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RESEARCH PAPER

Infection-Responsive Drug Delivery from Urinary Biomaterials Controlled by a Novel Kinetic and Thermodynamic Approach

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ABSTRACT

Purpose The pH-dependent physicochemical properties of the antimicrobial quinolone, nalidixic acid, were exploited to achieve 'intelligent' drug release from a potential urinary catheter coating, poly(2-hydroxyethylmethacrylate) (p(HEMA)), in direct response to the elevated pH which occurs at the onset of catheter infection.

Methods p(HEMA) hydrogels, and reduced-hydrophilicity copolymers incorporating methyl methacrylate, were loaded with nalidixic acid by a novel, surface particulate localization method, and characterized in terms of pH-dependent drug release and microbiological activity against the common urease-producing urinary pathogen *Proteus mirabilis*.

Results The pH-dependent release kinetics of surface-localized nalidixic acid were 50- and 10-fold faster at pH 9, representing the alkaline conditions induced by urease-producing urinary pathogens, compared to release at pH 5 and pH 7 respectively. Furthermore, microbiological activity against *P. mirabilis* was significantly enhanced after loading surface particulate nalidixic acid in comparison to p(HEMA) hydrogels conventionally loaded with dispersed drug. The more hydrophobic methyl methacrylate-containing copolymers also demonstrated this pH-responsive behavior, but additionally exhibited a sustained period of zero-order release.

Conclusions The paradigm presented here provides a system with latent, immediate infection-responsive drug release followed by prolonged zero-order antimicrobial delivery, and represents an 'intelligent', infection-responsive, self-sterilizing biomaterial.

KEY WORDS anti-infective · nalidixic acid · pH-responsive · solubility · urinary catheter

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ABBREVIATIONS

AIBN	2,2′-azobisisobutyronitrile
CAUTIs	catheter-associated urinary tract infections
EGDMA	ethyleneglycol-dimethacrylate
HEMA	2-hydroxyethylmethacrylate
MHB	Mueller-Hinton broth
MMA	methyl methacrylate
PBS	phosphate-buffered saline
pHEMA	poly(2-hydroxyethylmethacrylate)

INTRODUCTION

Biomaterials have revolutionized the practice of modern medicine with indispensable diagnostic and therapeutic applications (1). The medical device market generates ~ $200 billion each year in the United States (2009 data) and, furthermore, has an annual growth rate of ~7.5% (1989 to 2009 data) (2). However, while employed to improve health, their susceptibility to infection means they frequently cause much patient morbidity and mortality (3). The catheterized urinary tract constitutes an ideal niche for bacterial colonization, and provides an exemplary case of the risks associated with implantation of medical devices; an estimated 80% of all nosocomial urinary infections are attributed to the presence of an indwelling catheter (4), and catheter-associated urinary tract infections (CAUTIs) represent the most common healthcare-acquired infection, with almost 500,000 cases reported annually in the United States (5).

As highlighted recently, catheters in current clinical use are almost identical to those introduced in the 1930s, with improvements in catheter design considered relatively insignificant compared to advancements reported within other medical fields (6). However, on account of the 2008 decision by the Centers for Medicare and Medicaid Services to hold

hospitals financially accountable for these "reasonably preventable" nosocomial infections, catheters with improved resistance to infection must be developed as a matter of urgency, otherwise the financial implications of CAUTIs may be serious (7).

The preference of planktonic bacteria to grow on surfaces has been known since 1943 (8), resulting in rapid bacterial colonization of medical devices following introduction into the human body (9). Bacteria form biofilm by growing in highly-regulated sessile communities encased in self-produced amorphous matrix substances, protected from innate immune defenses and demonstrating up to 1000-fold greater resistance to antimicrobial agents than their planktonic counterparts (10). Up to 50% of patients undergoing long-term catheterization experience recurrent catheter encrustation and blockage (11), reported by Stickler *et al.* (2008) as the most significant complication associated with colonization of implanted devices by urease-producing urinary pathogens, particularly *Proteus mirabilis* (6). The urease-catalyzed hydrolysis of urea into ammonia can elevate urine pH to pH 9.1, causing precipitation of magnesium and calcium phosphates, which subsequently become trapped in the biofilm matrix (12). Sequelae include trauma to the bladder and urethral epithelia upon removal of the encrusted catheter, or, alternatively, occlusion of the catheter lumen with consequential urinary retention, pyelonephritis, septicemia and possible mortality if the catheter is left *in situ* (6).

This pH elevation at the device surface, where biofilm begins to form, is exploited in this study to act as a trigger for 'intelligent' drug release in direct response to the onset of infection, without external intervention, to give a latent, responsive, self-sterilizing surface. Unlike previously reported pH-responsive drug delivery systems, where stimuli-induced physical or chemical changes in the polymer network are necessary for resultant drug release (13–15), this study relies on the drug itself modulating its own rate of release in response to the stimulus through exploitation of its significantly increased solubility in alkaline media in comparison to its relatively poor solubility at normal physiological urine pH (16). To achieve this, we exploit the particularly poor solubility of particulate nalidixic acid in a hydrogel in the absence of infection, and tune the hydrophobicity of that hydrogel to give prolonged release when infection is present and nalidixic acid can dissolve, and, subsequently, elute from the surface of the hydrogel with a resulting cidal effect.

MATERIALS AND METHODS

Materials

Nalidixic acid, 2-hydroxyethylmethacrylate, methyl methacrylate, ethyleneglycol-dimethacrylate, phosphoric acid, acetic acid, boric acid, hydrochloric acid, potassium chloride and sodium hydroxide were purchased from Sigma-Aldrich (Poole, Dorset, UK). 2,2′-Azobisisobutyronitrile was obtained from VWR International Ltd. (Lutterworth, UK). Phosphate-buffered saline and Mueller-Hinton broth were obtained from Oxoid Ltd (Hampshire, UK). Mueller-Hinton agar was adjusted to pH 9 by addition of sodium hydroxide.

Synthesis of Poly(2-Hydroxyethylmethacrylate) Polymer Films

Films of p(HEMA) were synthesized by dissolving ethyleneglycol-dimethacrylate (EGDMA, 1% w/w) and 2,2′-azobisisobutyronitrile (AIBN, 1% w/w) (crosslinker and initiator, respectively) at ambient temperature in 2-hydroxyethylmethacrylate (2-HEMA, 98% w/w) with stirring. The total mass of polymer produced was 10 g. The resulting solution was injected slowly into moulds comprising two glass plates, which were separated with medical grade silicone tubing with lumen diameter of 3 mm and wall thickness of 0.18 mm, lined with release liner, and clamped using spring clips. Following polymerization at 90°C for a period of 3 h, the formed films were soaked for 1 week in double distilled water to remove unreacted monomers and initiator, with the soaking medium refreshed daily, then dried to constant weight in an oven at 60°C to form xerogels prior to the drug loading stage. The removal of all monomers from the films was verified by monitoring acrylate absorption in the washing media at 220 nm.

Drug Loading Methods

A series of nalidixic acid-loaded polymers were prepared. For comparative purposes, polymers were first loaded with nalidixic acid by two conventional drug loading methods. In the first method the hydrogel was in a saturated nalidixic acid solution (denoted *post polymerization*) and in the second method the drug was added to the monomer mixture before polymerization (denoted *in situ* loading). The conventional methods were then modified to allow localization of particulate nalidixic acid on the surface of both the same pHEMA hydrogel (to modify release due to kinetics of dissolution of particulate drug) and a more hydrophobic copolymer of HEMA and MMA (to both modify release due to kinetics of dissolution and retard release towards more clinically-relevant durations) and subsequent assessment of the pH-responsive behavior.

Conventional Drug Loading Methods

Post-Polymerization: Equilibration in a Saturated Nalidixic Acid Solution

Hydrogels were loaded with nalidixic acid by swelling xerogel discs (1 cm^2) of p(HEMA) in a saturated nalidixic acid

solution, prepared in pH 7 universal buffer, for 72 h, a sufficient duration to ensure equilibrium uptake of drug, as previously described (17).

In Situ Loading: Preparation of 1% w/w Nalidixic Acid-Loaded p (HEMA)

Nalidixic acid (1% w/w) was added to a mixture of 2-HEMA (97% w/w) and EGDMA (1% w/w). The mixture was sonicated for 15 min before heating to 60°C to fully dissolve the drug. The initiator, AIBN (1% w/w), was then added, mixed, and the resulting solution injected into moulds as previously described for p(HEMA). The total mass of polymer produced was 10 g. Polymerization was carried out at 90°C for 3 h, after which the formed films were washed for 5 days in pH 5 universal buffer as for p(HEMA) films to remove unreacted monomers and initiator.

Novel Drug Loading Methods

Post-Polymerization: Equilibration in a Saturated Nalidixic Acid Suspension

Xerogel discs (1 cm^2) of p(HEMA) were soaked in a suspension of nalidixic acid (prepared by adding nalidixic acid (200 mg) to pH 7 universal buffer (100 mL) and sonicating for 20 min) for 72 h, a sufficient duration to allow equilibrium uptake of dispersed drug in addition to surface localization of particulate matter. Prior to the release studies the drug-loaded hydrogels were immersed in pH 7 universal buffer for 5 s to rinse off excess particulate drug from the disc surface.

In Situ Loading: Preparation of 1% Nalidixic Acid-Loaded 50 mol% p(MMA-co-HEMA)

Nalidixic acid (1% w/w) and EGDMA (1% w/w) were sonicated for 20 min with monomers 2-HEMA and methyl methacrylate (MMA) in an equimolar ratio. AIBN (1% w/w) was added and the mixture stirred at 30°C for 20 min. The resulting solution was injected into moulds, as described for p(HEMA), then polymerized at 90°C for 18 h. The total mass of copolymer produced was 10 g. The formed films were soaked for 5 days in pH 5 universal buffer as for p(HEMA) films to remove unreacted monomers and initiator. Discs (1 cm^2) of the formed copolymers were then soaked for 5 days in either a pH 5 saturated nalidixic acid solution to allow equilibrium uptake of dispersed drug, or a pH 5 saturated nalidixic acid suspension (prepared by adding nalidixic acid (50 mg) to pH 5 universal buffer (100 mL) and sonicating for 20 min) to additionally load surface particulate drug. Prior to the release studies the drug-loaded hydrogels were immersed in pH 7 universal buffer for 5 s to rinse off excess particulate drug from the disc surface.

In Vitro Release of Nalidixic Acid

Release kinetics of all drug-loaded polymers were examined at pH 5, pH 7 and pH 9 in universal buffer solutions having constant ionic strength at all pH values, prepared according to the formulation of Koller *et al.* (1992) and reported in Table I.

Pre-swollen drug-loaded p(HEMA) discs (1 cm^2), suspended on a needle, were placed in McCartney bottles containing either 10 or 20 mL pre-warmed universal buffer solution (pH 5, pH 7 or pH 9) and shaken at 37°C in an oscillating water bath (100 rpm). At predetermined intervals, the discs were transferred to fresh medium (10 or 20 mL, to ensure sink conditions) and the released drug quantified by UV-visible spectroscopy (λ=257 nm), with concentration dependence of nalidixic acid on absorbance determined from freshly prepared calibration curves ($r^2 >$ 0.999). Release experiments at each pH were performed in triplicate.

The mechanism of drug release at each pH was determined by fitting data to the Korsmeyer-Peppas model equation (Eq. 1):

$$F = K_{KP} t^n \quad (1)$$

where F represents the fractional drug release in time t, K_{KP} is a kinetic constant incorporating geometric and structural characteristics of the release system, and n is the release exponent used to characterize the mechanism of drug release (19).

Determination of Thermodynamic and Kinetic Solubility of Nalidixic Acid

Rates of nalidixic acid dissolution in pH 5, pH 7 and pH 9 media of constant ionic strength were determined at a temperature of 37°C and rotation speed of 100 rpm. Excess nalidixic acid was added to pre-warmed universal buffer solution of the required pH, prepared according to the formulation in Table I, and samples (3 mL) withdrawn at designated

Table I Formulation of Universal Buffer

pH	Stock (mL)[a]	2 M NaOH (mL)	KCl (g)	H_2O (mL)
5.0	62.475	21.9	3.165	759
7.0	62.475	32.775	1.935	857.25
9.0	57.12	38.88	0.144	864

[a] Stock solution (100 mL) contained 2.7 mL phosphoric acid, 2.29 mL acetic acid and 2.48 g boric acid dissolved in double distilled water (18)

intervals, filtered through 0.22 μm pore size filter membranes (Millex, Millipore Corporation, MA, USA), and filtrate concentrations determined by UV-visible spectroscopy as before, after the necessary dilutions, with reference to freshly prepared calibration curves at each pH. Kinetic estimates of nalidixic acid dissolution at pH 5, pH 7 and pH 9 were obtained from linear regression of the respective dissolution profiles, whereas equilibrium solubilities at each pH were determined from filtrate concentrations of samples shaken continuously for 4 days as previously described (20).

In Vitro Antibacterial Activity of Nalidixic Acid-Loaded p(HEMA)

Proteus mirabilis ATCC 35508 (LGC Standards, Middlesex, UK) was maintained on cryopreservative beads (Protect Bacterial Preservation System, Technical Service Consultants Ltd., UK) in 10% glycerol at −80°C and cultivated in Mueller-Hinton broth (MHB) at 37°C when required. Overnight broth cultures were centrifuged (3,000 rpm, 12 min) and re-suspended in phosphate-buffered saline (PBS) to an optical density (OD) of 0.1 at 540 nm, representing an inoculum concentration of approximately 1×10^8 cfumL^{-1}.

The microbiological performance of nalidixic acid-loaded materials was assessed, with the same materials devoid of nalidixic acid serving as positive growth controls, on pH 9 MHA inoculated with *P. mirabilis* according to the standardised Kirby-Bauer disk diffusion method (21). Inoculum (1.0×10^8 cfumL^{-1}, 100 μL) was added to a test tube of molten MHA (pH 9, 10 mL), vortexed, then poured into a sterile Petri dish. Once the agar had set and cooled, discs (1 cm^2) of the drug-loaded hydrogels were placed on the surface of the inoculated MHA plates and incubated at 37°C for 24 h. Each day, the diameter of the circular inhibition zone with no bacterial growth, including the disk diameter, was measured. Any discs exhibiting zones of growth inhibition were transferred to freshly seeded plates and the process repeated daily until no further inhibition was observed.

Statistical Analysis

The effect of pH on kinetic and thermodynamic solubility values of nalidixic acid and cumulative drug released at selected intervals, and the effect of drug loading method on microbiological activity of the polymers against *P. mirabilis*, demonstrated by bacterial zones of inhibition, were statistically evaluated by a one-way analysis of variance. Post-hoc comparisons between means of individual groups were performed using Tukey's honestly significant difference (HSD) test and in all cases differences were considered significant when $p<0.05$.

RESULTS

In this study, the pH dependence on both the rate of dissolution and ultimate solubility of nalidixic acid are exploited to trigger enhanced release from hydrogels at the onset of infection, when pH rises significantly.

These solubility parameters for the drug were determined, and release from hydrogels based on pHEMA and pHEMA-*co*-MMA loaded with both particulate and dissolved drug were compared to pHEMA materials loaded more conventionally with dissolved drug either pre- or post-polymerization.

The enhanced rate of dissolution of particulate drug at elevated pH (found at the onset of infection) serves to significantly enhance the release rate from the former compared to the conventional loading methods.

Effect of pH on Kinetic and Thermodynamic Solubility Values of Nalidixic Acid

Kinetic rates of nalidixic acid dissolution, obtained from linear regression of the respective dissolution plots, and the corresponding equilibrium solubilities at pH 5, pH 7 and pH 9 are recorded in Tables II and III respectively.

The kinetics of dissolution were governed by thermodynamic solubility values, which in turn were dependent on pH; increased pH was associated with more rapid dissolution, for example dissolution rates of 6.00 μgmin^{-1}, 39.5 μgmin^{-1} and 378 μgmin^{-1} were demonstrated in universal buffer of pH 5, pH 7 and pH 9 respectively, while low solubility was characteristic of acidic media and very high solubility was demonstrated in alkaline media: equilibrium solubilities were 41.2± 0.62 μgmL^{-1} and 2,258±11.0 μgmL^{-1} in pH 5 and pH 9 universal buffer respectively. Furthermore, the increase in solubility was even more significant upon raising pH from pH 7 to pH 9 than the corresponding increase from pH 5 to pH 7, with equilibrium solubility enhanced by factors of 10 and 6 respectively.

In Vitro Nalidixic Acid Release

The significant effect of pH upon kinetics of nalidixic acid release from the p(HEMA) hydrogels loaded in a saturated drug suspension post-polymerization is shown in Fig. 1. Release of nalidixic acid was almost instantaneous when

Table II Kinetic Rate of Nalidixic Acid Dissolution at pH 5, 7 and 9

pH	Dissolution rate (μgmin^{-1})	Dissolution rate relative to pH 5
5	6.00	1
7	39.5	6.59
9	378	63.1

Table III Thermodynamic Solubility of Nalidixic Acid at pH 5, 7 and 9

pH	Equilibrium solubility ($\mu g mL^{-1}$) (± SD)	Equilibrium solubility relative to pH 5
5	41.2 ± 0.62	1
7	236 ± 11.1	5.73
9	2258 ± 11.0	54.9

pH was elevated to pH 9, with time for release of 90% of loaded drug reduced by factors of 50 and 10 at pH 9 compared to release at pH 5 and pH 7 respectively.

Prolonged drug release was demonstrated from p(HEMA) co-polymerized with hydrophobic methyl methacrylate (MMA) and loaded with nalidixic acid *in situ* and post-polymerization, as shown in Fig. 2, in comparison to release from unmodified p(HEMA) loaded with nalidixic acid in a saturated drug suspension solely post-polymerisation; in pH 9 media ~60% of loaded drug was released from 1% nalidixic acid-loaded 50 mol% p(MMA-co-HEMA) copolymers additionally loaded with nalidixic acid post-polymerization after 1 week, whereas release from p(HEMA) conventionally-loaded with nalidixic acid solution was so rapid that >90% of the loaded drug was released in the initial 5 min. When loaded in the nalidixic acid suspension post-polymerization, comparatively more drug was localized on the surface compared with loading in the saturated nalidixic acid solution post-polymerization, and this was accounted for in the initial rapid burst, which was thereafter followed by prolonged Case II release of drug from the matrix interior. Dissolution of surface-localized particulate drug was primarily responsible for the pH-mediated response and thereafter release that was dependent on diffusion of dispersed drug through the hydrogel matrix occurred at similar rates at all pH values from both systems. Modeling of the release data with the Korsmeyer-Peppas equation (19) was not possible on account of the initial rapid burst of surface-localized drug, however, when applied to data excluding this initial burst, release exponents, n, were found to approximate unity at all pH values implying zero-order release in which release rates were independent of time.

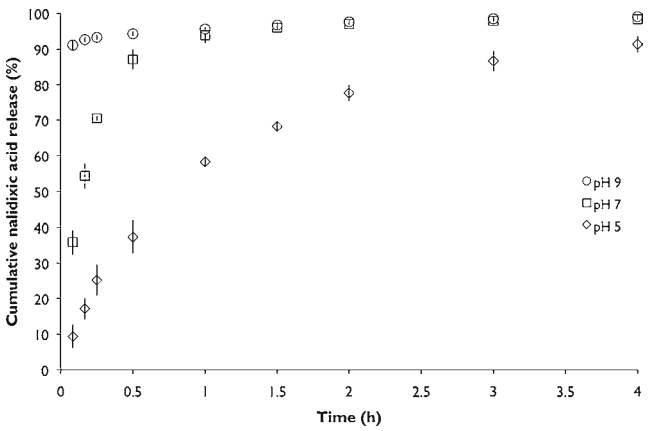

Fig. 1 The effect of pH on the mean (± s.d.) release of nalidixic acid from p(HEMA) loaded in a saturated drug suspension *via* novel method 1 at 37°C with shaking. *Circles*, *squares* and *diamonds* refer to release at pH 9, 7 and 5 respectively. Release approximates 100% at 24 h in all pH environments.

In Vitro Antibacterial Activity of Nalidixic Acid-Loaded p(HEMA)

All nalidixic acid-loaded p(HEMA) discs inhibited growth of *P. mirabilis* on MHA adjusted to pH 9 after 24 h incubation, however, significant differences were observed in both magnitude of growth inhibition zones and persistence of inhibitory activity depending on loading method, as illustrated in Fig. 3. After 24 h incubation, significantly larger inhibitory zones were displayed by p(HEMA) loaded in a saturated nalidixic acid suspension post-polymerization in comparison to hydrogels loaded *via* the two conventional methods, due to the burst release of surface-localized particulate drug. Hydrogels conventionally-loaded in a nalidixic acid solution rapidly lost their antibacterial activity with no growth inhibition by day 3, whereas with the exception of day 1, 1% w/w nalidixic acid *in situ*-loaded p(HEMA) displayed statistically similar antibacterial activity to that of p(HEMA) loaded in a saturated drug suspension post-polymerization. No zones of inhibition were observed around the p(HEMA) controls devoid of nalidixic acid, which implies that the observed microbiological activity is attributable solely to the release of drug.

DISCUSSION

Approximately 25% of all patients admitted to hospitals in the United States are catheterized with an indwelling urinary device (22), however, with the incidence of bacteriuria increasing by 5% each day of catheterization (23), insertion of a urinary catheter is ultimately associated with a significant risk of infection. Due to the nature of bacterial growth within highly coordinated biofilm communities, these infections are often difficult to treat resulting in much patient morbidity, mortality and increased healthcare costs (6). Novel approaches are urgently needed to obviate this problem and reduce the prevalence of these infections.

Much research has focused on either physicochemical surface modifications (24), or the local release of antibacterial agents, including antibiotics, metals, and antiseptics, which have been coated onto the device surface, or alternatively, directly incorporated within the biomaterial with the unanimous aim of preventing bacterial adherence (25–27). Clearly, it is desirable to deliver antibacterials locally to the required site and avoid systemic exposure, however, previously investigated approaches are united by the limitation of sub-optimal release kinetics. Commonly, there is an initial

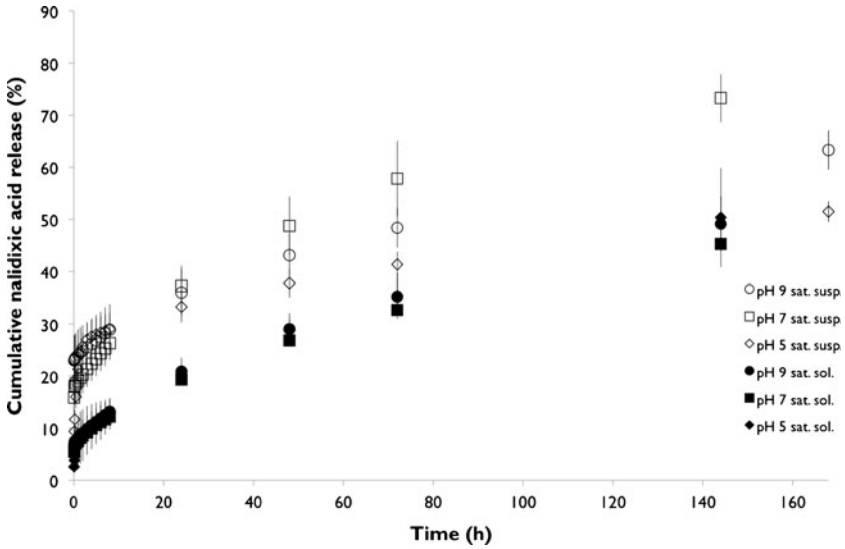

Fig. 2 The effect of pH on the mean (± s.d.) release of nalidixic acid from 1% nalidixic acid-loaded 50 mol% p(MMA-co-HEMA) at 37°C with shaking. *Open* and *closed symbols* refer to release from copolymers additionally loaded with nalidixic acid post-polymerization in a saturated nalidixic acid suspension and saturated nalidixic acid solution respectively.

rapid loss of agent from the surface limiting long-term effectiveness (28), followed by continuous sub-inhibitory antimicrobial release potentially augmenting bacterial resistance problems (29). Furthermore, all antimicrobial devices currently marketed in the United States with nitrofurazone or silver alloy coatings failed to demonstrate any clinical benefit with regard to variables such as patient morbidity, symptomatic urinary tract infections, secondary bacteremia, and financial savings in a recent randomized systematic review (30). Consequently, there has been significant interest in the literature in 'intelligent' drug delivery systems capable of tailoring drug release according to clinical need (14).

Previously reported pH-responsive drug delivery systems respond *via* physical induction of macroscopic changes in their polymer network, thereby controlling release of an entrapped drug (13,31), or, alternatively, *via* chemical cleavage of a labile linker between the drug and the polymer backbone (15). In this study, however, the drug itself, by responding to the pH trigger present at the onset of infection, was inherently responsible for its own release and not dependent on the polymer carrier to modulate release; we hold that this is a novel approach in the field.

The physicochemical properties of nalidixic acid are reported to be highly pH-dependent due to the presence of an ionizable carboxyl group in the quinolone molecular structure (Fig. 4). With a pKa of 6.0 ± 0.5 (32), nalidixic acid exists completely as its ionized moiety at alkaline pH, with resultant higher affinity for aqueous media. Nalidixic acid has previously been reported to dissolve more rapidly at pH values above pH 6.5 than in more acidic media (33), however, this study extends this observation by demonstrating the greater enhancement in solubility with increasing alkalinity: a 10-fold increase in both kinetic and thermodynamic solubility values of this therapeutic agent was demonstrated upon raising pH from pH 7 to pH 9 in comparison to the approximate 6-fold difference between pH 5 and pH 7. While the poor aqueous solubility and slow dissolution of nalidixic acid at physiological pH 7 is frequently regarded as an adverse physicochemical

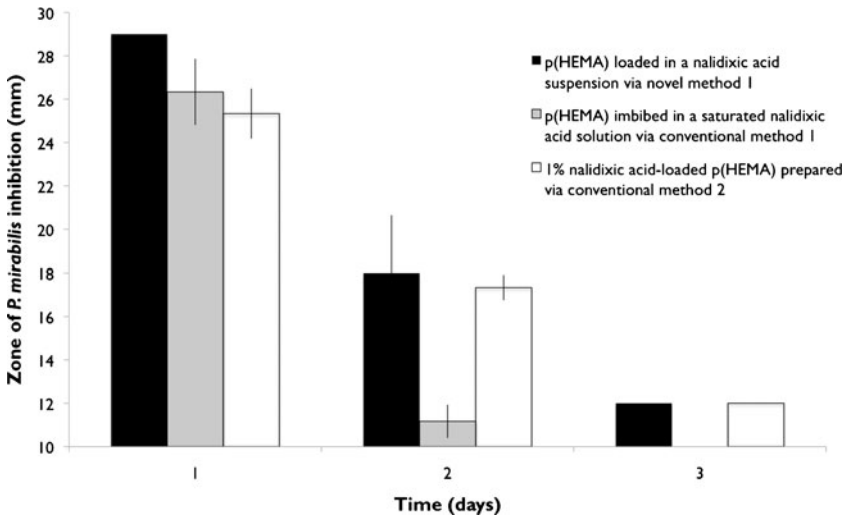

Fig. 3 Comparison of zones of growth inhibition of nalidixic acid-loaded p(HEMA) hydrogels against *P. mirabilis* as a function of time for each loading method used.

property, limiting the absorption, bioavailability and subsequent clinical use of this drug (34), we exploit this behavior as a key control mechanism to achieve rapid drug delivery responsive to conditions of elevated pH, such as the onset of urinary catheter infections, through design of a novel loading method involving the rational localization of particulate drug on the surface of a model hydrogel suitable for use as a urinary device coating, p(HEMA). The significance of this surface particulate drug loading, whereby resultant release was controlled specifically by drug dissolution rate and not limited by diffusion through the hydrogel matrix, was demonstrated by the almost instantaneous drug release at pH 9. Furthermore, a significant 50-fold reduction in time for release of 90% of loaded drug was demonstrated upon elevation of pH from normal physiological urine pH values ranging from pH 5 to pH 7 (35,36) to the pH typical of infected urine, pH 9 (12).

Fundamental to the application of these pH-responsive drug-loaded hydrogels as infection-resistant urinary biomaterials is their ability to inhibit growth of urinary pathogens, such as *P. mirabilis*. Accordingly, the antibacterial efficacy of the hydrogels loaded with surface particulate nalidixic acid post-polymerization was examined in comparison to p(HEMA) loaded *via* two conventional methods at pH 9, representing the alkaline conditions induced by typical urease-producing infecting microorganisms. Conventional molecular dispersion was achieved firstly through equilibrium partitioning, whereby drug was imbibed into cross-linked p(HEMA) discs by swelling in a saturated drug solution (17), and secondly, by *in-situ* drug loading during polymerization, which involved dissolving the drug in the hydrogel monomer prior to crosslinking (37). Zones of *P. mirabilis* growth inhibition were consistently larger around p(HEMA) loaded in the saturated nalidixic acid suspension post-polymerization than the zones surrounding the conventionally loaded hydrogels. Rapid release of particulate drug localized at the hydrogel surface was demonstrated by the significantly larger inhibitory zones displayed by this material after 24 h incubation at 37°C, with microbiological activity thereafter similar to discs of the 1% nalidixic acid-loaded p(HEMA) prepared *via* conventional *in situ* loading. Release from hydrogels imbibed in the saturated solution *via* the conventional post-polymerization method was so rapid that antibacterial efficacy was limited to the initial 24 h, with insufficient release thereafter.

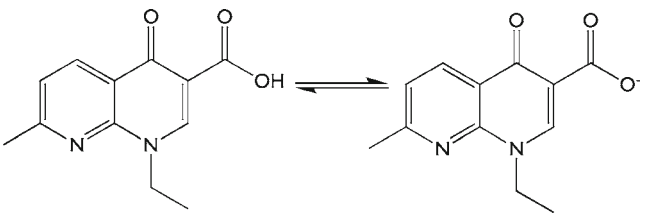

Fig. 4 Molecular structure of nalidixic acid in its unionised form (below its pKa) and its ionised form (above its pKa).

Long-term urinary catheters are typically *in situ* for up to 4 weeks before each scheduled change (6). Based on this release data, pH-responsive dissolution-controlled release of surface localized particulate nalidixic acid was an effective approach to achieve rapid drug release at pH 9, characteristic of infected urine, however, when combined with conventional dispersion of dissolved drug within a p(HEMA) matrix, the duration of prophylaxis against infecting pathogens was insufficient for long-term catheters implanted within the urinary tract. In contrast to the immediate dissolution-controlled pH-responsive release of particulate drug localized on the surface, the rapid release of molecularly dispersed drug from the p(HEMA) hydrogel matrix interior was a consequence of the high water wettability of p(HEMA) (38), in addition to the relatively insignificant amounts of nalidixic acid imbibed within the hydrophilic matrix interior. These two limitations were addressed by modifying both the loading method and polymeric chemistry, specifically through copolymerization with the hydrophobic monomer MMA, which reduced water wettability while simultaneously permitting higher quantities of nalidixic acid to be loaded into the copolymer network due to the introduction of hydrophobic binding sites (38). A prolonged duration of linear drug fractional release was demonstrated from the 1% nalidixic acid-loaded 50 mol% p(MMA-co-HEMA) copolymers prepared *via* novel *in situ* loading over a period of weeks in comparison to the complete release of nalidixic acid within a period of 1 to 4 h at all pH values from p(HEMA) loaded with nalidixic acid solely post-polymerization. The increased hydrophobicity of the polymeric carrier effectively counter-balanced normal Fickian diffusion by hindering release of the imbedded drug resulting in a prolonged period of zero-order drug delivery (37). Furthermore, cumulative release curves in Fig. 2 illustrate that the initial pH-responsive release of nalidixic acid was retained through subsequent localization of particulate drug on the copolymer surface after hydrophobic modification of the polymer matrix.

CONCLUSIONS

This study describes the rational design and development, drug release kinetics, and microbiological properties of a novel pH- and infection-responsive drug delivery system sensitive to the alkaline conditions generated at the onset of urinary catheter infections. Specifically, the inherent pH-dependent solubility characteristics of the antimicrobial quinolone, nalidixic acid, were exploited through surface particulate localization to achieve rates of drug release up to 50-fold faster at pH values reported during urinary catheter infections than at normal physiological urine pH. Furthermore, this initial pH-responsive release was both maintained and followed by a prolonged period of zero-order drug

delivery after localization of particulate nalidixic acid onto the surface of reduced-hydrophilicity 1% nalidixic acid-loaded 50 mol% p(MMA-co-HEMA) copolymers. The strategy presented represents a paradigm in infection-responsive drug delivery, particularly in respect of the objective of providing infection resistance for periods up to 4 weeks without intervention.

ACKNOWLEDGMENTS AND DISCLOSURES

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