The Prominence of Preservative Counter balancing in Parenteral Formulations for Sterilization Investigation

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Abstract - Anti-microbial preservatives (AMPs) has an important part in preventing the growth of microbes in multiple-dose parenteral formulations (PFs). Chemical preservation cannot keep products from spoiling, but they slow the spoiling process caused by microorganisms. Along with preservatives several excipients like buffers, polymers, antioxidantsetc., are added in the formulation. The preservative in the recovery agar or parenteral could artificially decrease the survival of viable cells thus the presence of preservative interfere with the sterility of PFs. It is important to counterbalance this residual activity to get accurate counts of survivors. The methods for inhibition of residual biocide are dilution and neutralization (dilution, chemical embarrassment, washing, and filtration) of the biocide. Neutralizer should inhibit the activity of AMPs and it should not become toxic later combining with the API. Several chemicals are used to inhibit the AMPs. The purpose of this research was to study the neutralization efficacy on a range of AMPs and its possible deadliness for microbes that are used for antimicrobial preservation testing.

Keywords: microbes, sterility, preservative, technique

INTRODUCTION:

The dosage forms which are given by bypassing the alimentary canal are called parenteral formulations (PFs) which have been using worldwide. PFs gives immediate action compares to oral dosage forms. PFs are available as a single dose and multiple-dose. Single-dose PFs do not require Anti-Microbial Preservatives (AMPs), whereas multi-dose formulations need them to encounter the growth of microbes. These preservatives diminish thegrowth of microbes which unintentionally enters into the containers during product withdrawal [1]. Multi-dose PFs have advantages over single-dose PFs viz., minimal product wastage, doses may be withdrawn from the container over some time without the microbial risks and require less packing material (both primary and secondary).

IMPORTANCE OF PRESERVATIVES

AMPs have been used since prehistoric times. Salt, sugar, vinegar and diatomaceous earth are preservatives, whereas freezing, pickling, smoking, and salting are preserving processes which were traditionally adopted [2]. The product with greater water content is a good environment for microbial growth essential for avoiding alteration and degradation by microorganisms during storage. Water containing PFs shelf life can be increased by using AMPs [3].

In pharmaceutical firms, a suitable AMPs is to be added to in all multiple dose PFsto inhibit the development of microbes which were accidentally introduced during the withdrawal of individual doses [4]. During filling operations if there is any inadvertent breach of asepsis, micro-organisms could be introduced and to prevent those AMPs may be added as a sterility assurance method to single-dose PFsthat are not terminally sterilized [5]. AMPs should not be used in formulations like injections into cerebrospinal fluids, eye or heart. The most common classical preservative agents are the weak organic acids viz., acetic, lactic, benzoic and sorbic acids [6]. AMPsretain consistency and texture of the product and also improve their nutritional possessions [7]. The common categories of additives [8] used in PFswere shown in **table 1.**

Table 1. Excipients used in parenteral formulations

Excipient	Role
Antioxidants	Protect the therapeutic agent in formulation from oxidative degradation
Buffers	Upholdessential pH
Chelating agents	Bind the traces of heavy metals
Preservatives	Prevent growth of microbes
Polymers	Controlprolong drug delivery
Solubilizes	Enhance active ingredient solubility
Solvents	To dissolve the active drug and other excipients in the formulation
Tonicity agents	Diminish the pain of injection

THE NEED OF PRESERVATIVES NEUTRALIZATION

Sterility testing of PFs maybe test carried out by filtering by membrane or by direct inoculation of the test sample into the culture media with the product to be examined [9]. In the later technique direct inoculation of a sample in a culture medium, and gestated for specifies a period for the cases of contamination [10]. If the PFs containing AMPs, which restrict with the real readings. In such cases, nullification of AMPs present in PFs is very necessary. It is necessary to neutralize this residual activity of AMPs in PFs. Dilution and chemical neutralization of the AMPs are the techniques used for inhibition of residual AMPs.

COUNTERBALANCING METHOD

First AMPs activity should be efficiently inhibited by a neutralizer, secondly the neutralizer should be nontoxic to the control microbes and lastly a toxic derivative should not form after the reaction [12-14]. In general neutralizers are used after suitable dilution of PF. AMPs which can be neutralized by dilution or addition of chemicals was represented in **table 2** [11], deactivated them by changing the pH was shown in **table 3** and the adsorbents to neutral was listed in **table 4**.

Table 2. List of preservatives and commonly used chemical or method for neutralization

Antimicrobial preservatives		Recommended neutralizing agents
Category	**Example**	
Alcohol	Benzyl alcohol	Dilution and Non-ionic surfactants (E.g., Polysorbates)
Aldehyde	Glutaraldehyde	Dilution, Glycine Sodium bisulfite, Sodium sulphite, and Sodium thioglycolate
Antiseptic	Chlorhexidine	Non-ionic surfactants (E.g., polysorbates), Lecithin and anionic surfactants
Formaldehyde donors	Imidazolidinyl urea	Dilution, Protein, Gelatine, Sodiumbisulfite, Histamine, Histidine, Non-ionic surfactants (polysorbates) and Lecithin
Isothiazolinones	Methylisothiazolinone	Dilution, Amines, Sulphites, Sodium bisulfite, Sodium thioglycollate, and Mercaptants
Mineral acids	Sulphuric and hydrochloric acids	Increasing pH and peptones
Nitrocompounds	Bronopol	Sulfhydryl compounds (E.g., Cysteine, thioglycollate, thiosulfate, and metabisulfite)
Organic acid preservatives	Benzoic acid and sorbic acid	Non-ionic surfactants(polysorbates), and increasing pH
Parabens	Methyl and propyl parabens	Lecithin, and Non-ionic surfactants (E.g., Polysorbates)
Phenolic compounds	Phenyl phenol, chlorophenol, cresols	Non-ionic surfactants (E.g., Polysorbates), Lecithin
Quaternary ammonium compounds	Benzalkonium chloride, benzethonium chloride	Lecithin, non-ionic surfactants (E.g., Polysorbates), protein and anionic surfactants

Table 3. Effect of pH on Preservative Efficacy

Name of the preservative		pH of optimum activity
Category	**Example**	
Aminobenzoate esters	Methyl Parabens and Propyl Paraben	4-8
Aryl acids,	Benzoic acid and salts	<4.5
Alkyl/Aryl alcohol	Benzyl alcohol, Chlorobutanol, 2-ethoxy ethanol	<5.0
Alkyl acids	Propionic acid	3.9
Alkyl acids	Sorbic acid/salts	4.5
Biguanides	Chlorhexidine	5-7
Formaldehyde donators	Bronopol	5-8
Formaldehyde donators	Imidurea	3-9
Nitrates	Thiomersal	Acidic pH
Phenols	Chloroxylenol	Little pH effect
Phenols	Chlorocresol	4-9
Phenyl mercuric salts	Acetate, borate	5-8
Phenolic compounds	Phenol	4-9
Quaternary ammonium compounds	Cetrimide	7-9
Quaternary ammonium compounds	Benzalkonium chloride and Benzethonium chloride	4-10

Table4. Preservatives with their adsorbents

Preservative		Adsorbent/substrate
Category	**Example**	
Cationic surfactant	Benzalkonium chloride	Hypromellose and Filter membranes
Carboxylic acid	Benzoic acid	Kaolin
Alcohol	Benzyl alcohol and chlorobutanol	Polyethylene and Natural Rubber
Quaternary ammonium compounds	Cetrimide	Bentonite
Biguanide	Chlorhexidine	Various polymeric excipients, E.g., Corboxymethyl Cellulose
Esters	p-aminobenzoate esters	Ion exchange resins and some plastics
Alcohol	Phenoxyethanol	Poly Vinyl Chloride and Cellulose based excipients
Salts	Phenyl mercuric salts	Suspending agents
Salts	Sorbic acid/sorbates	Polypropylene,PVC,Polyethylene
Organo mercury compound	Thiomersal	Polyethylene,plastics, and rubber

CONCLUSION:

This extensive study gives a clear picture about the various methodologies adopted for the neutralization of preservatives in parenteral formulations before they tested for their sterility.

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CONFLICT OF INTEREST:

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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