

TRIS AMINO[™]

Tromethamine Buffer Application Guide

TRIS AMINO™

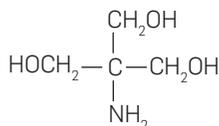
Tris Buffer

KEY PERFORMANCE ADVANTAGES

- Provides chemical stabilization in aqueous formulations
- Mild alkalinity for buffering at physiological pH
- Exhibits minimal salt effects

TRIS AMINO™ (2-amino-2-hydroxymethyl-1,3-propanediol) is a mildly alkaline chemical compound (Figure 1). Its chemical structure is that of a trihydric alcohol which also possesses primary amine functionality. Pure TRIS AMINO is readily available as a white crystalline solid, melting between 168°C and 172°C. Its molecular weight is 121.14. TRIS AMINO is readily water-soluble (up to 80 g/100 mL water) and possesses alcohol and glycol solubility as well.

FIGURE 1: TRIS AMINO™ Tris Buffer



C.A.S. 77-86-1
EINECS 201-064-4
m.w. 121.14
m.p. 168°-172°C

TRIS AMINO is well-known worldwide as a buffer in biochemical reactions. It can be used to buffer anywhere within the pH range of 7 to 9 by utilizing a combination of the free base and its salts; the hydrochloride salt is especially popular for this purpose. In fact, a buffer solution containing TRIS AMINO and its hydrochloride salt at a molar ratio of 1:3 is considered a standard buffer solution for use in the physiological pH range of 7.3 to 7.5.

TRIS AMINO AS A BUFFER

Molecular Weight	121.14
pka (20°C)	8.3
pka (37°C)	7.8
Δ pka/°C	-0.03
pH of 0.1M aqueous solution (20°C)	10.4
Δ pH/10X dilution	-0.05 to -0.10

The reasons for the widespread use of TRIS AMINO in this role include:

- Readily commercially available in highly purified form
- Chemically stable and moderately hygroscopic
- Formation of stable aqueous solutions
- Negligible metal binding with calcium, magnesium and manganese cations
- Insignificant light absorption between 240 and 700 nanometers
- Small salt effect and no interference from isotonic saline solution

Unlike zwitterionic buffers, TRIS AMINO is not excluded by biological membranes. TRIS AMINO has a significant temperature coefficient; however, its temperature coefficient more closely approximates that of whole blood and plasma than do those of phosphate and other buffers.¹ Moreover, its purity is considered high enough to serve as an acidimetric standard for calibration of instruments.² Its alkalinity is mild enough to show compatibility and utility with enzyme systems; it has been used to stabilize enzymes in aqueous solution³ and also to enhance their effects.⁴

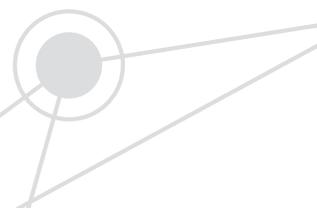
TRIS AMINO is the subject of monographs in the United States Pharmacopoeia Volume 24, Pharmacopoeia Europa 3. Edition, and Martindale: The Extra Pharmacopoeia, Vol. 29. In addition, TRIS AMINO is described in Japanese Standards of Pharmaceutical Ingredients and the German Pharmazeutische Stoffliste. This material is referred to under several different names in technical literature.

Nomenclature:

- 2-amino-2-hydroxymethyl-1,3-propanediol
- Tris (hydroxymethyl) aminomethane
- TRIS AMINO (ANGUS)
- Trometamol
- THAM
- Tris
- Tris Base
- Tromethamine (United States Pharmacopoeial Designation)

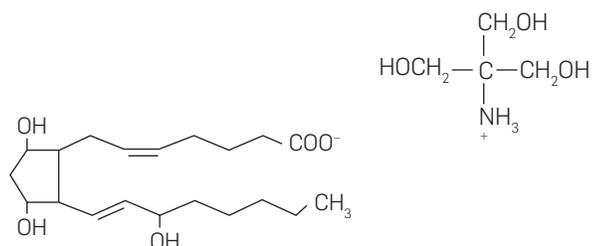
TRIS AMINO SALTS

At least six active pharmaceutical materials are listed in the Index Nominum International Drug Dictionary 1990/91, and/or the USAN and USP Dictionary of Drug Names, as being offered in the form of their TRIS AMINO salt. Dinoprost (Recommended International Non-Proprietary Name, INN), also known as Prostaglandin F_{2a}, is used in both human and veterinary medicine as an oxytocic agent for induction of labor and therapeutic abortion (Figure 2). It is also a powerful ocular hypotensive agent in animals and man. In the form of the TRIS AMINO salt, it becomes a crystalline material and can be obtained in high purity. Study of the pH-solubility profile of this salt showed, at a pH slightly greater than 5, a higher increase in solubility than could



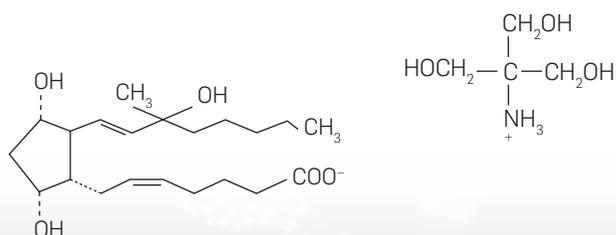
be attributable only to the ionization constant of the acid.⁵ The formation of micelles was shown to account for this behavior, with the critical micelle concentration (CMC) increasing as a function of degree of ionization, and hence of pH.

FIGURE 2: Dinoprost Tromethamine



Another prostaglandin, closely related in structure to the previously-described dinoprost, is known as carboprost (Rec. INN) (Figure 3). Also an oxytocic, carboprost is sold in the form of the TRIS AMINO salt. A recent clinical trial of carboprost tromethamine showed value against hemorrhagic cystitis resulting from cyclophosphamide chemotherapy. Administered intravesically in such cases, it can apparently resolve gross hematuria without systemic complications.⁶ Dinoprost tromethamine and carboprost tromethamine are each the subject of a monograph in Volume 23 of the U.S. Pharmacopoeia, in both active and injection forms.

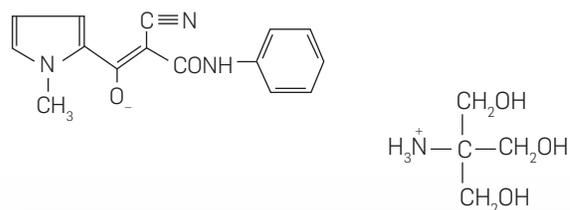
FIGURE 3: Carboprost Tromethamine



Prinomide (INN) is an anti-inflammatory and anti-arthritic compound (Figure 4). Its sodium, potassium and calcium salts are not crystalline.⁷ Its crystalline TRIS AMINO salt has been shown to have lower acute toxicity than do either the free acid or its triethanolamine (trolamine) salt. Among ten test animals, no deaths were noted at a dose of 2.4 millimoles/Kg p.o. (932 mg/Kg) of the TRIS AMINO salt, whereas a dose of 2.4 millimoles/Kg p.o. (1000 mg/Kg) of the triethanolamine salt produced 50% mortality and 2.4 millimoles/Kg p.o. (641 mg/Kg) of the free acid produced 40% mortality.

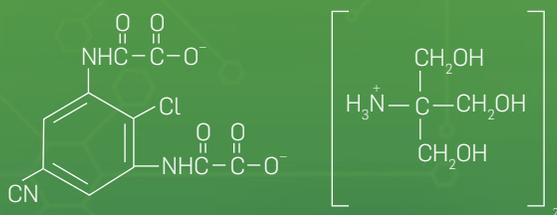
Active pharmaceutical materials are sometimes used as the triethanolamine salt. The use of the primary amine TRIS AMINO as a neutralizing moiety, unlike triethanolamine, will not lead to the formation, either incidental or through metabolic conversion, of N-nitroso derivatives. Indeed, the presence of TRIS AMINO in a system has been shown to prevent the formation of N-nitrosamines under conditions which favor nitrosation of available nitrosatable species.⁸ In several countries, there are already existing regulations against the use of nitrosatable amines in pharmaceutical preparations; TRIS AMINO should be free from such concern.

FIGURE 4: Prinomide Tromethamine



Lodoxamide Trometamol (British Approved Name, BAN) is an anti-allergy drug (Figure 5). It is a biologic of disodium cromoglycate (DSCG), but lodoxamide trometamol has been shown to have much greater intravenous activity than does DSCG, as well as possessing significant oral activity.⁹ It acts by preventing histamine release, through inhibiting the calcium flux into mast cells which normally triggers such release. While the ready solubility of di-TRIS AMINO salt permits its administration by several routes, the diethyl ester of lodoxamide is not sufficiently soluble for any but oral administration. In animal and human models of acute testing, lodoxamide trometamol has appeared to be very active in preventing bronchoconstriction. However, this markedly enhanced effect shown in clinical trials had not been repeated in long-term human trials.¹⁰

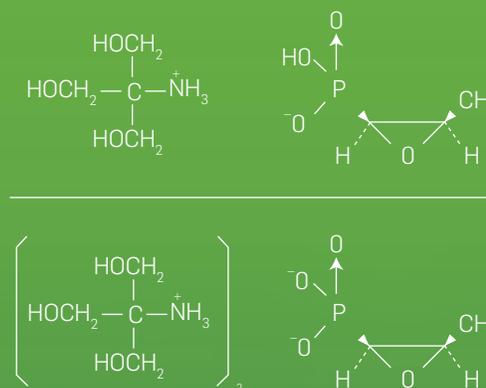
FIGURE 5: Lodoxamide Trometamol



Fosfomicin (Rec. INN) is a phosphonic acid compound, known as a useful antibiotic for urinary tract infections (Figure 6). Its tendency to act at comparatively high serum levels requires injection of the disodium salt for optimal dosing, since the orally-administered calcium salt shows limited gastro-intestinal absorption. The mono-TRIS AMINO salt of fosfomicin shows a six-fold increase in rate and extent of absorption over that of its calcium salt during the first two hours following dosing, and the TRIS AMINO salt values

are three to four times greater than those of the calcium salt over a twelve hour post-dose period.¹¹ A detailed study of peak serum concentrations and other pharmacokinetic parameters showed better proportionality to the initial dose, as well as faster and greater absorption for the TRIS AMINO salt, across all dose ranges.

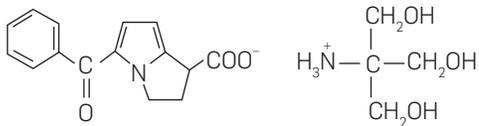
FIGURE 6: Fosfomicin Tromethamine



It can be noted from the structure of the free phosphonic acid that a 2:1 ratio of TRIS AMINO to acid is possible. This salt also shows advantages in bioavailability, as well as tolerability, over the calcium and sodium salts.¹² However, the mono-TRIS AMINO salt of fosfomicin produces aqueous solutions which are less viscous and hence easier to manipulate than does the di-TRIS AMINO salt, when compared on an equal activity basis.¹³

Ketorolac (INN) is a recently developed non-steroidal anti-inflammatory drug, whose systemic analgesic potency seems to be considerably greater than its anti-inflammatory activity (Figure 7). In single-dose treatment of moderate to severe pain, its analgesic response seems to be greater than that of morphine but with fewer side effects.

FIGURE 7: Ketorolac Tromethamine



It can be administered orally or intramuscularly. Ketorolac has also shown value in treatment of ocular inflammatory conditions following cataract surgery. Although animal experiments have relied mainly upon the free acid, the active material is administered clinically as the TRIS AMINO salt.¹⁴

The TRIS AMINO salt of ketorolac was shown to be non-hygroscopic even at high relative humidity levels, whereas its sodium salt showed appreciable hygroscopicity at relative humidities at or above 67%. The intrinsic dissolution rates and aqueous solubilities of the two salts are nearly identical.¹⁵

The 1992-93 Index Nominum lists two other materials offered in the form of their TRIS AMINO salt. Tromethamol Glucaldrate (Figure 8) is a gluconic-acid chelate of a hydrated aluminate, used as a stomach antacid. Desglugastrin Tromethamine (Figure 9) is a gastrin analog in which a gamma-carboxybutyryl residue has been introduced into the polypeptide. It is described as an indicator and stimulator of gastric-acid secretion.

FIGURE 8: Tromethamol Glucaldrate

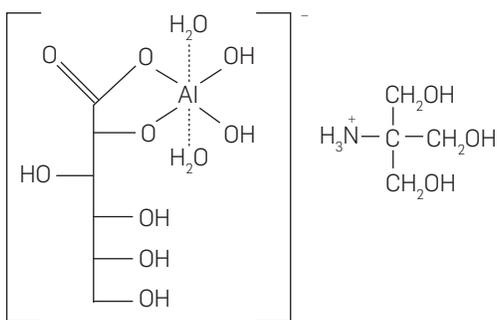
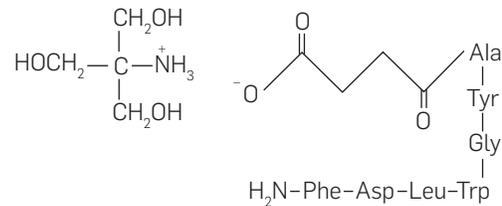
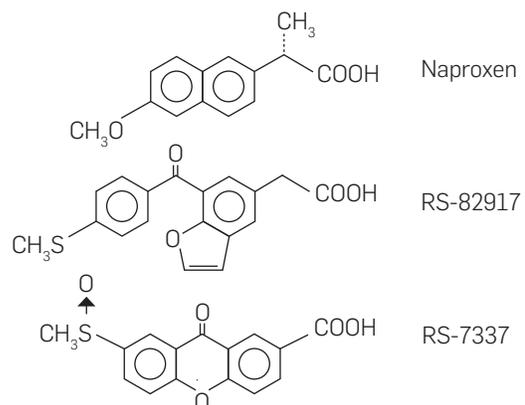


FIGURE 9: Desglugastrin Tromethamine



Comparisons of the sodium salts and the TRIS AMINO salts of three other analgesic anti-inflammatory agents (Figure 10) showed the TRIS AMINO salts of naproxen and RS-82917 to be totally non-hygroscopic up to 81% relative humidity, unlike their sodium salts.¹⁵ The TRIS AMINO salt of RS-7337, although slightly hygroscopic, was significantly less so than its sodium salt. The sodium salt of naproxen was approximately 12 times more water soluble than was the TRIS AMINO salt, while the sodium salt of RS-7337 was approximately four times less water soluble than was the TRIS AMINO salt; the sodium and TRIS AMINO salts of RS-82917 had equal water solubility. The intrinsic dissolution rates of the TRIS AMINO and sodium salts of RS-7337 and RS-82917 were equivalent, while in the case of naproxen, the sodium salt had a significantly greater intrinsic dissolution rate than did the TRIS AMINO salt.

FIGURE 10: Acid-Functional Analgesics



The advantages inherent in the TRIS AMINO salts of several other acid-functional active analgesics (Figure 11) have also been documented. TRIS AMINO provides a more satisfactory solubilizing cation for zomepirac than does sodium or potassium, enhancing solubility by 5,000 times over that of the free acid. The solubilization has been shown to be through a micellar mechanism, and the CMC determined to be 19 mg/mL (0.065M) of zomepirac.¹⁶ The TRIS AMINO salt of diflunisal shows a markedly increased aqueous solubility and dissolution rate over that of the free acid. In addition, pharmacokinetic profile comparisons demonstrate an enhanced absorption ratio for the diflunisal TRIS AMINO salt.¹⁷ The TRIS AMINO salt of tiaprofenic acid has produced a greater degree of analgesia in post-operative patients than either ketoprofen or lysine acetylsalicylate, with no evident adverse changes in other measured parameters.¹⁸

FIGURE 11: Acid-Functional Analgesics

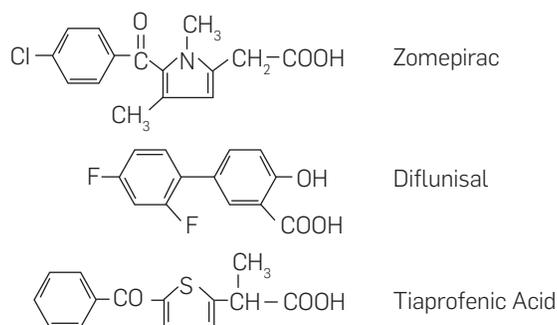
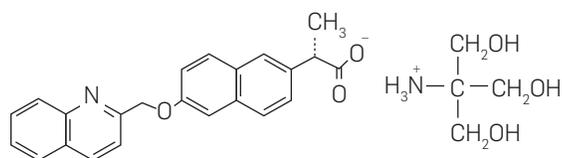


FIGURE 12: WY-50,295 Tromethamine



The TRIS AMINO salt of acetylsalicylic acid can be formed easily, in nearly quantitative yield, to give crystals with excellent water solubility.¹⁹ The TRIS AMINO salt has lower toxicity than does free acetylsalicylic acid and also offers enhanced bioavailability compared to that of the free acid. Additionally, the use of TRIS AMINO as a neutralizing cation instead of sodium will permit the exclusion of unwanted sodium from dietary intake.²⁰ Other non-steroidal anti-inflammatory agents which are documented to have shown enhanced aqueous solubility in the form of their TRIS AMINO salt, thus permitting their use in injectable solutions or ophthalmic preparations, include indomethacin and niflumic acid.²¹ These TRIS AMINO salts retain lipid solubility as well as now possessing water solubility, and their therapeutic action is as effective or more so than the parent free acids. These benefits are also expected from the TRIS AMINO salts of ibuprofen, ketoprofen, and similar carboxylic acid derivatives.

A further example of a valuable TRIS AMINO salt of an acid-functional active material is WY-50,295 tromethamine (Figure 12). This is an orally active 5-lipoxygenase inhibitor which shows anti-allergic activity and inhibition of bronchoconstriction. Although a portion of its structure is that of the naproxen molecule, WY-50,295 tromethamine is not metabolized into naproxen.²²



TRIS AMINO IN FORMULATED SYSTEMS

TRIS AMINO buffer demonstrates mild alkalinity, buffering capability and primary aminotriol functionality. These properties can offer the formulator advantageous combinations of active material solubilization into aqueous systems, chemical stabilization of actives against degradation and pH buffering of solutions. At the same time, the presence of TRIS AMINO in an aqueous formulation will not compromise favorable toxicological characteristics. These attributes of TRIS AMINO can be applied to topical, injectable, oral and ophthalmic systems.

TRIS AMINO is the buffer system of choice for a lyophilized dosage form of human recombinant interleukin-1. Not only is its pH-controlling capability for the target pH of 7.5 very good, even at low temperatures, but the polyol nature of TRIS AMINO permits it to participate in a hydration network surrounding the protein. This is considered a distinct advantage.²³ The polyol nature of TRIS AMINO buffer also offers storage stabilization against precipitation out of aqueous solution for somatotropin. In this case, the hydrochloride salt of TRIS AMINO is used to maintain the pH of the solution at about 5.7.²⁴

The polyhydroxy functionality of TRIS AMINO also provides solubility in alcohols, glycols and other polyols, thus offering a greater degree of flexibility to the formulator than do many other buffers. Tipredane is a novel corticosteroid which is more rapidly metabolized than are many other corticosteroids. A co-solvent topical delivery system for tipredane, consisting of propylene glycol, PEG 400 and water was developed.²⁵ These components were chosen to be non-stinging to broken skin. The formulation also contained, for stabilization of the active component, sodium metabisulfite and butylated hydroxytoluene as an antioxidant system, dipotassium EDTA dihydrate as a metal-chelating agent, and a buffer to maintain the pH between six and eight. The use of potassium citrate as the buffer permitted, through a common-ion effect, precipitation when the sodium metabisulfite was oxidized to form potassium sulfate. When TRIS AMINO was used as a buffer, no such precipitation occurred, due to its high solubility in the co-solvents used and to the lack of common-ion effect with the other components.

A similar co-solvent formulation was developed for an ophthalmic preparation containing pilocarpine nitrate.²⁶ Propylene glycol or PEG 300 were used as candidate viscosity-increasing agents in the aqueous solution. Since phosphate and acetate buffers are known to accentuate the hydrolytic decomposition of pilocarpine nitrate, test formulations were buffered to pH 5.2, 6.0, or 6.4 with sodium hydroxide and either TRIS AMINO maleate or sodium citrate. Four formulations buffered with the TRIS AMINO-containing combination were found to have favorable enough combinations of properties to warrant *in vivo* testing.

Neomycin undecylenate is often used in a propylene glycol or polyol vehicle to treat infections of the ear. Sodium metabisulfite is incorporated as an antioxidant, and the pH of the system is adjusted to 3-5 with benzoic acid. The incorporation of TRIS AMINO stabilized this formulation against formation of neomycin sulfate precipitate and loss of neomycin activity.²⁷ Although ethanolamines also provided similar stabilization, TRIS AMINO was preferred for its biological tolerance.

Mercury-based antimicrobial preservatives such as thiomersal are especially valuable in aqueous ophthalmic preparations. TRIS AMINO has the ability to prevent the deposition of such preservatives out of the aqueous system onto the walls of plastic containers used for such preparations.²⁸ At the same time, TRIS AMINO will stabilize the mercury-based preservatives themselves against chemical decomposition in aqueous solution, greatly lengthening the effective storage life of the preparations. The presence of TRIS AMINO will also stabilize the active non-steroidal anti-inflammatory drug sodium diclofenac in aqueous ophthalmic formulations and render it more tolerable by the eye.²⁸



Salts of folic acid or of leucovorin are formulated in water for injection in sealed doses. These formulations, whose optimum pH is about 8.0, require antimicrobial preservation. They are also prone to oxidative degradation in aqueous solution and are light-sensitive. Benzyl alcohol has been used in the past as the preservative, but it will not add any buffering action. A buffer/antioxidant combination of TRIS AMINO and monothioglycerol has been shown to impart superior stability against pH drift and degradation due to air or light.²⁹ The shelf life of the sealed doses was thereby greatly extended, and microbial testing showed that the presence of benzyl alcohol was now optional rather than required.

N-Nitrosourea anti-neoplastic agents are generally quite unstable in aqueous solutions. It is possible to extend the shelf-life of these nitrosourea anti-cancer drugs by including TRIS AMINO buffer in their formulation, instead of using carbonate buffers at the same pH.³⁰ The nitrosoureas have been shown to form a complex with the TRIS AMINO and the rate of hydrolytic degradation of the drug, in the form of this complex, is significantly slower than that of the nitrosourea alone. TRIS AMINO is also used in aqueous formulations of the anti-cancer drug, 5-fluorouracil to buffer the solution at pH 8.2.³¹

The arginine salt of fosfomicin has advantageous therapeutic properties, but its water-solubility is quite limited. However, when formulated together with TRIS AMINO, this amino acid-fosfomicin salt becomes soluble to the extent of about 14%, as well as becoming more bioavailable in its active form.³²

N-acetylcysteine is a mucolytic agent which offers other potential benefits, as well. When administered orally, its concentration in the blood is reduced by degradation in the intestines and liver. However, oral administration of a pharmaceutical composition of N-acetylcysteine and TRIS AMINO in a 1:1 molar ratio causes a significant increase in the plasma concentration of undegraded N-acetylcysteine.³³ This occurs without increasing the total amount of N-acetylcysteine absorbed by the body or other pharmacokinetic parameters. Weak-acid salts of TRIS AMINO can be used in place of free TRIS AMINO to achieve the same effect. Other alkaline compounds, such as sodium bicarbonate, lysine or glucosamine do not have this same effect of increasing plasma concentration of intact N-acetylcysteine while not materially affecting the total amount of drug absorbed.

Purpuromycin is an antibiotic useful in the treatment of infectious vaginitis, since it shows good efficacy against the causative bacteria, yeasts and protozoa. Its molecular structure contains three phenolic hydroxyl groups, the salification of which can produce a water-soluble purpuromycin salt. The use of strong bases for this purpose, however, can cause hydrolytic degradation of the molecule, while very weak bases cannot form stable salts with the hydroxyl function. Moreover, the base used must have adequate hydrophilic character to produce the required solubility of the salt. TRIS AMINO has been shown to be well-suited to this purpose,³⁴ with a purpuromycin:amine ratio of 1:1.5 being especially stable and also pharmaceutically acceptable. These water-soluble salts offer the

same excellent biological activity as does free purpuromycin, but the salts also show more favorable properties for formulation into suitable dosage forms.

Although most of the uses of TRIS AMINO buffer which demonstrate enhanced stability of active materials are in liquid media, TRIS AMINO can offer similar benefits in solid dosage forms. Estropipate, or piperazine estrone sulfate, is an estrogen which is unstable in acidic environments or when combined with the commonly used excipient lactose. Excess piperazine can be employed to assure both alkalinity in tablet dosage-forms and solubilization of the estrone sulfate uniformly in the granulating media. However, the volatility of piperazine renders it susceptible to loss during vacuum drying. Piperazine also reacts adversely with lactose. The stability of estropipate was enhanced when its formulation included TRIS AMINO as an additional alkaline buffering agent.³⁵ Under accelerated storage conditions, the active estropipate suffered greater than 50% degradation when no buffer was included, 3.5% degradation when piperazine

was used as the buffer and only 1.6% degradation when TRIS AMINO buffer systems were utilized.

Aqueous gels are a convenient means of delivering an active pharmaceutical ingredient to a specific site, especially for topical application. One pharmaceutically acceptable gellant for such systems is the series of crosslinked acrylic acid homopolymers known as carbomers. When neutralized with an alkaline material, these polymers become water soluble and swollen, imparting the desired viscosity to the aqueous solution. Concentrations of 1% by weight or less of the carbomers are ordinarily used for such purposes. Since poly(acrylic acid) is a weak acid, mild bases are preferable to strong bases for optimal pH adjustment (and hence viscosity adjustment) of the systems. Alkanolamines will produce satisfactory carbomer gels that demonstrate desirable shear-thinning properties and good clarity. In addition, alkanolamine-neutralized aqueous carbomer gels will accept significant levels of alcohols, which are sometimes used to aid in solubilizing active ingredients, without losing gel properties or clarity.

The presence of secondary amines may give rise to detectable levels of N-nitrosamines, a recognized class of carcinogens. Liquid tertiary amines, such as Trolamine, N.F., may contain secondary amines as technically unavoidable impurities. This leaves pure crystalline-solid primary alkanolamines as the most appropriate neutralizing agents for pharmaceutical gel products. A detailed survey of crystalline bases listed in pharmacopoeias produced meglumine as a candidate in addition to TRIS AMINO.³⁶ The flow curves of carbomers neutralized with each of these three materials are very similar. It has also been shown (Figure 13) that the flow curves of carbomers neutralized with TRIS AMINO buffer are very similar to those produced from the use of triethanolamine and isopropanolamines as neutralizing bases (Figure 14).³⁷ It has been concluded from these investigations that TRIS AMINO is a totally appropriate carbomer-neutralizing agent for the preparation of pharmaceutical gels.

FIGURE 13: TRIS AMINO™ in Hydroalcoholic Carbomer Gels

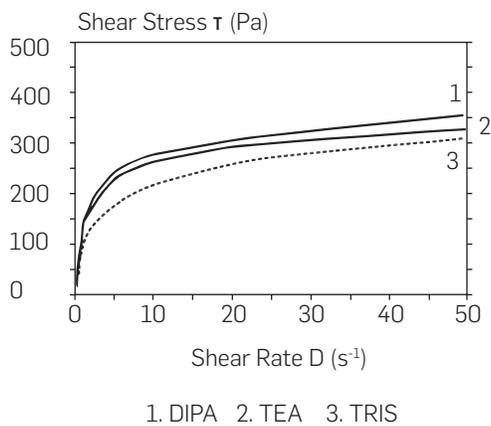
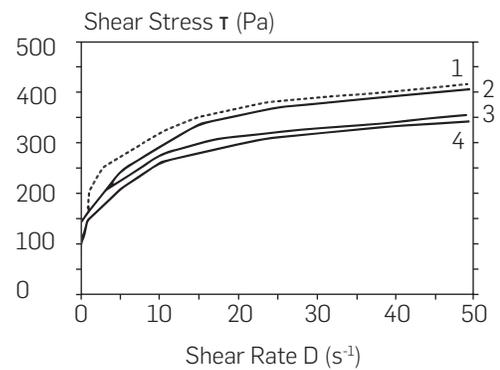


FIGURE 14: TRIS AMINO™ vs. Triethanolamine in Carbomer Gels



- | | |
|-------------------------|-----------------------------|
| 1. 1% Carbopol, 1% TEA | 3. 0.5% Carbopol, 0.5% TEA |
| 2. 1% Carbopol, 1% TRIS | 4. 0.5% Carbopol, 0.5% TRIS |



ANTIMICROBIAL ENHANCEMENT

The use of free TRIS AMINO buffer has been documented in the preparation of topical gel formulations of the analgesic ketorolac tromethamine.³⁸ These hydroalcoholic gels also contain skin-penetration enhancers, chelants and antioxidants. The final pH of the formulations is buffered at 4.2 with TRIS AMINO hydrochloride.

The cell walls of Gram-negative bacteria contain a layer of crosslinked peptoglycan which prevents cell lysis from internal osmotic pressure under external conditions of low osmolarity (osmotic shock). The chelating agent ethylenediaminetetraacetic acid (EDTA) has long been known to play a role in antibacterial attack through alteration of the cell wall. This enhanced permeability permits lysis of the cell,

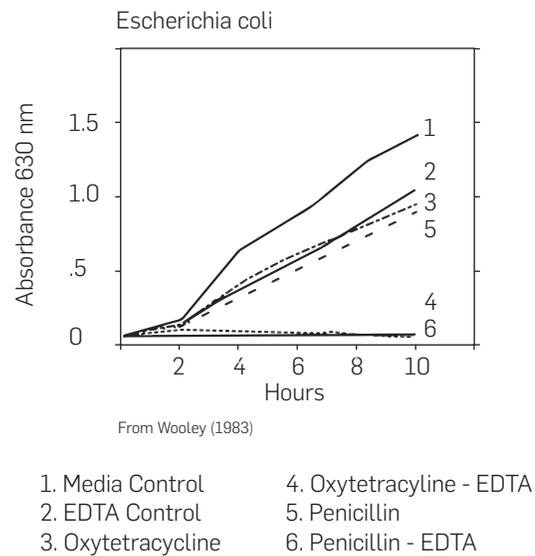
especially through the action of lysozyme. The combination of TRIS AMINO buffer together with EDTA has been shown to enhance this effect in *Pseudomonas aeruginosa*,³⁹ while no such toxicity to bacteria from TRIS AMINO alone, in the absence of EDTA, is seen. That is, TRIS AMINO acts as an organic cation with EDTA in increasing the permeability of the cell wall. A system concentration of 0.025 M TRIS AMINO and 0.25 mM EDTA depleted magnesium, calcium and manganese nearly 100% from cysts of *Azotobacter vinelandii*,⁴⁰ while magnesium and calcium were also removed from isolated cell walls of *Pseudomonas aeruginosa* by a TRIS AMINO-EDTA combination.⁴¹ A combination of 50 mM TRIS AMINO and 3.22 mM EDTA in growth medium prevented the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.⁴² The same system inhibited the growth of *Escherichia coli* and *Proteus vulgaris*. Against Gram-positive bacteria, the addition of 50-mM TRIS AMINO has been shown to greatly enhance the effect of diethylenetriamine pentaacetic acid (DPTA) against *Staphylococcus aureus* and *Klebsiella pneumoniae*.⁴³

The removal of multi-valent cations damages the cell wall by eliminating their ionic crosslinkages and rendering the wall more permeable to other compounds which may then penetrate to the cell interior. Subsequent cell exposure to magnesium ions may contribute to cell wall repair by re-sealing its outermost layer and partially reinstating its tensile strength.⁴⁴

The effect of TRIS AMINO combined with EDTA has been used to enhance the effect of known antibiotics. When oxytetracycline, gentamycin, polymyxin-B or triple sulfa were used together with 50 mM TRIS AMINO and

3.22 mM EDTA, significantly lowered (more than 85%) minimum inhibitory concentrations (MIC) against *Proteus vulgaris* were noted as compared to those of the antibiotics alone.⁴³ *E. coli* in the same test program was markedly more sensitive to penicillin, oxytetracycline and chloramphenicol when the antibiotics were used together with the TRIS AMINO-EDTA combination.

FIGURE 15: TRIS AMINO™-EDTA Antibiotic Enhancement

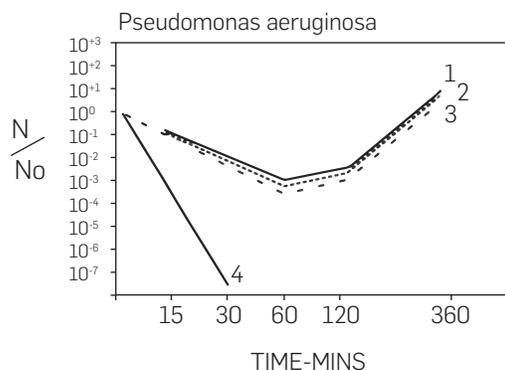


Further studies⁴⁵ (Figure 15) with concentrations of 50 mM TRIS AMINO and 250 mM EDTA confirmed the synergistic action of this combination with the three previous antimicrobial agents against *E. coli*, but showed slight or no synergism between the TRIS AMINO-EDTA combination and streptomycin, naldixic acid or triple sulfa. An antagonistic effect was noted with the chelant combination and polymyxin-B against *E. coli*.

Medical use of the TRIS AMINO-EDTA combination has been made to enhance the effect of gluconic acid salts of chlorhexidine (CHG) for infection treatment of urethral-catheter patients. When

instilled into the catheterized bladder, solutions of CHG alone at 0.02% can cause erosion of bladder tissue and other damage. Lower concentrations are ineffective in preventing infection. In vitro tests showed that, when 500 mg/L of disodium EDTA and 0.05 M TRIS AMINO were added to a 0.1% CHG solution, rapid kill of *Pseudomonas aeruginosa* (Figure 16), *E. coli* and *Proteus mirabilis* was achieved, even in the presence of urine.⁴⁶ When either TRIS AMINO alone or EDTA alone was combined with CHG, the killing action was only slightly enhanced over that of CHG alone. Somewhat less enhancement of effectiveness was seen in vitro against the Gram-positive bacterium *Streptococcus faecalis*. A further extension of this work⁴⁷ showed that the enhanced antimicrobial efficacy of CHG was not a pH-related phenomenon, and that the enhancement effect extended to levels of CHG as low as 0.0001% against Gram-negative bacteria. Bladder instillation of a commercial solution containing 0.1% CHG, 1.34 mM disodium EDTA and 0.01 M TRIS AMINO reduced significantly, over aseptic procedures, the incidence of bladder bacteriuria in catheterized patients. In this in vivo experience, Gram-positive as well as Gram-negative bacteria were adequately controlled.⁴⁸ This TRIS AMINO-EDTA-enhanced antibiotic solution was shown to be equal in efficacy and safety to kanamycin-colistin instillation solutions, and was preferred over the kanamycin system for its better ambient-temperature stability, lowered cost, and the decreased possibility of selecting antibiotic-resistant bacteria. This same CHG-TRIS AMINO product is also successfully used as topical antiseptic for treatment of infected ulcers in diabetic patients.⁴⁹

FIGURE 16: TRIS AMINO™-EDTA Antibiotic Enhancement



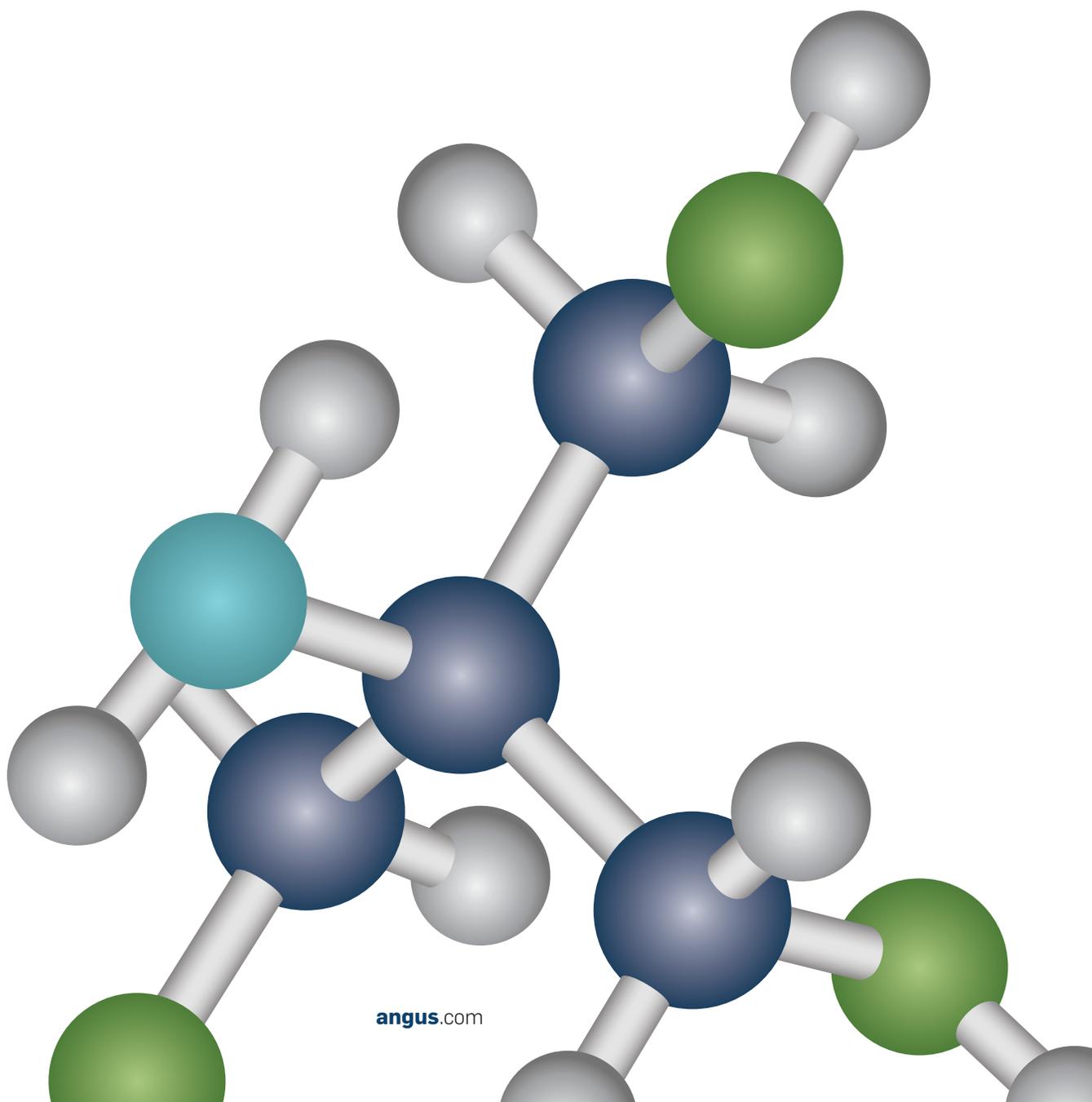
1. Chlorhexidine (0.01%) EDTA (500 mg)
2. Chlorhexidine (0.01%) Tris (0.05 M)
3. Chlorhexidine (0.2%) EDTA (500 M)
4. Chlorhexidine (0.1%) EDTA (500 mg) Tris (0.05 M)

From Harper (1983)

TRIS AMINO has shown similar value in enhancing antimicrobial effects in contact-lens disinfecting solutions. An isotonic solution with a pH of 7.4 containing 1.2% TRIS AMINO and 0.05% disodium EDTA showed significantly greater killing power toward *S. marcescens* and *C. albicans* than did similar solutions based on borate or phosphate buffers.⁵⁰ Moreover, the use of this TRIS AMINO-EDTA combination noticeably improved the disinfecting efficacy of several antimicrobial compounds, including chlorhexidine gluconate, hexetidine, polyhexamethylene biguanide, alexidine and polyquaternium-1. This enhancement of effects in contact-lens disinfecting solution was shown against Gram-positive and Gram-negative bacteria, molds, yeasts, and fungi. In similar fashion, the antimicrobial effect of a 50-ppm solution of hydrogen peroxide was greatly increased when used in a formulation together with 1.2% TRIS AMINO and 0.05% disodium EDTA.

CONCLUSION

The preceding data and examples have demonstrated various aspects of the usefulness of TRIS AMINO as a pharmaceutical auxiliary material. Its mild alkalinity easily permits buffering at physiological pH levels. It can provide benefits when used to make the isolated TRIS AMINO salt with acid-functional active pharmaceutical materials. In aqueous formulations, TRIS AMINO can provide chemical stabilization, solubilization of insoluble materials into water and pH buffering, while not compromising favorable toxicity properties. TRIS AMINO can be utilized to effectively neutralize and solubilize carbomer thickeners for aqueous or aqueous/alcoholic systems. Its use together with EDTA can enhance antimicrobial effects of antibiotics and antimicrobial products. Moreover, TRIS AMINO can be incorporated into pharmaceutical preparations to provide the benefits of amine functionality without raising concern for the possibility of nitrosamine formation. In the future, it is expected that the demonstrated value of TRIS AMINO as a pharmaceutically acceptable auxiliary and ingredient in these and similar applications will continue to increase.



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