concentration of tetracycline in the preparation solution, the samples prepared at pH 6 (the highest pH) had the lowest intercalated tetracycline compared with those prepared at pH 3 and 4. The higher the pH, the greater the net negative value of the tetracycline charge, thereby reducing the intercalation of tetracycline between the negatively charged laponite layers. However, it was found that despite the difference in the species of tetracycline between pH 3 and 4, there was no significant difference in the amount of tetracycline intercalated into laponite between these two pHs (Table 1). It should be noted that the p$K_a$ values of tetracycline are affected by changing the ionic strength of solution. In our study, the Na$^+$ ions released from laponite can increase the p$K_a$ values of tetracycline. Chang *et al.*[14] compared the tetracycline speciation under different pHs in aqueous solution (without clay) and in 1% Na-montmorillonite suspension. Based on their study, at pH 3 and 4 in 1% Na-montmorillonite suspension, tetracycline is in monocationic form and at pH 6 about 40% zwitterionic and 60% cationic. This can explain the reason that the amount of intercalated tetracycline at pH 3 and 4 were similar, and significantly higher than that at pH 6. At pH 3 and 4 the tetracycline in the preparation solution had similar speciation. Cationic tetracycline can be
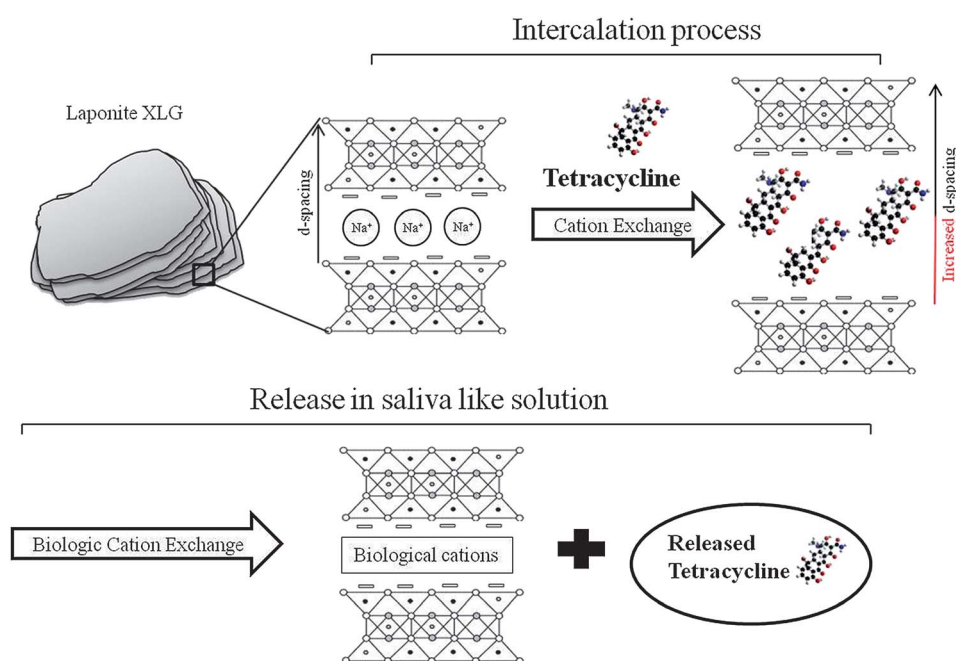


**Fig. 1** Illustration of the layered structure of laponite. The intercalation of tetracycline between these layers increases the layer spacing. Once the tetracycline-loaded laponite is placed in a biological fluid (*e.g.*, saliva-like fluid), the ion exchange from the biological fluid occurs: the biological ions such as Ca$^{2+}$, K$^+$, Mg$^{2+}$, Na$^+$ exchange with the intercalated tetracycline, releasing the tetracycline into the medium.

easier intercalated between negatively charged laponite layers than zwitterionic tetracycline. However it should be noted that zwitterions are able to rearrange themselves so that the negatively charged areas of tetracycline molecule have a minimal effect on the attraction between the negatively charged layers of laponite and the positive groups of zwitterionic tetracycline.[18]

Due to the layered structure of laponite particles (stack of layers or discs), the available surface between the laponite layers for tetracycline intercalation is substantially higher than the surface of laponite particles on which tetracycline can be adsorbed. Several studies reported the high total surface area of clay particles, suggesting that the main mechanism for drug uptake into clay is through intercalation.[11,14,26] However, it should be noted that a low percentage of tetracycline molecules might be indeed adsorbed onto the surface of particles and not being intercalated between the clay particles' layers.

### De-intercalation of tetracycline from laponite carrier

After stirring for 3 hours in a cationic solution (5% $K_2CO_3$), the amount of de-intercalated tetracycline released into the solution was calculated and is shown in Table 2. Depending on the pH and the concentration of tetracycline during sample preparation, the percentage of de-intercalated tetracycline varied between approximately 10–27%. The pH 6 samples demonstrated higher percentage of de-intercalated tetracycline than those prepared at pH 3 and 4. This shows that the tetracycline molecules in the samples prepared at pH 6 were relatively loosely attached to the laponite structure and therefore were easier released during the de-intercalation assay. This is in agreement with findings for the intercalation assay that the lowest intercalation rate of tetracycline was obtained for the samples prepared at pH 6. As expected, the amount of de-intercalated tetracycline increased by increasing the amount of tetracycline used in the sample preparation. No significant difference was observed in the de-intercalation results between the pH 3 and 4 samples. However, the pH 4 samples showed the highest intercalation and the lowest de-intercalation rates compared to the other groups (Tables 1 and 2).

### Interactions between tetracycline and laponite

**X-ray diffraction (XRD).** The XRD pattern for powder laponite and 0.3% L–Ts produced at pH 3, 4 and 6 are shown in Fig. 2.

**Table 2** The weight percentage of de-intercalated tetracycline (from the total intercalated tetracycline obtained from Table 1) under different pHs and tetracycline concentrations (w/v%) used in L–T preparation[a]

| Tetracycline (w/v%) used in sample preparation | 0.1% | 0.2% | 0.3% |
|---|---|---|---|
| pH 3 | 15.0 ± 0.4%[a] | 14.7 ± 1.7%[a] | 21.1 ± 0.5%[a] |
| pH 4 | 10.8 ± 0.8%[a] | 11.4 ± 2.1%[a] | 18.9 ± 1.3%[a] |
| pH 6 | 24.1 ± 3.1%[b] | 20.5 ± 1.5%[b] | 27.7 ± 4.3%[b] |

[a] Values (mean ± SD of three replicates) in the same column and not having the same superscript are significantly different ($p < 0.05$) post hoc (Tukey).
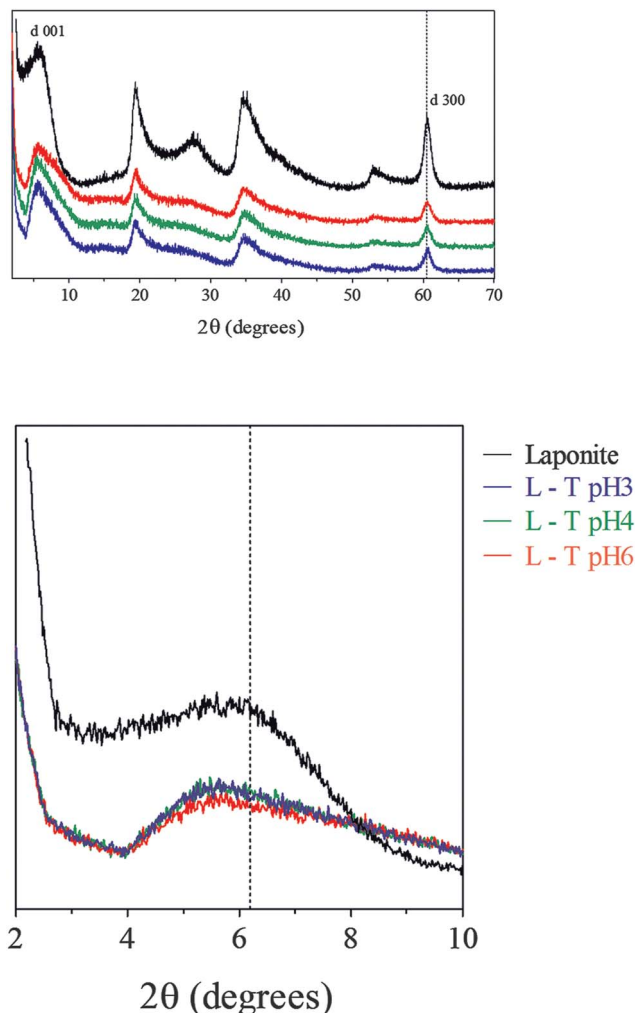


**Fig. 2** XRD pattern of laponite and 0.3% tetracycline–laponite composites prepared at pH 3, 4 and 6. The reflection corresponding to (001) laponite lattice plane at $2\theta = 6.2°$ was shifted to a lower degree in L–T samples.

Comparing these XRD patterns, it was found that the basal reflection corresponding to the (001) plane at $2\theta = 6.2°$ was shifted to a lower degree ranging from 5.36–5.5° in the L–T samples. Using the Bragg's equation eqn (3), the corresponding increases in the $d$-spacing of each L–T, which is the distance between 001 planes of the clay layers, are shown in Table 3. The increase in $d$-spacing in the L–T samples indicates that tetracycline has indeed been intercalated between the layers of laponite (Fig. 1). Subtracting the thickness of the silicate layer of laponite (9.6 Å) (ref. 27) from the $d$-spacing of the L–Ts prepared at pH 3, 4 and 6, gives inter-layer separation distances of 5.9 Å, 6.1 Å and 5.4 Å, respectively. Although, the inter-layer distance calculated here is smaller than the proposed conformation of tetracycline in acidic environments (12.9 Å long, 6.2 Å high and 7.5 Å thick),[28] it should be noted that the spacing obtained is not solely dependent on the dimensions of the intercalated molecule in its free state, but also on factors such as hydration and orientation.[29] The increase in the $d$-spacing of laponite was greatest for the pH 4 L–T sample (15.7 Å), showing that, at this pH, the intercalation of tetracycline was greatest, which is in

**Table 3** The zeta potential and *d*-spacing of 0.3% L–T produced at pH 3, 4 and 6. The zeta potential was measured at the pH in which the samples were made (*e.g.*, pH 3, 4, 6) and at pH 7 (the pH of the release study). The *d*-spacing of samples was calculated using XRD results

|  | *d*-Spacing (Å) | Zeta potential measured at the pH in which the sample was made (mV) | Zeta potential measured at pH 7 (mV) |
|---|---|---|---|
| 0.3% L–T pH 3 | 15.5 | −24.9 ± 4.5 | −27.3 ± 2.8 |
| 0.3% L–T pH 4 | 15.7 | −20.0 ± 3.4 | −24.9 ± 3.7 |
| 0.3% L–T pH 6 | 15.0 | −23.3 ± 1.5 | −30.0 ± 1.4 |
| Laponite | 13.9[a] |  |  |

[a] Significantly (*p* < 0.05) lower than the other groups (L–T samples).

agreement with the results in Table 1. It should be mentioned that larger *d*-spacing could be also due to the increased number of water molecules placed between laponite layers, therefore the higher the hydration, the larger the *d*-spacing.[30]

**Fourier transform infrared spectroscopy (FT-IR).** The FT-IR spectra of tetracycline, laponite and 0.3% L–T prepared at pH 4 are shown in Fig. 3. FT-IR was only carried out on samples prepared at pH 4, as this pH demonstrated the highest tetracycline intercalation. FT-IR spectrum of laponite showed one dominating peak at approximately 1000 cm$^{-1}$ and another peak at around 700 cm$^{-1}$, which are attributed to the Si–O stretching vibration and the O–Si–O bending vibration respectively.[31] The FT-IR spectrum obtained for tetracycline alone has several peaks within the measured range (Fig. 3). The peaks of tetracycline that were appeared in L–T sample were at 1664 cm$^{-1}$, 1612 cm$^{-1}$ and 1577 cm$^{-1}$, which correspond to the C=O vibration of the amide, C=O on the A ring and C=O on the C ring, respectively[15] (Fig. 3). In addition, the bands at 1517 cm$^{-1}$ and 1448 cm$^{-1}$ can be assigned to the NH$_2$ amide and the C=C skeleton.[15] Laponite does not have absorption band between 3000 and 1300 cm$^{-1}$ (expect water vibration at around 1630 cm$^{-1}$) providing a convenient window for the detection of tetracycline absorption bands. The main peak of laponite at around 1000 cm$^{-1}$ (Si–O stretching) slightly shifted in the L–T sample to the higher wavenumber. Based on our FT-IR observation, the slight shift of Si–O stretching of laponite structure can be attributed to the molecular interactions between laponite and tetracycline and it does not necessarily demonstrate a chemical bonding between tetracycline and laponite.

**Differential thermal analysis (DTA).** The DTA profiles of tetracycline, laponite and the 0.3% L–T prepared at pH 4 are depicted in Fig. 4. The DTA profile for tetracycline shows a strong peak at approximately 250 °C. This peak indicates major weight loss that corresponds to the decomposition of tetracycline. The DTA profile of laponite shows a large peak at approximately 80 °C, most likely due to the loss of water by evaporation. Laponite remains stable until 700 °C where a second weight loss is seen, attributed to the loss of the hydroxyl group. Looking at the DTA profile for the L–T samples, a small peak is seen at approximately 320 °C. This peak was assumed to be due to the decomposition of tetracycline, as laponite undergoes no mass changes near this temperature. The shift in tetracycline decomposition to higher temperatures can be due to the intercalation of tetracycline between laponite layers, which actually increases the thermal stability of the intercalated tetracycline by shielding the tetracycline molecules. The L–T undergoes a second mass loss at approximately 700 °C due to the decomposition of the laponite, a similar temperature to that of laponite alone.

**Zeta potential.** The zeta potential of the 0.3% L–T samples was measured at the pHs in which they were made (the pH in which tetracycline was intercalated between laponite layers) and at pH 7.2 (the pH of the release study), see Table 3. Although the
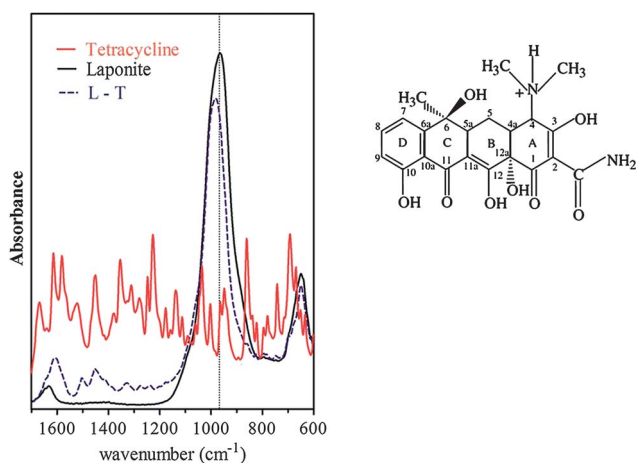


**Fig. 3** (Left) FT-IR spectra of laponite, tetracycline and tetracycline–laponite composites from 600 cm$^{-1}$ to 1700 cm$^{-1}$. (Right) Schematic drawing of a fully protonated tetracycline molecule.
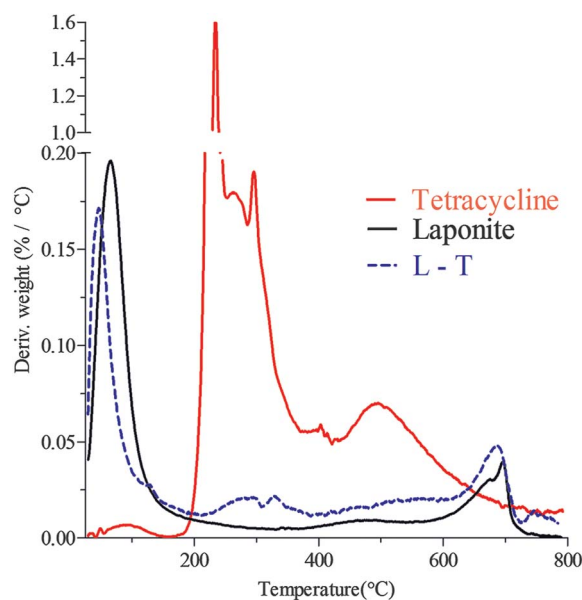


**Fig. 4** Differential thermal analysis of tetracycline, laponite and 0.3% tetracycline–laponite composite prepared at pH 6.

net negative charge of the pH 4 samples seemed to be the lowest, no significant difference was observed among the L–T samples, mainly due the large standard deviation of the results. The relatively lower negative charge of the pH 4 L–T samples could explain the slightly higher percentage of tetracycline released from this group (Fig. 5). A lower negative charge of the clay particles may decrease the attractive force between the clay particles and the positively charged tetracycline molecules, in turn enabling tetracycline to escape from the clay lattice more easily. It has been previously suggested that the release of intercalated drug from a clay carrier occurs when the drug molecules are exchanged with cations from solution,[13] *i.e.* $Ca^{2+}$ and $K^+$ in the saliva-like solution used in this study. Due to electrostatic attraction between these cations and the negatively charged laponite layers, these cations ($Ca^{2+}$ and $K^+$) can enter between laponite layers and push the tetracycline molecules out from the clay.

### Release profile of tetracycline from laponite

The release profiles of 0.3% L–Ts prepared under pH 3, 4 and 6 are shown in Fig. 5A. An increased release of the drug was observed in the first two hours for all L–T samples. The percentage of intercalated tetracycline being released in the saliva-like solution from the L–Ts prepared at pH 3, 4 and 6 in the first two hours was respectively 7.3%, 9.8% and 7.5%. This burst release of drug from smectite clay was consistent with findings from previous studies on this type of carrier.[16,17] This initial increased release has been suggested to be due to the elution of the drug adsorbed onto the clay particles.[32] As shown

in Fig. 5A, the release rate of tetracycline was reduced after 2 hours incubation. The total amount of the released drug continued to gradually increase until day 3 reaching a plateau after 3 days. The maximum percentage release of tetracycline at the end of the study period (72 h) was 15.4% for L–T prepared at pH 4. The pH 6 and 3 L–T samples demonstrated 14.6% and 14.7% tetracycline release, respectively. While in our study the extent of drug release from the L–T composites seemed to be lower than those reported for other drug/smectite clay systems,[13,16,17] it should be noted that in the majority of these studies, the drug release was performed under acidic condition (pH 1.2) to mimic the gastric environment. Since the pH of the release medium (saliva-like solution, pH 7.2) and the type of drug intercalated into the clay structure substantially affect the drug release profile and these two parameters varied from study to study, direct comparison between the outcomes of previous findings and this study is not possible. Fig. 5B shows the release of tetracycline from L–T samples (prepared at pH 4) in acidic (pH 5.2) saliva-like solution (SLS). It can be seen that decreasing the pH of SLS from 7.2 to 5.2 led to a substantial increase in tetracycline release from 15.4% to 52.3% after 72 h incubation. Therefore, acidic pH that occurs due to bacterial metabolic activities may enhance the drug release from clay by simply increasing clay solubility. In addition to pH, the presence of cations in the saliva-like solution also influences the drug release profile from laponite. Cations ($Ca^{2+}$, $K^+$) in the saliva-like solution can be exchanged for the intercalated tetracycline (cation exchange) pushing tetracycline out of the laponite layers, thereby increasing the release of tetracycline (Fig. 1). The smectite clay family, such as laponite, has advantages for use as a drug carrier due to the layered structure of smectite mineral, thereby having a high total surface area, adsorptive capacity, surface reactivity, and cation exchange capacity.[33] The cation exchange capacity of laponite helps the release of drug from the carrier in biological fluid due to the presence of cations such as $Ca^{2+}$, $K^+$ and $Mg^{2+}$ (Fig. 1).

### Antimicrobial activity of released tetracycline

Table 4 shows the results of the antibacterial activity assays of released tetracycline from laponite on *E. coli* and *S. aureus* as
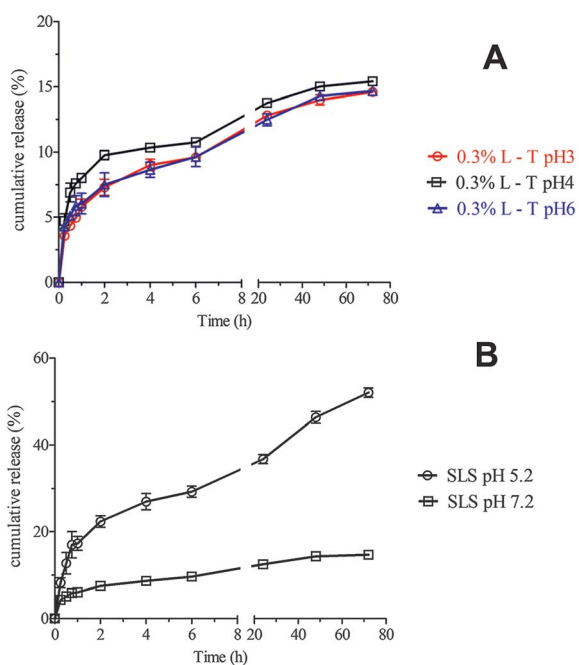


**Fig. 5** (A) 72 hour release profile of tetracycline in saliva-like solution from 0.3% laponite–tetracycline (L–T) composites prepared at pH 3, 4 and 6. (B) The release profile of tetracycline from L–T sample prepared at pH 4 in saliva-like solution and saliva-like solution in acidic condition (pH 5.2).

**Table 4** Antimicrobial activity assays for *S. aureus* and *E. coli versus* tetracycline, composites and extracts: diameter of zone of inhibition (well diffusion assay) *versus* tetracycline, tetracycline–laponite composite and laponite alone and minimum inhibitory concentration (MIC) of tetracycline, extract from tetracycline–laponite composite and extract of laponite

| | *S. aureus* | | *E. coli* | |
|---|---|---|---|---|
| | MIC ($\mu g\ mL^{-1}$) | Inhibition zone diameter (mm) | MIC ($\mu g\ mL^{-1}$) | Inhibition zone diameter (mm) |
| Free tetracycline | 0.468 | 36 | 1.875 | 29 |
| Tetracycline–laponite | 0.468 | 34 | 1.875 | 29 |
| Laponite | — | 0 | — | 0 |

determined by the agar well diffusion and the tube dilution minimum inhibitory concentration (MIC) methods. In the well diffusion assay, the diameter of the zones of inhibition indicated no significant difference between tetracycline alone and the L–T sample. This confirmed that the intercalation process did not have any negative consequences on drug activity and the antimicrobial activity of tetracycline was maintained after its release from the laponite carrier. Laponite alone as a negative control did not show any antibacterial activity. The MIC's of the extracts obtained from L–T samples, the extracts from laponite alone, and tetracycline solution (positive control) are presented in Table 4. This shows that the MIC of the extract from the L–T samples was similar to that of the tetracycline solution, whereas the extract from laponite alone did not inhibit bacterial growth. The MIC of tetracycline has been reported to be in the range of 0.006–124 µg mL$^{-1}$,[34] which is in accordance with the MIC of tetracycline in our study. *Staphylococcus aureus* is more susceptible to tetracycline than *Escherichia coli*,[35] which was reconfirmed by our results (Table 4).

## Conclusion

We observed an increase in the *d*-spacing of the L–T composites compared with laponite alone, suggesting the intercalation of tetracycline between the laponite layers. This was supported by FT-IR and the DTA analyses, indicating that the tetracycline successfully intercalated between the layers of the laponite clay. Tetracycline released from laponite was biologically active and inhibited growth of *S. aureus* and *E. coli*, producing the same zones of inhibition in well diffusion and similar MIC in tube dilution antibiotic assays, as for tetracycline alone. As demonstrated by the release study, the extent of tetracycline release was slightly higher for the samples prepared at pH 4 compared to pH 3 and 6. This was in accordance with the charge of L–T samples prepared at these pH conditions, that is the lower the net negative charge of the L–T samples, the higher the extend of release of tetracycline. The study showed that laponite could be a suitable carrier material for local delivery of tetracycline in treatment of periodontal disease, due to the intercalation of tetracycline between the laponite layers. The study also demonstrated that acidic environment increases the extent of release of tetracycline from laponite carrier.

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## References

1 S. P. Vyas, V. Sihorkar and V. Mishra, *J. Clin. Pharm. Ther.*, 2000, **25**, 21–42.

2 N. J. Medlicott, M. J. Rathbone, I. G. Tucker and D. W. Holborow, *Adv. Drug Delivery Rev.*, 1994, **13**, 181–203.

3 C. Walker and K. Karpinia, *J. Periodontol.*, 2002, **73**, 1188–1196.

4 K. Schwach-Abdellaoui, N. Vivien-Castioni and R. Gurny, *Eur. J. Pharm. Biopharm.*, 2000, **50**, 83–99.

5 N. Jain, G. K. Jain, S. Javed, Z. Iqbal, S. Talegaonkar, F. J. Ahmad and R. K. Khar, *Drug Discovery Today*, 2008, **13**, 932–943.

6 Z. Iqbal, N. Jain, G. K. Jain, S. Talegaonkar, A. Ahuja, R. K. Khar and F. J. Ahmad, *Recent Pat. Drug Delivery Formulation*, 2008, **2**, 58–67.

7 F. Tamimi, J. Torres, R. Bettini, F. Ruggera, C. Rueda, M. Lopez-Ponce and E. Lopez-Cabarcos, *J. Biomed. Mater. Res., Part A*, 2008, **85**, 707–714.

8 A. M. Young, P. Y. J. Ng, U. Gbureck, S. N. Nazhat, J. E. Barralet and M. P. Hofmann, *Acta Biomater.*, 2008, **4**, 1081–1088.

9 G. I. Maze, R. A. Reinhardt, J. B. Payne, C. Maze, R. A. Baker, O. J. Bouwsma, N. C. Damani, J. Fitzgerald, J. C. Hamlin and R. W. Gerlach, *J. Clin. Periodontol.*, 1996, **23**, 1133–1136.

10 S. Senel, G. Ikinci, S. Kas, A. Yousefi-Rad, M. F. Sargon and A. A. Hincal, *Int. J. Pharm.*, 2000, **193**, 197–203.

11 C. Aguzzi, P. Cerezo, C. Viseras and C. Caramella, *Appl. Clay Sci.*, 2007, **36**, 22–36.

12 D. L. Sorby, *J. Pharm. Sci.*, 1965, **54**, 677–683.

13 G. V. Joshi, B. D. Kevadiya, H. A. Patel, H. C. Bajaj and R. V. Jasra, *Int. J. Pharm.*, 2009, **374**, 53–57.

14 P. H. Chang, Z. H. Li, W. T. Jiang and J. S. Jean, *Appl. Clay Sci.*, 2009, **46**, 27–36.

15 Z. H. Li, P. H. Chang, J. S. Jean, W. T. Jiang and C. J. Wang, *J. Colloid Interface Sci.*, 2010, **341**, 311–319.

16 J. P. Zheng, L. Luan, H. Y. Wang, L. F. Xi and K. D. Yao, *Appl. Clay Sci.*, 2007, **36**, 297–301.

17 J. K. Park, Y. Bin Choy, J.-M. Oh, J. Y. Kim, S.-J. Hwang and J.-H. Choy, *Int. J. Pharm.*, 2008, **359**, 198–204.

18 M. Loginov, N. Lebovka and E. Vorobiev, *J. Colloid Interface Sci.*, 2012, **365**, 127–136.

19 H. Jung, H.-M. Kim, Y. B. Choy, S.-J. Hwang and J.-H. Choy, *Appl. Clay Sci.*, 2008, **40**, 99–107.

20 H. Nakayama, N. Wada and M. Tsuhako, *Int. J. Pharm.*, 2004, **269**, 469–478.

21 B. Sieck, S. Takagi and L. C. Chow, *J. Dent. Res.*, 1990, **69**, 1261–1265.

22 J. M. Cowley, *Diffraction physics*, North-Holland, Amsterdam, 1975.

23 C. Perez, M. Pauli and P. Bazerque, *Acta Biologiae et Medecine Experimentalis*, 1990, **15**, 113–115.

24 N. C. f. C. L. Standards, NCCLS, 1996, vol. Approved standard M2–A6.

25 M. E. Parolo, M. C. Savini, J. M. Valles, M. T. Baschini and M. J. Avena, *Appl. Clay Sci.*, 2008, **40**, 179–186.

26 P. Kulshrestha, R. F. Giese and D. S. Aga, *Environ. Sci. Technol.*, 2004, **38**, 4097–4105.

27 A. A. Tzialla, I. V. Pavlidis, M. P. Felicissimo, P. Rudolf, D. Gournis and H. Stamatis, *Bioresour. Technol.*, 2010, **101**, 1587–1594.

28 F. Gambinossi, B. Mecheri, M. Nocentini, M. Puggelli and G. Caminati, *Biophys. Chem.*, 2004, **110**, 101–117.

29 J. E. Gieseking, *Soil Sci.*, 1939, **47**, 1–13.

30 E. Ferrage, B. Lanson, B. A. Sakharov and V. A. Drits, *Am. Mineral.*, 2005, **90**, 1358–1374.

31 H. Palkova, J. Madejova, M. Zimowska and E. M. Serwicka, *Microporous Mesoporous Mater.*, 2010, **127**, 237–244.

32 N. Meng, N. L. Zhou, S. Q. Zhang and J. Shen, *Int. J. Pharm.*, 2009, **382**, 45–49.

33 S. S. Ray and M. Okamoto, *Prog. Polym. Sci.*, 2003, **28**, 1539–1641.

34 H. D. Isenberg, *Clinical microbiology procedures handbook*, American Society for Microbiology, Washington, DC, USA, 1992.

35 L. P. Garrod and F. O'Grady, *Antibiotic and chemotherapy*, E. & S. Livingstone, London, 3rd edn, 1971.