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Stabilizing Effect of Inorganic Phosphate Salts on Antibiotic–Steroid Ophthalmic Preparations

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Abstract □ Drocinonide phosphate potassium forms an insoluble complex with neomycin sulfate in aqueous solution. Dibasic sodium phosphate can be employed in an ophthalmic formulation to prevent the formation of this precipitate without affecting the stability of the steroid or the bioactivity of the antibiotic. Other phosphate steroid salts behaved in a like manner.

Keyphrases □ Ophthalmic formulations—antibiotic–steroid preparations, stabilizing effect of inorganic phosphate salts, drocinonide phosphate potassium–neomycin sulfate complex studied □ Steroid–antibiotic ophthalmic preparations—stabilizing effect of inorganic phosphate salts □ Antibiotic–steroid ophthalmic preparations—stabilizing effect of inorganic phosphate salts □ Drocinonide phosphate potassium—complex with neomycin sulfate, prevention of precipitation by use of inorganic phosphate salts □ Inorganic salts—as stabilizers of antibiotic–steroid ophthalmic preparations

Neomycin sulfate is a polybasic antibiotic which ionizes in aqueous solution to give a positively charged species of neomycin. A wide range of anionic compounds were studied to assess their compatibility with the antibiotic (1). Approximately 50% of the high molecular weight anionic compounds tested precipitated with neomycin sulfate as an addition complex of low solubility. The stability of neomycin was investigated in several pharmaceutical preparations, and it was found that the antibiotic was relatively stable in solution over pH 2.0–9.0 (2). All products studied showed a minimum stability of 1 year at room temperature; tablets, troches, and ointment bases were stable for 2 years.

The present report describes a method for overcoming the incompatibility of neomycin sulfate with corticosteroid drugs in aqueous ophthalmic preparations. As a model, the soluble steroid chosen for investigation was drocinonide phosphate potassium[1]. To formulate this steroid with neomycin sulfate in an ophthalmic solution, the incompatibility of the two drugs must be overcome to produce a clear, particle-free solution in which the drug remains stable and active.

EXPERIMENTAL

Materials—The following were used: neomycin sulfate[2], drocinonide phosphate potassium[1], anhydrous disodium hydrogen phospate[3], anhydrous dipotassium hydrogen phosphate[3], sodium borate[3], sodium bisulfite[3], dexamethasone sodium phosphate[4], prednisolone sodium phosphate[4], polyvinylpyrrolidone[5], sodium formaldehyde sulfoxylate[6], sorbitol[7], polymyxin B sulfate[8], and thimerosal[9].

Stability Assay of Drocinonide Phosphate Potassium—A quantitative paper chromatographic method (3) was employed, using the solvent system of 1-butanol–ethanol–2 *N* ammonium hydroxide (5:1:2). Prior to the application of the sample, the paper strips were washed in alcohol and dried (4). The zones of steroid were located by use of a guide-strip spray reagent technique, and the zones on the untreated strips were eluted with water. An aliquot of the eluate was mixed with concentrated sulfuric acid, and the resulting fluorescence was measured spectrophotofluorometrically[10].

Microbiological Assay of Neomycin Sulfate—The microbiological activity of neomycin sulfate was determined by the cylinder-plate method described in USP XVIII (5).

[1] Also known as tetrahydrotriamcinolone acetonide dipotassium phosphate.
[2] Squibb, New Brunswick, N.J.
[3] Mallinckrodt, St. Louis, Mo.
[4] Merck, Rahway, N.J.
[5] Plasdone C-30, GAF Corp., New York, N.Y.
[6] Fine Organics, Lodi, N.J.
[7] Atlas, Wilmington, Del.
[8] Pfizer, Groton, Conn.
[9] Lilly, Indianapolis, Ind.
[10] Aminco-Bowman.

Table I—Composition of Formula I

Ingredient	Amount, mg
Neomycin sulfate	3.26
Drocinonide phosphate potassium	3.4
Disodium hydrogen phosphate	6.0
Polyvinylpyrrolidone[a]	8.0
Sodium formaldehyde sulfoxylate	2.0
Thimerosal	0.02
Sorbitol, 70% (w/v)	0.01 ml
Purified water, *q.s.*	1.0 ml

[a] PVP-C-30.

Preparation of Formulas I–IV—These formulas appear in Tables I and II. The neomycin sulfate was dissolved in 60% of the purified water. The soluble phosphate buffer agent was dissolved in 15% of the water and was then added to the solution of neomycin sulfate. The steroid salt plus other additives was dissolved in 15% of the water and added slowly to the neomycin sulfate–phosphate buffer solution. Then the solution was adjusted to volume.

Stoichiometry of Antibiotic–Steroid Complex—To determine the stoichiometry of the complex, 0–10 ml of a 0.05 *M* solution of neomycin sulfate was pipetted in increments of 0.5 or 1 ml into 20-ml vials. Enough 0.05 *M* steroid solution was added until the volume of each vial totaled 10 ml. The mixtures were swirled and permitted to equilibrate at 25°; after 24 hr, the contents of each container were filtered. The precipitated complex was dried and weighed. The data obtained are plotted in Fig. 1, according to the method of Job (6). Data points represent the average of duplicate experiments.

RESULTS AND DISCUSSION

The ideal agent used to overcome the incompatibility of drocinonide phosphate potassium and neomycin sulfate in an ophthalmic solution should exhibit little or no physiological activity and be effective in very small concentrations. Various materials were studied for their effectiveness in solubilizing the precipitate or in preventing the formation of the low solubility complex.

Preliminary screening studies showed that the solubility of the antibiotic–steroid complex was dependent on pH and that the addition to the mixture of strongly acidic or basic amino acid salts produced clear solutions. However, the addition of amino acid salts closer to neutrality was unsuccessful. Sodium borate, which has been used to solubilize a number of drugs (*e.g.*, riboflavin), proved reasonably effective when used in a great concentration (20 mg/ml) at pH 9.2. However, when the preparation was buffered to a pH range desirable for ophthalmic solutions (pH 6–8.5), a precipitate slowly appeared.

An agent successful in overcoming the incompatibility of drocinonide phosphate potassium and neomycin sulfate was disodium hydrogen phosphate. Singh (7), who studied the interaction of various antibiotics with condensed phosphates, found that neomycin possessed the strongest binding capacity for these higher phosphates. Disodium hydrogen phosphate proved an ideal agent for overcoming the incompatibility of drocinonide phosphate potassium and neomycin sulfate, because it was a good buffer agent and was effective in small concentrations. The pH of a solution of disodium hydrogen phosphate (7.8–8.0) may be lowered by the addition of sodium dihydrogen phosphate or dilute phosphoric acid to produce the monobasic salt *in situ*.

Table I shows the composition of a prototype formulation containing the two active ingredients in therapeutic amounts. This product was packaged in 30-ml amber-glass containers and stored at various temperatures to assess the stability of the steroid. Soluble steroid salts have been shown to undergo hydrolysis in aqueous solution, the process being accelerated at temperatures above room temperature. Saccharin sodium (8), creatinine, and niacinamide (9) have been shown to slow this hydrolytic process.

Preliminary studies[11] of stability had shown that polyvinylpyrrolidone was effective in preventing the hydrolysis of drocinonide phosphate potassium, and it was included in Formula I as a stabilizing agent. The data in Table III show that the stability of the steroid was maintained at 33° after 6 months but had declined at 40° after the same period.

[11] Unpublished data.

Table II—Formula Assayed Microbiologically for Neomycin Activity

Ingredient	Formula II	Formula III	Formula IV
Neomycin sulfate	3.26 mg	—	3.26 mg
Drocinonide phosphate potassium	3.4 mg	3.4 mg	—
Disodium hydrogen phosphate	6.0 mg	6.0 mg	6.0 mg
Purified water, *q.s.*	1.0 ml	1.0 ml	1.0 ml

To assess the influence of the steroid on the bioactivity of neomycin, three formulations were prepared (Table II). To eliminate interference with the neomycin assay, the other additives listed in Table I were omitted from the formulation. The data in Table IV show that the steroid did not interfere with the antimicrobial activity of neomycin and that the steroid itself possessed no antimicrobial activity.

To characterize the complex further, its stoichiometry was determined, as outlined under *Experimental*. From Fig. 1, it can be seen that maximum interaction between the two drugs occurred when the mole fraction of neomycin (X_A) to steroid (X_B) was 1:3. The level of disodium hydrogen phosphate required to maintain a clear solution proved to be dependent on the concentration of neomycin sulfate and not on that of drocinonide phosphate potassium.

For a concentration of neomycin sulfate equivalent to 2.5 mg/ml as neomycin base, the minimum recommended level of disodium hydrogen phosphate was 6 mg/ml. At lesser concentrations of buffer (*e.g.*, 4 mg/ml), it was also possible to obtain a crystal-free solution if both reactants were well diluted. However, when both solutions had been frozen at −20° for 12 hr, the preparation containing disodium hydrogen phosphate at 6 mg/ml produced a clear solution after being shaken at room temperature, whereas the preparation containing buffer at 4 mg/ml contained crystals. These crystals did not dissolve at room temperature, but their solubility increased as the temperature was raised; all crystals were in solution at 50°.

NMR spectroscopy was employed to investigate the system further. The primary amino groups on the neomycin molecule were suspected to be the sites of interaction with the steroid. The buffer agent and the two active ingredients were each lyophilized from deuterated water solutions to ensure the deuteration of the water of hydration. The NMR spectra obtained from deuterated water solutions of neomycin sulfate in the presence of the disodium hydrogen phosphate, with and without the steroid, indicated no interaction among the species. This fact, plus the fact that neomycin was bioactive in the presence of the steroid, suggested that the two active constituents were present as individual moieties and would be bioavailable to exert the desired therapeutic effect, but this assumption has yet to be proven *in vivo*.

Disodium hydrogen phosphate was also investigated for its ability to overcome the incompatibility that occurred when polymyxin

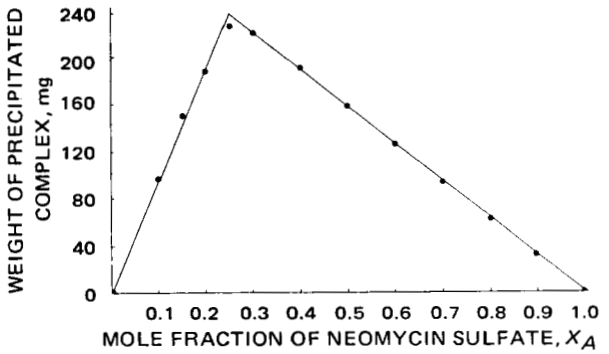

Figure 1—*Job (6) plot showing stoichiometric relationship between neomycin sulfate and drocinonide phosphate potassium. (See text for details.)*

Table III—Stability of Drocinonide Phosphate Potassium in Formula I[a]

Temperature	1 Month	2 Months	3 Months	6 Months
5°	97.0	99.4	97.7	96.4
25°	95.2	96.2	100.3	93.5
33°	94.7	95.3	99.7	97.9
40°	94.1	95.9	95.6	80.0

[a] Expressed as percent undegraded steroid.

B sulfate was added to the drocinonide phosphate potassium–neomycin sulfate system to increase the antibacterial activity of a combination ophthalmic preparation. Increasing the buffer concentration an additional 25% and adding the steroid slowly to the disodium hydrogen phosphate–antibiotic mixture in dilute solution made it possible to prepare a solution of the three drugs in concentrations considered desirable for therapeutic effect.

A very slight haze in the product developed immediately after preparation. Filtration yielded a crystal-clear solution that remained clear. The haze also appeared in the product formulated without neomycin sulfate. (The haze might be due to a small amount of impurity in polymyxin that was not protected by disodium hydrogen phosphate and was, therefore, precipitated by the steroid.) The bioactivity of polymyxin in these systems has not been investigated and certainly warrants further investigation.

The ability of disodium hydrogen phosphate to counteract the incompatibility of neomycin sulfate with other steroids, such as dexamethasone sodium phosphate and prednisolone sodium phosphate, was also investigated. As with drocinonide phosphate potassium, solutions of these steroids in the presence of neomycin sulfate were clear and free of crystals.

A currently marketed ophthalmic solution of dexamethasone sodium phosphate and neomycin sulfate contains sodium bisulfite as an antioxidant to help overcome the incompatibility between the steroid and the antibiotic. Studies in these laboratories have shown sodium bisulfite to be effective in the drocinonide phosphate potassium–neomycin sulfate system; small concentrations of bisulfite produced a crystal-free solution. When this solution was purged with oxygen, the bisulfite was oxidized to bisulfate, and rosette crystals began to grow on the walls of the container. The advantage to using disodium hydrogen phosphate rather than bisulfite was the stability of phosphate in the presence of oxygen, which permitted packaging of the product in a plastic squeeze-bottle container.

Dipotassium hydrogen phosphate exhibited properties similar to those of the disodium salt. No studies have been made of the stability or bioactivity of drocinonide phosphate potassium and neomycin sulfate in the presence of the dipotassium salt, but no marked difference is expected between the two salts.

SUMMARY AND CONCLUSIONS

Dibasic phosphate salts can be satisfactorily utilized to overcome the chemical incompatibility between neomycin sulfate and soluble corticosteroid phosphate salts.

Of the many mechanisms that could play a role in this "salting-in" process, two are: (*a*) phosphate ions from disodium hydrogen phosphate exert a common-ion effect, *via* ion-pair formation, or a cosolute effect (10); and (*b*) phosphate ions from disodium hydrogen phosphate interact with reactive sites on neomycin and sterically hinder the chemical reaction between the antibiotic and the steroid salt. By this latter mechanism, the bonding of phosphate ions to the reactive site(s) on neomycin would probably be *via* weak electrostatic bonds, since NMR spectra suggested that both neomycin sulfate and drocinonide phosphate potassium are present as separate moieties. The stoichiometry of the antibiotic–steroid complex (1:3) seems to indicate that the complex may not be formed by a simple ionic interaction between the two high molecular weight species. Further studies of the salting-in mechanism are currently underway.

The stability and bioactivity of drocinonide phosphate potassium and neomycin sulfate in a disodium hydrogen phosphate solution were not adversely influenced by the presence of the other drug. The use of disodium hydrogen phosphate as a simple and efficient way to overcome the chemical incompatibility between neomycin sulfate and soluble phosphate salts of corticosteroids is strongly recommended for ophthalmic formulations where some combination of these drugs is desirable.

Table IV—Bioactivity[a] of Neomycin in Formulas II, III, and IV Stored at Various Temperatures

Time	Temperature	Formula II	Formula III	Formula IV
Initial	25°	99.5	0	96.3
1 month	5°	99.1	0	98.0
	25°	97.3	0	98.0
	33°	98.0	0	97.3
	40°	96.4	0	96.4
3 months	5°	99.5	0	97.7
	25°	99.1	0	96.8
	33°	99.5	0	97.7
	40°	96.8	0	95.9
6 months	5°	97.7	0	97.2
	25°	97.3	0	97.2
	33°	100.0	0	96.3
	40°	90.9	0	90.0

[a] Expressed as a percent of the predetermined potency of the neomycin sulfate used in the formulation. Confidence limits of less than 4% (2 days) were used for all assay values.

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