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Antimicrobial Preservatives Part One: Choosing a Preservative System

Antimicrobial Preservatives Part Two: Choosing a Preservative

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- Print

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Introduction

Excipients are included in medicinal products to facilitate manufacture, consumption or administration, or to enhance stability/absorption. They can also facilitate product differentiation, enhance aesthetic appearance, and improve compliance. They are generally considered as inert viz not possessing intrinsic biological activity [1]. Antimicrobial preservatives might be considered exceptions to such categorization, being added to help improve antimicrobial stability and hence requiring antimicrobial activity. Their presence is mandated for multidose liquid and semi solid products and performance standards are defined in compendial monographs [2,3]. Compliance with such requirements is not easy. This review, being Part One of a three-part review discusses the biological mode of action of preservatives, options for selection, and the requirements for preservative efficacy testing.

Preservative Availability

Table 1 - Common Preservatives for Pharmaceutical Products

Medicinal Product	Preservative	Chemical Class
Oral [7]	Methyl, ethyl, propyl parabens and combinations	amino aryl acid esters
	Sodium benzoate, benzoic acid	aryl acids
	Sorbic acid, potassium sorbate, propionic acid	alkyl acids
	Methyl paraben/sodium benzoate combination	amino aryl acid esters/ organic acid
Topical (including nasal) [8]	Benzalkonium chloride, cetrimonium bromide, benzethoniun chloride, alkyltrimethylammonium bromide	quaternary ammonium compounds (QACs)
	QACs e.g. benzalkonium chloride/EDTA	QACs/ metal chelator
	Methyl, ethyl, propyl, butyl parabens and combinations	amino aryl acid esters
	Benzyl alcohol, cetyl alcohol, steryl alcohol	Alkyl / aryl alcohols
	Benzoic acid, sorbic acid,	Alkyl / aryl acids (and salts)
	Chloroactamide, trichlorocarban	Alkyl / aryl amides
	Thimerosal	organomercurials
	Imidurea, bronopol	Formaldehyde donators
	Chlorhexidine	biguanides
	4-Chlorocresol, 4-chloroxylenol, dichlorophene, hexachlorophene	phenols
Parenteral (including vaccines) [9]	Benzyl alcohol, Chlorbutanol, 2-ethoxyethanol	Alkyl / aryl alcohols
	Methyl, ethyl, propyl, butyl parabens and combinations	amino aryl acid esters
	Benzoic acid, sorbic acid	Alkyl / aryl acids
	Chlorhexidene	biguanides
	Phenol, 3-cresol	phenols
	Thimerosal, phenylmercurate salts	Organic mercurials
Ophthalmic [10]	QACs e.g. benzalkonium chloride (and others)	QACs
	QACs/EDTA	QACs / metal chelator
	Thimerosal, phenylmercurate salts	Organic mercurials
	Benzoic acid, sodium benzoate, sorbic acid, potassium sorbate	Alkyl / aryl acids
	Chlorhexidine, Polyaminopropylbiguanide, polyhexamethylbiguanide	biguanides
	Imidurea	Formaldehyde donators

Preservatives are widely used in cosmetic and food products. A more limited number are used in medicinal products, if monographs in the major pharmacopoeias are the yardstick [4,5,6]. Preservatives comprise a range of chemical classes as illustrated in Table 1.

The listings in Table 1 illustrate the limitations available to the pharmaceutical formulator. Availability for specific products or routes of administration is further constrained by the requirements listed in Table 3. Furthermore, there are no preservatives possessing sufficient efficacy, safety and non-irritancy to allow inclusion in medications instilled into ocular or intrathecal tissue [3]. Such products must be preservative-free.

Other agents have also been used as preservatives, and can still be found in mature products [2]. These include many of the older quaternary ammonium compounds (QACs), such as cetrimide, cetalkonium chloride, cethexonium bromide, as well as hexachlorophene, ethyl alcohol, sodium metabisulphite and sulphur dioxide. The phenols, although generally discouraged in oral and to a lesser extent in topical products still have a major role to play in preserving parenteral products, particularly biologicals [10]. Similar comments apply to the organo mercurials, although their use in vaccines is still subject to ongoing controversy. The use of formaldehyde-donors e.g. imidurea and bronopol has decreased in topical and ophthalmic products due to concerns about formaldehyde sensitization. Hexachlorophene is an excellent disinfectant, but its use as a preservative has declined because of concerns over neurotoxicity [8]. Typically, the use of these older preservatives in new products has been largely discontinued because of safety considerations.

Concentrated sucrose solutions can be effective preservatives in oral liquids because of the high osmotic pressure they exert. However, sucrose has fallen from favor as a preservative or sweetener in liquid oral medications (concerns regarding dental caries) despite its almost ubiquitous presence in food and confectionary products. Propylene glycol is often used as an adjunct to other preservatives because of its good solvent properties (superior to glycerin).

Modes of Action of Preservatives

Preservatives generally offer limited protection against viral contamination. Bactericides and fungicides may evince their effects on a variety of microbial cellular targets, for example; the cell wall, the cytoplasmic membrane or the cytoplasm. It is often difficult to assign a precise target for a specifi c class of preservative; the target can and does change with preservative concentration. As a consequence, preservatives can often interfere with several different microbial cellular mechanisms (Table 2).

Table 2 - Site of Preservative Activity in Microbial Cell

Cell Wall	Cytoplasmic membrane	Cytoplasm
Phenols	2-Phenoxyethanol	2-Phenoxyethanol and other organic alcohols
Aryl and alkyl acids	Parabens	Aryl and alkyl acids
Organo mercurials	Organo mercurials	Halogenated preservatives
EDTA (edetic acid)	EDTA	
Chlorhexidine, cetrimide	Chlorhexidine, hexachlorophene	Chlorhexidine (high concentrations)
Glutaraldehyde	Formaldehyde donators e.g. bronopol, imidurea	Formaldehyde donators e.g. bronopol, imidurea
Anionic surfactants	Benzalkonium chloride (BKC)	

Such cytotoxicity may also affect mammalian cells. Hence inclusion levels should be minimal, consistent with adequate preservation. There is a regulatory expectation that the reason for preservative inclusion, proof of efficacy, safety information, control methods in fi nished product and details of labeling in the fi nished product should all be addressed by the applicant [11]. Mechanisms for activity at the locations listed in Table 2 can also differ with each preservative as discussed below:

Cell wall activity may involve lysis due to enzyme inhibition, as is the case with phenols and organo mercurials. In contrast glutaraldehyde evinces its effect by irreversible cross-linking at the cell wall [2, 3].

Cytoplasmic membrane activity may be due to effects on membrane potential, membrane enzymatic function or general membrane permeability [2, 3]. Cetrimide, chlorhexidine, hexachlorophene, 2-phenoxyethanol, parabens and phenols affect membrane permeability allowing ‘leaking’ of essential cell constituents leading to cell death. Sorbic acid inhibits transport mechanisms across the cytoplasmic membrane and suppresses fumarate oxidation [3]. Chlorhexidine also inhibits membrane ATPase, thereby inhibiting cellular anaerobic activity. At higher concentrations it induces precipitation of cytoplasmic nucleic acids and related proteins. Other biguanides induce phase separation and the formation of domains in the phospholipid bi-layer. Chelators such as edetic acid (EDTA) compromise the integrity of the cytoplasmic membrane by chelating Ca^{2+} and Mg^{2+}, making these ions unavailable to the microbial cell and potentiating other anti-microbial agents, e.g. 4-chloroxylenol [12]. Quaternary ammonium compounds bind strongly to the cytoplasmic membrane evoking general cytoplasmic membrane damage (and subsequent leakage), but particularly targeting the phospholipid bi-layer.

Cytoplasmic activity may concern uncoupling of oxidative and phosphorylation processes or interference with active transport mechanisms, as is the case with weak carboxylic acid and alcoholic preservatives. Other preservatives can inhibit electron transport chains, thereby inhibiting metabolic activity in aerobic bacteria [13]. Benzoic acid and the parabens inhibit folic acid synthesis [3]. Bronopol and other organo-mercurials target thiol enzymes [3] in the cytoplasm (as do silver compounds); whereas, formaldehyde donators e.g. imidurea act on the

carboxylic and amino enzymes in the cytoplasm. Phenols cause protein denaturation [12], as do the alcohols [3].

There is the potential to select specifi c preservatives to address a particularly troublesome organism associated with a manufacturing site or process; however, eradication of these organisms is the remit of GMP (good manufacturing practice) and the preservative system should not be used to address defi ciencies in manufacturing processes. There are also possibilities for synergistic combinations to provide the requisite spectrum of activity in a particular system or product. However, the selection constraints that are discussed in Part 2 of this review, present other barriers to use of combinations of preservatives.

Choosing a Preservative

In concept, the preservative system protects the product against microbial proliferation but does not compromise product performance. In practice, this means that it must:

- exert a wide spectrum of antimicrobial activity at low inclusion levels.
- maintain activity throughout product manufacture, shelf life and usage.
- not compromise the quality or performance of product, pack or delivery system.
- not adversely affect patient safety or tolerance of the product.

Table 3 - Performance Requirements for Preservatives

Property	Performance Requirement
Antimicrobial activity	Active against bacteria (Gram +ve/Gram –ve), molds, yeasts and fungi at low inclusion levels
Aqueous solubility	Solubility exceeds minimum inhibitory concentrations (MIC) over anticipated product pH range
Partitioning Behavior	Remains essentially in the continuous aqueous phase in multi-phase products
Stability properties	Chemically and physically stable during manufacture and at end of product shelf-life.
Non-irritant properties	Non-irritant at concentration used in product, especially germane for treatment of sensitive mucosal membranes, e.g. nose, eye, etc
Organoleptic properties	Odor and taste acceptable where product is administered orally, intranasally or by inhalation (the latter two routes of administration still have a significant 'swallowed' fraction)
Compatibility properties	Does not react or reacts minimally with other product components, including the proposed container closure.

Table 3 illustrates such requirements. Relevant microbiology textbooks provide more extensive background [2,3].

It will be evident; from the performance criteria in Table 3 that the limited list of acceptable materials is likely to be further reduced by these other considerations. Physicochemical and organoleptic properties may limit choice for some product types while possibilities for interactions with the active ingredient or excipients, the pack or delivery device must also be considered. Such properties and performance criteria could form the basis for a Target Product Profi le (TPP) with respect to the preservation system that can then be addressed in the formulation design program.

The Preservative Challenge Test (Antimicrobial Effectiveness Test)

Pharmacopoeial antimicrobial effectiveness tests (AET) or preservative efficacy tests (PET) involve challenging a product with a defi ned number of colony forming units (cfu) of a variety of test microorganisms (bacteria, yeasts and fungi), enumeration at time zero and then monitoring kill / survival rate at defi ned time intervals up to 28-days [14-16]. Test organisms that are recommended by all of the pharmacopoeias include,

- Gram positive coccus, Staphylococcus aureus.
- Gram negative rod, Pseudomonas aeruginosa.
- Fungi / mold, Aspergillus niger.
- Yeast, Candida albicans.

In addition, USP [14] and Ph. Eur. [15] recommend the use of *E. coli.* The list may be supplemented by additional organisms that may be associated with a particular process, facility or material, e.g. *Burkholderia cepaceia* an opportunistic pathogen often isolated in manufacturing environments, *Bacillis subtilis* a spore-forming bacteria, etc.

Acceptance criteria for USP [14] and JP [16] are broadly similar with some differences between product type and presentation. All require satisfactory reduction for each challenge organism with no subsequent increase from the initial count after 14- and 28-days. However, it is widely recognized that the criteria of the Ph. Eur. [15] are the more stringent and challenging to meet. The Ph. Eur. requires a specifi ed reduction in bacterial count within the fi rst 14-days with no subsequent increase from the initial count after 14- and 28-days. A comparison of the relative approval criteria of the USP and Ph. Eur (EP) are shown in Table 4 and 5.

Table 4 - Comparison of USP and EP Criteria for Preservative Efficacy, I) Total Viable Bacteria

Pharmacopoeia	Time points (1. Bacterial Log Reductions)						
	6-hours	24-hours	2-days	7-days	14-days	21-days	28-days
USP Category 1				≤ 1.0	≤ 3.0		No increase (vs. 14-day count)
USP Category 2					≤ 2.0		No increase (vs. 14-day count)
USP Category 3					≤ 1.0		No increase (vs. 14-day count)
USP Category 4					No increase (vs. initial count)		No increase (vs. 14-day count)
EP Category A1	≤ 3.0	No recovery	No recovery	No increase	No recovery	No recovery	No recovery
EP Category B2		≤ 1.0		≤ 3.0			No increase
1 - suggested 2 - minimum							

Table 5 - Comparison of USP and EP Criteria for Preservative Efficacy, II) Total Viable Fungi / Yeasts

	6-hours	24-hours	2-days	7-days	14-days	21-days	28-days
USP Category 1				No increase (vs. initial count)	No increase (vs. initial count)	No increase (vs. initial count)	No increase (vs. 14-day count)
USP Category 2					No increase (vs. initial count)	No increase (vs. initial count)	No increase (vs. 14-day count)
USP Category 3					No increase (vs. initial count)	No increase (vs. initial count)	No increase (vs. 14-day count)
USP Category 4					No increase (vs. initial count)	No increase (vs. initial count)	No increase (vs. 14-day count)
EP Category A1				≤ 2.0	No increase	No recovery	No recovery
EP Category B2					≤ 1.0	No increase	No increase
1 - suggested 2 - minimum							

These AET tests form part of the preservative optimization studies. They also need to be performed at the end of product shelf-life to confi rm adequate preservation over the total duration of the product's use. Some regulatory authorities also require confi rmation that the product is adequately preserved during its in-use period, when it is being routinely opened, dispensed and closed and the potential for microbial contamination is highest.

High-sensitivity test systems are being explored as possible replacements for the cumbersome, time-consuming and rather unreliable pharmacopoeial tests. Techniques include ATP bioluminescence, electrical impedance and chemiluminecence [17]. Such approaches offer the potential for automation of testing and high throughput screening of formulations during development. Inevitably, much development, validation and corroboration would be required before adopting any replacement technique. In the meantime, some issues could be addressed to ensure a more sensible approach is taken with the current monographs. These could include:

Harmonized Compendial Monographs

There is no clear evidence that the USP performance criteria have led to poorly preserved products within the US [18]. In the light of such experience and the great difficulty in getting some products to meet Ph.Eur requirements (with attendant cost and delays to product development), it would be benefi cial for the ongoing pharmacopeial harmonization initiatives to be completed as soon as possible.

Align test duration with product usage

Some oral liquid products are manufactured as lyophilized solids that are constituted with water prior to use. Shelf life in the liquid state is typically constrained by drug instability. 7 or 10 day use periods are common. Performance criteria for preservatives in such products should refl ect this. Running the test for 30 days, when the product may fail the test for other reasons e.g. loss of preservative through hydrolysis or sublimation makes little scientifi c sense. Kill / re-growth criteria should refl ect the product's in-use shelf life.

Preservative Free Formulations

Preservative-free cosmetics, medications and food / beverages are frequently promoted as superior products. Such publication fuels demands that preservatives be omitted from medicinal

products. It is true that signifi cant progress has been made in technologies for "microbiologically clean" manufacture and packaging, as well as in good manufacturing practice such that gross microbial contamination can be avoided. At the same time, many materials used as excipients, being of biological provenance cannot realistically be sterile. Furthermore, their nature or physical properties ensure that they cannot readily be sterilized before or during incorporation in product. Terminal sterilization may not be feasible for the same reasons. The presence of low levels of microbes cannot be obviated in such cases and, if the vehicle allows or encourages microbial growth (over the necessarily long shelf life that pharmaceutical products must possess) the inclusion of a preservative in the product is simply a prudent way to assure an important quality attribute and safeguard the patient.

In practical terms, removing preservative from a medicinal product (even if technically feasible) would require a comprehensive re-think of quality systems throughout manufacture to provide a product that is essentially microbe-free (essentially sterile) . Packs and delivery devices must also ensure that the quality built in at manufacture is maintained throughout the product's shelf-life, in particular, during its in-use period. Such an approach might involve the following practices:

- Controls of microbial levels in product components (drug, excipients and especially water, container / closure) incorporating vendor certification, testing, validation of packaging and storage in appropriately clean areas.
- Product manufacture in a microbe-free environment. In some cases it may be possible to reduce or eliminate contamination by procedures during or at end of manufacture, as is possible with some food and confectionary products. However, there is a general regulatory reluctance to use preservatives to address poor manufacturing practices, to reduce viable microbial population of a non-sterile product or to control the bio-burden prior to sterilization of a multi-dose sterile product [14]. Such "post-manufacture sterilization" may deal with residual microbes but endotoxins remain and pose additional quality and safety problems.
- Product is packaged in units that maintain closure integrity during shelf life e.g. blow fill seal ampoules. Single-use units e.g. BFS are the most frequently used preservative-free container-closure systems, but are difficult to use, particularly for geriatric patients and are more costly.

Several preservation-free intranasal devices are available for commercialization [19], but there are relatively few multi-use preservative-free nasal products. It should be stressed that even using a preservative free device, the manufacturer still needs to minimize microbial contamination during manufacture / storage; there is a need to protect the nozzle during the within-use period and preventing contamination through air-intake after device actuation [19]. Strategies for preservative-free nasal devices include the use of mechanical seals, the use of embedded anti-microbial agents e.g. silver on the interior of the nozzle, filtering systems within the device to remove microbial contamination and the use of negative pressure containers to prevent ingress of micro-organisms post actuation. Some companies are developing a multi-use nasal device with self sealing nozzle that could maintain sterility after repeated microbial challenge tests [19].

Similarly, there are several novel ophthalmic container / closure systems that utilize either a 0.2 micron filter or a preservative (e.g. bezalkonium chloride) adsorbed onto a filter to maintain sterility during the in-use period, and several have been commercialized [20].

The ABAK®(Laboratoires Théa, France) and COMOD®(Ursapharm, Germany) container-closure systems have been used for several different ophthalmic products, such as common beta-blockers e.g. timolol and carteolol [21].

Technologies are generally available to assure such quality, controls and treatments being product-specific. Many pharmaceutical products already utilize such approaches. The in-use period where patients use the product still remains the Achilles heel, when the closure needs to be broached or penetrated so that a dose can be withdrawn and more importantly where there is potential for microbial contamination. There is no universally reliable way that this can be achieved for each and every product type. Getting it wrong at any stage of the supply chain, manufacture, storage or in-use will almost certainly result in a return to earlier health concerns caused by microbial contamination of preservative free multi-use 'sterile' medicines [22].

Conclusions

Preservatives, either singly or in synergistic combinations remain necessary to prevent microbial contamination of multi-use liquid or semi-solid medicinal products, particularly from opportunistic pathogens. Non-inclusion can result in serious patient health consequences. There are a limited number of regulatory approved preservatives that can be included in these multi-use medicinal oral or topical products and the number is constrained even further in parenteral products. Furthermore, it may be time to revisit the tests and performance requirements that products must undergo before being considered to be adequately preserved. Performance criteria and assessment techniques, based on product type, dose, environmental history in manufacture and experience during patient usage might be more appropriate than applying a single quality standard defined in pharmacopoeias that may well represent "overkill" in a microbiological and commonsense context for many products. Preservative-free approaches are still in their infancy and much more research is required before they can be considered on an equal footing with preserved approaches. However, several preservative-free intranasal and ophthalmic devices are available and do offer some promise.

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Introduction

The second article in this series deals with the many constraints that face the pharmaceutical scientist tasked with developing preservation systems for multi-use oral, topical and parenteral medicinal products. The key role that pH plays in antimicrobial efficacy, as well as general stability considerations (both chemical and physical), will be covered.

Evaluating Performance

Compendial tests [1-3] for antimicrobial efficacy set high performance standards. It is also a regulatory requirement to assess the antimicrobial efficacy of the drug product (in its final container) at the end of the product's proposed shelf-life. Activity needs to be broad spectrum, encompassing bacteria (Gram-positive and Gram-negative), yeasts, fungi and molds; but not viruses. An effective preservative must reduce a microbial population significantly and prevent subsequent re-growth and these effects must be both microcidal and microstatic in nature.

Combining preservatives that act synergistically may help meet performance standards. Benzalkonium chloride (BKC) is ineffective against some strains of *Pseudomonas aeruginosa*, *Mycobacterium* and *Trichophyton* [4], but combinations with EDTA, benzyl alcohol, 2-phenylethanol or 3-phenylpropanol enhances anti-*Pseudomonad* activity [5]. Synergy is also observed in combination with cetrimide, 3-cresol, chlorhexidine and organo mercurials [6,7].

The amino benzoic acid esters (parabens) are more active against Gram-positive, than Gram-negative bacteria, and more active against yeasts and molds than bacteria. Activity increases with increased alkyl chain length (butyl > propyl > ethyl > methyl) but aqueous solubility

commensurately decreases, and consequently the parabens are also often used in combination, e.g. methyl and propyl paraben. Parabens also show some synergy with EDTA [8], 2-phenylethanol [9] and imidurea [10].

The complexity of multi-phase dermal products, formulated as creams, lotions or ointments mean that adequate microcidal efficacy may not be attainable. The best that can be achieved is a microstatic effect. In practical terms such performance may be perfectly acceptable. If the bioburden is low most preservative systems can adequately kill or attenuate growth of most organisms. Current GMP (good manufacturing practice) standards, encompassing operating and sampling procedures, controls on input materials, use of clean room and automated technologies in manufacturing and packaging, when viewed holistically ensure that high standards of microbial cleanliness can be routinely achieved in fi nished products. Additionally, the state of the art in packaging technology is now such that contamination, prior to use is unlikely. Hence the risk of contamination is probably greatest during patient use of multi-dose liquid products. Microstasis may be an acceptable performance standard for non-parenteral products at this stage, if the in-use period is short (< 1-month).

Influence of Product pH

Table 1 - Effect of pH on Preservative Efficacy

Preservative	pH of optimum activity	Reference
Aminobenzoate esters e.g. Parabens	pH 4-8	[13]
Quarternary ammonium compounds (QACs) e.g. Benzalkonium Chloride (BKC), Benzethonium chloride	pH 4-10	[4,14]
Aryl acids e.g. Benzoic acid / salts	<pH 4.5	[15]
Aryl alcohols e.g. Benzyl alcohol	<pH 5.0	[16]
Quarternary ammonium comounds (QACs) e.g. Cetrimide	pH 7-9	[17]
Biguanides e.g. Chlorhexidine	pH 5- 7	[18]
Chlorocresol	pH 4-9	[19]
Chloroxylenol	Little pH effect	[20]
Formaldehyde donators e.g. Imidurea	pH 3-9	[21]
Formaldehyde donators e.g. Bronopol	pH 5 - 8	[22]
Alkyl acids e.g. Propionic Acid	pH 3.9	[23]
Alkyl acids e.g. Sorbic acid / salts	pH 4.5	[24]
Phenolic compounds e.g. Phenol, m-cresol	pH 4-9	[25,26]
Phenylmercuric salts e.g. acetate, borate, nitrate	pH 5-8	[27,28,29]
Thiomersal	acidic pH	[30]

pH can affect the rate of growth of microbes, the interaction of the preservative with cell wall components and the MIC (minimum inhibitory concentration) of many preservatives [11,12]. In general, microbial growth is optimal between pH 6-8. Outside this range growth rate signifi cantly declines. In contrast, the product pH may refl ect the intrinsic pH of the active pharmaceutical ingredient (API), or the product may require pH modifi cation to enhance product solubility, stability, palatability or optimal microbial effectiveness (MIC_{max}). Specifi c excipients may also infl uence product pH. Hence, pH adjustment to regions less favorable to microbial viability i.e. away from pH 6-8, may not be feasible or must take account of competing effects on overall product quality versus the activity of the preservative system. Table 1 lists pH ranges for optimum activity for common preservatives.

Such pH effects refl ect the chemical composition of the active moiety in the preservative molecule. For instance, if activity is associated with the non-ionized moiety (acids, alcohols and phenols) the effect is usually optimal at acidic pH but ultimately refl ects the pKa of the individual agent. However and almost inevitably, there are exceptions. For example, phenol is most active in acidic solutions, despite its high pKa (10.0). Substituted alcohols are also less reliant on pH. Bronopol (2-bromo-2-nitro-1, 3-propanediol) is not markedly infl uenced by pH in the range 5.0-8.0, perhaps refl ecting that its main activity is via release of formaldehyde, whose microcidal activity is not signifi cantly infl uenced by pH [22].

Phenolic preservatives tend to be active over a wider pH range than alcohols or acids. Chlorocresol [19] is most effective at acidic solutions but can retain activity at pH regions up to its pKa (9.2). Similarly, m-cresol [25] is also effective at pHs below its pKa (9.6). Solution pH does not have a marked effect on the anti-microbial efficacy of 4-chloroxylenol [31].

Esterifi cation of acids can extend the pH span of activity. The parabens are active over the range pH 4-8. Efficacy decreases at higher pH due to the formation of phenolate ion (pKa ca. 8.4). Efficacy increases with the longer alkyl chain, but conversely aqueous solubility decreases as hydrophobicity increases [13].

In contrast to acid preservatives, the quaternary ammonium compounds (QACs) such as benzalkonium chloride (BKC) and benzethonium chloride have anti-microbial efficacy over a wide pH range (pH 4-10), activity being associated with the ionized (cationic) moiety and being optimal at high pHs [4,11,14]. Efficacy is also linked with alkyl chain length (C18 > C16 > C14 > C12). Cetrimide [17] has a slightly narrower effective pH range (7-9), probably caused by the presence of a methyl rather than a benzyl moiety (less effective at stabilizing the charge). High pH causes the microbial cell wall to be negatively charged, thereby favoring the binding of cationic species.

There are no reported pH constraints on the permeation enhancing capability of EDTA, probably a consequence of its multiple pKa's. However, its limited intrinsic anti-microbial efficacy means that it is rarely used on its own, but in combination with other preservatives [32,33].

pH-related effects can sometimes be more complex than those summarized in Table 1. The antifungal activity of benzoic acid is less susceptible to pH than are its antibacterial effects [15]. The substituted benzoic acid derivative thiomersal, which has a pKa of 3.1 is bacteriostatic and

fungistatic at neutral and even mildly alkaline pHs. However, the microcidal activity of organic mercury also needs to be taken into account [30]. A similar effect is evident with propionic acid [23] and sorbic acids [24], which have appreciable antifungal but little or no antibacterial activity at pH 6.0.

Biguanide anti-microbials are active over the pH range 3-9. However, chlorhexidine is effective over a narrower pH range (5-7), and above pH 8.0 the base may precipitate from aqueous solutions [18]. Imidurea is effective over this whole pH range (3-9), although optimum efficacy is seen at acidic pH [21].

Organo mercurial preservatives, for example, phenylmercurate salts, have broad spectrum bactericidal and fungicidal activities, being more potent with increasing pH. Efficacy against *Pseudomonad's* have also been demonstrated at pH 6 or below [27,28,29]. These preservatives have been utilized in several eye drop product having acidic pH values. Activity is enhanced at acidic pH in the presence of sodium metabisulphite, which can enhance activity at low pH, but has the opposite effect at alkaline pH [34,35]. In topical products phenylmercurate salts have been reported as being active at pH 5-8 [36].

Factors that Compromise Preservative Efficacy

Preservatives are no different from any other group of organic compounds. They possess reactive functional groups and may have pH-solubility profiles that need to be considered on a case-by-case basis when formulating the drug product. Preservative efficacy can be compromised by interactions with active ingredients, excipients, container / closures or by other physicochemical behaviors. Deterioration can occur during manufacture or throughout the product shelf life or use. Effects can be ascribed to:

- interactions with other components within the product (drug, excipients, pack or delivery device)
- chemical instability
- physical losses or changes

Possibilities for degradation are manifold, but the risk can be mitigated at the outset by a thorough knowledge of all the product components and by appropriate pre-formulation studies to determine interaction propensity. It is important that such awareness be available at the product design stage, i.e. a QbD (Quality by Design) approach. Pharmaceutical products generally have much longer shelf life requirements than food or beverage products and quality must obviously be retained over such periods. The relatively insensitive nature of preservative efficacy tests [1-3] may mean that modest but inexorable deterioration of effectiveness during storage may take time to be considered significant. A consequent reformulation and evaluation program having deleterious effects on development timelines.

Chemical Stability of Preservatives

In addition to antimicrobial effectiveness testing (AET), it is a regulatory requirement to monitor the chemical stability of the drug product (in its final container) throughout the product's proposed shelf-life. It should not surprise that most acidic preservatives e.g. benzoic, sorbic and propionic acids are incompatible with strong bases [15,23,24]. Strong oxidizing agents degrade sorbic acid [24], 2-phenylethanol [37], hexetidine [38], EDTA [32], thimerosal [30], propyl gallate [39] and butylated hydroxyanisole [40]. This latter material (BHT) is particularly unstable in the presence of peroxides and permanganates and interaction may even result in spontaneous combustion [40]. The deliberate inclusion of such potent materials in a dosage form might be unusual (although benzoyl peroxide is formulated as lotions to treat acne), but excipients such as povidone, crospovidone, polyethylene glycol and polysorbates may contain residual peroxides [41]. Residues may be low but a high excipient-to-preservative ratio may be sufficient to fuel interactions.

The antimicrobial efficacy of several preservatives is compromised by surface-active agents:

- benzalkonium chloride [4], benzethonium chloride [14] and cetrimide [17], all being cationic in nature are incompatible with anionic surfactants.
- benzyl alcohol [16], 2-phenoxyethanol [42], 4-chloroxylenol [20] and m-cresol [25] should not be formulated with non-ionic surfactants. Chlorobutanol [43] and 2-phenylethanol [37] are adversely affected by the presence of non-ionic surfactants, e.g. polysorbate 80.

Such interactions may not involve conventional chemical transformation, but concern more subtle phenomena e.g. hydrogen bonding and complex formation. Thus the overall level of preservative in the product may not change, but unless the preservative is available in the "free" form its efficacy may be compromised. Determination of preservative efficacy is therefore mandated [1-3].

Some preservatives interact with other preservatives, for example: EDTA [32] interacts with thimerosal, propyl gallate and phenylmercuric salts; chlorhexidine [18] can interact with benzoic acid and cetrimide [17] is incompatible with phenyl mercuric nitrate.

Most of the available preservatives seem ostensibly to be stable structures. This may explain why reports on intrinsic chemical instability (i.e. that do not involve interaction with other product components) of preservatives are less widespread than those interactions discussed above. Paraben [13,44,45] preservatives are susceptible to base-catalyzed ester hydrolysis, degrading by classic pseudo-first order kinetics, with shorter chain analogues being least stable [13]. Stability in solution is not markedly affected by pH up to about pH 6.5, but degradation rates increase significantly at pH 7.5 and above [46]. As parabens are reputedly active over the pH range 4-8 it would seem that caution is advised if product pH is likely to be higher than neutral. In the light of the predictable degradation kinetics of these agents scientifically relevant accelerated (high temperature) stability studies at the formulation development stage may well predict long term stability (or instability) in the final product. The formulation scientist may need to include excess parabens to compensate for chemical instability of the preservative system, including losses during manufacture, and this is allowable from a regulatory perspective. The guiding principle

however is to minimize levels in the formulation commensurate with adequate preservative efficacy at the end of shelf-life [47].

Despite its many advantages as a preservative and its undoubted stability in the solid state, sorbic acid is unstable in semi-solid and liquid preparations. The principal degradation pathway is auto-oxidation resulting in acetaldehyde and β-carboxyacreloin end-products; as well as numerous other volatile aldehydes, e.g. malonaldehyde, acrolein, crotonaldehyde and related furans (2-methylfuran, 2-acetyl-5-methylfuran, 2,5-dimethylfuran) [48]. Sorbic acid may be stabilized by phenolic anti-oxidants, for example 0.02% w/w propyl gallate [39].

Macromolecules can be adversely affected by preservatives. Benzyl alcohol causes aggregation of rhIFN (recombinant human interferon), while several commercial biopharmaceutical products specify that diluent for constitution must not contain preservative(s) because of potential adverse effects on the protein [49].

Preservatives for insulin preparations must be chosen carefully. Insulin zinc suspensions cannot contain phenol as it destroys the crystallinity of the insulin and mixtures of parabens are used instead. In contrast neutral protamine insulin requires the use of phenol or meta-phenol to form and preserve the crystal form that provides the long-acting effect [50].

Physical Stability of Preservatives

Preservative content in products can be depleted during manufacture, storage or use.

The parabens [13,45,46], benzoic acid [15], benzyl alcohol [16], 2-phenoxyethanol [42], m-cresol [25], chlorocresol [19] and chlorbutanol [44] are all volatile to greater or lesser extents. This renders them susceptible to losses by sublimation or evaporation during manufacture or throughout product life. m-Cresol [25] and phenol [26] are not suitable as preservatives for preparations that need to be lyophilized due to their volatility. In addition, if any of the container / closure components are permeable to gases, e.g. plastic bottles or elastomeric closures, then this can result in the depletion of volatile preservatives.

Polyvalent ions may cause precipitation of preservative from solution e.g.:

- sorbic acid [24] and chlorhexidine [18] can be “salted out” by Ca^{2+} ions.
- chlorobutanol [44] and chlorhexidine [18] interact with Mg^{2+} ions.
- bronopol [22] and phenylmercuric nitrate [29] can be precipitated by Al^{3+} ions.
- Fe^{3+} ions can salt out butylated hydroxyanisole [40] and butylated hydroxytoluene [51].
- EDTA [32] is precipitated by most polyvalent cations.

The overall level of the preservative in the product may remain unchanged but solution concentration is diminished, as a consequence of precipitation, leading to reduction of microbiological efficacy. Analytical techniques to monitor preservative content need to refl ect such considerations, viz assessing the free versus bound concentrations within the product.

Table 2 - Examples of Preservatives Susceptible to Adsorption

Preservative	Adsorbent / Substrate	Reference
Benzalkonium chloride	Hypromellose	
Filters	[52]	
[53]		
Benzoic acid	Kaolin	[54]
Benzyl alcohol	Polyethylene, natural rubber	[55]
Cetrimide	Bentonite	[17]
Chlorbutanol	Polyethylene	[56]
Chlorhexidine	Various polymeric excipients e.g. Na carboxymethylcellulose	[57]
Parabens	Ion exchange resins, some plastics	[58,59]
Phenoxy ethanol	PVC plastic, Cellulose-based excipients	[60,61]
Phenylmercuric salts	Various suspending agents	[62-65]
Sorbic acid / sorbates	Plastics (polypropylene, PVC and polyethylene)	[24]
Thiomersal	Polyethylene / other plastics and rubber	[66,67]

Adsorption onto excipients, especially those with large surface areas or on to container / closure systems can also remove preservative(s) from solution. Table 2 lists some documented examples.

Antacid formulations illustrate that physical and chemical interactions can combine to make preservation difficult. pH of such products is usually neutral to slightly alkaline, where intrinsic preservative activity can be low. Additionally, the presence of polyvalent cations (e.g. Al^{3+}, Ca^{2+}, Mg^{2+}) associated with the actives can lead to precipitation. Adsorption of the preservative on to the insoluble antacid substrate is also possible. All contribute to the overall loss of preservative efficacy. Antacid suspensions are notoriously difficult to preserve to the standards defi ned in pharmacopoeias because of such behaviors. This is refl ected in the lowered acceptance criteria for Antacids (category 4 products) in USP <51> [1], i.e. 'No increase (in bacteria, yeasts and molds) from the initial calculated count at 14 and 28 days'.

Multiphase products such as creams and lotions, as well as some parenteral and nasal / opthalmic products, can have aqueous and oily phases maintained in equilibrium by surface active agents. Viscosity enhancers may also be included as suspending agents. Such agents can interact with the preservatives as articulated above. The chlorinated preservatives, e.g. chlorobutanol [43], chloroxylenol [20] and chlorhexidine [18] can partition to or migrate on to polymeric suspending agents by competitive displacement of water of solvation. Similarly, the antimicrobial efficacy of 2-phenoxyethanol [42] is reduced in the presence of the cellulosic suspending agents, methylcellulose, sodium carboxymethyl cellulose and hydroxypropyl methylcellulose [61].

Preservatives will also distribute between oil and aqueous phases and at the interface containing the surface active agent, depending on distribution coefficient. Aqueous concentration, where the

antimicrobial effect is required, is thereby reduced. Such behaviors reduce the efficacy of the parabens preservatives, particularly the longer chain analogues such as butyl paraben [46]. Chlorhexidine activity can also be reduced because of micelle formation [18]. Some preservatives can form ion-pairs with the corresponding API, e.g. timolol and sorbic acid. Whilst this has been proposed as a mechanism for enhancing the ocular bioavailability of timolol, the impact on the efficacy of the preservative system has not been reported [68].

The possibilities for reduced anti-microbial efficacy in multi-phase systems, has engendered efforts to devise *in silico* predictive approaches to determine the impact of formulation parameters on preservative activity. The infl uence of partition coefficients, binding constants (surfactants and polymers), and oil-in-water ratios have all been investigated but with limited success [12]. The pragmatic approach, involving optimizing the preservation system and inclusion levels by conventional assessment techniques therefore remains the desired approach for the present.

Conclusions

Preservatives, either singly or in synergistic combinations remain necessary to prevent microbial contamination of multi-use liquid or semi-solid medicinal products, particularly from opportunistic pathogens. Non-inclusion can result in serious patient health consequences. There are a limited number of regulatory approved preservatives that can be included in these multi-use medicinal oral or topical products and the number is constrained even further in parenteral products. The optimal conditions for preservative efficacy (pH, physical and chemical stability) are rarely the same as for the product itself and as such compromises are often necessary to ensure an optimal product shelf-life.

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