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Preservatives: an uncertain future?

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There are a limited number of approved preservatives available for multi-use oral products, and the number is even less for other routes of administration. The limited number can be further restricted by dose, pH-solubility profile, incompatibility, toxicity and other relevant factors. The existing preservative palette is coming under scrutiny from regulators, scientists, and in some instances the media. This is based on a number of concerns, not all of which are firmly rooted in good science. It should be clearly understood that there are no new anti-microbial preservatives currently being developed that have a better risk-to-benefit ratio than the existing portfolio. In addition, new preservatives take significant time, effort and money to gain regulatory approval, by any organisation, and this effort is not to be undertaken lightly. Removal of preservatives in cosmetic or pharmaceutical multi-use products leads to an increased risk of contamination by opportunistic microbial pathogens, resulting in potential health and/or life threatening consequences. The recent development of a preserved multi-use intra-nasal formulation highlights some of the real issues facing development groups. The drug substance was the potassium salt of a selective inhibitor of the α-4 chain of the VLA-4 integrin, being developed for the treatment of seasonal rhinitis. The drug substance had extremely good solubility at pH 7 (200 mg/ml), but this decreased rapidly with decreasing pH (< 2mg/ml at pH 5). The proposed clinical dose was 15 mg/ml. The combination of solubility and pH requirements constrained the use of many of the more common preservatives. In addition to the unacceptable pH-solubility profile, the anionic nature of the drug substance was also problematical from an incompatibility perspective. Cloudy solutions, indicative of both benzalkonium and benzethonium chloride being precipitated out of solution at the desired concentration, were observed. Addition of a number of pharmaceutically acceptable co-solvents did not address the issue. The synergistic mixtures of parabens and 2-phenyethanol enhance the antimicrobial activity of parabens alone. This preservative combination was tried, and although it successfully complied with the USP AET criteria, it failed the more stringent requirements of the EP. In addition, the parabens accentuated the poor taste of the formulation and were discounted from additional evaluation. The organomercurials were discounted from a toxicity and hypersensitivity perspective. EDTA was compatible with the drug substance but there was concern that it could block the binding of the drug to α-4 chain of the VLA-4 integrin (pharmacological activity relies on drug binding to Ca^{2+} ion at the active centre). Thus a combination of dose, pH-solubility profile, incompatibility (due to the anionic nature of drug substance), toxicity and some pharmacological considerations rapidly depleted the entire anti-microbial preservative palette that have been approved for intranasal administration. Given the limited choices that are currently available to serve as preservatives, as well as the years of demonstrated clinical safety, a careful risk-to-benefit analysis should be considered before discounting preserved multi-dose formulations.

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Development of a novel model for the assessment of encrustation of urinary biomaterials

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Encrustation of indwelling urinary catheters is widely recognised as a key limiting factor in the long term management of these devices (Hukins 2005). Crystalline

deposits primarily consisting of Struvite [$MgNH_4PO_46H_2O$] and Hydroxyapatite [$Ca_{10}(PO_4)6(OH)_2$] occur in conjunction with biofilms of urease producing bacteria such as *Proteus Mirabilis*. Urease plays a key role in encrustation development by cleaving urea within urine to form ammonia, which elevates urinary pH and therefore reduces the solubility of Ca^{2+} and Mg^{2+} salts within urine. Blockage of the drainage eyelets or lumen of Foley catheters may lead to urinary retention with painful bladder distension and may ultimately result in pyelonephritis if prompt removal of the device does not occur, with encrustation around the balloon potentially leading to severe damage to the uroepithelium on removal. Many models exist for the assessment of urinary encrustation but there is a need for a simple model utilising commercially available apparatus which will facilitate the testing of potential materials, taking into account the key role of urease producing bateria. This study therefore details the development of a novel model for the assessment of urinary encrustation utilising a laboratory biofilm reactor system (the CDC biofilm reactor), which allows biofilm growth under moderate shear stress. The model developed allows for the testing of 24 sample coupons per batch over defined time periods with controlled shear stress and continual flow of replacement solutions. Artificial urine similar in composition to that described (Jones et al 2006) containing 5% Mueller Hinton Broth is continually fed into the reactor via a peristaltic pump at with a residual volume of 350 ml being retained within the reactor mimicking the residue of urine around the catheter balloon in vivo. A commercially available strain of *Proteus Mirabilis* (NCTC strain 11938) is added as an innoculum to the reactor and allowed to equilibrate for 1 hour prior to the initiation of flow of artificial urine. The resulting encrustation on sample material is demonstrated to be reproducible and independent of the rods position within the reactor or the coupon position within the rod. Characterisation of the encrustation produced by X-ray diffraction and Infra-red spectroscopy shows primarily struvite and hydroxyapatite in a ratio similar to that observed in clinical isolates.

Table 1 The effect of sample placement within the reactor on encrustation development

Position Within reactor	Calcium encrustation (mcg/cm2)	Magnesium encrustation (mcg/cm2)
Top	547 ± 37	483 ± 28
Middle	531 ± 26	454 ± 32
Bottom	506 ± 33	437 ± 29

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Examination of the effect of urine conditioning film on bacterial adherence and encrustation development on urinary biomaterials

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The adherence of bacteria to biomaterials is the first step in the process leading to device related infection. In urinary catheters this process progresses to the formation of a biofilm on the surface of the material and ultimately the development of encrustation around the eyelets and balloon of the device (Bonner et al 1997). The initial adherence of the bacterial cell may be influenced by surface characteristics of the material such as charge or hydrophilicity and by the characteristics of the adhering bacteria. On insertion of a polymeric biomaterial within the urinary tract the precursor to bacterial adherence is recognised to be the deposition of a conditioning film consisting of proteins, electrolytes and other unidentified organic molecules (Denstedt et al 1998). It is noted that the deposition of this conditioning film may mask the underlying surface chemistry of materials and therefore the performance of materials in-vitro may differ markedly from the resistance to adherence and encrustation in-vivo. This study focuses on the effect of the conditioning film on a range of performance characteristics of a library of potential urinary biomaterials including 2-Hydroxyethyl methacrylate (HEMA) hydrogels alone or containing 20% w/w methacrylic acid (MAA), (Diethylamino) ethyl methacrylate (DEAMA) and trifluoroethyl methacrylate (TFEMA). Materials tested were prewashed in deionised water to remove unreacted monomer, dried at 60°C to constant mass and then soaked in either PBS (pH 7.2) or human urine for 24 h before testing. Measurements of dynamic contact angle, zeta potential and the resistance to bacterial adherence and encrustation were performed. Modification of the properties of materials are noted with increased adherence of *Proteus mirabilis* (NCTC strain 11938) in copolymer systems containing DEAMA and MAA in conjunction with HEMA. Adherence to HEMA alone is significantly lower in the presence of the conditioning film although encrustation is increased when tested using an encrustation model containing Jack Bean urease spiked artificial urine (Jones et al 2006). Treatment of silicone surfaces with urine reduced significantly the mean advancing water contact angle from 93.26° to 49.31° rendering the surface significantly more hydrophilic with a similar trend observed for treatment of the HEMA/TFEMA copolymer system. This work demonstrates the need to mimic in-vivo conditions when testing potential urinary biomaterials to ensure that all conclusions are based on actual potential performance rather than being skewed by experimental parameters.

Table 1 The effect of soaking urinary biomaterials in human urine on the 24h adherence of *P. mirabilis*

Material	Adhered bacteria ($\times 10^6$)	
	PBS soaked	Urine soaked
Silicone	1.67 ± 0.65	1.83 ± 0.62
100% HEMA	2.17 ± 0.74	0.87 ± 0.25
80% HEMA, 20% MAA	1.67 ± 0.61	4.17 ± 1.36
80% HEMA, 20% DEAMA	0.97 ± 0.31	1.82 ± 0.70
80% HEMA, 20% TFEMA	8.67 ± 3.12	9.17 ± 3.81

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