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An Overview of Antimicrobial Preservatives: Definitions, Properties, Classifications, and Safety Concerns

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Abstract: *Excipients known as antimicrobial preservatives are used in pharmaceutical preparations that are administered in many doses and may be sterile or non-sterile. Preservatives with antimicrobial properties are primarily used to stop bacteria from growing during a product's usage. For certain excipients, the effectiveness of antimicrobial preservatives must be established. This article covers the fundamentals of the preservative effectiveness test, the most crucial factors to take into account when choosing preservatives, and the applicability of regulatory criteria for preservative effectiveness testing in the practice of medicine. To determine the efficiency of preservative systems in multidose products, the producer must conduct antimicrobial effectiveness testing.*

Keywords: Antimicrobial Effectiveness Testing, Preservative Efficacy.

I. INTRODUCTION

To avoid product instability and customer infection, aqueous pharmaceuticals must be microbiologically preserved. Antibacterial agents are used in these products.

Antimicrobial preservatives protect non-sterile dosage forms against bacteria that may have entered during manufacture or microbiological development. These goods include preservatives, which are used in cosmetics and drugs. Sterile products in multiple-dose containers utilize antimicrobial preservatives to prevent germ growth after repeated dosage removal.

Antimicrobials are harmful and should be avoided. To enhance patient safety, the final packaged product's proven-active preservative concentration should not harm people. If the active ingredients are naturally antibacterial, the antimicrobial preservative concentration may be minimized. It may be effective whether the antibacterial action is from a preservative or the product's composition.

Properties of the Ideal Preservative

- It is effective against a broad range of species even at low concentrations, which is a plus.
- Chemical and physical stability under typical usage settings throughout a broad pH and temperature range are important considerations.
- At the concentrations necessary, it is soluble in water.
- It is compatible with a broad range of pharmaceuticals & excipients.
- Does not have an odor, taste, color, or stinging sensation.
- Toxic & sensitizing properties are not present at the recommended dose.
- It could not be a source of irritation.
- A substance that has no reaction with the container or the closure.
- Reasonable pricing.

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Uses of Preservatives

When microbial contamination and growth are possible during product production or patient use, preservatives should be used.

Antimicrobials are needed for these dose forms:

- **Aqueous emulsions:** Because the watery phase promotes microbe growth, all emulsions require antimicrobials.
- **Suspensions:** For the purpose of preventing contamination by bacteria, yeast, and fungi, suspensions "intended for any route of administration" may contain a suitable antimicrobial agent.
- **Oral Solutions:** Additionally, antimicrobial compounds are frequently incorporated to impede the growth of bacteria, yeast, and mildew.
- **Ophthalmic solutions:** Each solution must contain a material or combination of substances that will inhibit or kill microorganisms accidentally introduced when the container is opened during use. Despite being sterile, antibacterial ophthalmic solutions for surgical operations cannot be used. Antibacterial agents may irritate eyes.
- **Ophthalmic Ointments:** Unless otherwise stated in the monograph or the formula is bacteriostatic to inhibit microorganism growth, ophthalmic ointments may contain a proper substance or mixture of substances to prevent accidental bacteria introduction.
- **Parenteral Products:** A appropriate substance or mixture of chemicals might be added to multiple-dose injectable solutions to inhibit germ proliferation. This applies whether the preparation is sterilized by heat, cold, or other methods.
- **Preservatives are utilized to:** 1- Stop germs from attacking the product. 2- Enhance medication efficacy 3- Extend product shelf life. 4- Protect the product from germs during storage to increase stability.

Preservatives are unnecessary in these cases:

The product shall be used immediately upon receipt. The product is prepared and administered using methods that reduce contamination.

No water is available. Tablets, powders, and hydrocarbon ointments do not function as growth medium because bacteria require water to flourish. Ocular and non-aqueous injections do.

The mean pH is neutral or slightly alkaline. < 3, or > 9. Despite the pH range suppressing development for most microbes, certain resistant molds thrive at pH < 3.

One or more antimicrobial compounds provide the product antibacterial properties.

Preservatives are contraindicated when:

1. Infants and babies.
2. Ophthalmic solutions for eye surgery, corneal transplantation, or intraocular injection.
3. Large-volume parenterals > 30 ml.

Classification of Preservatives

Preservatives are categorized by mechanism, chemical type, and source.

Classification based on mechanism of action

Antioxidants: They prevent active medicinal components and excipients from oxidizing. Self-reducing agents prevent environmental oxidation by oxidizing themselves. This affects oxygen-oxidizing components. They protect against deterioration by considering oxygen and sunshine, the most important factors. Vitamin E, C, BHA, and butylatedhydroxytoluene.

Antimicrobial antibiotics destroy gram-positive and gram-negative bacteria and degrade pharmaceuticals.

Sorbates chelating agents protect the medication. Pharmaceutical formulations include EDTA, polyphosphates, and citric acid.

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Classification based on chemical class

Alcohols & glycols:

- **Ethyl alcohol:** Pharmaceuticals and cosmetics often include ethanol or aqueous ethanol. Ethanol has bactericidal and antibacterial preservative characteristics beyond solvents. Propylene glycol is used in many cosmetic and pharmaceutical goods as a solvent, disinfectant, stabilizer, and water-miscible co-solvent. The antiseptic is equivalent to ethanol, however it is somewhat less effective against molds.
- **Glycerin:** Oral, otic, ophthalmic, topical, and parenteral medications include glycerin. As an antimicrobial preservative, co-solvent, emollient, humectant, plasticizer, sweetener, tonicity agent, and solvent.
- **Benzyl Alcohol:** Cosmetics, foods, and medications include antibacterial preservative benzyl alcohol. Benzyl alcohol is bacteriostatic and antimicrobial, killing Gram-positive bacteria, molds, fungi, and yeasts. Benzyl alcohol weakly inhibits most Gram-positive bacteria. However, certain Gram-positive bacteria are very susceptible to the antibacterial characteristics. According to the literature, benzyl alcohol is less effective against Gram-negative than Gram-positive pathogens. Molds and yeasts resist benzyl alcohol, despite its antibacterial properties.
- **Isopropyl Alcohol:** Cosmetic and pharmaceutical goods employ isopropyl alcohol as a solvent and cosmetic ingredient. Besides other uses, it disinfects and preserves. At concentrations over 70% (vol/vol), isopropyl alcohol kills bacteria. It is more effective than ethanol 95% by volume as an antibacterial preservative.
- **Phenol:** Parenteral pharmaceutical products like intravenous fluids use phenol as an antibacterial preservative. Topical medications and cosmetics have also used it. Its antiseptic and disinfecting uses are varied.
- **Chlorocresol:** Chlorocresol is an efficient antibacterial preservative in cosmetics and pharmaceuticals. Chorocresol kills Gram-positive and Gram-negative bacteria, spores, molds, and yeasts, including Pseudomonas aeruginosa. It's antibacterial and antifungal against molds and yeasts.

Organic acids:

- **Benzoic Acid:** Cosmetics, foods, and pharmaceuticals use benzoic acid to inhibit microbial development. Whitfield's Ointment and other topicals include benzoic acid as an antifungal. Only the undissociated acid is bactericidal, and its action depends on medium pH. Benzoic acid has significant bacteriostatic effect against most Gram-positive bacteria but less against Gram-negative bacteria. This chemical also inhibits molds and yeasts.
- **Sodium Benzoate:** Except for pharmaceuticals, sodium benzoate is used as an antimicrobial preservative in cosmetics, foods, and pharmaceuticals. It also lubricates tablets and capsules. Its greater solubility makes it preferable to benzoic acid in certain conditions. Sodium benzoate is used in various products because it is antibacterial and antifungal.
- **Potassium Benzoate:** In drinks, foods, and medicines, potassium benzoate is used as an antibacterial preservative to limit germ growth. In low-sodium applications like food preparation, potassium benzoate is replacing sodium benzoate. Food is often made using non-toxic and non-irritant phosphoric acid (KOH).
- **Sorbic Acid:** In pharmaceuticals, foods, enteral preparations, and cosmetics, sorbic acid is an antimicrobial preservative with antibacterial and antifungal properties. Its antibacterial properties make it beneficial in additional applications than antifungal ones. When synergistic effects are seen, sorbic acid is used with other antimicrobial preservatives or glycols due to its limited stability and efficiency against bacteria.
- **Potassium Sorbate:** Potassium sorbate is an antibacterial and antifungal preservative used in pharmaceuticals, foods, enteral preparations, and cosmetics.

Preservative potassium sorbate is used in cosmetics. Potassium sorbate works well with other substances.

Because antimicrobial preservatives and glycols synergize, they may be avoided. Chemical compound potassium sorbate. Due to its increased water solubility and stability, sorbic acid is employed in twice as many medical formulations than citric acid.

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XEsters of p-Hydroxybenzoic Acid (Parabens):

Parabens are effective against yeasts and molds due to their broad antibacterial spectrum and pH range. Paraben salts, particularly sodium salt, are used in cosmetics to boost paraben efficacy owing to their low solubility. Parabens are more antifungal and antibacterial than bacteria. They also function better against Gram-positive than Gram-negative bacteria, which is good. Synergistic mixed parabens improve product action. Paraben activity increases with alkyl moiety length but decreases with solubility. Thus, methyl-, ethyl-, propyl-, and butylparaben combinations are prevalent. Methylparaben and Propylparaben dominate.

- **Methylparaben:** Antibacterial preservative methylparaben is used in cosmetics, food, and medicines. It can treat various skin disorders alone or with other parabens or antimicrobials. It is the least active paraben, but alkyl moiety chain length makes it the most active antibacterially. Methylparaben and propylparaben helped several pharmaceuticals last longer.
- **Methylparaben Sodium:** Methylparaben sodium may substitute methylparaben in certain applications due to its higher water solubility.
- **Propylparaben:** Because of its antimicrobial qualities, propylparaben is utilized in cosmetics, food, and drugs. It may be useful alone or with other paraben esters. With 25% of cosmetics uses, it is a popular preservative.
- **Propylparaben Sodium:** Propylparaben sodium is an antibacterial or antifungal preservative included in many water-based cosmetics and oral pharmaceuticals. Other paraben esters are commonly used alongside this one.

Organic mercurial derivatives:

- **Phenylmercuric salts:** At medication preservation dosages, phenylmercuric salts hinder development in a broad number of ways. Their bactericidal and fungicidal effects emerge slowly. It is mostly used as an antibacterial preservative in ophthalmic preparations, although it is also found in cosmetics, injections, and topicals. Phenylmercuric salts kill bacteria and fungi at several pH levels.
- **Phenylmercuric Acetate:** Phenylmercuric acetate may be used as an alternative antibacterial preservative in cosmetics and medications with mercury concentrations below 0.007%. It is chosen over phenylmercuric nitrate due to its solubility. Phenylmercuric Acetate has several applications. Preservative phenylmercuric acetate, like nitrate, exhibits broad-spectrum antibacterial and delayed bactericidal and fungicidal properties.
- **Phenylmercuric Nitrate:** Phenylmercuric salts are utilized in cosmetics, parenteral medicines, and topical pharmacy as antimicrobial preservatives for ophthalmic solutions.
- **Phenylmercuric Borate:** Phenylmercuric boreate is used as an antibacterial preservative instead of acetate or nitrate. Phenylmercuric acetate and nitrate are more irritating than methylmercuric nitrate. Phenylmercuric borate, like nitrate, has modest but consistent bactericidal and fungal activity.
- **Thimerosal:** Preservative Since the 1930s, biological and pharmaceutical products have employed thimerosal. Parenteral and topical medicines employ it as an antimicrobial preservative. Bacteriostatic and fungal activities make this preservative a good benzalkonium chloride alternative. Due to increased knowledge of mercury's and other mercury compounds' toxicity, concerns over thimerosal's pharmacy usage have grown. Increasing reports of adverse reactions, notably hypersensitivity, indicate that thimerosal should not be used as a preservative in eye drops or immunizations. Thimerosal is also utilized in cosmetics and soft contact lens solutions.

Salts of quaternary ammonium base:

- **Benzalkonium Chloride:** Like cetrimide, it is used as an antibacterial preservative in pharmaceuticals. It is a quaternary ammonium compound used as an antibacterial preservative like cetrimide. With one-third of ophthalmic treatments using it, it is a popular preservative. This preservative or excipient is used with disodium edetate to boost its antibacterial activity against Pseudomonas strains. Benzalkonium chloride solutions kill many bacteria, yeasts, and fungi. More active against Gram-positive bacteria than Gram-negative

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bacteria, and less against endospores and acid-fast bacteria. Benzalkonium chloride's antibacterial activity depends on the chemical combination's alkyl content. It is also used in nasal and otic versions, often with thimerosal to boost efficacy. Benzalkonium chloride is an efficient preservative in small-volume parenteral formulations.

- **Benzethonium Chloride:** Pharmaceutical formulations include this quaternary ammonium component to prevent bacterial development. Typically used in injectable, ophthalmic, and oral preparations. The wetting and solubilizing agent benzethonium chloride and topical disinfection are further uses for this chemical. Benzethonium chloride preserves cosmetics like deodorants. It kills germs. It has traditionally been used therapeutically as a disinfectant and topical anti-infective. Due to the availability of more strong antimicrobials, it is currently largely utilized as a stabilizer or preservative in a few pharmaceutical and cosmetic compositions.
- **Cetylpyridium Chloride:** Pharmaceutical and cosmetic compositions use the quaternary ammonium cationic surfactant cetylpyridinium chloride as an antibacterial preservative. Also known as cetylpyridinium chloride. Its antiseptic properties are employed in oral and throat care. It works alone or with additional drugs. It is used in oral, inhaled, and nonparenteral forms. Cetylpyridinium chloride mouthwashes decrease plaque. The company says it kills Gram-positive bacteria but modestly kills Gram-negative microorganisms. Cetylpyridinium chloride kills oral germs and is antimicrobial.

Classification based on source

- **Natural Preservative:** These agents come from plants, minerals, animals, etc. Neem oil, salt (sodium chloride), lemon, and honey are common food preservatives.
- **Preservatives synthesized in a laboratory:** These items include chemically manufactured preservatives such benzoates, sodium benzoate, sorbates, propionets, and nitrites (Table 1).

Table 1. Some common preservatives used in pharmaceutical formulations

Preservatives	% Concentration in preparations			
	Oral Liquid	Ointments / Creams	Opthalmic / Nasal	Parenteral
Methylparaben	0.25	0.001-0.2	0.1	0.01-0.5
Ethylparaben	0.1- 0.25	0.001-0 .2	0.1	0.01-0.5
Propylparaben	0.5- 0.25	0.001- 0.2	0.1	0.005-0.02
Butylparaben	0.1- 0.4	0.001- 0.2	0.1	0.015
Benzyl Alcohol	3.0			0.5-10
Chlorobutanol	0.5	0.5	0.5	0.25-0.5
Phenol	0.1-0.5	0.25-0.5		0.065-0.02
Meta cresol	0.15-0.3	0.1-0.3		0.1-0.25
Chlorocresol	0.2	0.1-0.3		0.1-0.18
Benzoic acid	0.1-0.2			
Sorbic acid	0.1-0.2			
Thiomersal	0.1	0.01	0.01	0.01
Phenylmercuric nitrate	0.002-0.1	0.002	0.004	0.002
Propylene Glycol	15-30			
Benzylkonium Chloride	0.002-0.02	0.01	0.004-0.02	0.01
Benzethonium Chloride	0.01-0.02	0.01	0.004-0.01	0.01

Safety and Side Effects of Preservatives

Consider negative side effects while selecting a preservative. These factors may be evaluated against low concentration exposure and harm risk. Preservatives should only affect microorganisms and not mammalian cells. Our experience

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reveals that most preservatives influence microbial and human cells, broadening this task. Alcohol is safe. The risk of lethal toxic syndrome in low-weight babies renders parenteral benzyl alcohol unacceptable. Rarely, benzyl alcohol sensitivity is topical. Cetyl and stearyl alcohols seldom sensitize. Phenylethanol may irritate skin, eyes, and mucous membranes.

Benzoic acid and other carboxylic acids may irritate skin, eyes, and mucous membranes. Some consider moderate sensitivity normal. Sorbic acid sensitivity is rare. However, allergic dermatitis and conjunctivitis are infrequent. There are no systemic toxicity reports. Parabens irritate and should not be used in parenteral and ophthalmic products. Multiple delayed hypersensitivity reactions to topical usage have been reported by the FDA. However, such responses are rare.

Production's low inclusion levels may explain phenol-related side effects' rarity. Ten-hour doses could not exceed 50mg. Chlorocresol is less toxic than phenol but may irritate skin, eyes, and mucous membranes. Not authorized for intrathecal, intracisternal, or peridural injections. Cross-sensitivity to chloroxylenol noted. Compared to chlorocresol, chloroxylenol is less irritating. Neurotoxicity concerns have decreased hexachlorophene usage. QACs like benzalkonium chloride (BKC) might aggravate pre-existing dermatitis, even if sensitivity is rare. BKC was omitted from contact lens solutions because it might bind to soft lenses and cause eye pain. For ophthalmic and parenteral formulations, 0.02 percent benzethonium chloride is allowed. BKC may restrict asthmatics' bronchi. Solution overdoses exceeding 0.03 percent need medical treatment.

Organo-mercurial preservatives in topical and parenteral formulations are common. Mercurial chemicals are seldom used intra-vaginally due to toxicity concerns. Phenylmercuric salts in eye drops may produce mercurialetis. Although rare and does not affect vision, the drug is not recommended for long-term usage. Phenylmercuric salt concentrations over 0.1 percent cause skin irritation. High dosages of sensitizer thimerosal are applied topically. Up to 10% of soft contact lens wearers may have allergies. Eye drops and vaccines should avoid it due to hypersensitivity. The European Medicines Agency and FDA recommend phasing out thimerosal in vaccines.

Some preservers use EDTA. In nebulizer solutions, it induces dose-related bronchoconstriction, hence avoid. EDTA salts may induce nephrotoxicity in renally compromised people. Avoid them. Because it chelates calcium, EDTA may deplete calcium. Although toxic, EDTA is usually harmless.

Antimicrobial Effectiveness Testing (AET)

Chemical preservative testing is usually included in specifications, however antimicrobial preservatives may be effective throughout development, scale-up, and shelf life. Antibacterial testing is required for multi-dose preservatives. To prove antibacterial, multi-dose products without preservatives must pass. This is tested under USP38. The company claims this test analyzes a preservative system's biological activity. Development employs the test to evaluate product effectiveness, whereas stability uses it to ensure preservative system stability. Standard quality control release tests are unsuitable for this test. This test cannot substitute manufacturing standards.

To test product efficacy, microorganisms are added to a specified amount. Try original package. Before usage, the containers must incubate at room temperature for 28 days out of direct sunlight. Compendial recommendations relate death rates to standards. Inspection is necessary to maintain the product's preservation system from amassing harmful germs. To show a product's effectiveness throughout its life cycle, testing must occur throughout development and stability.

Four sets of compendial articles are tested (Table 2). These drugs' antimicrobial efficacy depends on administration. Preservative-containing formulations in multi-dosage or unit dose containers must fulfill basic efficacy criteria. Tables 3 and 4 include microorganisms, medium, and testing criteria. Not a typical quality control release test. Never let the test replace good production. A known quantity of microorganisms are inoculated into a known amount of product to determine its effectiveness. Assays are done in original containers whenever feasible. The containers are incubated at room temperature for 28 days out of direct sunshine before use. Calculate and compare 28-day mortality to compendial guideline approval criteria.

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Table 2. Compendial product categories

Category	Product Description
1	Administered through intramuscular injection or other parenteral routes, as an example intravenously or intravenously via the skin
2	Nonsterile nasal products, emulsions, and other topically utilized aqueous-based products, as well as those applied to mucosal membranes
3	Non-antacid oral products, as an example those prepared using water-based bases or vehicles.
4	Aminoglycosides that are based on water.

Table 3. Microorganisms and media used

Bacteria	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*	Tryptic Soy Agar (TSA)
Yeast	*Candida albicans*	Sabouraud Dextrose Agar (SDA)
Mold	*Aspergillus niger*	Sabouraud Dextrose Agar (SDA)

Table 4. Criteria for tested microorganisms

For Category 1 Products	
Bacteria	A 1.0-log decrease at 7 days was followed by a 3.0-log decrease at 14 days & a 0 log gain at 28 days relative to the baseline count.
Yeast & mold	No increase in day count from the original calculation at 7, 14, or 28 days
For Category 2 Products	
Bacteria	As of day 28, there was a 2.0 log decrease from the 14-day count & no rise from the 14-day count.
Yeast & mold	At 14 & 28 days, there was no change from the baseline total.
For Category 3 Products	
Bacteria	A 1.0 log decrease from the initial 14-day count and no increase in the 14-day count at the end of the 28-day period.
Yeast & mold	At 14 & 28 days, there was no change from the baseline total.
For Category 4 Products	
Bacteria, Yeast, & Molds	At 14 & 28 days, there was no change from the baseline total.

II. CONCLUSION

The risk of microorganism contamination is decreased when antimicrobial preservatives are added, regardless of whether the product is being used or stored. It is of the utmost importance to demonstrate that the agents function as intended and, if possible, are secure for human consumption. Antimicrobial products are additionally subject to efficacy testing to determine their capacity to eliminate microorganisms. Antimicrobial efficacy tests are a prerequisite for the prospective sale of all multi-dose products. The use of preventative selection is vital. A number of factors, including pH, dose form, and any additional excipients that may interact with the preservative, influence the selection of preservatives.

REFERENCES

[1]. Moreton C. Functionality and Performance of Excipients in a Quality-by-Design World: Part VII. Explor Investig Discov. 2010:32-5.

[2]. Fassihi RA. Preservation of medicines against microbial contamination. Disinfect Steriliz Preserv Philadelphia Lea Febiger. 1991;871–86.

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[3]. Ahmed N, Singh J, Kour H, Gupta P. Naturally occurring preservatives in food and their role in food preservation. Int J Pharm Biol Arch. 2013;4:22–30.

[4]. Anand SP, Sati N. Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. Int J Pharm Sci Res. 2013;4(7):2496–501.

[5]. Council of Europe. European pharmacopoeia: Efficacy of Antimicrobial Preservation. European Directorate for Quality of Medicines, Strasbourg, France. 2010.

[6]. Sutton SVW, Porter D. Development of the antimicrobial effectiveness test as USP chapter <51>. PDA J Pharm Sci Technol. 2002;56(6):300–11.

[7]. Gabel LF. The relative action of preservatives in pharmaceutical preparations. J Am Pharm Assoc. 1921;10(10):767–8.

[8]. World Health Organization, UNICEF. Good practices guidance handbook for national TB surveys: how to apply good clinical and good data management practices for national TB surveys. 2022.

[9]. ICH Expert Working Group. Stability data package for registration applications in climatic zones III and IV Q1F. EMEA, London. 2003;4.

[10]. Barr M, Tice LF. The preservation of aqueous sorbitol solutions. J Am Pharm Assoc (Scientific ed). 1957;46(4):221–3.

[11]. Shaikh SM, Doijad RC, Shete AS, Sankpal PS. A Review on: Preservatives used in Pharmaceuticals and impacts on Health. PharmaTutor. 2016;4(5):25–34.

[12]. Chiori CO, Ghobashy AA. A potentiating effect of EDTA on the bactericidal activity of lower concentrations of ethanol. Int J Pharm. 1983;17(2–3):121–8.

[13]. Rowe RC, Sheskey PJ, Quinn, ME. Handbook of pharmaceutical excipients. Sixth Edition. Pharmaceutical Press & American Pharmacists Association; 2009.

[14]. Krzyzaniak JF, Raymond DM, Yalkowsky SH. Lysis of human red blood cells 2: effect of contact time on cosolvent induced hemolysis. Int J Pharm. 1997;152(2):193–200.

[15]. Grissom CB, Chagovetz AM, Wang Z. Use of viscosigens to stabilize vitamin B12 solutions against photolysis. J Pharm Sci. 1993;82(6):641–3.