Optimization of a Lozenge-Based Sensor for Detecting Impending Blockage of Urinary Catheters.

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KEYWORDS

Lozenge; pH-sensitive polymer; CAUTI; diagnostics; *P. mirabilis*; dye-release.

ABSTRACT

Catheter-associated urinary tract infections resulting from urease-positive microorganisms are more likely to cause a urinary catheter blockage owing to the urease activity of the microbes. Catheter blockage can be dangerous and increases the risk of severe infections, such as sepsis. Ureases, a virulence factor in *Proteus mirabilis,* cause an increase in urine pH - leading to blockage. An optimised biosensor "lozenge" is presented here, which is able to detect impending catheter blockage. This lozenge has been optimised to allow easy manufacture and commercialisation. It functions as a sensor in a physiologically representative model of a catheterised urinary tract, providing 6.7 h warning prior to catheter blockage. The lozenge is stable in healthy human urine and can be sterilized for clinical use by ethylene oxide. Clinically, the lozenge will provide a visible indication of impending catheter blockage, enabling quicker clinical intervention and thus reducing the morbidity and mortality associated with blockage.

1. INTRODUCTION

Catheter-associated urinary tract infections (CAUTI) are the most common nosocomial infections, affecting approximately 150-250 million patients globally per year (Zowawi et al., 2015). The use of a urinary catheter increases the risk of urinary tract infections by bypassing the patient's natural defences, such as the ability to completely void the bladder. This results in a pool of residual urine remaining within the bladder, providing nutrients for bacterial colonisation (Stickler and Feneley, 2013). Although some CAUTIs are asymptomatic, those arising from urease-positive microorganisms, such as *Proteus mirabilis* (*P. mirabilis*), increase the likelihood of catheter blockage (Nicolle, 2014). Urease causes catheter blockage by metabolising urea into ammonia and carbonic acid (Norsworthy and Pearson, 2017). Ammonia subsequently increases the pH of the urine, causing the formation of apatite and struvite crystals (Stickler and Feneley, 2010). This crystallisation, and the ability of *P. mirabilis* to form biofilms, results in a matrix of crystalline biofilms which form on and around the lumen of the catheter, ultimately leading to blockage and the formation of bladder stones (Armbruster et al., 2017; Stickler and

Feneley, 2010). Upon removal of the catheter, the infection can often remain within the bladder owing to the formation of bladder stones; this infection can ascend to the kidneys, forming kidney stones (Choong et al., 2001; Norsworthy and Pearson, 2017). Patients using long-term catheters often fall into two groups: 'blockers' and 'non-blockers'. Those whose catheters frequently block are often found with urease-positive infections, caused by bacteria such as *P. mirabilis* and *Providencia stuartii* (Kunin, 1989). *P. mirabilis*, a gram-negative bacterium, is the most common urease-positive bacterium found to cause blocked catheters (Nicolle, 2014). Catheter blockage can force infected urine up the ureters towards the kidneys, causing pyelonephritis and increasing the risk of urosepsis (Armbruster and Mobley, 2013).

There is no competing technology currently in routine clinical use which monitors impending catheter blockage, currently diagnostic 'dipsticks' may be used when changing the urine bag, although this requires high patient/carer compliance and is not routine in the United Kingdom. Stickler et al., in 2006 developed a device for catheter monitoring based on a bromomethyl blue indicator, however it was not adopted in the clinic, possibly due to having poor diagnostic specificity (Long et al., 2013; Stickler et al., 2006).

The development of a hydrogel-lozenge, which predicts impending blockage of a urinary catheter, was developed by Milo et al. (Milo et al., 2018). A fluorescent dye, 5(6)-carboxyfluorescein (CF), was contained within a poly(vinyl-alcohol) (PVA) hydrogel, which was coated with a pH sensitive polymer EUDRAGIT® S 100 (Milo et al., 2018). Upon increase in pH, owing to urease activity and the production of ammonia, the EUDRAGIT® S 100 polymer dissolved, releasing the CF dye. This hydrogel-based lozenge was placed within the drainage bag of *in vitro* bladder models and provided 14.5 hr of warning prior to catheter blockage (Milo et al., 2018). Here we describe the optimisation of the hydrogel-lozenge technology for easy manufacture and patient use.

2. EXPERIMENTAL

2.1. Lozenge manufacture:

The tablet component of the lozenge was manufactured using a TDP 0 Desktop Tablet Press (LFA Machines LTD, UK). Powder mix containing 45% (w/w) Firmapress (LFA Machines LTD, UK), 20% (w/w) SF (Merck, Germany), 5% (w/w) Talc (Johnson and Johnson, USA), and 30% (w/w) lactose (Merck, Germany), was pressed into small tablets. The tablet mix was optimized to produce a mix that would flow through the tablet press without clogging and would not cake on the dies of the press. The tablets had to be biconvex (where the top and bottom surface of the tablet is domed), hence the importance of preventing caking of tablet mix onto the dies. Tablets were drum-coated, by Evonik, Germany, with EUDRAGIT® S 100 polymer, to achieve a coating of 10 mg/cm^2. **Table 1**, shows the composition of EUDRAGIT® S 100 polymer, prepared following manufacturing guidelines. A total of 250 g of tablets were coated, this produced a lozenge with a diameter of approximately 7 mm **(Fig. 1a)**.

Table 1: Composition of the EUDRAGIT® S 100 polymer solution.

Component	Mass /g
EUDRAGIT® S 100 (Evonik, Germany)	25.6
Dibutyl sebacate (Merck, Germany)	2.6
Magnesium stearate (Merck, Germany)	10.2
Acetone (Merck, Germany)	131.5
Isopropanol (Merck, Germany, UK)	197.2
Deionised water (produced onsite at Evonik, Germany)	16.4

2.2. Functionality testing of CF release:

Standard curves allow the concentration of SF released from the lozenge to be determined. The graphs were prepared by measuring the mass of SF mixed into 10 mL of the different pH buffers. Aliquots of 200 µL were taken and placed in 96-well black plates (Corning, UK). Aliquots were removed and the fluorescence measured at excitation and emission wavelengths: 485 nm and 520 nm respectively (SPECTROstar Omega BMG LabTech, Germany)**(Supplementary Fig. 1)**. Buffers of different pH were prepared, pH 5.0: 0.1 M sodium acetate: sodium acetate (0.0673 M) and acetic acid (0.0343 M), pH 6.0: 0.1 M sodium phosphate: sodium dihydrogen phosphate (0.0863 M) and disodium hydrogen phosphate heptahydrate (0.0137 M), pH 7.5: 0.1 M sodium phosphate: sodium dihydrogen phosphate (0.0754 M) and disodium hydrogen phosphate heptahydrate (0.0246 M), and pH 8.0: 0.1 M tris: tris base (0.099 M), adjusted with HCl. The pH was checked and adjusted, if necessary, to the desired pH ± 0.1 (Jenway 3505 pH meter). To measure the release of SF, lozenges were submerged in 10 mL of the buffer. The buffers were shaken (to represent movement of a drainage bag attached to the leg) at 30 min intervals for the first 3 h then hourly intervals, 200 µL aliquots were taken and the fluorescence measured. Aliquots were returned to the original 10 mL of buffer after the fluorescence was read, unless the sample required diluting for fluorescence readings - in which case only 10 µL aliquots were removed. Using the calibration curves, the concentration of SF released was determined **(Supplementary Fig. 1)**.

2.3. Functionality testing using *P. mirabilis*:

P. mirabilis B4 was recovered from freezer stocks LB media with 15% (v/v) glycerol) by streaking onto non-swarming LB agar plates (10 g/L tryptone, 5 g/L yeast extract, 15 g/L bacteriological agar) and growing statically at 37 °C overnight. A single colony was picked and grown in 10 mL LB broth overnight at 37 °C with agitation (200 rpm). Overnight samples were centrifuged (3100 *g*, 10 min, at 4 °C, 5810 R Eppendorf centrifuge). The supernatant was removed, and the pellet resuspended in artificial urine. Artificial urine was prepared according to Nzakizwanayo et al., (Nzakizwanayo et al., 2019). An overnight culture gives approximately 7.2×10^9 CFU/mL of *P. mirabilis* which was diluted using artificial urine to target 1×10^7 CFU/mL. The lozenge was added to the culture, pH was measured every hour and samples were taken for determining the quantity of bacteria present, using the Miles and Misra technique (Miles et al., 1938). Lozenge release was observed visually and photographs were taken. During the experiment, three overnight cultures were used which were diluted separately into two different tubes. This allowed the pH measurement and CFU/mL samples to be taken from separate tubes, thus preventing contamination of the CFU/mL tube arising from use of the pH probe.

2.4. Testing the lozenge on *in vitro* bladder models:

In vitro bladder models were set up as described by Nzakizwanayo et al., (Nzakizwanayo et al., 2019). Bladders were temperature-controlled to 37 °C and the artificial urine flow set to 0.75 mL/min. The bladders were inoculated with 1×10^8 CFU/mL of bacteria, to represent a late-stage CAUTI. Overnight cultures of *P. mirabilis* B4 and *Escherichia coli (E. coli)* NSM59 were grown up as described above (*E. coli* is grown on LB agar plates instead of non-swarming LB). They were diluted with artificial urine to achieve the desired inoculation. The following variables were measured: pH of the drainage bag, CFU/mL of the drainage bag, and visual release of the lozenge placed in the drainage bag. CFU/mL of the drainage bag was corrected to the volume of the drainage bag, as this consistently increased during the experiment. To determine the warning provided by the diagnostic lozenge for catheter blockage, time-lapse photography was carried out to determine the point at which the lozenge payload was released. A Nikon D3100 camera was programmed to take photographs every 2 min from approximately 2-3 h into the experiment until the catheter blocked. Lozenge release was observed visually.

2.5. Stability of the lozenge:

The stability of the lozenge was assessed during the *in vitro* bladder model experiments described above. Lozenges which released in the control and *E. coli* bladder models were described as false positives. The stability of the lozenge was also tested in human urine. Ethics approval from the University of Bath, REACH committee was granted to allow the donation of human urine from healthy volunteers (reference number: EP 19/20 089). Informed consent was given by 12 healthy adults; healthy was defined as not currently taking antibiotics or using a urinary catheter. A morning donation was taken so that all urine samples were collected at a similar time point. Urine was tested on the day of collection and discarded 48 h later. Urine was transported in sterile falcon tubes, refrigerated upon arrival at 3-5 °C, 10 mL was transferred to a new falcon and a lozenge was added, it was now incubated at room temperature. At regular intervals 200 μL was removed and the fluorescence measured as described above. Samples were returned to the falcon tubes after the measurements so that the volume in the sample remained constant. The positive control consisted of a lozenge released in 0.1 tris, pH 8, fluorescence measured at 24 h. The pH of the urine was measured regularly by means of a probe solely used for biological samples, lozenge release was observed visually and with photographs.

The stability of the lozenges during ethylene oxide (EO) sterilization was determined, sterilization was carried out by means of 32 lozenges that were packaged separately into individual pouches - representing individual sterilisation, with a further 100 lozenges packed together. EO sterilization was carried out by Sterigenics, A Sotera Health company, on a fully qualified cycle. Integrity was checked visually followed by functionality testing using buffers with different pH, as described above.

3. RESULTS AND DISCUSSION

3.1. Optimisation of the lozenge and functionality testing:

The limitations of the hydrogel-lozenge previously described by Milo et al., were fragility, predisposition to damage, and variability between hydrogels (Milo et al., 2018). To overcome these limitations the lozenge was developed as a solid tablet instead of a PVA hydrogel impregnated with CF dye. The CF dye was replaced with sodium fluorescein (SF), a less expensive alternative suitable for mass clinical use, which also provided significant fluorescence. Tablet formulation was optimised to allow easy manufacture using a manual tablet press, ensuring the following properties were met: a solid tablet with good integrity that did not easily crumble; a tablet mix that did not block the tablet press with clumping; and a tablet

mix that produced biconvex tablets. A biconvex tablet is one which has a domed surface on its top and bottom, consistent with the shape of the dies which push the tablet into place. With the addition of excipients, lactose and talcum powder, along with a binder Firmapress (LFA Machines LTD, UK), a composition was obtained that reduced caking onto the tablet press dies and produced solid, biconvex, tablets **(Fig. 1a)**. The production of the lozenge in tablet form allows straightforward scale-up to commercial manufacture, while keeping the cost of the lozenge low.

The hydrogel-lozenge described by Milo et al., had been dip-coated in EUDRAGIT® S 100; however the new tablet-based lozenge could now be coated via the commercial process of drum-coating (Milo et al., 2018). EUDRAGIT® S 100 was sprayed onto the tablets whilst they were mixed and air dried; this prevents the tablets sticking together and allows an even coating of the polymer to be applied. The lozenges were drum-coated by Evonik (Darmstadt, Germany), providing a 10 mg/cm^2 EUDRAGIT® S 100 coating (**Fig. 1a**). To test whether the optimised tablet-based lozenge was able to release SF at varying pH, lozenges were placed in different pH buffers and the fluorescence was measured over 24 h (**Fig. 1b**). The dissolution threshold pH for EUDRAGIT® S 100 is pH 7.0, therefore release should be observed in buffers with a pH > 7. **Figure 1b** illustrates the stability of the tablet-based lozenge with no release of SF at pH 5 and 6, whilst release was observed at pH 7.5 and 8.0.

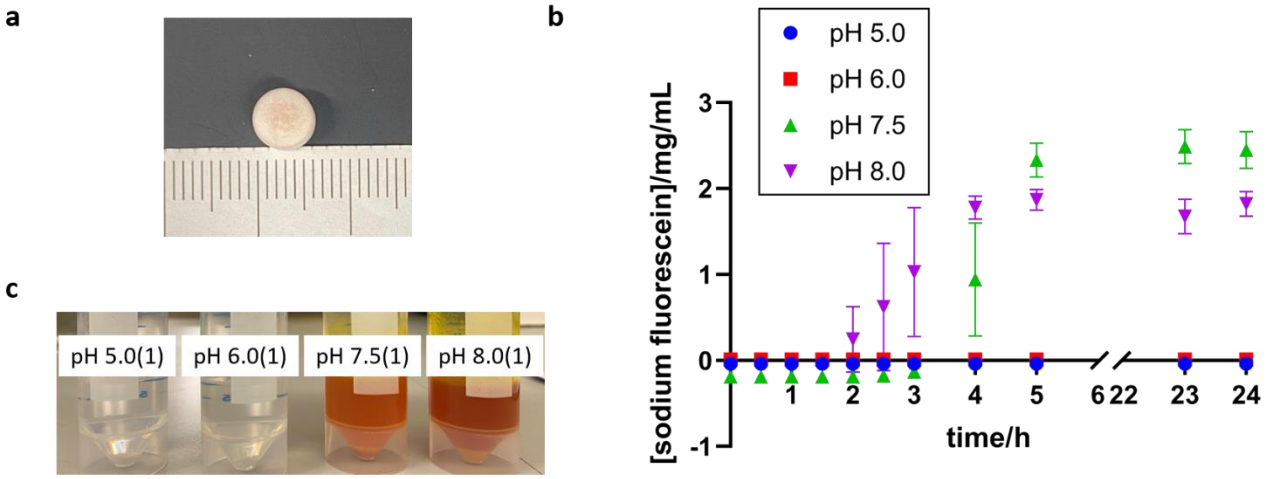

Figure 1. Functionality testing of the tablet-based lozenge. **(a)** A coated tablet-based lozenge, scale in mm. **(b)** Concentration of SF released from lozenge in buffers of varying pH (5.0, 6.0, 7.5 & 8.0), sodium fluorescein quantified using calibration curves (**Supplementary Fig.1**). **(c)** The lozenge was placed in the falcon tubes, visual analysis of SF released from the lozenge release buffers of varying pH (5.0, 6.0, 7.5 & 8.0). Plots were prepared using GraphPad Prism version 9. Mean from four independent experiments are shown with error bars representing the standard deviation.

Analysis of long-term catheter users who identified as ‘blockers’ were found to have a more alkaline urine, with a voided pH (pH of urine passed out of the urethra) greater than 7 vs non-blockers whose urine was more acidic (pH < 7); therefore in the latter patients, the tablet-based lozenge should not release (Choong et al., 1999; Kunin, 1989; Mathur et al., 2006). The SF fluorescence release was also visually observed, which is important as the lozenge is designed to be placed within the catheter drainage bag and the SF release needs to be clearly observed by the patient or carer **(Fig. 1c)**. SF fluorescence undergoes quenching at high concentrations, hence the orange colour at the high pH. The quenching is not a problem for the application of the lozenge as this still provides a clear colour change (Liu et al., 2014). The tablet-based lozenge takes

approximately 4 h to fully release SF; however, release is visually observed after 3 h and the higher the pH, the quicker the release **(Fig.1b)**. **Figure 1** indicates that the lozenge is functioning as expected and providing a clear colour change at pH >7.

3.2. Testing the functionality of the lozenge using *Proteus mirabilis*

The functionality of the lozenge was assessed in regard to bacterial bioburden. It was important to establish that the increase in pH was due to an increase in urease concentration, arising from the increase in the bacterial load of *P. mirabilis*. The bioburden (signifying infection) at which the lozenge released was investigated to ensure that the lozenge would release SF prior to catheter blockage, but not too early – in order to prevent unnecessary catheter changes. *P. mirabilis* was cultured in artificial urine with a lozenge over time, the pH, viable cell count of *P. mirabilis*, and visual analysis of SF release was assessed **(Fig. 2)**. **Figure 2a**, indicates that the increase in the bacterial load correlates with an increase in pH. The viable count of *P. mirabilis* remained constant for the first 3 h; the lag phase is slightly elongated in artificial urine as the lag phase for *P. mirabilis* in Luria Bertani (LB) media is 2.5 h (Milo et al., 2021). From 3-7 h, the growth was in the exponential phase, and at 7 h the growth rate plateaued, where the bacteria were in the stationary phase. **Figure 2a** shows that overall, as *P. mirabilis* grew, the pH of the artificial urine increased. There is a lag in the increase of the pH, suggesting a delay in the metabolism of urea to ammonia. **Figure 2b**, shows that after 8 h the lozenge has begun releasing SF, correlating to an increase of pH > 7 and a higher bacterial titre. Importantly, the lozenge did not release during the early hours of growth, where the pH (pH < 7) and the bacterial bioburden (<10^8 CFU/mL) was low.

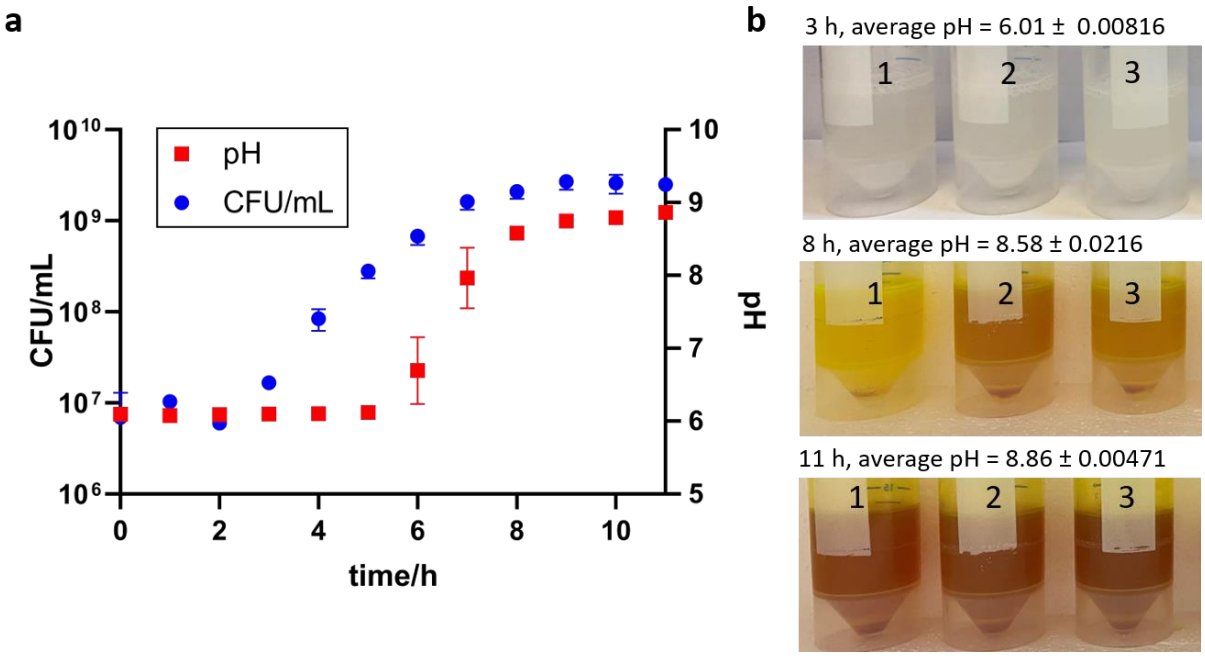

Figure 2. (a) Quantification of the pH and viable cell count of *P. mirabilis* in artificial urine over time. **(b)** Visual analysis of the lozenges at timepoints: 3, 8, and 11 h, showing release of the lozenge at pH > 7. Plots were prepared using GraphPad 9. Mean from three independent experiments are shown with error bars representing the standard deviation.

3.3. Testing the lozenge using *in vitro* bladder models of the catheterized urinary tract

The lozenge was tested in a physiologically representative model of the catheterised urinary tract using *in vitro* bladder models (Nzakizwanayo et al., 2019). This tested the functionality and stability of the lozenge alongside its early warning capabilities. *In vitro* bladder models were inoculated with 1×10^8 CFU/mL of *P. mirabilis* and *E. coli*. *E. coli* is the most common microorganism associated with urinary tract infections and is often found in CAUTI (Dhakal et al., 2008; Tambyah et al., 1999). As *E. coli* is urease negative and does not cause catheter blockage with crystalline biofilms, it served as a urease-negative control (Milo et al., 2016). *In vitro* bladder models were inoculated with a high starting-inoculum of bacteria (10^8 CFU/mL) to mimic a late-stage CAUTI (Milo et al., 2021). The pH and the CFU/mL of the drainage bags were measured throughout the experiment **(Fig. 3)**.

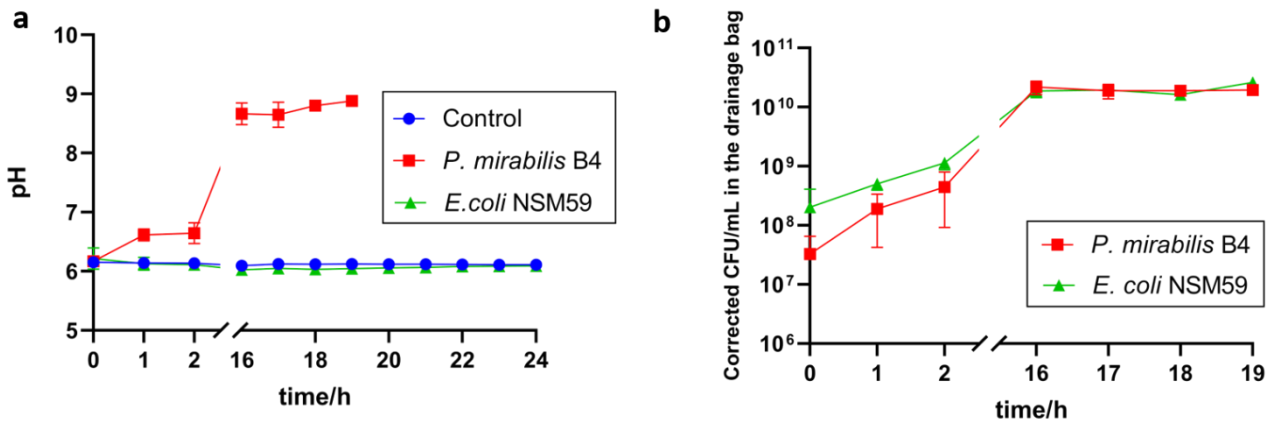

Figure 3. *In vitro* bladder model experiments. **(a)** pH measured from the drainage bag of control (no bacteria), *P. mirabilis*, and *E. coli*, over time. **(b)** Viable cell count (CFU/mL) of *P. mirabilis* and *E. coli* in the drainage bag of the bladder models, corrected for the increase in volume in the drainage bag over time. Plots were prepared using GraphPad 9. Mean from three independent experiments are shown with error bars representing the standard deviation.

The pH rose exclusively in the *P. mirabilis* bladder models, whilst the control (no bacteria) and *E. coli* models remained static (**Fig. 3a**). Quantification of *P. mirabilis* and *E. coli* within the drainage bag showed no significant difference between the quantity of *P. mirabilis*, (2.19×10^{10} CFU/mL, n = 3) and *E. coli* (1.87×10^{10} CFU/mL, n = 3) after 16 h (unpaired t-test with Welch's correction, $p = 0.7242$) **(Fig. 3b)**. However, the measured pH of the drainage bag displayed significant differences between the bladders infected with *P. mirabilis* (pH = 8.67, n = 3) and *E. coli* (pH = 6.02, n = 3) at 16 h (unpaired t-test with Welch's correction, $p = 0.0363$). On average, it took 18 h (±1.41) for the *P. mirabilis* infected bladders to block, whilst the control and *E. coli* bladder models continued to flow freely until experiment end at 24 h. Time-lapse photography was used to monitor the drainage bags during the night **(Supplementary video)**. The average early warning of impending catheter blockage provided by the lozenge was 6.7 h (±0.58). This is an optimum warning time, allowing the patient a sufficient interval in which to have their catheter changed prior to catheter blockage, preventing the serious clinical outcomes associated with catheter blockage.

There is a visual difference in the appearance of the drainage bags 24 h post-bacterial inoculation (**Fig. 4**). The lozenge has released SF into the drainage bag connected to the bladder infected with the urease-positive microbe *P. mirabilis*. However, it has not released in the control bladder or the urease-negative *E. coli* bladder. The use of physiologically representative *in*

vitro bladder models indicate that the lozenge is functioning as an early warning diagnostic tool for impending catheter blockage. The distinctive color change of the *P. mirabilis* drainage bag vs the control and *E. coli* bags suggest that the lozenge would unambiguously indicate impending catheter blockage to the patient or carer.

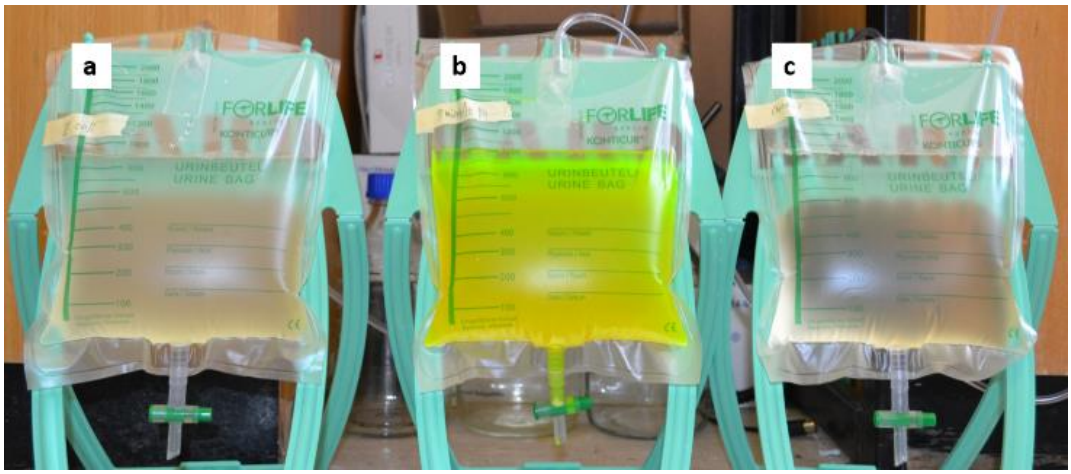

Figure 4. Visual analysis of the different drainage bags from the *in vitro* bladder models. **(a)** Drainage bag of: bladder infected with *E. coli*, **(b)** bladder infected with *P. mirabilis*, and **(c)** control bladder with no bacteria.

3.4. Investigating stability of the lozenge

The lozenges were manufactured using a manual tablet press to form the solid tablet prior to drum-coating with EUDRAGIT® S 100 polymer. This method was chosen as it allows for easy manufacturing and scale-up to bulk production. The stability of the tablet was assessed to ensure there was little variation in the tablet production or performance. A total of 30 tablets were evaluated, where lozenges were placed within the drainage bags of the *in vitro* bladder models and assessed periodically for failure over a 24 h period **(Supplementary Table 1)**. A failed lozenge was defined as a lozenge which released SF in the control or *E. coli* bladder models or early in a *P. mirabilis* bladder compared to the biological repeats. Overall, 80% (24/30) of lozenges were stable over 24 h. The failure rate of the lozenges is likely due to the manual manufacture of the solid tablet, as the tablets occasionally did not cap correctly. Variation in the surfaces of the tablets would have led to unequal polymer coating during the drum-coating process. To improve the stability of the lozenge, bulk manufacture using a motorised tablet press will lead to less variation in the tablets produced. The polymer weight gain of 10 mg/cm^2 could also be increased. This would ensure more stability however, it would take longer for the EUDRAGIT® S 100 polymer to dissolve, therefore reducing the warning time interval provided by the lozenge.

The lozenge, a diagnostic tool used to identify impending catheter blockage within the human bladder, was tested for stability in healthy human urine. The release of SF at a high pH (> 7) would indicate impending catheter blockage, thus the lozenge should not release in healthy human urine (below pH 7) (Kaye, 1968; Landry and Bazari, 2012). A morning sample of urine was donated by 12 healthy volunteers. The pH of the donor's urine remained constant and below pH 7 over 26 h **(Fig. 5a)**. The healthy human urine was acidic (average pH at 0 h: 5.63 ± 0.30), in agreement with the literature (Kaye, 1968; Landry and Bazari, 2012). Furthermore, the fluorescence intensity remained constant over time and was significantly lower compared to the control (t-test, Welch's correction $p = 0.0001$). Therefore, it was concluded that no SF was released from

any of the lozenges **(Fig. 5b,c)**. The lozenge remained stable, suggesting that there were no components of human urine which could cause release of the dye. Thus, the lozenge is unlikely to lead to false positive results during clinical application.

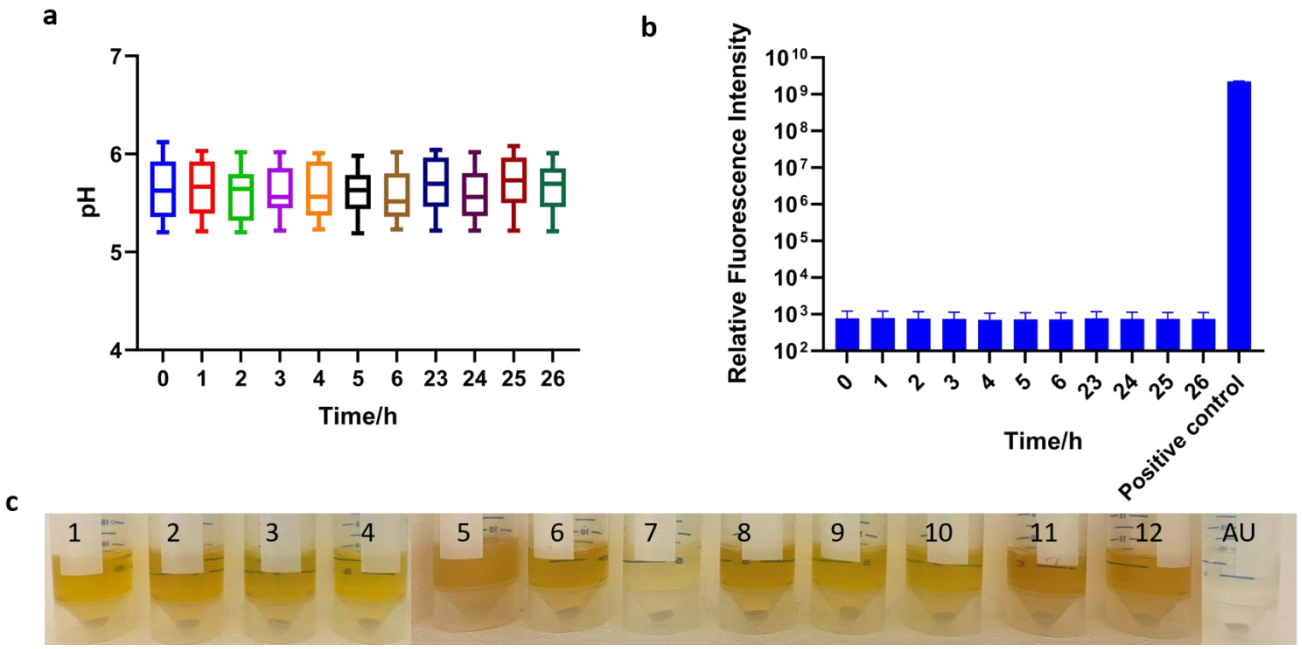

Figure 5. pH variation in sterile human urine. **(a)** Shows the variation in pH over 26 h with box and whisker graphs showing the mean and variation between the human urine samples. **(b)** Variation in fluorescence intensity of the urine over time. Positive control represents fluorescence after 24 h of being incubated in pH 8 Tris buffer, error bars represent standard deviation in 12 independent repeats. **(c)** Visual analysis of the urine with the lozenge 24 h after the lozenge was added. 1-12 are urine samples and 13 is artificial urine (AU). Plots were prepared using GraphPad 9. Mean from 12 independent experiments.

3.5. Investigating sterilisation of lozenges

Medical devices are often sterilized with EO. EO is toxic to all microorganisms and is used to sterilize urinary catheters and drainage bags, as it can be carried out at low temperatures. For clinical application, the lozenge would be packaged within the drainage bag. Therefore, the lozenge must not release SF during EO sterilization, and the sterilization process must not disrupt the integrity of the polymer coating. In this study, 132 lozenges were sterilized by EO sterilization; 32 lozenges were packaged separately into individual pouches to represent individual sterilization and a further 100 lozenges were packed together for group sterilization. Post-sterilization examination showed no SF release, suggesting the EUDRAGIT® polymer-coating remained intact. The stability and functionality of the sterilized lozenges were tested using various buffers at different pH **(Supplementary Fig. 2)**. **Supplementary Figure 2** shows that the lozenges were functioning as expected post-EO-sterilization. Therefore, the EO sterilization did not affect the ability of the lozenge to release SF at pH > 7, thus enabling the lozenges to be sterilized prior to use.

4. CONCLUSION

The original design for the lozenge has been optimised to allow for easier manufacturing on a large scale, while remaining robust, and functional as a sensor for impending catheter blockage. This study demonstrated that the lozenges performed as anticipated and were successful in predicting impending blockage of a urinary catheter. The technology provides a 6.7 h warning, allowing users a sufficient time period for a catheter change prior to blockage while preventing subsequent serious clinical outcomes. The lozenge remained stable during *in vitro* bladder model experiments and is stable in healthy human urine. The principal shortcoming of the technology is that it has not yet been tested in the urine bags of patients and for this, clinical studies will be required to assess the diagnostic performance of the technology in catheterised patients. Such a study is planned to commence before 2023.

Supporting Information

PDF document containing Supplementary Figure 1-2 and Table 1.

Mov. File contains the time-lapse photography, supplementary video.

Author Statement:

RH wrote the manuscript, designed, and conducted the experiments. MB helped conduct the experiments, NH and LG coated the tablets. JU helped to organize and setup the sterilization experiments. LG, BP, J.M-C, NT, ML, and ATAJ helped designed the experiments, and assisted in project management. All authors reviewed the manuscript and approved the submitted version.

Conflicts of Interest:

The authors declare no competing financial interests.

ACKNOWLEDGMENTS

RH would like to thank the Annette Trust and EPSRC IAA project for funding, LG thanks grant MR/N0137941/1 for the GW4 BIOMED DTP, awarded to the Universities of Bath, Bristol, Cardiff and Exeter. BP would like to thank the James Tudor Foundation and Mr and Mrs A. Watson for their funding. We also wish to thank Evonik, Darmstadt for drum-coating the tablet-based lozenges and Mediplus Ltd, for sterilizing the lozenges with ethylene oxide.

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