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Review Article

Use of Nonaqueous Solvents in Parenteral Products

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THE PRACTICE OF incorporating naturally occurring nonaqueous solvents such as fixed oils and glycerin in pharmaceuticals has been common for many years. Water is always the solvent of choice. However, when it is not possible for physical or chemical reasons (such as limited solubility and hydrolytic reactions) to use a wholly aqueous system, nonaqueous solvents aid the formulator in developing stable, convenient parenteral dosage forms. A parenteral solution avoids the disadvantages inherent in suspensions, such as nonuniform dosage, caking, and possible slow release of the medicament when it is not desired.

A formulator encounters many problems once he determines that an aqueous system is unsatisfactory. The chosen solvent must be nontoxic, nonirritating, and nonsensitizing. It also must exert no pharmacologic activity of its own, nor adversely affect the action of the medicament. There are reported instances in which a solvent potentiated the activity of the medicament necessitating a change of dosage level. This will be discussed in greater detail later in this review.

In addition to being pharmacologically acceptable, the chemical and physical properties of the solvent must be taken into account. Thus, the ideal solvent should not be affected by acids or alkalies and it should be generally stable under normal conditions of pharmaceutical use. The viscosity must be such as to allow for ease of injection, and the solvent must remain fluid over a fairly wide temperature range. It is advantageous if the solvent has a sufficiently high boiling point to allow heat sterilization. Additional considerations are water and body fluid miscibility, the degree of flammability, availability, source of supply, and constant purity.

Obviously, no such individual solvent presently exists. Thus, the selection of a nonaqueous solvent for a parenteral vehicle is a compromise among the many influencing factors. The advent of modern chemical technology has produced many new synthetic solvents in addition to the naturally occurring ones.

This review presents the toxicity, chemical and physical properties, and applications of some of the more commonly used nonaqueous solvents, as well as some specialized and rarely used solvents in pharmaceutical formulations.

FIXED OILS

The U.S.P. (1) recognizes the use of fixed oils as parenteral vehicles. Fixed oils are mainly mixtures of esters of unsaturated fatty acids which are fluid at 20°. The fluidity is generally due to the presence of the oleic acid esters of glycerin. The most commonly used fixed oils are corn oil, cottonseed oil, peanut oil, and sesame oil (2). Castor oil and olive oil have been used occasionally. While the toxicities of vegetable oils are relatively low, some patients exhibit allergic reactions to specific vegetable oils. Therefore, when such oils are used as vehicles, the label must state the specific oil contained in the product. Fixed oils have been known to cause undesirable

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local tissue reactions, such as cysts, foreign-body granulomas and, occasionally, nerve injury.

The requirements for fixed oils to be used in parenteral products are specified in the U.S.P. (1). The use of fixed oils is limited because of the low solubility of most drugs in these solvents. Since these oils are not miscible with water, dissolved drugs may exhibit a sustained-release effect with a possible diminution of absorption. Aqueous insolubility also precludes the use of fixed oils in unemulsified intravenous products.

Cottonseed oil has been used in intravenous fat emulsions administered to surgical patients as reported by Lehr and co-workers (3) and a commerical product is available.

Drugs which are incorporated in oils are mainly the steroid hormones, dimercaprol, calciferol, and menadione. Since fixed oils contain unsaturated fatty acids, oxidative changes take place which may necessitate the use of oil soluble antioxidants such as propyl gallate, butylated hydroxyanisole, butylated hydroxytoluene, and tocopherols.

A list of the official U.S.P. (1) and N.F. (4) parenteral solutions using fixed oils as solvents is given in Table I.

TABLE I.—OFFICIAL INJECTIONS IN OIL

Desoxycorticosterone acetate U.S.P.
Dimercaprol U.S.P.
Estradiol benzoate U.S.P.
Estradiol cyclopentylpropionate N.F.
Estradiol dipropionate U.S.P.
Estrone U.S.P.
Progesterone U.S.P.
Testosterone propionate U.S.P.
Diethylstilbestrol dipropionate N.F.
Menadione N.F.

A typical intramuscular formula for a testosterone propionate oil solution, 50 mg./ml., is (5)

	Gm./1000 ml.
Testosterone propionate	50.0
Benzyl alcohol	21.0
Sesame oil	869.0

Since sesame oil becomes turbid on cooling, a "winterized" or treated oil should be used, so that the oil remains clear when cooled to 5°.

ETHYL OLEATE

The "British Pharmacopoeia" (6) recognizes ethyl oleate as an alternative vehicle in injections of deoxycortone acetate, estradiol monobenzoate, progesterone, and testosterone propionate.

It is a yellowish oily liquid which is insoluble in water and miscible with alcohol, ether, and fixed oils. It has properties similar to fixed oils, except that it is less viscous, is a superior solvent, and is more rapidly absorbed by the tissues (7). Unlike untreated sesame oil, ethyl oleate remains clear at 5°, but it has the disadvantage of discoloring on standing.

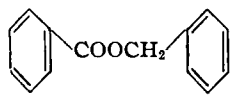
There are indications of increased hormone activity when ethyl oleate is used in place of sesame oil as a parenteral hormone vehicle. Studies by Dekanski and Chapman (8) demonstrated improved intensity and duration of action of testosterone phenylpropionate and testosterone propionate in ethyl oleate over that of the same androgens in sesame oil.

ISOPROPYL MYRISTATE

The use of isopropyl myristate as a vehicle for parenteral injections has been reported by Platcow and Voss (9). It is an oil miscible, water immiscible, chemically stable substance, not susceptible to rancidity and having a specific gravity of 0.852 (10). It consists mainly of isopropyl myristate and a small amount of isopropyl esters of other saturated fatty acids. Acute toxicity studies indicate a very low order of toxicity, but attempts to establish an LD_{50} in mice failed when dosages equivalent to 100 ml./Kg. did not affect the test animals. Isopropyl myristate shows a very low degree of irritability and exhibits no sensitizing properties in rabbits and guinea pigs following topical and parenteral administration. In experiments on ovariectomized rats it compared favorably with sesame oil as a repository vehicle for estrogens (9). The external pharmaceutical use has been evaluated by Donovan, *et al.* (11), who found it a useful intermediate solvent for the incorporation of phenol, cocaine, resorcinol, and salicylic acid into liquid petrolatum.

BENZYL BENZOATE

Benzyl benzoate (12) is a colorless, oily liquid with a pleasant aromatic odor. It has a specific gravity of 1.118, boils at 323°, and is insoluble in water or glycerin, but is miscible with alcohol, chloroform, ether, and fixed oils. Its structural formula is

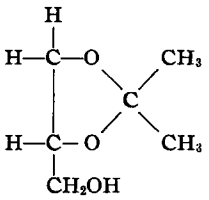


Benzyl benzoate has found some use as a cosolvent in oleaginous injectables such as dimercaprol injection, and in commercial preparations of hydroxyprogesterone benzoate where it is present in concentrations of 30% for the 125-mg. product in sesame oil, and 46% for the 250-mg.

product in castor oil. The formula for the injection of dimercaprol B.P. (1958) is dimercaprol 5.0 Gm., benzyl benzoate 9.6 ml., and peanut oil, to make 100 ml.

DIOXOLANES

The dioxolanes represent a new and interesting group of synthetic solvents for pharmacists. These substances are the reaction products of glycerin with ketones in the presence of a dehydrating agent (13). The least toxic member of the group is 2,2-dimethyl-1,3-dioxolane-4-methanol (14). This structural formula is



This compound [also known as Solketal, isopropylidene glycerol, and glycerol dimethylketal (15)] is reported to be a nontoxic, nonirritating solvent, miscible with water, alcohol, esters, aliphatic and aromatic hydrocarbons, and virtually all other organic solvents. It is a water-white, practically odorless liquid of medium viscosity, stable on storage, and unaffected by alkalies. Its boiling point is 82–83°, and has a specific gravity of 1.064. It is, however, hydrolyzed by strong aqueous acid solutions to acetone and glycerin (15–19).

Berger (14) reported the dioxolanes (as a class) produce transient muscular relaxation and paralysis. These effects were due to a depressant action on the central nervous system and not to a peripheral curare-like action. The mean paralyzing dose, ED_{50}, and the mean lethal dose, LD_{50}, for 2,2-dimethyl-1,3-dioxolane-4-methanol after intraperitoneal administration in mice was reported to be greater than 2.112 Gm./Kg. (16.0 mM/Kg.).

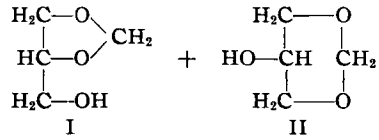
Teuber (20, 21) reported that the oral LD_{50} in mice is 4.0 to 7.2 Gm./Kg. (30–55 mM/Kg.). Dermatologic tests on rabbits produced no untoward reactions after 3 weeks of application.

Guinea pigs exposed to aerosol sprays for 1 hour a day for 3 days exhibited no inflammation of mucous membranes. Huber (22) reported on the use of antibiotic aerosol sprays utilizing this solvent as a carrier for penicillin and oxytetracycline.

In this country, 2,2-dimethyl-1,3-dioxolane-4-methanol has been reported in the patent literature as a water-miscible solvent in gelatin capsules (23) and as a parenteral vehicle for a tetracycline preparation (24).

GLYCEROL FORMAL

Glycerol formal is a condensation product of glycerol and formaldehyde consisting of 3-hydroxymethyl-1,3-dioxolane (I) (25%) and 5-hydroxy-dioxane (II) (75%). The structural formulas are

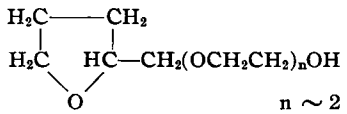


I II

It is a chemically stable, colorless, odorless liquid of low viscosity and is miscible with water in all proportions. Sanderson (25) reported that the LD_{100} for rats by intraperitoneal administration was 3000 mg./Kg. and that the maximum symptomless dose was 1500 mg./Kg. The use of glycerol formal as a nontoxic solvent in toxicity testing has been suggested. It has been used as an industrial solvent and no toxic effects have been reported (25).

GLYCOFUROL

Glycofurol is a Hoffmann-LaRoche trade name for a tetrahydrofurfuryl alcohol polyethylene glycol ether containing an average of two ethylene glycol units per molecule. It is a colorless liquid, miscible with water in all proportions and soluble in methanol, ethanol, *n*-propanol, and glycerin. It has a boiling point of 80–155° and a specific gravity of 1.078. The structure is (26)

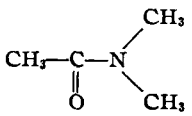


Spiegelberg and co-workers (26) extensively studied the pharmacology of this material and reported on its use as a parenteral solvent. Irritating when administered undiluted, it is nontoxic and nonirritating when diluted with water. The intravenous LD_{50} in the mouse is 3.78 Gm. (3.5 ml.)/Kg., and its tolerability equals that of propylene glycol. Acetylcholine chloride is reported to be stable in glycofurol solutions, while it is not stable in propylene glycol.

DIMETHYLACETAMIDE

Dimethylacetamide (DMA), also known as acetic acid dimethylamide and acetyldimethylamine, is an interesting solvent which warrants some discussion. It is a clear neutral liquid

having a boiling point of 165.5°, a specific gravity of 0.943, and a molecular weight of 87.12. The structural formula is



This solvent is miscible in all proportions with water and alcohol and very soluble in organic solvents and mineral oil (27).

Davis and Jenner (28) studied the acute toxicities of dimethylacetamide, dimethylformamide (DMF), and propylene glycol after single doses were administered intraperitoneally to mice. A 50% solution of DMA was used; however, the toxicity results are for the DMA content of the solution. The DMF and propylene glycol were administered undiluted. These results are as follows: the LD_{50} for DMF was 1122 mg./Kg., for DMA 3236 mg./Kg., and for propylene glycol 11,400 mg./Kg. The LD_{100} for DMA is 5012 mg./Kg.

Horn (29) investigated the chronic toxicity of DMA by dermal application in dogs at dosage levels of 0.1 to 4.0 mg./Kg. and by exposing both rats and dogs to an atmosphere containing DMA at concentrations of 40.0, 64.4, 102, and 195 p.p.m. All experiments were of 6-month duration unless obvious toxicity occurred. Liver damage occurred at all levels greater than 0.1 ml./Kg. dermally and 40 p.p.m. by inhalation.

The patent literature mentions the use of a 50% DMA solution as a vehicle for a preconstituted oxytetracycline (30) solution and as a solvent in soft and hard gelatin capsules (31). Its use as an anti-inflammatory agent in topical formulations is also reported (32).

Hammer, *et al.* (33), reported on a preconstituted intramuscular solution of oxytetracycline which consisted of a solution of an ethanolammonium magnesium salt of oxytetracycline in 50% N,N-dimethylacetamide. After testing in animals and humans, this formulation was found to be well tolerated and produced effective antibiotic serum levels. The stability was satisfactory for 2 years at room temperature.

A 50% solution of DMA is used as a solvent for a 250-mg./ml. chloramphenicol intravenous formulation, but it must be diluted with normal saline or 5% dextrose before administration.

A commercially available reserpine intramuscular product contains 10% DMA as a cosolvent (34).

DMA, when used as a drug solvent and administered to 15 patients with advanced malignancies produced hallucinations when given at levels above 400 mg./Kg. of body weight per day for 3 days or more (35). However, the normal parenteral level for DMA is equivalent to 30 mg./Kg. per day. Thus, in normal use this hallucinagenic effect would not be expected.

An oxytetracycline 50-mg./ml. formula (30) was reported to have been composed of oxytetracycline, 50 mg.; magnesium chloride, hexahydrate 1.7%; ethanolamine, 20% aqueous 1.5%; sodium formaldehyde sulfoxylate 0.2%; lidocaine 2%; and N,N-dimethylacetamide 50%, to make 1.00 ml.

N-(β-HYDROXYETHYL)-LACTAMIDE

N - (β - Hydroxyethyl) - lactamide (36), also known as lactic acid carboxamide, is a clear, colorless, syrupy liquid which is water miscible. The specific gravity of the pure compound is 1.192. It is used as a 50% solution and has the following formula: $CH_3CHOHCONHCH_2CH_2OH$. This compound is the reaction product of methyl acetate and 2-aminoethanol. The acute subcutaneous, LD_{50}, toxicity (37) for a 50% w/v N-(β-hydroxyethyl)-lactamide solution is 15.8 Gm. lactamide/Kg. in mice and 16.1 Gm. lactamide/Kg. in rats. This compound has been used in Europe as a solvent for a preconstituted oxytetracycline solution. Neumann (37) reported that this product was stable for several years and showed improved tissue tolerability.

Dimmling (38) has also reported on the use of N-(β-hydroxyethyl)-lactamide as a solvent for oxytetracycline. After 24 hours, a detectable serum level was found after a single dose of 250 mg. in ten healthy persons. Following a second injection of 250 mg. after an interval of 24 hours, the levels showed a cumulative increase. Further studies (39) on the serum concentrations confirmed the previous findings.

The results of Seeliger's (40) investigation with oxytetracycline intramuscular in N-(β-hydroxyethyl)-lactamide solution in patients have confirmed the previous values obtained in healthy individuals. A single injection of 250 mg. gave an effective serum concentration for over 24 hours. Following repeated injections on consecutive days, marked cumulative effects were observed. The clinical effect was in accordance with blood level determinations. Survey of local tolerability showed practically no pain in 93.7% of the 380 injections performed; slight and tolerable local reactions, which in no case persisted for more than 2 or 3 hours, were found in 6.3%.

Hupe (41) reported that in 90 major surgical cases 250 mg. of oxytetracycline intramuscular in this solvent, once a day, was effective and well tolerated.

The patent literature also refers to the use of N-(β-hydroxyethyl)-lactamide as a solvent for oxytetracycline injectables (30, 42). The use of other alkylol amides such as the amides of β-hydroxybutyric acid, succinic acid, adipic acid, tartaric acid, glycolic acid, and salicylic acid, was also mentioned (42). A typical formula for a 250 mg./3 ml. oxtyetracycline product is

	Gm./100 ml.
Oxytetracycline hydrochloride	9.62
Magnesium chloride-hexahydrate	4.00
Sodium formaldehyde sulfoxylate	0.20
Water, pyrogen-free	44.20
N-(β-hydroxyethyl)-lactamide	50.00
Monoethanolamine	2.30

ETHYL LACTATE

Ethyl lactate, ethyl α-hydroxypropionate, $CH_3CH(OH)COOCH_2CH_3$, is a colorless liquid with a specific gravity of 1.042 which is miscible with water and has a characteristic odor. In aqueous solution some decomposition takes place (43).

Latven and Molitor (44) determined the acute toxicity of ethyl lactate in mice by subcutaneous and intravenous administration, and their results are shown in Table II.

TABLE II.—ACUTE TOXICITY OF ETHYL LACTATE

	LD_0	LD_{50}	LD_{100}	Minimum Sympt. Dose	Maximum Nonsympt. Dose
Subcutaneous, ml./Kg.	2.0	2.5	3.0	1.0	0.8
Intravenous, ml./Kg.	0.2	0.6	1.0	0.3	0.2

Ethyl lactate was irritating on intradermal injection in guinea pigs and on application to the eyes of rabbits.

Ethyl lactate (10–100%) solubilizes an esterone injection in castor oil to a concentration of 3.5 to 6.5 mg./ml. (45). This product is stable at room temperature (46). It has been used as an industrial solvent and no toxic effects from its use have been recorded (47).

ETHYL CARBONATE

Ethyl carbonate, diethyl carbonate, $CH_3CH_2OCOOCH_2CH_3$, has also been used as a solvent for erythromycin, but there is a paucity of literature on its use and toxicity. It is a liquid immiscible with water but miscible with alcohol and ether and has a specific gravity of 0.975 and a boiling point of 126° (48). This compound has also been used as an industrial solvent with no reported toxic effects (49).

POLYETHYLENE GLYCOLS

The polyethylene glycols (PEGs), as the name implies, are polymers of ethylene oxide (50) with the general formula: $HOCH_2(CH_2OCH_2)_nCH_2OH$, where n represents the average number of ethylene oxide groups. These polymeric products are designated by a number which represents the average molecular weight (51). Polyethylene glycol 200, 300, 400, and 600 are moderately viscous, colorless, somewhat hygroscopic liquids. They are less volatile than glycerin and do not hydrolyze or deteriorate (52). They dissolve in water in all proportions to form clear solutions (51). Polyethylene glycol 1000, 1540, 4000, and 6000 are white waxy solids (Table III).

For the purpose of this discussion, we will confine our comments to the liquid polyethylene glycols which are more likely to be used in parenteral products. The literature abounds with papers discussing and describing measurements of the toxicity of the various PEGs by oral or topical routes (50–58). However, there is a scarcity of material on the parenteral administration. PEG 300 and 400 are better described than the other two members of the liquid PEG series. Only the parenteral LD_{50}'s (as shown in Table IV) were found by the authors. Oral and dermal toxicity data are given in Tables V and VI.

Subcutaneous dosages of PEG 400 up to 10 ml. (ten times the human dose) in rats caused no permanent damage. The reactions were described as "blanching of the skin and scab formation in 48 hours." The test results were reported to be the same as with propylene glycol. PEG 300 and 400 do not elicit a foreign body reaction in animals (52). In dogs, the removal of PEG

TABLE III.—PHYSICAL PROPERTIES OF POLYETHYLENE GLYCOLS (51)

PEG	Av. Mol. Wt.	Sp. Gr., 20° C.	Freezing or Melting Range, ° C.	Viscosity, Cps. at 210° F.	Comparative Hygroscopicity Glycerol = 100
200	190–210	1.125	Super Cools	4.3	70
300	285–315	1.125	−15 to −8	5.8	60
400	380–420	1.125	4 to 8	7.3	55
600	570–630	...	20 to 25	10.5	40
1000	950–1050	1.151	37 to 40	17.4	35
1540	1300–1600	1.151	43 to 46	25–32	30
4000	3000–3700	1.204	53 to 56	75–85	...
6000	6000–7500	...	60 to 63	700–900	...

TABLE IV.—PARENTERAL TOXICITY OF POLYETHYLENE GLYCOLS

PEG	Animal	Route	Dose	Value, mg./Kg.	Ref.
300	Female rat	i.v.	LD_{50}	7,979	52
300	Rat	i.p.	LD_{50}	19,125	50
400	Mouse	i.p.	LD_{50}	4,200	59

TABLE V.—ORAL AND DERMAL TOXICITY OF POLYETHYLENE GLYCOLS IN MICE (60)

PEG	LD_{50} Mouse, oral ml./Kg.	Sublethal Dose Mouse, oral ml./Kg.	Sublethal Dose Mouse, s.c. ml./Kg.	LD_{100} Mouse, oral ml./Kg.
200	38.3	30.0	6.5	55
600	47.0	16.0	8.0	60

TABLE VI.—ORAL TOXICITY OF POLYETHYLENE GLYCOLS IN RATS (51)

Polyethylene Glycol	Single Oral LD_{50} Rats Gm./Kg.	No-Effect Dose with Repeated Feedings, Rats Gm./Kg./Day
200	28.9 ml. (32.51 Gm.)	0.88 (2 yr.)
300	31.7 ml. (35.66 Gm.)	5.4 (90 days)
400	43.6 ml. (49.05 Gm.)	0.96 (2 yr.)
600	38.1 ml. (42.86 Gm.)	2.42 (90 days)

from the site of injection is rapid, since the material diffuses freely into surrounding tissue.

When PEG 400 was injected intravenously into humans, 77% was recovered in the first 12 hours. Intramuscular injections in rats of five to ten times the expected human dosage levels of PEG 300 produced ischemic necrosis of the muscle fibers when the dose infiltrated a muscle bundle. The tissue response was one of mild chemical inflammation (50).

Lee and Anderson (61) determined the toxicity of vancomycin in 50% PEG 200 and of PEG 200 alone. Their results indicated that PEG 200 produced no apparent toxic effects when given to dogs at 1.0 ml./Kg. per day for 80 days intramuscularly, or of 0.5, 1.0, 2.5, and 5.0 ml./Kg. as single intravenous doses. Venous carbon dioxide content, blood nonprotein-nitrogen, and blood alkaline phosphatase were normal, no gross or microscopic abnormality was found in the kidneys, circulatory system, or other organs.

A dosage form for intravenous use containing nitrofurantoin was marketed some years ago containing PEG 300. In a study by McCabe, *et al.* (62), it was found that daily administration of 240 mg. of nitrofurantoin in PEG 300 to 30 patients caused severe metabolic acidosis and nephropathy in seven resulting in two deaths. These damaging effects were attributed to the PEG rather than nitrofurantoin and that dosage form was withdrawn from the market.

It should be noted that drugs dissolved in the PEGs may well present a toxicity and drug level much different from those reported in aqueous solutions or suspensions (51).

Swanson and co-workers (63) investigated the effect on the activity and toxicity of sodium amobarbital and sodium secobarbital by the addition of 60 and 70%, respectively, of polyethylene glycol 200. Their findings indicated that the addition of PEG 200 showed approximately the same potency and toxicity as aqueous solutions of these barbiturates. The median anesthetic dose (AD_{50}) by vein in rats was 75.0 ±3.5 mg./Kg. for sodium amobarbital and 39.0 ±1.2 mg./Kg. for sodium secobarbital. The median lethal doses (LD_{50}) were approximately twice as large as the AD_{50}'s. This ratio of nearly 2:1 is common to most barbiturates in use. It was also reported that subacute toxicity experiments show that both barbiturates in PEG 200 produced no obvious pathological changes. When injected intramuscularly in rabbits the aqueous solutions of the two barbiturates produced more irritation in tissues than solutions in PEG 200.

Bodin and Taub (64) investigated the stability of sodium pentobarbital in aqueous solutions containing 0 to 60% of polyethylene glycol 400. Their results indicated that the pH influences the concentration of polyethylene glycol 400 required for optimum stability. At a concentration of 30% and at a pH of 10, aseptic formulation of a stable product is possible. The addition of 10% ethanol permits sterilization by autoclaving without discoloration. It is also possible to prepare formulations containing 60% of the glycol and 10% ethanol at a pH as low as 8. These solutions are also stable to autoclaving.

Linde (65) studied the extent to which a number of sodium salts of 5,5-disubstituted barbiturates such as phenobarbital, barbital, aprobarbital, amobarbital, and pentobarbital could be stabilized by propylene glycol, polyethylene glycol 400, glycerin, and alcohol. Both the glycols, as well as alcohol, showed the same stabilizing activity. Glycerin had considerably lower stabilizing properties. With glycols as stabilizers, the various barbiturate derivatives studied displayed great differences in stability. The concentration of the solvent added to the solution was found to be the factor governing the stabilizing effect. The stability increased as the concentration was increased. A concentration of

50% was the highest used. The author (65) also established that stability of barbiturates dissolved in pure propylene glycol and polyethylene glycol 400 was very good, and that a lowering of pH in alcohol solutions decreases the rate of deterioration.

The use of 10% polyethylene glycol 300 as a solubilizing agent for a 2.5 mg./ml. injection of reserpine has been reported by Leyden, *et al.* (66). Commercial reserpine products containing 10–30% PEG 300, as well as 25% PEG 400, are available.

Higuchi and Lach (67) reported that although pentobarbital and barbital have little or no tendency to complex with polyethylene glycols, phenobarbital forms stable and stoichiometric molecular compounds with these macromolecular substances. Analysis indicates that one phenobarbital molecule is bound by two ethylene oxide units of the polyether chain. They have also shown that phenolic compounds are bound by the polyethers in the same manner, the higher molecular weight polymers exhibiting greater complexing tendency than those of lower degrees of polymerization. Several organic acids, such as salicylic acid and *p*-hydroxybenzoic acid, are only weakly bound.

An erythromycin ethyl succinate intramuscular product in a PEG vehicle is available, as is a secobarbital parenteral product in a 50% PEG vehicle. The specific PEG used was not indicated in either case.

GLYCERIN

Glycerin (68) is a clear, viscous, high-boiling liquid, which is miscible with water and alcohol and is a good solvent for many compounds. It is hygroscopic and will absorb several times its weight of water under conditions of high humidity. Glycerin tends to supercool rather than crystallize at lower temperature; its aqueous solutions resist freezing.

The toxicity (69) is low with oral administration. No deleterious effects were observed in man with 110 Gm. of glycerin per day for 50 days. Normal growth and reproduction occurred in rats with 41% of glycerin added to the food for 40 weeks, and in dogs with 35% added for 50 weeks. Drill (70) reports the oral LD_{50} in rats for undiluted glycerin to be 25 Gm./Kg. and the intravenous LD_{50} is 5 to 6 Gm./Kg.

There appears to be some controversy regarding the use of glycerin in parenterals, as indicated by reports that its administration to animals has caused hemoglobinurea (71, 72), hypotension (73, 74) and central nervous disturbances (74), and weight loss (75). Hanke (76) has given an excellent review of the physiologic action of glycerin.

There are, however, references to its use in human parenteral therapy. No toxic effects were noted after the administration of very small amounts of glycerin by intra-arterial administration in the treatment of elephantiasis (77).

Sloviter (78, 79) reported that the intravenous administration of solutions containing 5% glycerin to experimental animals and to man caused no toxic or other undesirable effects. Humans were given intravenously 50 Gm. of glycerin in a liter of solution which also contained 50 Gm. of glucose and 9.0 Gm. of sodium chloride. No disturbances of cardiorespiratory or central nervous system function occurred, and no significant hemolytic effects occurred. Sloviter (78, 79) further stated that the previously reported toxic effects of parenterally administered glycerin were not observed, and that there were indications that these toxic effects were due to the osmotic disturbances produced by the injection of solutions of high glycerin concentrations. In the dog, the rapid injection of a concentrated solution produced a transient drop in blood pressure which the author believed to be due to a peripheral vasodilating effect. It was also suggested that intravenously administered glycerol may be useful as a nutritional agent as well as for a solvent in preparations of drugs for intravenous administration (79).

Large parenteral doses cause convulsant and paralytic symptoms through direct action on the central nervous system. The blood corpuscles are also laked, probably caused by an osmotic effect as previously mentioned. The glycerin remains for a time, unabsorbed, and in high concentration at the site of injection, and the corpuscles are probably laked during their passage through this area (69).

Lachaux (80) states that aqueous solutions of up to 30% glycerin are well tolerated intramuscularly. Absorption is good and there is rapid dilution by the body fluids.

Latven and Molitor (44) determined the intravenous and subcutaneous toxicity in white mice. Their results are shown in Table VII.

TABLE VII.—ACUTE TOXICITY OF GLYCERIN

	LD_0	LD_{50}	LD_{100}	Min. Sympt. Dose	Max. Non-Sympt. Dose
Subcutaneous, ml./Kg.	8.0	10.0	12.0	8.0	7.0
Intravenous, ml./Kg.	5.0	6.0	7.0	3.0	2.0

Krause and Cross (81) have found that the solubility of phenobarbital in alcohol was enhanced by the addition of glycerin. The maximum solubility of phenobarbital in alcohol–glycerin mixtures was reached at a level of 80% alcohol and 20% glycerin.

Linde (65), in his work on sodium salts of barbiturates, found that glycerin has considerably lower stabilizing properties than propylene glycol, polyethylene glycol, and alcohol.

Husa and Jatul (82) found that after heating a sodium phenobarbital solution in 50% glycerin for 30 minutes at 115°, deterioration was 8% compared to 9% in pure water solution.

The U.S.P. (1) allows the use of glycerin in injections of deslanoside and digitoxin. A commercial product of deslanoside contains 15% glycerin.

ETHANOL

Ethanol (alcohol), ethyl alcohol, finds occasional use in parenteral products, particularly the digitalis glycosides. A commercial digitoxin preparation for intramuscular or intravenous use contains 49% alcohol. This produces pain on intramuscular administration. The U.S.P. (1) states that such a product can contain 5–50% alcohol. A digoxin preparation containing 10% alcohol, as allowed by the U.S.P., may be used for intramuscular or intravenous administration. A deslanoside product containing 7.4% alcohol is also available.

Alcohol injected subcutaneously causes considerable pain followed by anesthesia. If injection is made close to nerves, neuritis and nerve degeneration may occur. Injection in or near nerves is deliberately used to cause anesthesia in the treatment of severe pain. The intravenous anesthetic dose is 2–3 ml. of 95% alcohol/Kg. (83).

Latven and Molitor (44) have reported the LD_{50} in mice to be 1973 mg./Kg. intravenously, and 8285 mg./Kg. subcutaneously.

A commercial hydrocortisone intravenous product contains 50% alcohol. Mephensin injection B.P. (1958) contains 25% alcohol, its formula is mephenesin 10 Gm.; alcohol (95%) 25 ml.; propylene glycol 15 ml.; and water, pyrogen-free, to make 100 ml.

The formula for digoxin injection B.P. (1960 Supplement) is digoxin 25 mg.; alcohol (80%) 12.5 ml.; propylene glycol 40 ml.; citric acid 75 mg.; sodium phosphate 0.45 Gm.; and water, pyrogen-free to make 100 ml.

PROPYLENE GLYCOL

Seidenfeld and Hanzlik (84) in 1932 stated that propylene glycol, which was first described in 1895, has commanded little or no interest in chemistry or medicine even though it is available commercially. Today, it is one of the most widely used nonaqueous solvents.

Propylene glycol, 1,2-propanediol, is a viscous hygroscopic liquid with a specific gravity of 1.036. It freezes at −59° and boils at 188° and is miscible with water, acetone, and chloroform, but is immiscible with fixed oils. Under ordinary conditions it is very stable but at high temperatures it is oxidized to propionaldehyde, lactic acid, pyruvic acid, and acetic acid (85, 86).

The toxicity of propylene glycol has been extensively studied (44, 84, 87–91). Seidenfeld and Hanzlik (84) reported the intravenous minimum fatal dose for white rats to be 1.68 Gm./Kg. and for rabbits, 5.25 Gm./Kg. The intramuscular minimum fatal dose was 14.7 Gm./Kg. for rats and 7.5 Gm./Kg. for rabbits. Mice are more sensitive to propylene glycol (92) and have a lower toleration for this solvent. The LD_{50} for mice by intraperitoneal injection was found to be 9.7 Gm./Kg. (92). The subcutaneous and intravenous LD_{50} for mice was reported to be 18.5 Gm./Kg. and 8.0 mg./Kg., respectively (44). The action of propylene glycol on the central nervous system in dogs is similar to that of ethyl alcohol; however, its activity is about one-third that of ethyl alcohol (93).

Brittain and D'Arcy (94) reported that intravenous injections of propylene glycol in saline solution up to 50% in rabbits had no effect on the red blood cell count, packed cell volume, hemoglobin, or total white blood cell count. There was, however, an increase in the number of circulating polymorphs and a decrease in the lymphocytes. Blood clotting time was markedly decreased.

Heine, *et al.* (95), have given several formulas illustrating the use of propylene glycol in the preparation of barbiturate solutions for intramuscular or intravenous use

Sterile Solution of Sodium Pentobarbital 0.150 Gm./ml.	
Sodium pentobarbital	15.0 Gm.
Benzyl alcohol	2.0 ml.
Propylene glycol	60.0 ml.
Water for injection, to make	100.0 ml.

Sterile Solution of Sodium Amobarbital 0.250 Gm./ml.	
Sodium amobarbital	25.0 Gm.
Benzyl alcohol	2.0 ml.
Propylene glycol	60.0 ml.
Water for injection, to make	100.0 ml.

Sterile Solution of Sodium Phenobarbital 0.150 Gm./ml.	
Sodium phenobarbital	15.0 Gm.
Benzyl alcohol	2.0 ml.
Propylene glycol	60.0 ml.
Water for injection, to make	100.0 ml.

Brass (96) prepared a quinidine formulation for intramuscular use composed of 10.0 Gm. of quinidine hydrochloride and 75.0 ml. of propylene glycol. This solution showed no signs of discoloration or crystallization over a period of 6 months. When injected in man, the evidence of the action of quinidine is detectable in 15 minutes and persists for approximately 2 hours.

Gluck, *et al.* (97), tested a 20% solution of quinidine sulfate in propylene glycol for its local irritant effects by intramuscular injection and for its action on the auricle in auricular fibrillation and flutter. The results indicated negligible local reaction and a dose response similar to that obtained with oral administration—peak effect being reached in an average of about 3 hours, about half of the effect wearing off in about 8 hours, and the effect disappearing completely in 24 hours or less.

The use of propylene glycol as a vehicle for the intravenous administration of desoxycorticosterone acetate in a concentration of 10 mg./ml. has been discussed by McGavack and Vogel (98). Since this compound crystallizes out when diluted with water, a technique is reported whereby the preparation is injected slowly, not to exceed a rate of 2.5 ml. per minute. This assures its rapid dilution and prevents the formation of any acicular precipitate.

Ganz and co-workers (99) have studied the intramuscular administration of digoxin in 40% propylene glycol and 10% ethanol in patients with auricular fibrillation and reported satisfactory results.

The comprehensive use of propylene glycol is reviewed in articles by Parisi (100), Gialdi and Baruffini (101), Brown (102), and Heine, *et al.* (95).

Gershenfeld and Witlin (103) investigated the use of propylene glycol as a solvent for iodine and suggested that iodine 2% and sodium iodide 2.4% in distilled water containing 25 to 50% propylene glycol would be a suitable formula.

Linde (65) reported that the stability of barbiturates dissolved in pure or diluted propylene glycol was satisfactory.

Peterson and Hopponen (104) determined the solubility of phenobarbital in propylene glycol–alcohol-water systems. The solubility reached a maximum at a 50% mixture of propylene glycol and alcohol. Propylene glycol, 100%, dissolves more phenobarbital than 100% alcohol. Upon addition of water to the solvents, the solubility falls off more quickly with propylene glycol than with alcohol.

Propylene glycol was found to potentiate the preservative action of the parabens (105). Mehta and Drommond (106) determined the stability of various medicaments in propylene glycol after storage at 25°.

Marcus and Taraszka (107) reported that the specific hydrogen ion–catalyzed degradation of chloramphenicol in solutions containing up to 50% (v/v) of propylene glycol was qualitatively similar to degradation in purely aqueous systems. The reactions remain pseudo-first order with respect to chloramphenicol and the direct dependence of the rate upon acid concentration is maintained. Their results show that it is unwise to assume that replacement of all or a portion of the water in a vehicle will always enhance the stability of the active ingredient.

Huttenrauch (108) has shown that propylene glycol enhances the stability of L-ascorbic acid in tests run for 120 weeks at room temperature.

Weinstein, *et al.* (109), reported that oxytetracycline intramuscular solution in propylene glycol was well tolerated and produced prompt, prolonged, satisfactory blood levels. A 250 mg./-2 ml. oxytetracycline intramuscular formula would be

Oxytetracycline	250 mg.
Lidocaine	2%
Magnesium chloride, hexahydrate	6%
Sodium formaldehyde sulfoxylate	0.5%
Ethanolamine	4.2%
Propylene glycol	67%
Water	16.8%

This formula is stable for 2 years at room temperature. Without the use of propylene glycol, the stability is of 2-day duration only.

Hanson (110) compared the amount of tissue reaction produced by subcutaneous and intramuscular injections of a number of parenteral preparations of broad-spectrum antibiotics. The smallest amount of tissue reaction was produced by propylene glycol solutions of oxytetracycline.

1,3-BUTYLENE GLYCOL

1,3-Butylene glycol, 1,3-butanediol, is a colorless, viscous liquid having a specific gravity of 1.005, a boiling point of 204°, and is soluble in water and alcohol (111). It is possible to modify the effect of drugs by choosing the proper solubilizing agent. In this manner, we can cause an increase or decrease in the effect of a drug. Bornmann, *et al.* (112), demonstrated that 1,3-butylene glycol can prevent the toxic reactions of pentamethylenetetrazol. It was also shown that the action of morphine hydrochloride, meperidine hydrochloride, and methadone hydrochloride in 1,3-butylene glycol, as compared to aqueous solutions, gave stronger and more prolonged effects

in a shorter time. Thus, by the use of this solvent, the dose may be lowered and undesirable side effects eliminated.

Bornmann and Loeser (113) reported on the use of propylene glycol and 1,3-butylene glycol as solvents for meprobamate. The 1,3-butylene glycol preparation was found to be slightly more toxic than the propylene glycol product, but both were suitable as solvents for the drug.

Bornmann (114–116) in his review of glycol toxicity, stated that 1,3-butylene glycol had a slight acute as well as a slight chronic toxicity. The LD_{50} subcutaneously in mice is 16.51 ml./-Kg. and in rats is 20.06 ml./Kg.

SUMMARY

A large number of organic solvents are currently available to the pharmaceutical formulator The three most commonly used nonaqueous solvents are fixed oils, propylene glycol, and the polyethylene glycols. The remaining solvents discussed are of minor importance and are used only in specific instances.

The use of nonaqueous solvents in parenteral products offers greater latitude in the formulation of new dosage forms. Such solvents, however, should be used only if a definite need is established. It must be recognized that any formulation containing a nonaqueous solvent is potentially a new entity and must be adequately tested.

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Research Articles

Nonclassical Antimetabolites XII

Bridge Principle of Specificity with Exo-Alkylating Irreversible Inhibitors V. Differences in Specificity of Enzymic Nucleophilic Sites as Detected by the Carbophenoxy Group

By B. R. BAKER and R. P. PATEL

Investigation of four carbophenoxyamino derivatives of salicylic acid and oxanilic acid has shown that all four reversibly bind to lactic dehydrogenase (LDH) and glutamic dehydrogenase (GDH). Three of these compounds showed irreversible inhibition of GDH with no irreversible effect on LDH. Since 4-(iodoacetamido)-salicylic acid had previously been shown to inhibit both enzymes irreversibly, the specificity shown by the carbophenoxyamino group is attributed to its specificity for reaction only with a primary amino group in the nucleophilic site of an enzyme.

RELATIVELY LARGE molecules—compared to the substrate—have been found that can inhibit an enzyme and have accordingly been called nonclassical antimetabolites (3, 4), in contrast to classical antimetabolites that have only a small change in structure compared to the substrate. Based on the nonclassical antimetabolite theory, it was possible to propose the concept of irreversible inhibition by an exo-alkylation mechanism (3); a properly designed inhibitor such as 4-(iodoacetamido)salicylic acid can reversibly complex with glutamic dehydrogenase (GDH),[1] then become irreversibly bound within the complex by alkylation of the enzyme adjacent to the active site. Strong experimental evidence to support this exo-alkylation phenomenon has been presented (5, 6).

Experimental observations pertinent to the exo-alkylation phenomenon were subsequently

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A preliminary communication on part of this work has appeared (2).
For previous paper in this series see Reference 1.

[1] LDH, lactic dehydrogenase; GDH, glutamic dehydrogenase; DPNH, reduced diphosphopyridine nucleotide; Tris, tris-(hydroxymethyl)aminomethane hydrochloride buffer; 4-ISA, 4-(iodoacetamido)salicylic acid.