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Synthesis and release kinetics of polymerisable ester drug conjugates: towards pH-responsive infection-resistant urinary biomaterials

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ABSTRACT

Herein we report the synthesis, characterisation and hydrolytic release kinetics of a suite of novel, polymerisable ester quinolone conjugates with varying alkenyl chain lengths. Hydrolysis was shown to proceed up to 17-fold faster upon elevation of pH from neutral to pH 9.29, making these conjugates attractive for the development of 'designer' infection-resistant urinary biomaterials exploiting the increase in urine pH reported at the onset of catheter-associated infection to trigger drug release.

Implantation of medical devices, with their inherent susceptibility to bacterial colonisation, has been implicated in over half of all hospital-acquired infections,[1] hence there is currently a significant interest in the development of antimicrobial biomaterials. Currently marketed drug-eluting medical devices rely on diffusive release of therapeutic agents from the device surface,[2] however, clinical performance is limited by suboptimal release kinetics: an initial burst of surface-localised agent restricting long-term effectiveness, followed by continuous antimicrobial elution in subtherapeutic levels potentially augmenting bacterial resistance problems.[3] Furthermore, voids left by release of drug from within the polymer matrix may compromise mechanical performance.[4] The ideal drug-eluting polymer system would be engineered to display tunable release properties and, in the context of infection-resistant devices, be capable of achieving a precise 'on–off' response.[5] Recently, much interest has been generated in the application of labile-drug polymer conjugates to three-dimensional drug delivery systems.[6] This strategy provides an additional level of control over drug release, mediated by cleavage of the therapeutic from pendant groups attached to the polymer main chain, while simultaneously preserving structural integrity.[2]

Herein we describe the synthesis of a series of polymerisable, vinyl-functionalised derivatives of nalidixic acid (**1**), a naphthyridinone antibiotic with a wide spectrum of activity against common Gram-negative urinary pathogens,[7] via a two-step procedure involving the preparation of a reactive nalidixic anhydride intermediate **2** and subsequent esterification with a range of deprotonated alkenols **3**–**8**. The design of the conjugates was predicated upon the availability of two key components: firstly, an appropriate hydrolysable linkage to allow release of the incorporated drug and, secondly, a functional group amenable to free radical co-polymerisation with vinyl monomers to allow permanent incorporation within biomaterials. Characterisation of hydrolysis kinetics across a full pH range (pH 2–pH 12) informs the use of these novel ester drug conjugates in the development of a chemically-triggered release system exploiting the elevation in urine pH caused by urease-producing urinary pathogens, such as *Proteus mirabilis*,[8] to trigger antimicrobial cleavage, specifically through controlled hydrolysis of an ester bond.

Nalidixic acid adopts a very stable pseudo six-membered ring structure, resulting either from strong intramolecular hydrogen bonding between the carbonyl oxygen of the naphthyridine ring carbonyl group with the hydrogen of the carboxylic acid group, effectively rendering the carboxylic acid group chemically inert,[9] or from conjugation of the carboxylic acid carbonyl group with the ring nitrogen. Direct esterification is therefore difficult and, in addition, synthesis of a reactive acyl chloride derivative is hindered by the concomitant chlorination of the aromatic methyl group by thionyl chloride or oxalyl chloride.[9] Amides of nalidixic acid have, however, been successfully synthesised using mixed carboxylic–carbonic anhydrides as intermediates.[10,11] This strategy has been adapted to the esterification of nalidixic acid during examination of the effect of neighbouring group participation on ester hydrolysis rates,[12] and is extended herein to demonstrate the effects of spacer chain length on the release of nalidixic acid from polymerisable ester drug conjugates.

The mixed anhydride {[(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbonyl) amino] acetic acid ethyl ester} (**2**) formed from the reaction of 1-ethyl-7-methyl-4-oxo-1,4-dihydro-

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1,8-naphthyridine-3-carboxylic acid (nalidixic acid) (**1**) with ethyl chloroformate in the presence of triethylamine at ambient temperature, as shown in Scheme 1, was isolated in 85% yield.[13] Subsequent coupling with the appropriate deprotonated alkenol **3–8**, as summarised in Scheme 1, yielded conjugates **9–14** in yields of 32–62%, following work-up and purification by column chromatography.[14]

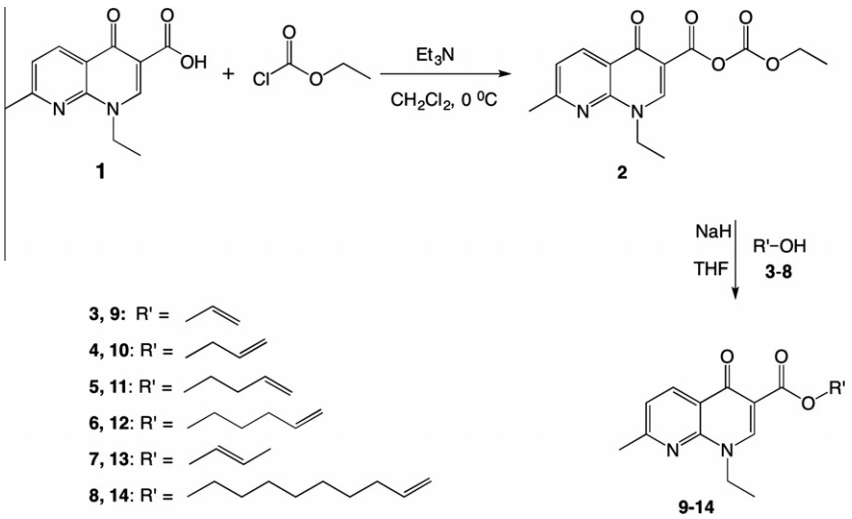
To determine the effects of pH and spacer length on the hydrolysis behaviour of the ester drug conjugates, solutions of each derivative **9–14** were prepared in aqueous buffers with pH values ranging from pH 2 to pH 12 and a constant ionic strength of 154 mmol, prepared according to Table 1, and analysed by UV–visible spectroscopy. Verification of the pH was performed throughout the study. Prior to hydrolysis, conjugates **9–14** show an absorbance maximum at 258 nm due to the n–π^* transition of the carbonyl group and a less intense band at 330 nm with a shoulder at 320 nm corresponding to the extended π-system of the nalidixate chromophore, as previously reported for derivatives of nalidixic acid.[15] Hydrolysis proceeds in an analogous manner to literature reports,[12] with a bathochromic shift of 4 nm of the longest-wavelength band with a shoulder to a single band of lower intensity. Figure 1 shows overlaid UV–visible spectra of conjugate **10** after various times during the course of hydrolysis as a representative example of the behaviour of **9–14**. The similar spectral changes observed for all conjugates at pH values greater than pH 9 together with the isosbestic point at 334 nm indicate that **9–14** hydrolyse by a similar process involving a single reaction: cleavage of the ester bond between the nalidixic moiety and the alkenyl spacer, with no side reactions. Furthermore, nalidixic acid (**1**) possesses the same aromatic π-system as the ester drug conjugates **9–14**, therefore no significant spectral changes were expected during hydrolysis; the 4 nm bathochromic shift observed upon liberation of nalidixic acid (**1**) may be attributed to the loss of a minor stabilising interaction resulting from the small degree of overlap between the vinyl moiety and this π-system.[12] Liberation of free nalidixic acid (**1**) was confirmed by identification of the reaction products following chromatographic separation. Conversely, UV–visible spectra of the conjugates in universal buffers below pH 9 exhibit no appreciable evolution of nalidixic acid after a similar time (30 h). The slight decrease in intensity of the longest-wavelength band over time, shown by the overlaid spectra of **10** at pH 6.15, Figure 2, suggests that ester hydrolysis does occur at neutral and acidic pH, albeit at a very slow rate.

Table 1
Formulation of universal buffer

pH	Stock[a] (μL)	2 M NaOH (μL)	KCl (mg)	H_2O (mL)
2.3	1111	67	71.0	10.60
2.8	1000	180	61.8	10.62
4.01	1000	245	55.6	11.20
5.03	833	292	42.2	10.12
6.01	833	350	35.3	10.65
7.04	833	437	25.8	11.43
7.98	714	429	8.6	10.29
9.05	714	486	1.8	10.80
9.98	714	557	0	11.44
11.3	625	530	0	10.31
11.96	625	601	0	10.95

[a] Stock solution (100 mL) contains 2.7 mL of H_3PO_4, 2.29 mL of AcOH and 2.48 g of H_3BO_3 dissolved in deionised H_2O.[19]

The progress of the hydrolytic reactions for **9–14** in aqueous buffer solutions of the appropriate pH was monitored by quantification of the first-order rate constant using spectral data at 320 nm. Conjugates **9–14** demonstrate a similar hydrolytic behaviour with negligible hydrolysis below pH 7 during the study period of 144 h. Of particular significance to the development of pH-triggered drug-eluting urinary biomaterials is the approximate order of magnitude logarithmic increase in hydrolytic rate between pH 7 and pH 9, and 1.5–2.5 orders of magnitude logarithmic increase between pH 7 and pH 12, as shown in Table 2. The regression coefficients describing the linear relationship between rates of hydrolysis and pH for each conjugate confirm the mechanism of reaction as general base-catalysed hydrolysis. Steric and electronic effects are both acknowledged to contribute to variation in hydrolytic rates.[16–18] The major variant between the studied compounds in this study is the length of the alkenyl spacer, which is electronically insulated from the nalidixyl chromophore. The monomeric materials presented are likely to be less influenced by steric factors than their polymeric or co-polymeric counterparts; indeed the observed hydrolysis rate constants in Table 2 show no significant correlation with chain length in solution. Following co-polymerisation with an appropriate hydrogel backbone, variation in steric hindrance and subsequent accessibility to attacking nucleophiles is expected to modulate the rate of the ester hydrolysis according to the length of the spacer chain, making it possible to both predetermine and sustain subsequent release kinetics of the selected



Scheme 1. Synthesis of **2**, a mixed anhydride of nalidixic acid (**1**) and vinyl drug conjugates **9–14**.