

REVIEW

Excipient–Drug Interactions in Parenteral Formulations

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ABSTRACT: Excipients are added to parenteral formulations to enhance or maintain active ingredient solubility (solubilizers) and/or stability (buffers, antioxidants, chelating agents, cryo- and lyoprotectants). Excipients also are important in parenteral formulations to assure safety (antimicrobial preservatives), minimize pain and irritation upon injection (tonicity agents), and control or prolong drug delivery (polymers). These are all examples of positive or synergistic interactions between excipients and drugs. However, excipients may also produce negative effects such as loss of drug solubility, activity, and/or stability. This review article will highlight documented interactions, both synergistic and antagonistic, between excipients and drugs in parenteral formulations. The reader will gain better understanding and appreciation of the implications of adding formulation ingredients to parenteral drug products. © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association *J Pharm Sci* 91:2283–2300, 2002

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INTRODUCTION

Well-referenced and useful publications are available listing every formulation component in all marketed parenteral drug products^{1–5} (Food and Drug Administration web site^a). The information in these publications has been invaluable to parenteral formulation scientists developing soluble, stable, resuspendable, manufacturable, and deliverable parenteral dosage forms. Formulation component precedence takes on high stature in the sterile product world because of significant toxicological and regulatory concerns. In other words, it is usually better to use a component that has a track record of relatively safe use in injectables and is likely not to raise concerns on the part of regulatory reviewers.

^awww.fda.gov/cder/drug/iig/default.htm

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This review article will not cover all excipients used in parenteral formulations because the aforementioned publications already do so. What this review article presents are examples of synergistic and antagonist interactions that have been reported for excipients used in parenteral formulations. Although extensive, this review will not be exhaustive in the effort to cite all published references on parenteral drug–excipient interactions. *Pharmaceutical Excipients 2000*⁶ was a very helpful text in obtaining valuable information about drug–excipient interactions and compatibilities.

When one studies stability and compatibility issues in parenteral drug formulation, the packaging system also must be considered. Potential interactions between excipients and rubber closures in finished products are as much a concern as interactions between excipients and drugs. Therefore, some drug–sterile packaging interactions will be covered in this article.

Although this article will focus on chemical and physical compatibilities of drugs and excipients used as injectable products, readers must also be

aware of the potential for any excipient and drug, either alone or in combination, when injected intravenously to cause certain problems. As Yalkowsky et al.⁷ pointed out, some formulation-related problems associated with intravenous drug delivery include hemolysis, precipitation, phlebitis, and pain. Therefore, as scientists develop sterile product formulations, not only must they be concerned with physical and chemical interactions that may occur *in vitro*, but they must also be concerned with the potential for formulation-related problems occurring *in vivo*.

Drug–excipient interactions are studied in two basic ways. One is to perform traditional preformulation studies using full factorial or Plackett Burman type of experimental designs. A good example of this approach for parenteral formulation development is a preformulation study published by Peswani and Lalla.⁸ Analytical methods such as differential scanning calorimetry (DSC),⁹ isothermal microcalorimetry,^{10,11} and fourier transform infrared (FT-IR) spectroscopy¹² are excellent tools for predicting drug–excipient interactions. The other approach for studying drug–excipient interactions is to conduct both short-term and long-term stability studies on various formulations of the drug and measure both chemical stability (usually by chromatographic techniques) and physical stability (e.g., by microscopic, electronic particle analysis, and circular dichroism techniques).

This review is organized according to major functions of parenteral excipients (solubilization, stabilization, preservation, and drug delivery aids). Several excipients serve more than one function [e.g., polyvinylpyrrolidone (PVP) as a complexing agent and as a freeze-drying bulking agent], so such excipients may be referenced in more than one segment of the article.

Table 1 lists all the major pharmaceutical excipients used in parenteral formulations. Table 2 provides a listing of lesser-used excipients that are found in 1–2 commercial parenteral formulations. References^{1–5} provide much greater detail about the specifics of these excipients (e.g., concentration) and the products in which they are components (e.g., brand names, manufacturer).

Solubility Effects

Many parenteral formulations require additives, either solvent or solute excipient, to increase and/or maintain solubility of the active ingredient in the solution. Sweetana and Akers¹³ summarized

seven basic approaches for solubilization of parenteral drugs as follows:

1. Salt formation
2. pH adjustment
3. Use of co-solvents
4. Use of surface-active agents
5. Use of complexation agents
6. Change formulation from solution to a dispersed system, oily solution, or a more complex formulation such as a microemulsion or liposome
7. “Heroic” approaches involving the use of non-commercially approved types and/or concentrations of solvents or excipients

This section will highlight some of the interactions between drugs and solubilizing agents, focusing on co-solvents, surfactants, suspending and emulsifying agents, complexation agents, and oils or lipids. Some examples of unpredicted interactions of excipients and drugs to enhance drug solubility are listed in Table 3.

Co-Solvents

There are approximately 20 different co-solvent agents used in approved parenteral products. However, the most commonly used co-solvents in parenteral formulations are ethanol, glycerin, propylene glycol, sorbitol, polyethylene glycol (both 300 and 400), dimethylacetamide, Cremophor EL, and *N*-methyl-2-pyrrolidone.

Glycols are widely used solubilizing agents, but can cause some stability or compatibility problems. Glycerol is used not only as a co-solvent for improving solubility of poorly water-soluble drugs, but also as a tonicity-adjusting agent (e.g., in insulin formulations). In freeze-dried formulations, glycerol can serve as a plasticizer, lowering the glass transition temperature of the product without the significant change in water content or activity.¹⁴ In certain formulations containing unstable peptides, the presence of glycerol will increase the mobility of the freeze-dried formulation matrix, leading to peptide deamidation. Sorbitol has been reported to increase the degradation rate of penicillins in neutral and aqueous solutions.¹⁵ On a more positive note, propylene glycol will potentiate the antimicrobial activity of the parabens in the presence of nonionic surfactants and prevents the interaction of methylparaben and polysorbate 80.¹⁶

Table 1. A Listing of Major Excipients Used in Sterile Product Formulations (Both Commercial and Developmental)

Solvent systems
Co-solvents
Propylene glycol
Glycerin
Ethanol
Polyethylene glycol (300 and 400)
Sorbitol
Dimethylacetamide
Cremophor EL
Oils
Sesame
Soybean
Corn
Castor
Cottonseed
Peanut
Arachis
Ethyl oleate
Isopropyl myristate
Glycofurol
Petrolatum
Solubilization agents
Co-solvents
See above
Surface-active agents
Polyoxyethylene sorbitan monooleate (Tween 80)
Sorbitan monooleate
Polyoxyethylene sorbitan monolaurate (Tween 20)
Lecithin
Polyoxyethylene-polyoxypropylene copolymers (Pluronic [®])
Complexation agents
Hydroxypropyl- β -cyclodextrin
Sulfobutylether- β -cyclodextrin (Captisol [®])
Polyvinylpyrrolidone
Amino acids (arginine, lysine, histidine)
Stabilization agents
Buffers
Acetate
Citrate
Tartrate
Phosphate
Triethanolamine (TRIS)
Antioxidants
Ascorbic acid
Acetylcysteine
Sulfurous acid salts (bisulfite, metabisulfite)
Monothioglycerol
Chelating agents
Ethylenediaminetetraacetic acid (EDTA)
Sodium citrate
Cryo- and lyoprotectants and bulking agents
Mannitol
Glycine
Sucrose

Table 1. (Continued)

Lactose
Trehalose
Dextran
Povidone
Sorbitol
Competitive binding agents
Serum albumin
Heta-starch
Tonicity-adjusting agents
Sodium chloride
Glycerin
Mannitol
Dextrose
Antimicrobial preservative agents
Phenol
Meta-cresol
Benzyl alcohol
Parabens (methyl, propyl, butyl)
Benzalkonium chloride
Chlorobutanol
Thimerosal
Phenylmercuric salts (acetate, borate, nitrate)
Delivery polymers
See Table 6

Co-solvents are known to cause hemolysis. In a study conducted by Fuet et al.¹⁷ comparing the hemolytic effects, both *in vitro* and *in vivo*, of a variety of co-solvents (ethanol, propylene glycol, polyethylene glycol, dimethylisorbide, and dimethylacetamide), complexing agents (nicotinamide), and surfactants (Pluronic L64 and emulphor EL-719), solutions most prone to elicit a hemolytic response were those containing propylene glycol, dimethylisorbide, and nicotinamide). However, these authors found that the hemolytic effects of propylene glycol can be alleviated by the addition of either a tonicifying agent or polyethylene glycol 400.

Cremophor EL (polyoxyl 35 castor oil) has been approved as a solvent in commercial injectable dosage forms containing paclitaxel, diazepam, propanidid, and alfaxalone. It is compatible with many organic solvents and aqueous solutions. However, compounds containing phenolic hydroxyl groups may cause precipitation of Cremophor EL.

Surfactants

Surfactants serve a variety of very important functions in parenteral formulations. Among the most important are stabilizing proteins against aggregation. Tween 20 (polyoxyethylene sorbitan monolaurate) was shown to greatly reduce the

Table 2. Examples of Special or Uncommon Excipients Used in Injectable Drug Products

Excipient	Product	Manufacturer
Acacia	Tuberculin Old Test (ID)	Lederle
Acetone sodium	Talwin (IM)	Sanofi Winthrop
Aluminum monostearate	Solganal	Schering
Benzenesulfonic acid	Tracrium	Glaxo Smith Kline
Benzyl benzoate	Depo-testosterone	Pharmacia
Cyclodextrin (alpha)	Alprostadil	Schwarz
Diethanolamine	Bactrim	Roche
Desoxycholate sodium	Fungizone	Bristol Myers Squibb
Formaldehyde	Some vaccines	Lederle, Connaught, Merck
Gelatin, hydrolyzed	Some vaccines	Merck
Gelatin, purified	Lupron Depot	TAP
Hydroxypropyl- β -cyclodextrin	Itraconazole	Janssen
Imidazole	Kogenate	Bayer
Monoethanolamine	Terramycin (IM)	Roerig
<i>N,N</i> -dimethylacetamide	Vuman	Bristol Myers Squibb
	Busulfan	Orphan
Polyoxyethylated fatty acid	AquaMephyton	Merck
PEG 40 castor oil	Monistat	Janssen
PEG 60 castor oil	Prograf	Fujisawa
Sodium lauryl sulfate	Proleukin	Cetus
Sulfobutylether- β -cyclodextrin	Ziprasidone mesylate	Pfizer
Triacetin	Prepidil Gel (ICV)	Pharmacia

rate of formation of insoluble aggregates of recombinant human factor XIII caused by both freeze thawing and agitation stresses¹⁸ (Fig. 1). Maximum protection occurs at concentrations close to the critical micelle concentration of Tween 20, independent of initial protein concentration. In another report, Tween 20 at a 1% (w/v) concentration caused precipitation of a relatively hydrophobic protein (*Humicola lanuginosa* lipase) by inducing non-native aggregates.¹⁹

Tween 80 is well known to protect proteins against surface-induced denaturation.²⁰ Tween 80 was demonstrated to reduce hemoglobin aggregation in solution by preventing the protein from reaching the air-liquid interface or the liquid-surface interfaces.²¹ Polyoxyethylene surfactants

such as Tween 80 can form peroxide impurities after long-term storage. Knepp et al.²² concluded that Tween 80 and other nonionic polyether surfactants undergo oxidation during bulk material storage and subsequent use and the resultant alkyl hydroperoxides formed can contribute to the degradation of proteins. In such formulations, they further reported that thiols such as cysteine, glutathione, and thioglycerol were most effective in stabilizing protein formulations containing peroxide-forming nonionic surfactants.

The nonionic surfactant octoxynol 40 (ethoxylated alkyl phenol, Igepal CA897, GAF), was found to solubilize an otherwise insoluble complex of a nonsteroidal anti-inflammatory drug and a quaternary ammonium antimicrobial preservative

Table 3. Examples of Esoteric Excipients Used as Solubilizers

Generic Name	Brand Name	Manufacturer	Excipient
Ciprofloxacin	Cipro IV	Bayer	Lactic acid
Doxorubicin HCl	Adriamycin RDF	Pharmacia	Methyl paraben
Ergotrate Maleate	Ergonovine maleate	Lilly	Ethyl lactate
Polyestradiol phosphate	Estradurin	Wyeth	Niacinamide
Zomepirac	Zomax	McNeil	Tromethamine ¹⁰⁵

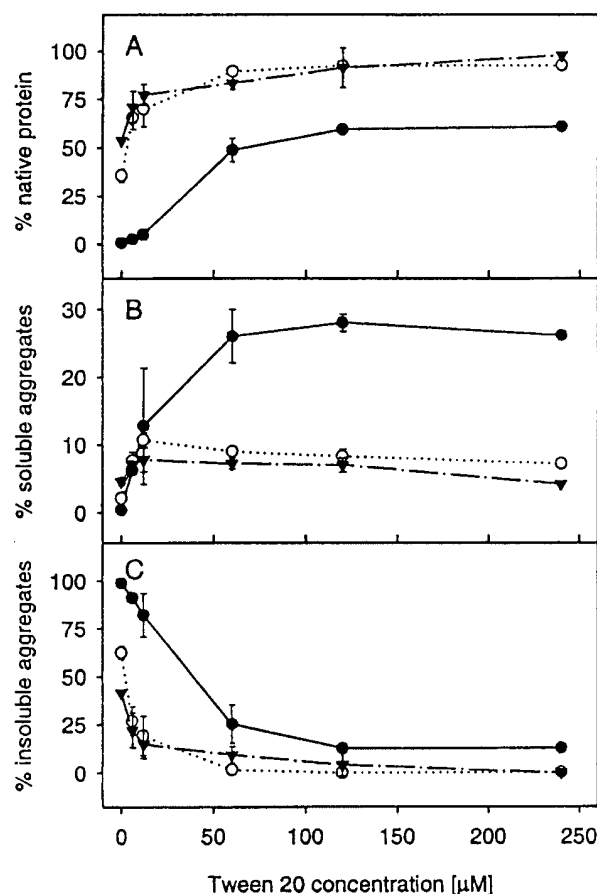


Figure 1. Recovery of native rFXIII (A) and formation of soluble (B) and insoluble (C) aggregates after 10 freeze–thaw cycles of 1 mg/mL (●), 5 mg/mL (○), and 10 mg/mL (▼) as a function of Tween 20. (From Krielgaard et al., *J Pharm Sci*, 87, 1593–1603, © 1998 John Wiley & Sons, Inc., reproduced with permission.)

mixture in an ophthalmic formulation.²³ This is a rather unique drug–excipient interaction in which the interaction of the excipient involves not only a drug, but also a drug–preservative combination that is otherwise incompatible.

Complexing and Dispersing Agents

Cyclodextrins have emerged as very effective additive compounds for solubilizing hydrophobic drugs. In the parenteral dosage form area, modified cyclodextrins, such as hydroxylpropyl- β -cyclodextrin and sulfobutylether- β -cyclodextrin have been reported to solubilize and stabilize many injectable drugs, including dexamethasone, estradiol, interleukin-2, and other proteins and peptides²⁴ without apparent compatibility problems.²⁵ There are still only a few approved

products worldwide that contain cyclodextrins (see Table 2). However, based on the literature and scientific meeting presentations, there will be a higher number of cyclodextrin-containing injectable formulations in the future.

The only reports of incompatibilities with cyclodextrins involve certain antimicrobial preservatives, primarily parabens.^{26,27} One preliminary report described both sulfobutylether- β -cyclodextrin and hydroxylpropyl- β -cyclodextrin accelerating the degradation of an unidentified water-soluble drug to its insoluble degradant form.²⁸ The authors concluded that both the type and degree of substitution of the proximal hydroxyl groups in the cyclodextrin cavity will influence the potential for cyclodextrin additives to accelerate chemical degradation of drugs. As cyclodextrins become more prominent in injectable drug product development, there likely will be more reports of incompatibilities along with the expected reports describing solubility and stability enhancements.

Cyclodextrin-containing formulations (either 0.1 M sulfobutylether- β -cyclodextrin or 0.1 M hydroxylpropyl- β -cyclodextrin) were shown to cause less damage to venous epithelial cells at the site of injection compared with formulations containing organic co-solvents.²⁹ PVP (povidone) is a generally compatible polymeric excipient. However, it can form molecular adducts (a positive reaction with respect to iodine therapy topically) and will complex with some preservatives such as thimerosal.⁶ Lecithin is a commonly used emulsifying and stabilizing agent in intramuscular and intravenous injections, primarily the intravenous fatty or lipid emulsions used in parenteral nutrition. Lecithin also is a component of some liposomal formulations. Polaxamers (e.g., Poloxamer 188, BP) are nonionic polyoxyethylene–polyoxypropylene copolymers used as emulsifying agents in intravenous fat emulsions. They have also been used in several patented protein formulations as stabilizers and sustained release injectables in development as solubilizing and stabilizing agents.³⁰ Polaxamers, like the polysorbates, can form peroxide impurities over time and are incompatible with antimicrobial preservatives such as phenol and paraben.

Oils/Lipids

Many commercially available parenteral products contain lipophilic or oleaginous solvents. Examples of injectable lipid solvents include ethyl

oleate, isopropyl myristate, glycofurol, and fixed vegetable oils (e.g., peanut, corn, cottonseed, sesame, soybean, castor, arachis). There are several oily injectable solutions and suspensions used as sustained-release formulations (Clopixol[®], Haldol Decanoate[®], Deca-Durabolin[®], Modecate[®], Depixol[®], and others).³¹ Oils are generally compatible with lipophilic drugs and excipients. However, formulation scientists must be aware of the potential for oils to be absorbed by rubber closure materials.^{32,33} Oils can contain unacceptable impurities (e.g., gossypol in cottonseed oil, saturated fatty materials, unsaponifiable materials, other organic residuals), but the United States Pharmacopeia and other compendia specify limits on these impurities.

Highly purified sesame oil was found to improve the long-term stability of lidocaine.³⁴ Lidocaine in sesame oil that was not purified was degraded and formed crystals because of oxidation products such as hydroperoxides and impurities such as mono- and di-glycerides, free fatty acids, plant sterols, and the colorants chlorophyll and carotene).

Soybean oil is the preferred oil in parenteral fatty emulsions. Soybean oil emulsions have been studied extensively and have been found to be incompatible with calcium chloride, calcium gluconate, magnesium chloride, phenytoin sodium, tetracycline hydrochloride, and potentially many other drug substances and intravenous infusion solutions.³⁵

Petrolatum is a commonly used ointment base for topical ophthalmic ointments. There are no known incompatibilities with petrolatum.³⁶

Stability Effects

This section highlights both classical and more recent publications that report on both positive and negative effects of excipients on drug stability in parenteral formulations. Examples of some esoteric effects (the stabilization of the drug by the excipient was not predicted or expected) of excipients stabilizing certain drugs are given in Table 4.

Table 4. Esoteric Examples of Excipient Stabilizers

Drug or Brand Name	Stabilizing Agent
Dexamethasone acetate	Creatine or creatinine
Cardiotec	Gamma cyclodextrin
Albumin	Sodium caprylate
Stelazine/compazine	Sodium saccharine

Buffers

Buffer components in parenteral formulations can cause stability problems. Phosphate buffer, particularly the dibasic phosphate anion, serves as a nucleophile that can attack electrophilic centers like ester or amide carbonyl groups or polarized carbon-nitrogen double bonds.³⁷

Hasegawa et al.^{38–42} published several articles describing the use of pharmaceutical phosphate buffer solutions in the presence of calcium and/or aluminum by the use of EDTA in pH ranges of 5 to 9, carboxylic acids such as citric acid and maleic acid in acidic to neutral solutions, and pyrophosphate and lysine hydrochloride in alkaline solutions. Of course, the best approach is to eliminate metal contaminants in solutions or additives by techniques such as ion exchange, but often this is not practical.

Zhu et al.⁴³ presented a poster describing a study in which an unstable drug needed to be buffer stabilized at pH 3. Four buffer systems (citrate, glycinate, maleate, and tartrate) were studied using the same pH; only one buffer (glycinate) did not catalyze drug degradation (see Fig. 2). The authors did not speculate on why glycinate buffer did not catalyze the degradation of the drug although low concentrations (0.1 M) of all buffers would be acceptable. In a similar study, Nakamura et al.⁴⁴ found that

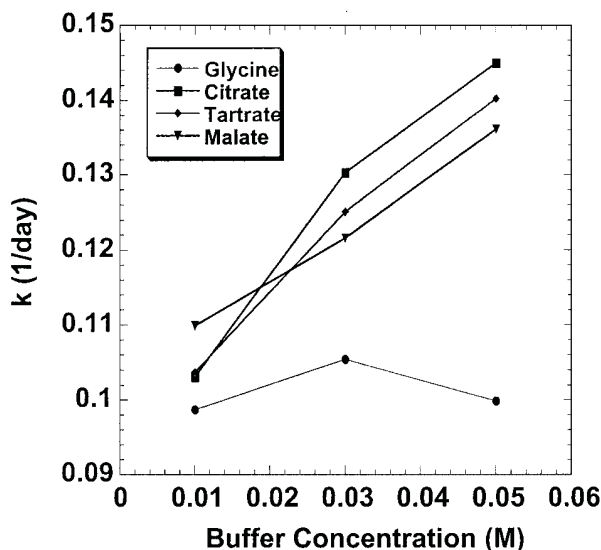


Figure 2. Rate of hydrolysis of GW280430 (0.2 mg/mL) as a function of buffer type and concentration at 60°C. (From Zhu, Merserve, & Floyd, Drug Dev Ind Pharm, 28, 135–142, © 2002 Marcel Dekker, Inc., reproduced with permission.)

Table 5. Stability Data for Minodronic Acid in Different Buffer Systems⁴⁴

Buffer	pH	Potency (%) After 4 Weeks at 60°C	Number of Particles >2 μ m in 100 mM Buffer After 4 Weeks at 60°C
Glycine HCl	3.0	78.1	1000–9999
	5.1	95.5	> 10,000
Citrate	3.0	99.5	0–99
	5.0	101.8	0–99
Succinate	3.1	82.4	1000–9999
	5.0	94.4	> 10,000
Acetate	3.1	74.8	100–999
	5.0	88.5	1000–9999
Tartrate	3.0	93.4	0–99
	5.0	95.7	0–99
Lactate	3.1	86.0	100–999
	4.4	93.9	1000–9999
Maleate	3.1	87.3	1000–9999
	5.0	80.1	> 10,000

citrate and tartrate buffers maintained both chemical and physical (fewer particles) stability of minodronic acid in a parenteral aqueous solution whereas glycine, succinate, acetate, lactate, and maleate buffers did not (see Table 5). Li et al.⁴⁵ compared the stability of tezacitabine in three buffer systems (phosphate, glycine, and TRIS) and found phosphate to be superior. Higher concentrations of phosphate also improved drug stability (see Fig. 3a and b).

Tris buffer, when used in a peptide formulation and stored at 70°C, will degrade to liberate formaldehyde.⁴⁶ Although this was not seen at lower temperatures, formulators need to be aware of this possible instability when using this common biological buffer.

Tris buffer will form a stable complex with *N*-nitrosourea anticancer agents and retard the degradation of these agents.⁴⁷ However, Tris buffer will degrade 5-fluorouracil, causing the formation

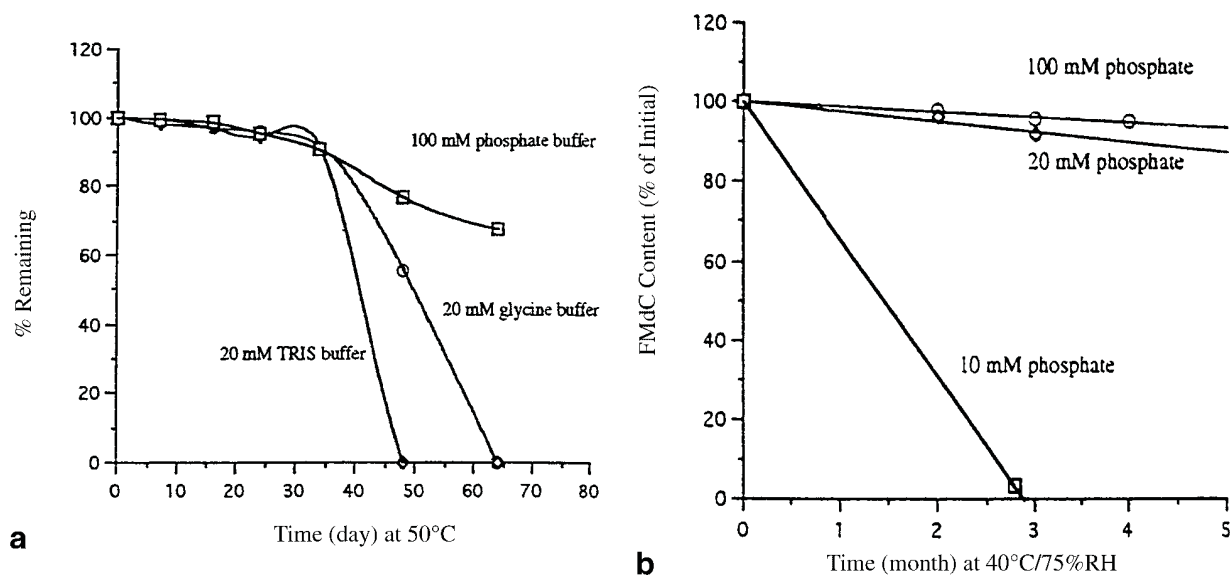


Figure 3. (a) Effect of buffer on stability of FMdC solution at 10 mg/mL. (b) Effect of phosphate buffer concentration on stability of FMdC solution at 10 mg/mL. (From Li, Chang, Pan, & Jones, AAPS Pharm Sci, (S) 3, © AAPS Denver, reproduced with permission.)

of two degradation products that can cause serious-to-lethal cardiotoxicities.⁴⁸

Sometimes the cationic species in a buffer system matters. Sarciaux et al.⁴⁹ found that a sodium phosphate buffer system consistently resulted in more turbid reconstituted solutions of bovine immunoglobulin (IgG) than a potassium phosphate buffer system at the same concentration. The authors believed that this effect was not attributable to a pH shift sometimes seen during freezing as a result of crystallization of sodium phosphate. This is because sodium chloride-containing formulations also showed substantially higher levels of aggregation compared with potassium chloride-containing formulations. Bovine IgG will denature at the ice/freezing-concentrate interface that is irreversible after freeze-drying and reconstitution. This ice/freezing-concentrate interfacial denaturation is dependent on the amount or percentage of protein residing at the ice/freezing-concentrate interface and the surface area of the freeze-dried solid. Formulations containing sodium salts showed a higher surface area of dried solids than formulations containing potassium salts. The higher the surface area, the more drug is exposed to the interfacial area, resulting in a higher degree of denaturation and resultant aggregation. When phosphate buffers are frozen, selective precipitation of the less-soluble buffer component (dibasic sodium phosphate) and subsequent pH shift may induce protein denaturation.⁵⁰ In the case of monomeric and tetrameric β -galactosidase, both sodium and potassium phosphate buffers caused significant secondary structural perturbations, greater for

sodium phosphate samples. The addition of sucrose was able to minimize this freeze-dried denaturation in phosphate buffers, even if there remained large-scale changes in solution pH during freezing.

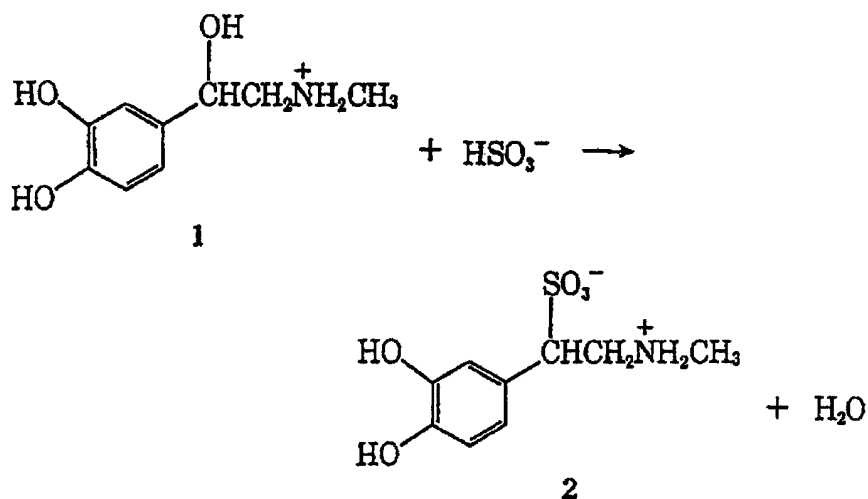
Histidine was used as a buffer system in an experimental formulation containing humanized IgG2 monoclonal antibody.⁵¹ Histidine underwent oxidation and the oxidation products caused a significant loss of potency of the monoclonal antibody. The antibody also degraded in citrate buffer, although not as much as in histidine buffer. Histidine buffer oxidizes in the presence of peroxides and the source of peroxides in these formulations presumably originated from Tween 80 also present. Nitrogen overlay inhibited the histidine buffer oxidation and enhanced antibody potency.

Antioxidants

Ascorbic acid has been reported to be incompatible with certain drugs such as penicillin G.⁵² However, this is not a direct incompatibility of two organic molecules, but rather an incompatibility caused by the pH effects of ascorbic acid,⁵³ as specified by Stella.³⁷

Sodium bisulfite and sodium metabisulfite are strongly nucleophilic antioxidants capable of catalyzing drug degradation.³⁷ The well-known interaction of bisulfite and epinephrine leads to degradation of epinephrine. (Scheme 1)

Epinephrine reacts with bisulfite to form the sulfonic acid derivative.⁵⁴ However, the addition of sodium borate complexes the parahydroxybenzyl



Scheme 1.

derivatives and prevents the reaction between epinephrine and bisulfite.⁵⁵ Zinc, copper, and iron also will not catalyze any reaction between epinephrine and bisulfite.⁵⁶ Interestingly, aluminum (III) will catalyze epinephrine degradation via a complexation effect not seen with these other metal ions.⁵⁶

Sodium bisulfite also has been reported to dehalogenate uracil-type molecules⁵⁷ and cause ester hydrolysis.⁵⁸ Bisulfite will react with physostigmine in aqueous solutions rapidly and reversibly, which is dependent both on the total bisulfite added and pH.⁵⁹ Bisulfite will attack physostigmine on the optically active carbon-10a,⁶⁰ which is reversed if the mixture is diluted and pH adjusted to values greater than pH 6. This pH dependency suggested that the reaction involved both SO_3^{2-} and HSO_3^- reacting with the protonated form of physostigmine.

Sodium metabisulfite inactivates cisplatin in solution⁶¹ and is incompatible in ophthalmic solutions containing phenylmercuric acetate, especially when autoclaved.⁶² If dextrose and sodium metabisulfite are combined in aqueous solution, metabisulfite stability declines.⁶³

Ascorbic acid is a frequently used antioxidant in parenteral formulations. Formulators must be aware that ascorbic acid generally is incompatible with alkaline solutes, heavy metals, phenylephrine hydrochloride, pyrilamine maleate, salicylamide, sodium nitrite, sodium salicylate, and theobromine salicylate.⁶⁴ Kerwin et al.⁶⁵ found that ascorbate ion in sufficient concentrations reacted with oxygen producing superoxide that in turn caused chemical modification and aggregation of recombinant deoxy hemoglobin. Lower levels of ascorbate and oxygen and lower solution pH combined to eliminate this problem.

Ethetic acid and its salts are used as metal chelating agents to aid in stabilization of drugs sensitive to metal-catalyzed oxidation and/or photolysis. They also can serve to enhance antimicrobial activity of formulations. Ethetic salts are incompatible with zinc insulin, thimersosal, amphotericin, and hydralazine hydrochloride.⁶

Bulking Agents and Lyoprotectants

Several mechanisms have been proposed to explain why excipients serve as cryo- or lyo protectants. The most widely accepted mechanism to explain the action of cryoprotection is the preferential exclusion mechanism.⁶⁶ Excipients that will stabilize proteins against the effects of freez-

ing do so by not associating with the surface of the protein. Such excipients actually increase the surface tension of water and induce preferential hydration of the protein. Examples of solutes that serve as cryoprotectants by this mechanism include amino acids, polyols, sugars, and polyethylene glycol.

For lyoprotection, that is, stabilization of proteins during the drying stages of freeze drying and during storage in the dry state, two mechanisms have been generally accepted. One is the water-substitute hypothesis⁶⁷ and the other is the vitrification hypothesis.⁶⁸ Both are legitimate theories, but both also have exceptions, i.e., neither fully explain the stabilization of proteins by excipients during dehydration and dry storage.⁶⁹ The water-substitute hypothesis states that a good stabilizer is one that hydrogen bonds to the protein just as water would do were it present and, therefore, serves as a water substitute. Sugars are good water substitutes^b which is why many freeze-dried protein formulations contain sucrose or trehalose.

The vitrification hypothesis states that excipients that remain amorphous (glass formers) form a glassy matrix with the protein with the matrix serving as a stabilizer. Acceptance in this hypothesis requires formulators to determine glass transition temperatures of formulations to be freeze dried and to develop freeze-dry cycles that maintain drying temperatures below the glass transition temperature. Studies have been published showing that excipient stabilizers that crystallized during storage caused degradation (typically aggregation and loss of potency) of the protein.⁷⁰⁻⁷²

Freeze-dried formulations typically contain one or more of the following bulking agents: mannitol, lactose, sucrose, trehalose, dextran 40, and povidone. These excipients may also serve as cryo- and/or lyoprotectants in protein formulations. Fakes et al.⁷³ studied these bulking agents for moisture sorption behavior before and after freeze drying. Moisture uptake certainly can affect drug stability in the freeze-dried state, particularly with proteins. They reported the following observations for each excipient:

^bIt may at first appear contradictory that sugars can serve both as cryoprotectants because of being excluded from the surface of the protein and as lyoprotectants that hydrogen bond to the protein. However, keep in mind that the excluded solute concept involves a frozen aqueous system whereas the water-substitute concept occurs in a dry system.

Mannitol

Crystalline and nonhygroscopic both before and after freeze drying

Total moisture contents of 0.1 to 0.3% w/w between 10 and 60% relative humidity (RH)

Lactose

Crystalline and nonhygroscopic before lyophilization

Moisture content 0.86% before lyophilization

Amorphous after lyophilization with moisture content of 1.6%

Absorbed moisture rapidly upon exposure to high RH

Converted to crystalline form at 55% RH after absorption of 10% moisture

Desorption of all moisture sorbed by the amorphous form

Sucrose

Crystalline and nonhygroscopic before lyophilization

Moisture content 0.15% before lyophilization

Amorphous after lyophilization with moisture content of 2.5%

Absorbed moisture rapidly upon exposure to high RH

Converted to crystalline form at 50% RH after absorption of 4.5% moisture

Desorption of all moisture sorbed by the amorphous form

Trehalose

Crystalline and nonhygroscopic before lyophilization

Moisture content 9.2% before lyophilization

Amorphous after lyophilization with moisture content of 1.2%

Absorbed moisture rapidly upon exposure to high RH

Converted to crystalline form at 50% RH after absorption of 10% moisture

Desorption of all moisture sorbed by the amorphous form

Dextran

Amorphous and hygroscopic both before and after freeze drying

Sorbed 10–20% moisture at 50% RH

PVP

Amorphous and hygroscopic both before and after freeze drying

Sorbed 10–20% moisture at 50% RH

When selecting a bulking agent, these properties, particularly the tendency for moisture uptake, must be considered by the formulation

scientist in developing an optimally stable freeze-dried formulation.

Several excipients can serve as stabilizers for proteins that are unstable during the drying phases of freeze drying and/or during long-term storage in the dry state. Typically, additives that will crystallize during lyophilization (e.g., mannitol) or will remain amorphous but unable to hydrogen bond to the dried protein (e.g., dextran) are not effective lyoprotectants for proteins. Excipients that will crystallize during freeze drying will also be relatively ineffective as was shown with sucrose in *Humicola lanuginosa* lipase formulations¹⁹ (Fig. 4). However, these authors also reported that sucrose crystallization could be inhibited by decreasing the mass ratio of sucrose to protein and by minimizing the moisture content that serves to decrease the glass transition temperature during storage.

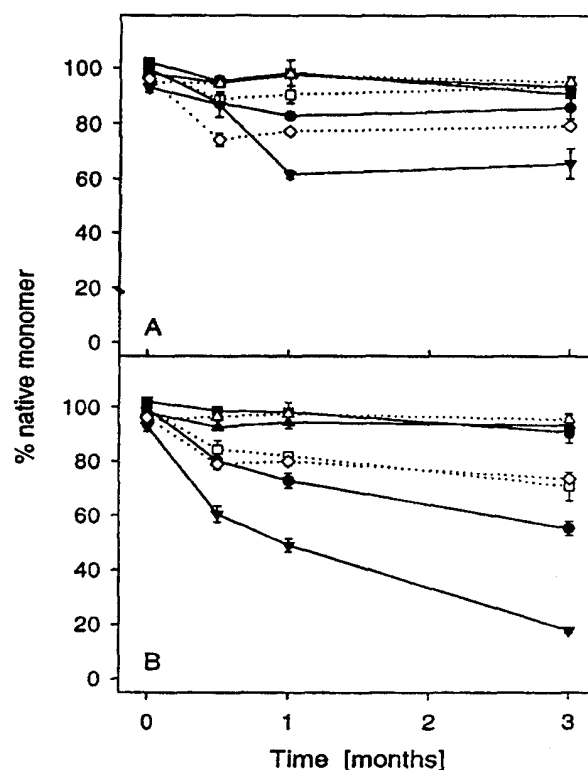


Figure 4. Recovery of native, monomeric HLL as a function of time as determined by size exclusion chromatography. Formulations stored at 40°C (A) or 60°C (B) in the absence of additives (●) or in the presence of 300 mM mannitol (▼), 5% (w/v) dextran (◇), 50 mM trehalose (▲), 300 mM trehalose (△), 50 mM sucrose (■), or 300 mM sucrose (□). (From Krielgaard, Frokjaer, Flink, Randolph, and Carpenter, *J Pharm Sci*, 88, 281–290, © 1999 John Wiley & Sons, Inc., reproduced with permission.)

The reverse can also be true for certain small molecules. For example, excipients (mannitol or sodium bicarbonate) that promoted the crystallization of cyclophosphamide during freeze drying stabilized the final product whereas excipients that did not allow crystallization (e.g., lactose) destabilized the final product.⁷⁴

Costantino et al.⁷⁵ studied the effects of a variety of parenteral excipients on stabilizing human growth hormone in the lyophilized state. Mannitol, sorbitol, methyl α -D-mannopyranoside, lactose, trehalose, and cellobiose all provided significant protection of the protein against aggregation, particularly at levels (131:1 excipient-to-protein molar ratio) to potentially satisfy water-binding sites on the protein in the dried state. At higher excipient-to-protein ratios, mannitol and sorbitol crystallized and were not as effective in stabilizing the protein compared with low levels in which they remained in the amorphous, protein-containing phase.

Reducing sugars may not be as effective as other bulking agents, cryoprotectants, or lyoprotectants because they may potentially react with proteins via the Maillard reaction. For example, glucose will form covalent adducts with side-chain amino acids lysine and arginine of human relaxin.⁷⁶ In addition, a significant amount of serine cleavage from the C-terminal of the B-chain of relaxin was formed when glucose was used as the excipient. These reactions did not occur if mannitol and trehalose replaced glucose in the lyophilized formulation.

Lactose will react with primary amines in the well-known Maillard-type condensation reaction to form brown-colored degradation products.¹² Thus, lactose is known to be incompatible with amine-containing compounds such as aminophylline, amphetamines, and amino acids. This reaction occurs more readily with amorphous lactose than crystalline lactose.

Mannitol is probably the most widely used bulking agent in lyophilized formulations because of its many positive properties with respect to crystallinity, high eutectic temperature, and matrix properties. However, some lots of mannitol can contain reducing sugar impurities that were implicated in the oxidative degradation of a peptide in a lyophilized formulation.⁷⁷ Mannitol, at or above certain concentrations and volumes in glass vials, is well known to cause vial breakage because of the unique crystallization properties of mannitol-ice during the primary drying states of freeze drying.^{78,79}

Other Freeze-Dry Excipients

High-molecular-weight carbohydrates, such as dextran, have higher glass transition temperatures than proteins. Therefore, when mixed with proteins, the overall glass transition temperature presumably can be increased with resultant increases in protein storage stability. Typically, carbohydrates (sucrose, trehalose, or dextran) alone do not result in appreciable increases in storage stability of proteins. However, combinations of disaccharide and polymeric carbohydrates do tend to improve protein storage stability.⁸⁰

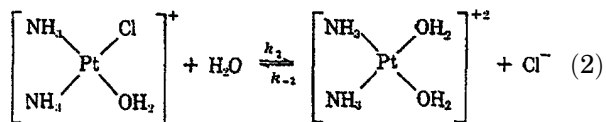
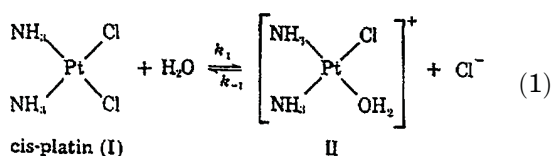
However, singular carbohydrates (sucrose or trehalose at 60 mM) were just as effective in stabilizing a model recombinant humanized monoclonal antibody as combinations of sucrose and mannitol or trehalose and mannitol. Interestingly, with this monoclonal, mannitol alone at 60 mM provided less protection during storage than sucrose or trehalose alone. A specific sugar/protein molar ratio was sufficient to provide storage stability for this particular monoclonal antibody.^{81,82}

Low-molecular-weight additives, such as osmolytes (*N,N'*-dimethylglycine, trehalose, and sucrose) or salts (sodium chloride, sodium phosphate, ammonium sulfate, and sodium citrate) were found to be highly effective in stabilizing keratinocyte growth factor, both against thermal denaturation and enhancing long-term storage stability.⁸³

PVP and glycine were found to stabilize lyophilized sodium prasterone sulfate whereas dextran 40 or mannitol did not.⁸⁴ PVP and glycine stabilized the pH of the reconstituted solution by neutralizing the acidic degradation product, sodium bisulfate, formed by the hydrolysis of prasterone sulfate. Dextran 40 or mannitol were ineffective because of no buffer capacity. Buffering agents, such as phosphate-citrate buffer and some neutral and basic amino acids (L-arginine, L-lysine, and L-histidine), also stabilized prasterone sulfate. L-cysteine is an example of an amino acid that did not stabilize the drug, presumably because of its weak buffer capacity.

Tonicity Agents

Sodium chloride will significantly stabilize the solution stability of cis-platin because of the presence of chloride ion that shifts the equilibria shown in eqs. 1 and 2 in favor of eq. 1.⁸⁵



(Equations 1 and 2 from Hincal, Long, and Repta, *J Parenter Sci Technol*, 33, 107–116, © 1979 Parenteral Drug Association, reproduced with permission.)

Consequently, the use of other chloride-containing aqueous solutions, such as lactated Ringer's or Ringer's injections, will also stabilize cis-platin. Sodium bicarbonate and alkaline solutions in general will enhance degradation of cis-platin whereas mannitol or dextrose have no effect on the rate or extent of loss of cis-platin.

The potential salting-out effects of marginally soluble drugs and excipients must be considered with any usage of sodium chloride. For example, methylparaben will salt out in the presence of sodium chloride in solution.⁸⁶

Dextrose solutions of various concentrations (5% isotonic) are major intravenous diluents. In intravenous therapy, many drugs and drug products are combined with dextrose solutions for controlled infusion of these drugs. Trissel's book³⁵ contains compatibility information for all drugs and dextrose as well as all other diluents. Drugs that are known to be incompatible with dextrose include cyanocobalamin, kanamycin sulfate, novobiocin sodium, warfarin sodium, erythromycin gluceptate, B-complex vitamins, and gemcitabine. Formulators wishing to use dextrose as a nonionic tonicity-adjusting agent must be aware of its degradation upon excessive heating⁸⁷ and its potential for reacting with amine drugs (browning reaction).

Antimicrobial Preservative Effects

Structurally, most antimicrobial preservative agents are volatile and very reactive with many different types of organic molecules. Literature refers to interactions of various preservatives with drugs, excipients, packaging, and filter materials. Benzalkonium chloride, the most common ophthalmic product preservative system, is well known to adsorb onto various filter membrane surfaces.⁸⁸

Benzyl alcohol antimicrobial activity is reduced in the presence of nonionic surfactants, such as polysorbate 80, although this reduction is less than what is seen with hydroxylbenzoate esters (parabens)⁸⁹ or quarternary ammonium compounds (benzalkonium chloride).⁹⁰ As noted previously, propylene glycol will prevent the incompatibility between paraben and polysorbate 80.¹⁷

Benzyl alcohol is incompatible with methylcellulose and can be adsorbed by rubber closures composed of natural rubber, neoprene rubber, and butyl rubber.⁹¹ Plastic materials, such as polyethylene or polystyrene, should not be used with solutions containing benzyl alcohol, although plastics such as polyvinyl alcohol and polypropylene are compatible with benzyl alcohol.⁹² Plastic materials also will adsorb the parabens although low- and high-density polyethylene containers do not.⁹³

Meta-cresol has been reported to be incompatible with chlorpromazine⁹⁴ and, like benzyl alcohol and possibly other antimicrobial preservatives, the antimicrobial activity of meta-cresol is reduced in the presence of nonionic surfactants.⁶

Chlorobutanol, a commonly used ophthalmic antimicrobial preservative, will complex with polymers such as polyethylene, carboxymethylcellulose, and polysorbate 80, and will be sorbed by the polymers of plastic vials.⁹⁵ The binding (sorption) of chlorobutanol into rubber closures is well known.⁹⁶

Thimerosal, no longer a preferred antimicrobial preservative in newly developed parenteral multi-dose formulations, is still a component of older formulations. Thimerosal is adsorbed by rubber closures,⁹⁷ is incompatible with sodium chloride solutions,⁹⁸ and is incompatible with a wide range of antioxidants, chelating agents, proteins, and other antimicrobial preservative agents.⁶

Phenylmercuric salts (acetate, borate, nitrate) also are no longer preferred antimicrobial preservatives because of their mercury content. These salts have many incompatibilities including many suspending agents, antioxidants such as sodium metabisulfite, disodium edetate, amino acids, and many rubber and plastic materials.⁶

Any formulator of a protein or peptide dosage form likely has experienced compatibility problems in attempting to develop multi-dose biopharmaceutical products. However, very few articles have been published describing these problems. Lam et al.⁸² are among the few who have published their experience with an incompatibility

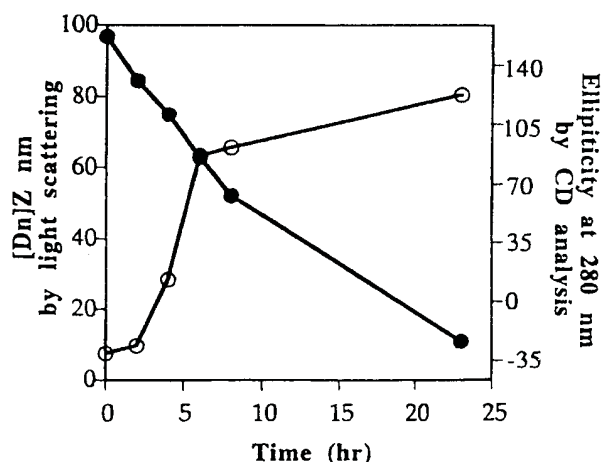


Figure 5. Time course of aggregate formation of 1.0 mg/mL rhIFN-gamma in 5 mM succinate, pH 5.0 in the presence of 0.9% benzyl alcohol as determined by dynamic light scattering (○) and by circular dichroism analysis (●). (From Lam, Patapoff, & Nguyen, *Pharm Res*, 14, 725–729, © Kluwer Academic Publishers, reproduced with permission.)

between a protein and an antimicrobial preservative. They found that benzyl alcohol caused the aggregation of recombinant human interferon gamma (Fig. 5).

Various preservatives (benzyl alcohol, methylparaben, propylparaben, chlorobutanol, phenol, and m-cresol) were formulated with a humanized monoclonal antibody and tested for compatibility and antimicrobial activity.⁹⁹ The protein precipitated in the presence of phenol or m-cresol, but was not adversely affected in the presence of the parabens and low concentrations of chlorobutanol. Benzyl alcohol causes aggregation at high concentrations, but was the most effective antimicrobial preservative agent against bacterial and fungal challenges. However, a combination of methylparaben and chlorobutanol produced a synergistic effect with respect to antibacterial (although no improvement against fungi) activity while still maintaining stability of the protein.

Several marketed protein dosage forms specify that the protein should not be reconstituted with a diluent containing a preservative, presumably because of adverse effects of the preservative on the stability of the protein. Examples include Activase[®], Proleukin[®], and Leukine[®].

Antimicrobial preservatives serve a unique function in insulin formulations.¹⁰⁰ Insulin zinc suspensions (the Lente series) are preserved

with methylparaben because phenol will destroy the crystals. However, neutral protamine insulin (NPH) formulations require the use of phenol or phenol derivatives (e.g., meta-cresol) to form the tetragonal oblong crystals characteristic of this widely used prolonged insulin formulation.

Delivery Effects

It is beyond the scope of this review to consider the huge variety of excipient materials used in drug-delivery formulations for sustained and controlled release of injectable drugs (see Table 6). Some polymers used to control drug delivery are discussed herein. The reader is referred to recent publications edited by Sanders and Hendren¹⁰¹ and Senior and Radomsky¹⁰² for information on excipients used in injectable drug-delivery formulations.

Topical ophthalmic formulations use viscosity-inducing agents such as methylcellulose, hydroxypropylmethylcellulose, and polyvinyl alcohol to enhance drug bioavailability simply by increasing contact time with the corneal epithelium and retard drug loss via the tearing mechanism. Formulators must recognize that the cellulosic excipients are generally incompatible with a wide variety of antimicrobial preservatives (e.g., chlorocresol, phenol, cetylpyridinium chloride, and the parabens). Polyvinyl alcohol is less interactive, but at high concentrations ($\geq 5\%$) will precipitate from solutions containing sulfate and phosphate salts.⁶

Carbomers (carbopols) are acrylic acid polymers used in ophthalmic formulations for sustained-release drug delivery.¹⁰³ They are incompatible with phenol and potentially other antimicrobial preservative agents, cationic polymers, strong acids, and high levels of electrolytes.⁶ Lactide/glycolide homo-polymers, poly(lactic acid) or poly(glycolic acid), or the copolymers, poly(lactic acid-co-glycolic acid) are the most commonly used biodegradable polymers in injectable drug delivery formulations (e.g., Lupron Depot[®], Zoladex Depot[®]).

Hydrogels used for injectable cell delivery vehicles include alginates, poly(aldehyde guluronate) and adipic acid dihydrazide.¹⁰⁴ The latter two polymers have a wide range of mechanical stiffness and controllable degradation rate. Alginate gels exhibit a limited range of mechanical properties and have uncontrollable disintegration times.

Table 6. Examples of Excipients Used To Sustain and/or Control the Released of Drugs in Injectable Drug Delivery Systems¹⁰²

Synthetic polymers for nanosphere and microsphere products
Polyesters
Polylactide
Polyglycolide
Poly(lactide-co-glycolide)
Polycaprolactones
Polyanhydrides
Poly(biscaroxyphenoxy) propane-cosebacic acid
Polyphosphazenes
Poly(dichlorophosphazene)
Polymer blends
Polycaprolactone + poly(hydroxybutyric acid + poly(lactide-co-glycolide)
Nano/Micropheeres from natural polymers
Albumin
Collagen
Fibrinogen
Gelatin
Alginate
Cellulose
Chitan and Chitosan
Dextran
Hyaluronate
Starch
In situ gelling systems
Polyethylene oxide–polypropylene oxide (PEO–PPO) (Pluronic [®] F 127)
<i>N</i> -isopropylacrylamide (NIPA)
Glycerol monooleate (GMO)
Atrigel [®] (PLA, PLGA, and copolymers of DL-lactide with caprolactone)
Sucrose acetate isobutyrate (SAIB)
Acrylate-terminated polymers
Acrylate-terminated PEG–PLA
Liposomes
Hydrogenated soy phosphatidylcholine
Distearoylphosphatidylglycerol
Dioleoyl phosphatidylethandamine (DOPE)
Dioctadecylamidoglycylspermine (DOGS)
Other general polymers
Polyvinyl alcohol
Polyamides
Poly(ethylene oxide)
Poly(ethylene glycol)

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