REVIEW ARTICLE

The Potential Use of Cyclodextrins in Parenteral Formulations

MARCUS E. BREWSTER*†△, JAMES W. SIMPKINS†‡, MANINDER SINGH HORA§, WARREN C. STERN* and NICHOLAS BODOR†

Pharmatec, Inc., Alachua, Florida; College of Pharmacy, University of Florida, Gainesville, Florida; and Cetus Corporation, Emeryville, California

ABSTRACT: The general use of cyclodextrins in drug formulations is reviewed. The ability of cyclodextrins to form reversible inclusion complexes with many drugs can eliminate various undesirable physicochemical properties. While β-cyclodextrin is extremely useful in many of these applications, it is toxic when given parenterally, precluding its use in i.v. and other formulations. Chemically modified cyclodextrins such as 2-hydroxypropyl-β-cyclodextrin are amorphous isomeric mixtures which are potent complexing agents and innocuous when administered i.e., either acutely or subchronically. The use of these modified cyclodextrins in parenteral formulations and to solubilize and stabilize various proteins and peptides is presented.

Introduction

The inability to formulate poorly water soluble or stable drugs intended for parenteral administration is a vexing and longstanding concern. Several methods have been developed to address the problems of aqueous incompatibility of pharmacologically active agents including the use of prodrugs, organic cosolvents, emulsions, liposomes, and micelles (1–4). Unfortunately, the absolute requirement of low toxicity for parenteral delivery systems has frustrated the application of some or all of these approaches. An alternative to these methods is the use of cyclodextrins. Cyclodextrins, by virtue of their ability to form inclusion complexes with many drugs, can substantially increase the aqueous solubility of pharmaceuticals. Unfortunately, some cyclodextrins are nephrotoxic precluding their application to parenteral formulations. One potentially useful class of these cyclic starches, i.e., hydroxyalkylated-β-cyclodextrins appear to lack the toxic potential of unsubstituted or alkylated cyclodextrins and as a result may be useful in i.v. and other products. As these hydroxyalkyl cyclodextrins, specifically 2-hydroxypropyl-β-cyclodextrin, effect molecular encapsulation using forces identical to those employed by the natural cyclodextrins, these unsubstituted starches will be first considered. The inappropriateness of these natural and other cyclodextrins for parenteral administration will be discussed and the specific physiochemical and toxicological advantages of hydroxyalkyl cyclodextrins described. This is not intended to be an exhaustive review of cyclodextrins as excellent contributions have already been published in the literature. Rather, this work attempts to illustrate the potential use of one class of cyclodextrins in parenteral formulation. Finally, three examples of such application to parenteral administration will be presented. These include: the use of 2-hydroxypropyl-β-cyclodextrin complexes of glucocorticoids as replacements for 21-phosphate prodrugs of these materials, the use of a 2-hydroxypropyl-β-cyclodextrin complex of an estradiol chemical delivery system in human clinical trials and the use of 2-hydroxypropyl-β-cyclodextrin as a solubilizing and/or stabilizing excipient for peptides and proteins.

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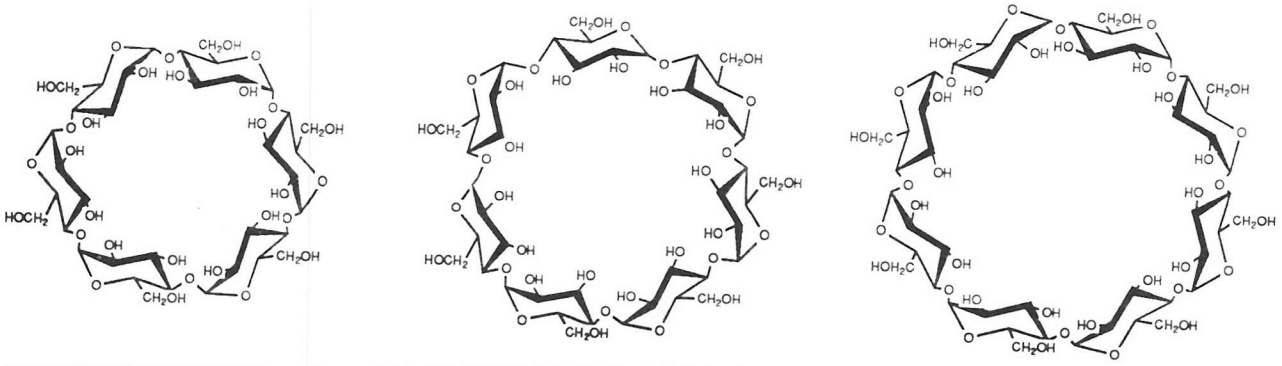
* Pharmatec, Inc., P.O. Box 730, Alachua, FL 32615.
† Center for Drug Design and Delivery, College of Pharmacy, University of Florida, Gainesville, FL 32610.
‡ Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 32610.
§ Cetus Corporation, Emeryville, CA 94608.
△ Author to whom correspondence should be addressed.

Cyclodextrins

Cyclodextrins are oligomers of glucose which are produced by enzymatic (cyclodextrin transglycosylase, CTG) degradation of starch. Cyclodextrins are classified by the number of α-1,4-linked glucose units which occur in their molecular structure. Alpha(α)-cyclodextrin(α-CD) has six such units, β-cyclodextrin (β-CD) has seven, and γ-cyclodextrin (γ-CD), eight (5–8). In these compounds, the C-1 chain conformation of the glucose monomers imparts to the molecule a cone-like structure in which the hydroxy groups are oriented on the exterior of the torus (Table I). The narrower end of the cone contains the primary hydroxy functionalities while the wider face contains the secondary hydroxy groups. This arrangement makes the cyclodextrin exterior decidedly hydrophilic. The secondary hydroxy groups can, however, interact via hydrogen bonding to stabilize the crystalline lattice (9). This reduces to a large extent the solubility of cyclodextrins, especially β-CD, in water. Most importantly, the interior of the cyclodextrin cone is hydrophobic due to the presence of the skeletal carbons and ethereal oxygens which line the cavity. The result of this architecture is a lipoidal microenvironment which can solubilize non-polar

TABLE I. Structure and Physical Properties of Various Cyclodextrins[a]



	Alpha (α)	Beta (β)	Gamma (γ)
Molecular weight	972	1135	1297
Glucose monomers	6	7	8
Internal cavity diameter Å	5	6	8
Water solubility (g/100 mL; 25°C)	14.2	1.85	23.2
Surface tension (mN/m)	71	71	71
Melting range (°C)	255–260	255–265	240–245
Water of crystallization	10.2	13–15	8–18
Water in cavity	6	11	17

[a] Modified from References (7) and (9).

compounds. Taken as a whole, cyclodextrins are water soluble compounds which can form reversible complexes with poorly water soluble molecules resulting in a soluble molecular inclusion complex. In this solubilization, steric considerations are important in that the molecule must fit wholly or at least partially into the cyclodextrin cavity. Various physical and dimensional characteristics for α-, β-, and γ-CD are given in Table I.

The amphiphatic nature of cyclodextrins has been exploited to camouflage undesirable physicochemical properties of several pharmacologically active agents. One of the most important improvements afforded by complexation, as previously indicated, is an increase in aqueous solubility. In investigations aimed at studying this phenomenon, an excess of drug is added to a solution of an appropriate cyclodextrin, equilibrated for some time and filtered. The filtrate may be maintained as a clear solution or can be freeze-dried to yield the solid complex. This highly stable complex can be easily reconstituted in water to generate an aqueous solution of the drug. When the inclusion complex of the cyclodextrin drug combination is diluted in a sufficiently large volume of water or blood, it dissociates rapidly releasing the sequestered agent.

Various properties of the inclusion complexes formed between cyclodextrins and drugs can be determined by examining the effect of solubilizer concentration on drug solubility. The slope and intercept (i.e., the aqueous solubility of the compound in the absence of a solubilizer) of the resulting phase-solubility profile can give valuable information on both the type of complex formed in terms of stoichiometry and its solution stability. According to the definitions provided by Higuchi and Connors, the two main types of solubility profiles are A and B (Fig. 1) (10). A-type curves indicate the formation of soluble inclusion complexes while B-type relationships indicate the formation of complexes with limited solubility. Each type is further subdivided. If a plot of cyclodextrin concentration (mM) versus the concentration of drug solubilized (mM) is linear, an A_L-type system is obtained. Positive or negative deviations from linearity give A_p- and A_n-type responses, respectively. A_p-systems generally reflect high order complexation at higher cyclodextrin concentrations meaning that more than one cyclodextrin molecule is complexing with the guest. If a complex of a drug and cyclodextrin is not soluble, a B_I-type curve is generated and complexes of limited solubility give B_s type relationships. For A and B_s-type systems, the initial linear portion of the curve can be useful in examining the efficiency of complexation. Table II gives the slope values obtained by

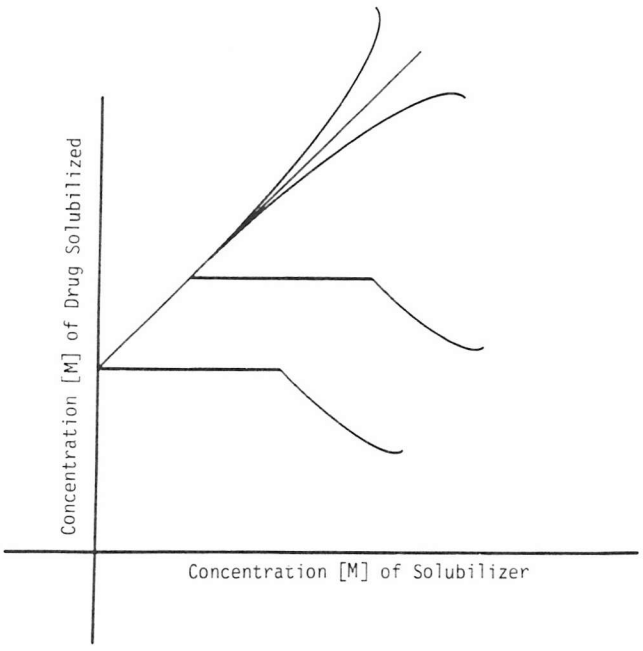


Figure 1—Phase-solubility relationships.

TABLE II. Slopes of the Interaction Isotherms of Selected Drugs with α- and β-Cyclodextrins[a]

Drug	α-CD	β-CD
Sulphadiazine	0.0051	0.084
Tetracycline	0.0081	0.093
Morphine	0.025	0.237
Sorbic acid	0.911	0.454
Propylparaben	0.295	0.687
Butylparaben	0.357	0.747
Aspirin	0.133	0.772
Benzocaine	0.567	0.795
p-Aminobenzoic acid	0.211	0.981
Salicyclic acid	0.305	0.885
Ethylparaben	0.510	0.896
Vanillin	0.567	1.010
Benzoid acid	1.004	1.044
Methylparaben	0.911	1.044
N-acetyl-*p*-aminophenol	0.395	1.100
p-Aminosalicyclic acid	0.211	1.111
p-Hydroxybenzoic acid	0.813	1.135
m-Hydroxybenzoic acid	1.085	1.192
Ephedrine	1.101	1.588

[a] Slopes refer to the solubility increase (M) as a function of cyclodextrin concentration (M) adopted from Reference (6), p. 223.

phase-solubility analysis of a number of drugs for α- and β-CD. The steeper the slope, the better is the complexation and at values of 1.0, 1.0 mole of cyclodextrin is complexing 1.0 mole of drug, i.e., the process is 100% efficient. At slope values greater than one, higher order complexation is occurring. More quantitative information can also be obtained from these diagrams. The stability constant (K) for the 1:1 complex of a drug and cyclodextrin can be estimated from the following equation:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

for the initial linear portion of an A- or B_s-curve (10). S_0 refers to the solubility of the drug in the absence of cyclodextrin (y-intercept). For A_p-type relationships, $K_{1:2}$ constants can be obtained from phase-solubility curves which can be fit to a quadratic equation from the expression:

$$S_t = S_0 + K_{1:1}S_0(\text{CD}) + K_{1:1}K_{1:2}S_0(\text{CD})^2$$

where S_t is the total drug solubilized and CD is the cyclodextrin concentration (10).

The enhanced solubility afforded by cyclodextrins has been demonstrated in the case of cardiac glycosides including digoxin, steroid hormones, barbiturates, chloramphenicol, sulfonamides, phenytoin, benzodiazepines, vitamins, amphotericin B, and many others (6, 7, 11–13). The improved solubility of drug complexes increases their rate of dissolution and, as a result, may enhance their bioavailability. This was shown in several instances including complexes of ibuprofen, prazosin, barbiturates, phenytoin, cinnarizine, and spironolactone (14–19).

Complexation of compounds can also stabilize chemically weak points in a molecular structure assuming that the sensitive portion of the molecule has penetrated into the cyclodextrin cavity. Prostaglandins E_1 and E_2 (PGE_1 and PGE_2) are known to be exquisitely sensitive to dehydration resulting in a mitigation of their biological activity. The formation of α-, β-, and γ-CD complexes of these agents greatly increases the stability of these drugs and provides for rapid *in vivo* dissolution (20, 21). Digitoxin is readily hydrolyzed in acidic media to result in partial inactivation. β-CD almost completely inhibits this degradation (20). It is important to note that while cyclodextrins, as pointed out above, can stabilize molecules, they are also efficient catalysts for certain reactions and as a result can accelerate unwanted side reactions. In fact cyclodextrins have long been used as models for various enzymes (22–24).

Cyclodextrin complexes of drugs may act to lower their vapor pressure and reduce undesirable odors or tastes. Chloral hydrate, chloramphenicol, and fenbufen exhibit improved aesthetic properties when complexed (6, 7). Also, oils or liquids such as clofibrate, fat soluble vitamins, nitroglycerin, and methyl salicylate can be converted to free flowing powders by complexation (6, 8). Cyclodextrins can even decrease toxic side effects of pharmaceutical agents. Incorporation of indomethicin and flurbiprofen into β-CD can decrease the tendency of these nonsteroidal anti-inflammatory agents to cause stomach ulcerations (6, 7). Chlorpromazine when complexed with β-CD is less likely to produce the photosensitivity associated with this agent (20, 25). In addition, β-CD complexes of chlorpromazine are less toxic to skeletal muscles when given i.m. than is the unmanipulated drug (26, 27). Thus, cyclodextrins have been shown to be applicable to a wide variety of pharmaceutical problems. Of the "natural" cyclodextrins available, β-CD appears to be the most useful complexing agent due to its size, availability, and other properties.

Several important limitations have prevented the widespread use of β-CD with drugs intended for parenteral use. Cyclodextrins are limited in their aqueous solubility. Thus, it is an unfortunate situation that the solubilizing agent is itself only sparingly soluble in water. This low aqueous solubility has toxicological ramifications which are manifested after parenteral, *but not oral*, dosing. Administration of β-CD to rats by either i.p. or i.v. routes causes an increase in blood urea nitrogen (BUN), a decrease in the rate of body weight gain, and a decrease in the weight of the liver in rats and mice (28, 29). Most striking is the ability of β-CD to increase both the absolute and relative weight of the kidney and to decrease the activity of a number of kidney enzymes. Histologically, nephrosis occurs as indicated by cytoplasmic vacuolation, cellular distinction, and lysosome formation (30). The necrosis occurs as a result of tubular reabsorption of β-CD which, after concentration in vacuoles, precipitates due to its low aqueous solubility. These series of events impart to β-CD a relatively low LD_{50}, estimates of which have ranged from 300–800 mg/kg (6, 30, 31).

Several chemical modifications have been performed on β-CD to increase its low aqueous solubility and, therefore, its usefulness. Since the low solubility is due in large part to intramolecular hydrogen bonding, methods for disrupting this process have been attempted. Methylation of β-CD has given rise to two important compounds: hep-

TABLE III. Physicochemical Properties of Various Chemically Modified β-Cyclodextrins Including heptakis(2,6-di-O-methyl)-β-cyclodextrin (DMCD), heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (TMCD), 2-hydroxyethyl-β-cyclodextrin (2-HECD), 2-hydroxypropyl-β-cyclodextrin (2-HPCD), 2-hydroxypropyl-β-cyclodextrin (3-HPCD) and 2,3-dihydroxypropyl-β-cyclodextrin (2,3-HPCD)[a]

Host Molecule	Substituent	Degree of Substitution	[α]	Surface Tension (mN/m)	Aqueous Solubility (g/100 mL)
		Alkylated Cyclodextrins			
DMCD	—OCH_3	14	160	62	57
TMCD	—OCH_3	21	158	53	31
		Hydroxyalkylated Cyclodextrins			
2-HECD	—OCH_2CH_2OH	2.67	138	71	>50
2-HECD	—OCH_2CH_2OH	4.56	134	71	>50
2-HECD	—OCH_2CH_2OH	5.00	129	70	>50
2-HECD	—OCH_2CH_2OH	6.07	130	71	>50
2-HECD	—OCH_2CH_2OH	9.00	121	68	>50
2-HECD	—OCH_2CH_2OH	10.55	118	68	>50
2-HPCD	—$OCH_2CH(OH)CH_3$	2.5	144	69	>50
2-HPCD	—$OCH_2CH(OH)CH_3$	5.6	146	64	>50
2-HPCD	—$OCH_2CH(OH)CH_3$	6.8	139	61	>50
2-HPCD	—$OCH_2CH(OH)CH_3$	8.0	137	60	>50
2-HPCD	—$OCH_2CH(OH)CH_3$	11.3	98	52	>50
3-HPCD	—$OCH_2CH_2CH_2OH$	1.8	140	71	>50
3-HPCD	—$OCH_2CH_2CH_2OH$	2.8	136	70	>50
3-HPCD	—$OCH_2CH_2CH_2OH$	4.5	128	71	>50
3-HPCD	—$OCH_2CH_2CH_2OH$	6.1	125	70	>50
		Dihydroxyalkylated Cyclodextrins			
2,3-HPCD	—$OCH_2CH(OH)CH_2OH$	2.6	126	71	>50
2,3-HPCD	—$OCH_2CH(OH)CH_2OH$	4.7	128	71	>50
2,3-HPCD	—$OCH_2CH(OH)CH_2OH$	5.9	114	71	>50
2,3-HPCD	—$OCH_2CH(OH)CH_2OH$	9.3	108	70	>50

[a] From References (9), (32), and (39).

takis(2,6-di-O-methyl)-β-cyclodextrin (DMCD) and heptakis(2,3,6-tri-O-methyl-β-cyclodextrin (TMCD) (6). The methylation of the secondary (2′ or 3′) hydroxy groups paradoxically increases the aqueous solubility of DMCD manyfold. Table III gives the physicochemical characteristics for a series of chemically modified β-CD. In addition to inhibiting hydrogen bonding, methylation extends the hydrophobic cavity of the cyclodextrin and provides a greater surface area for complexation. As a result, complexation constants ($K_{1:1}$) for DMCD are generally higher than those for β-CD. The conversion of reactive hydroxy groups to less active methyl ethers also decreases the tendency of DMCD to induce degradation. DMCD has most of the beneficial characteristics of β-CD and has been used in many similar applications. The main drawback of DMCD is its relatively high lipophilicity compared to β-CD. When this compound is dissolved in water it decreases the aqueous surface tension. This surface activity as well as the ability of DMCD to complex with and extract cholesterol and proteins from red blood cells, causes the cyclodextrin to be hemolytic at relatively low concentrations (9, 32). This effect is translated *in vivo* to a low LD_{50} for DMCD which has been determined to be less than 200 mg/kg (31). In addition, DMCD is irritating to mucus membranes and muscles (9).

Hydroxyalkylated β-Cyclodextrins

Another type of chemical modification is non-selective hydroxy- or dihydroxyalkylation (33, 34). This chemical reaction generates amorphous mixtures of literally thousands of geometric and optical cyclodextrin isomers. This process has several advantages over simple alkylation. First, hydroxyalkyl cyclodextrins are, in and of themselves, water soluble and hydrophilic. Secondly, the non-selective nature of the modifications generates many chemically distinct species whose solubility is independent of even close structural analogs. The mixture produced is therefore highly water soluble (usually >100% w/v) and can be rapidly dissolved (35). The derivatizing moieties are the same used to generate such food additives as hydroxypropylcellulose.

In the preparation of these compounds, O-alkylation is achieved by addition of an appropriate epoxide or chloride to a basic solution of the cyclodextrin (36). In the case of 2-hydroxypropyl-β-cyclodextrin (2-HPCD), propylene oxide is used (34). The resulting product is purified by ion exchange chromatography, acetone extraction, and lyophilization. These cyclodextrin mixtures are characterized by soft-ionization mass spectral techniques including Californium-252 plasma desorption or fast atom bom-

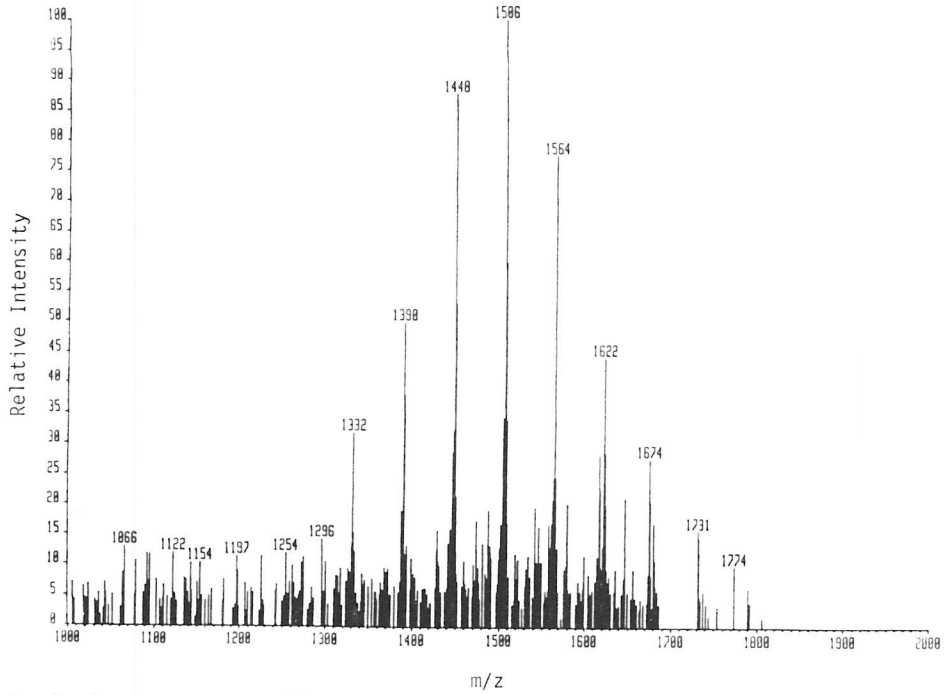


Figure 2—Fast atom bombardment mass spectra (FAB-MS) of 2-hydroxypropyl-β-cyclodextrin. The sample was immobilized in a glycerol matrix. Mass spectral determination were made using a Kratos MS 80RFA double focusing instrument.

bardment. Figure 2 gives a representative spectrum for 2-HPCD. The observed symmetrical distribution of isomers is commonly observed for this type of derivatization (36). The average degree of substitution (DS), i.e., the number of hydroxypropyl groups per cyclodextrin nucleus, is characteristic for a particular cyclodextrin batch and is determined by summing the products of the individual masses of the isomers and their intensities and dividing by their intensities. It is interesting to note that, in general, the hydroxyalkylation is not symmetrical with respect to the cyclodextrin ring. When, for example, a sample of 2-HPCD (DS = 6.2) was hydrolyzed in hot concentrated hydrochloric acid and analyzed by MS, only 34% of the glucose subunits were monohydroxypropylated. The remainder was bis- (33%), tri- (18%), and tetrakis- (15%) hydroxyalkylated, suggesting that multiple reactions had occurred on single glucose subunits or that a polypropylene side chain grew from one of the glucose moieties (36). To date, amorphous mixtures of 2-hydroxyethyl (HECD), 2-hydroxypropyl-(2-HPCD), 3-hydroxypropyl-(3-HPCD), 2-hydroxyisobutyl-, carboxamidomethyl, and 2,3-dihydroxypropyl-(2,3-HPCD)-β-cyclodextrin have been prepared and studied. Of these, the 2-HPCD has been most extensively examined. Table IV gives a comparison of the solubility enhancement for a number of drugs afforded by β-CD, DMCD, and 2-HPCD and Table V gives a list of apparent stability constants ($K_{1:1}$) for inclusion complexes of various β-CD derivatives. Finally, various physical characteristics of chemically modified β-CD are shown in Table III. These data indicate that the

TABLE IV. Solubility Enhancement Afforded by β-Cyclodextrin (β-CD), Heptakis(2,6-di-O-methyl)-β-Cyclodextrin (DMCD) and 2-Hydroxypropyl-β-cyclodextrin (2-HPCD)[a]

Drug	Aqueous Solubility (mg/mL)	Solubility in Various Cyclodextrins (μg/mL)					
		β-CD	Enhancement	DMCD	Enhancement	2-HPCD	Enhancement
Carmoful	90.0	437	4.9	1420	16	611	6.8
Diazepam	55.2	199	3.6	498	9.0	154	2.79
Digitoxin	15.5	422	27	6000	390	2330	150
Digoxin	66.5	5970	90	6140	92	3090	57
Flurbiprofen	44.0	108	2.4	1920	44	1250	28
Indomethacin	22.9	56.5	2.5	102	4.5	38.7	1.7
Isosorbide dinitrate	826	590	0.7	2130	2.6	1620	2.0
Phenytoin	28.0	282	10	350	13	403	14
Prednisolone	145	3440	14	3300	13	2210	9.0
Progesterone	13.2	41.5	3.1	2020	150	1160	88
Testosterone	31.4	86.0	2.7	2220	71	1580	50

[a] From Reference (9).

TABLE V. Apparent Stability Constants for Complexes of Various Drugs in β-Cyclodextrin and Several β-Cyclodextrin Derivatives[a]

Drug	β-CD	DMCD	HECD	2-HPCD	3-HPCD	2,3-HPCD
Diazepam	220	770	140	170		
Digitoxin	17000	84000	17000	18000	20000	14000
Digoxin	11000	37000	5600	7300		4900
Ethyl-4-biphenyl acetate	3000	12500	2400	4100	6000	2400
Prednisolone	1600	7000	820	1800	2000	760
Progesterone	13000	55000	7500	17000		16000
Testosterone	7500	29000	5100	12000		5200

[a] From References (9), (32), and (39).

complexing power of 2-HPCD is generally greater than or equal to that of β-CD and generally less than DMCD. Most important, however, is that the >50-fold improvement in the aqueous solubility of 2-HPCD compared to β-CD allows for the complexation of much greater quantities of drug. In addition, 2-HPCD tends to give A_L or A_P-type solubility relationships meaning the solubility of a drug is linearly or exponentially correlated with the cyclodextrin complexes. β-CD often generates poorly soluble (B_S) complexes in which the maximum solubility of the drug is obtained at β-CD concentration lower than its solubility limit (1.8 g/100 mL in water). These hydroxyalkylated derivatives significantly improve the solubility, stability, and aesthetic properties of a number of drugs in a manner greater than or equal to that of β-CD (34, 36–39).

One example of this is the application of 2-HPCD to Chemical Delivery Systems (CDS) (40–43). CDS are derivatives of known active drugs which act to selectively increase the concentration of the drug in the central nervous system (CNS). The method is based on attachment of a molecular carrier to the drug of interest. The carrier is generally a 1,4-dihydrotrigonellinate moiety which increases the lipophilicity of the conjugate and, therefore, allows the compound to better penetrate biological membranes such as the blood-brain barrier (BBB) (40). After systemic administration of the CDS's, the lipophile readily partitions into most body compartments including the brain. With time, the designed instability of the CDS is manifested and the attached dihydronicotinamide is converted through enzymatic oxidation to the charged trigonellinate species. The now polar conjugate is easily lost from the systemic circulation due to its ionic nature but trapped behind the lipoidal BBB where it can be hydrolyzed to yield the active drug in a slow but sustained fashion. While these events have been thoroughly validated *in vivo*, the formulation of these systems is problematic due to their high lipophilicity, low water solubility, and oxidative and hydrolytic instability. A 2-HPCD complex of a CDS for estradiol was therefore produced (44). The aqueous solubility of the unmanipulated estradiol chemical delivery system (E_2CDS) is only 62 ng/mL. Complexation of E_2CDS in a 40% w/v solution of 2-HPCD improved the solubility of the estrogen by over five orders of magnitude. In addition, the freeze-dried E_2CDS/2-HPCD complex was at least four times more stable than the uncomplexed drug in a temperature range between 23–80°C. This stabilization was observed in solutions as well. Solutions of ferricyanide salts are used as a potent oxidant used to study the stability of CDS. The addition of 2-HPCD to ferricyanide solution can decrease the rate of oxidation of the E_2CDS by over 90%. Other amorphous cyclodextrin mixtures such as 2,3-HPCD also have been shown to increase the stability of drugs (39). Reactions which are inhibited include the hydrolysis of prostacyclin and carmofur, the photolysis of nimodipine, the dehydration of prostaglandin E_1 (PGE_1), and the isomerization of prostaglandin A_1 (PGA_1).

One of the most important advantages of amorphous cyclodextrins is their hydrophilicity. These compounds, unlike DMCD, are much more compatible with biological environments. Hydroxyalkylated cyclodextrins cause hemolysis at much higher concentrations than DMCD (9, 32) as they are much less surface active. In addition, 2-HPCD and related compounds are much less irritating to muscle tissue after i.m. administration (9). Table VI gives the irritation index obtained after i.m. treatment of a 50 mg/mL solution of various β-cyclodextrin derivatives. In this rabbit muscle (*M. vastus lateralis*) model, it is apparent that DMCD is the most corrosive of the series.

The acute toxicity of 2-HPCD has been reported to be insignificant after oral, i.p., i.v., i.m., topical, and intracranial administration (34, 45). Recently, systemic acute (14 day) and subchronic (90 day) studies have been completed (46). In the acute study, two groups of 10 Sprague-Dawley rats (5 males and 5 females) were injected (tail vein) with either normal saline or with a solution of 2-HPCD in sterile water (200 mg/kg) every second day for 14 days. Twenty-four hours after the seventh dose, the animals were sacrificed and necropsied. During the period of drug administration, the animals were monitored for toxic reactions and macroscopic observations were made at necropsy. Histopathological samples were prepared

TABLE VI. Intramuscular Irritation of Various β-Cyclodextrins on Rabbit (*M. vastus lateralis*) Muscle. Scoring is According to the Method of Shintani (Maximum Irritation = 5)[a]

β-CD	0.25 ± 0.14
DMCD	3.50 ± 0.29
HECD	0.20 ± 0.12
2-HPCD	0.38 ± 0.24
3-HPCD	0.25 ± 0.14
2,3-HPCD	0.00 ± 0.00

[a] From Reference (39).

from various organs and blood was drawn for clinical chemistry evaluation. In a complementary study, cynomolgus monkeys (2/group, one male and one female) were injected i.v. with either normal saline or 2-HPCD in doses of 200 mg/kg. The pattern of drug administration, observation, sample collection, and histopathological investigation was the same as that used for the rats. In both species, *no* indication of toxicity of any kind was noted for animals receiving 2-HPCD. *No* differences in blood chemistry occurred and no differences in food intakes, body weights, or behavioral parameters were apparent. Also, *no* differences were observed after macroscopic or microscopic study of the control (saline) and treated groups. This was especially important in terms of the kidney as previously indicated. In the chronic study, Sprague-Dawley rats (20 animals per groups, 10 males and 10 females) and cynomolgus monkeys (8 animals per group, 4 males and 4 females) were treated with either normal saline or 200 mg/kg 2-HPCD every second day for 90 days. Study results were similar to the 14-day experiment and indicated there were *no* toxic effects elicited by the modified cyclodextrin. Finally, a high i.v. study was completed in monkeys to assay tolerance of animals to 2-HPCD. In the i.v. test, cynomolgus monkeys (four total, two males and two females) received a dose of 2 g/kg 2-HPCD in sterile water followed 48 hours later with a dose of 10 g/kg 2-HPCD. There was *no* mortality in the study and with the exception of some hematuria in two animals at the high dose, no material-related toxic manifestations.

Use of 2-Hydroxypropyl-β-Cyclodextrin in Parenteral Formulations

Several feasibility studies have been conducted in our laboratory to assess the practicality of using hydroxypropyl-β-cyclodextrin in parenteral formulations. In the first of these experiments, the ability of a 2-HPCD-dexamethasone complex (Dex-CD) to deliver dexamethasone to the blood stream following i.v. administration was examined in dogs and compared to the dexamethasone delivery after administration of a widely used dexamethasone prodrug, dexamethasone phosphate. In this preliminary examination, 3 dogs were injected with either 5 mg/kg dexamethasone phosphate or equimolar Dex-CD in a crossover study. Blood samples were obtained prior to drug administration and from 5–480 min post-drug administration. Plasma samples obtained after dexamethasone phosphate or Dex-CD dosing were analyzed by HPLC as previously described (47). The finding of this study indicated that there were no significant differences in various pharmacokinetic parameters for the disappearance of dexamethasone following either treatment (48). Parameters examined included the half-life of β-phase elimination, mean residence time, volume of distribution, and the total drug clearance. Interestingly however, plasma levels of dexamethasone were significantly higher in the first hour after administration of Dex-CD relative to dexamethasone phosphate indicating that the dissociation of the cyclodextrin complex was faster than enzymatic dephosphorylation of the dexamethasone prodrug (Fig. 3). In this experiment, no toxic reactions in dogs were observed.

A second use of 2-HPCD was as a component of a formulation for estradiol and for E_2CDS, both of which were examined in humans. In these Phase I studies, the safety of the estradiol-2-HPCD complex (E_2-CD) and the E_2CDS-2-HPCD complex doses were assessed (49). Doses of 2-HPCD as high as 30 mg/kg produced no untoward effects in either the men or post-menopausal women who made up the test sample. In addition, the pharmacological potency of the steroids tested was in no way mitigated by complexation as indicated by response of lutenizing hormone (LH) and follicle stimulating hormone (FSH) to the administered estrogens. A second human study using 2-HPCD has also been described. In a potentially life saving indication, 2-HPCD was employed to accelerate the elimination of Vitamin A in a child suffering from familial vitaminosis A (50). The treatment was effective in improving the normal elimination of the vitamin and did not cause noticeable toxicity even at a total injected dose of 30 g.

One final area where the hydroxyalkyl cyclodextrins may be useful is in stabilizing and/or solubilizing proteins intended for parenteral administration. The recent explosion in drugs resulting from genetic engineering as well as the numerous peptides now available in large quantities from natural sources has presented to the pharmaceutical scientists a wide range of novel formulation issues. For a well written and comprehensive discussion of the solubility, stability, and other formulation problems of proteins and peptides the reader is referred to the recent review by Wang and Hanson (51). In attempting to demonstrate the solubilizing potential of 2-HPCD, we have initiated preliminary studies on ovine growth hormone (OGH). This protein is practically insoluble in pH 7.6 buffer at room temperature. If OGH at a concentration of 2.5 mg/mL is added to a solution of 40% w/v 2-HPCD, complete dissolution of the protein is observed (52). Light absorption at 600 nm was used as an indication of clarity and as shown in Figure 4, cyclodextrin solutions are far less absorbent than those containing only buffer. Studies on interleukin-2 (IL-2) (Cetus Corporation, Emeryville, CA) were next

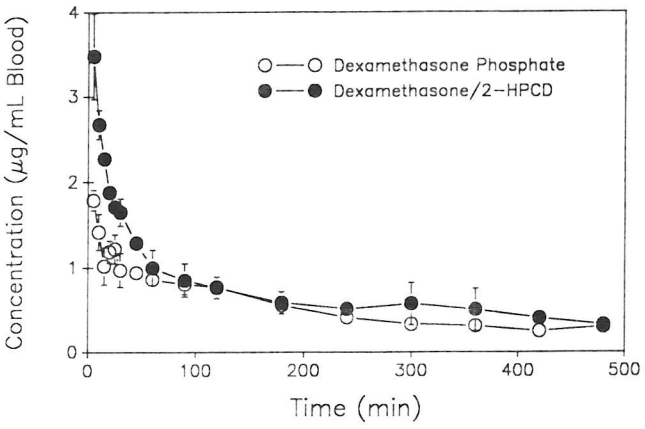


Figure 3—Concentration of dexamethasone in dog blood following i.v. administration of either dexamethasone phosphate or a 2-hydroxpropyl-β-cyclodextrin complex of dexamethasone.

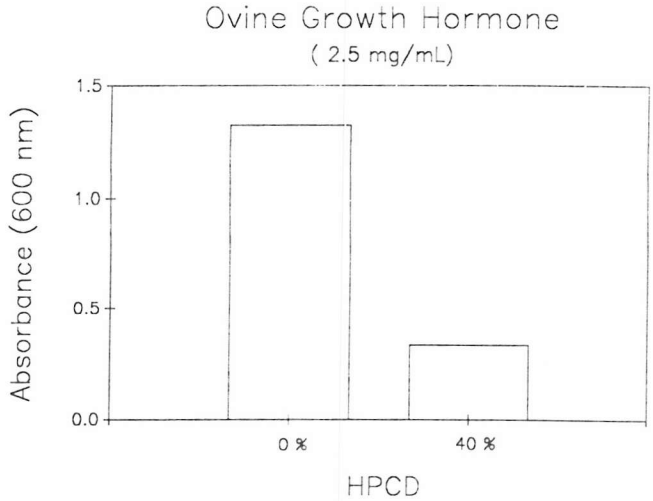


Figure 4—Light absorbance (600 nm) for a solution of 2.5 mg/mL of ovine growth hormone in pH 7.6 buffer with or without 2-hydroxypropyl-β-cyclodextrin (40%).

initiated. The purpose of these examinations was to generate formulations which would produce clear solutions upon reconstitution after lyophilization. In these studies, 12.5 and 25% w/v solutions of 2-HPCD were prepared, each containing 1 mg/mL of IL-2, and were lyophilized. The product was then reconstituted with 1 mL of water. On visual inspection, all solutions were clear. Light scattering using a fluorometric assay was also shown to be minimal (52). The lowest effective level of 2-HPCD was next identified by preparing IL-2 in solutions of sucrose, citrate buffer, and varying concentrations of the cyclodextrin (0–25% w/v). As illustrated in Figure 5, clear solutions (low light scattering) were obtained at 2-HPCD concentrations greater than 0.2%.

The effect of the 2-HPCD complexation on IL-2 biological activity was considered. In the assay used, the proliferation of HT-2 cells (MTT strain) was measured as previously described (53) and it was demonstrated that the peptide retained 100% of its biological potency in the presence of 2-HPCD (Fig. 6). Similar solubility/activity

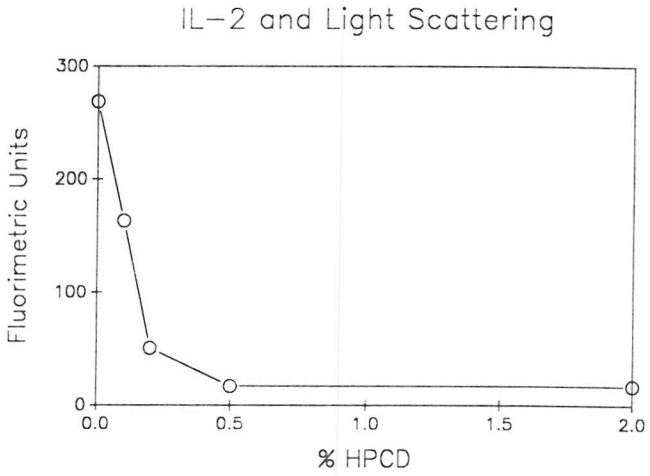


Figure 5—Light scattering (in arbitrary fluorimetric units) for a solution of 1 mg/mL IL-2 in 10 mM citrate buffer and 1% sucrose containing various concentrations (0–2%) of 2-hydroxypropyl-β-cyclodextrin.

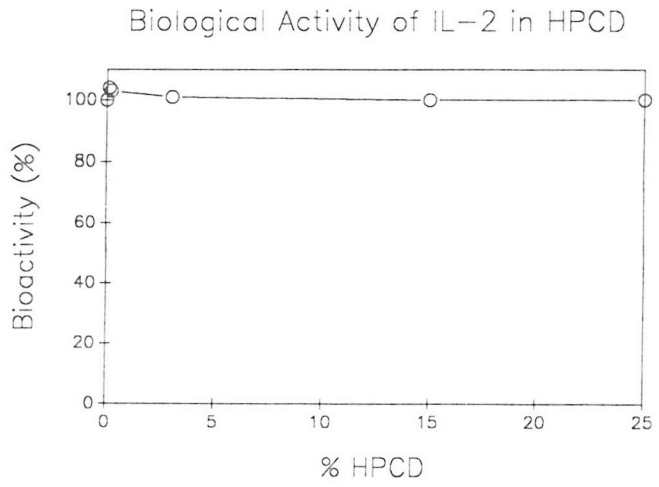


Figure 6—Biological activity of IL-2 formulated with or without various concentrations of 2-hydroxypropyl-β-cyclodextrin.

studies were performed on tumor necrosis factor (TNF) and macrophage colony stimulating factor (M-CSF). The solutions prepared using 2-HPCD for TNF and M-CSF were clear and both compounds retained 100% of their biological activity (54–56).

The ability of 2-HPCD to inhibit protein aggregation was examined. Insulin was chosen as a model because of its general importance and because there is a great need to produce an insulin product which is stable over a long period of time (51). The reason for this is that insulin delivery systems, which administer a metered portion of the drug as a function of time, often clog with precipitated protein and therefore require frequent cleaning and flushing. Bovine insulin (BI) readily dissolves in both pH 7.4 buffer and in buffer containing 40% 2-HPCD. Within two

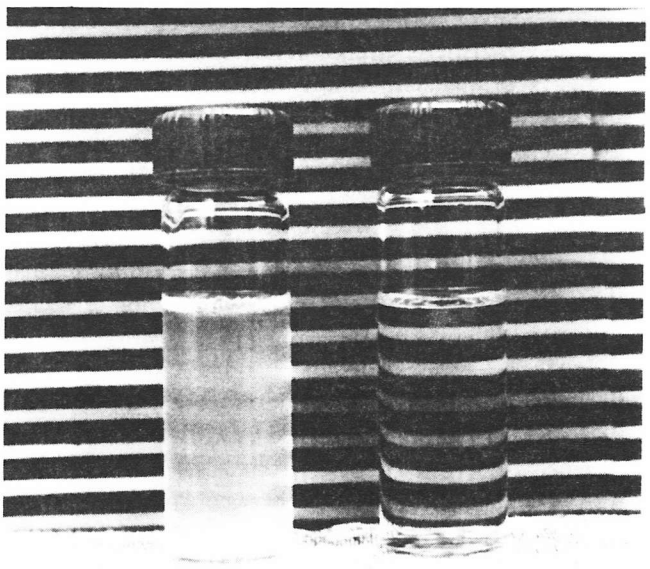


Figure 7—Effect of 2-hydroxypropyl-β-cyclodextrin on the stability of a 1 mg/mL insulin solution. The vial on the left contains insulin and pH 7.4 isotonic phosphate buffer. The vial on the right contains insulin, buffer, and 40% w/v (HPCD). These solutions were stored at room temperature, protected from light for four weeks.

weeks at ambient temperature, the 1 mg/mL solutions of BI in buffer had begun to precipitate and by 4 weeks the solutions were milky (57). The 2-HPCD containing samples were clear even up to 2 months post-preparation. This is illustrated in Figure 7.

In conclusion, 2-HPCD demonstrates many of the characteristics desirable in a parenteral excipient. It is non-toxic, it can improve the solubility and stability of many drugs and it is easily available via derivitization of β-cyclodextrin. Complexes formed between drugs and this starch appear to rapidly dissociate after i.v. administration and as a result there is no mitigation of the biopotency of drugs delivered using this technology. This preliminary evidence suggests, therefore, that this material is useful and should be pursued.

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References

1. Davis, S. S., and Illum, L., "Colloidal Delivery Systems: Opportunities and Challenges," In *Site-Specific Drug Delivery*, E. Tomlinson and S. Davis, Eds. J. Wiley and Sons, Chichester, England (1986), pp. 93–110.
2. Davis, S. S., Washington, C., West, P., Illum, L., Liversidye, G., Sternson, L., and Kirsh, R., "Lipid emulsions as drug delivery systems," *Ann. N.Y. Acad. Sci.*, **507,** 75 (1987).
3. Kirsh, R., Bugelski, P., and Poste, G., "Drug delivery to macrophages for the therapy of cancer and infectious diseases," *Ann. N.Y. Acad. Sci.*, **507,** 141 (1987).
4. O'Mullane, J., Artursson, P., and Tomlinson, E., "Biopharmaceutics of microparticulate drug carriers," *Ann N.Y. Acad. Sci.*, **507,** 120 (1987).
5. Uekama, K., "Pharmaceutical applications of cyclodextrin complexations," *Yakugaku Zasshi*, **101,** 857 (1981).
6. Szejtli, J., *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiado, Budapest, Hungary (1982).
7. Pagington, J. S., "β-Cyclodextrin: The success of molecular inclusion," *Chem. Brit.*, 455 (1987).
8. Parrish, M., "Cyclodextrins—A Review," Sterling Organics, Ltd., Newcastle, England.
9. Uekama, K., and Otagiri, M., "Cyclodextrins in drug carrier systems," *CRC Crit. Rev. Therap. Drug Carrier Systems*, **3,** 1 (1987).
10. Higuchi, T., and Connors, K., "Phase-solubility techniques," *Adv. Anal. Chem. Instrum.*, **4,** 117 (1965).
11. Szejtli, J., Bolla-Pusztai, E., Szabo, P., and Ferenczy, T., "Enhancement of stability and biological effect of cholecalciferol by β-cyclodextrin complexation," *Pharmazie*, **35,** 779 (1980).
12. Lach, J., and Cohen, J., "Interaction of pharmaceuticals with Schardinger Dextrins II," *J. Pharm. Sci.*, **52,** 137 (1963).
13. Vikmon, M., Stadler-Szoke, A., and Szejtli, J. "Solubilization of Amphotericin B with γ-cyclodextrin," *J. Antibiot.*, **38,** 1822 (1985).
14. Chow, D. D., and Karara, A. H., "Characterization, dissolution and bioavailability in rats of ibuprofen-β-cyclodextrin complex system," *Int. J. Pharm.*, **28,** 95 (1986).
15. Koizumi, K., and Kidera, Y., "Effect of α- and β-cyclodextrin on gastrointestinal absorption of barbituric acid derivatives," *Yakugaku Zasshi*, **97,** 705 (1977).
16. Tsuruoka, M., Hashimoto, T., Seo, H., Ichimasa, S., Ueno, O., Fujinaga, T., Otagiri, M., and Uekama, K., "Enhanced bioavailability of phenytoin by β-cyclodextrin complexation," *Yakugaku Zasshi*, **101,** 360 (1981).
17. Tokumura, T., Nanba, M., Tsushima, Y., Tatsuishi, K., Kayano, M., Machida, Y., and Nagai, T., "Enhancement of bioavailability of cinnarizine from its β-cyclodextrin complex on oral administration with DL-phenylalamine as a competing agent," *J. Pharm. Sci.*, **75,** 391 (1986).
18. Seo, H., Tsuruoka, M., Hashimoto, T., Fujinaga, T., Otagiri, M., and Uekama, K., "Enhancement of oral bioavailability of spironolactone by β- and γ-cyclodextrin complexation," *Chem. Pharm. Bull.*, **31,** 286 (1983).
19. Nambu, N., Shimoda, M., Takahashi, Y., Ueda, H., and Nagai, T., "Bioavailability of powdered inclusion compounds of nonsteroidal antiinflammatory drugs with β-cyclodextrin in rabbits and dogs," *Chem. Pharm. Bull.*, **26,** 2952 (1978).
20. Uekama, K., "Cyclodextrin inclusion compounds: Effects on stability and biopharmaceutical properties," *Topics Pharm. Sci.*, 181 (1987).
21. Uekama, K., Fujise, A., Hirayama, F., Otagiri, M., and Inaba, K., "Improvement of dissolution characteristics and chemical stability of prostaglandin E_1 by γ-cyclodextrin complexation," *Chem. Pharm. Bull.*, **32,** 275 (1984).
22. Akkaya, E., and Czarnik, A., "Synthesis and reactivity of cobalt(III) complexes bearing primary- and secondary-side cyclodextrin binding sites," *J. Am. Chem. Soc.*, **110,** 8553 (1988).
23. de Rossi, R., Barra, M., and de Vargas, E., "Catalysis by cyclodextrins in nucleophilic aromatic substitution reactions," *J. Org. Chem.*, **51,** 2157 (1986).
24. Ramamunthy, V., and Eaton D., "Photochemistry and photophysics within cyclodextrin cavities," *Acct. Chem. Res.*, **21,** 300 (1988).
25. Irie, T., and Uekama, K., "Protection against the photosensitized skin irritancy of chlorpromazine by cyclodextrin complexation," *J. Pharmacobio-Dyn.*, **8,** 788 (1985).
26. Irie, T., Otagiri, M., and Uekama, K., "Alleviation of the chlorpromazine-induced muscular tissue damage by β-cyclodextrin complexation," *J. Inclusion Phenom.*, **2,** 637 (1984).
27. Irie, T., Kuwahara, S., Otagiri, M., Uekama, K., and Iwamasa, T., "Reduction in the local tissue toxicity of chlorpromazine by β-cyclodextrin complexation," *J. Pharmacobio-Dyn.*, **6,** 790 (1983).
28. Perrin, J., Field, F., Hansen, D., Mufson, R., and Torosian, G., "β-Cyclodextrin as an aid to peritoneal dialysis. Renal toxicity of β-cyclodextrin in the rat," *Res. Commun. Chem. Path. Pharm.*, **19,** 373 (1978).
29. Hiasa, Y., Ohshima, M., Kitahori, Y., Yuasa, T., Fujita, T., Iwata, C., Miyashiro, A., and Konishi, N., "Histochemical studies of β-cyclodextrin nephrosis in rats," *J. Nara. Med. Assoc.*, **32,** 316 (1981).
30. Frank, D., Gray, J., and Weaver, R., "Cyclodextrin nephrosis in the rat," *Am. J. Pathol.*, **83,** 367 (1976).
31. Muller, B., and Brauns, V., "Solubilization of drugs by modified β-cyclodextrins," *Int. J. Pharm.*, **26,** 77 (1985).
32. Yoshida, A., Arima, H., Uekama, K., and Pitha, J., "Pharmaceutical evaluation of hydroxyalkyl ethers of β-cyclodextrin," *Int. J. Pharm.*, **46,** 217 (1988).
33. Gramera, R., and Caimi, R., U.S. Patent 3,459,731 (1969).
34. Pitha, J., Milecki, J., Fales, H., Pannell, L., and Uekama, K., "Hydroxypropyl-β-cyclodextrin: preparation and characterization; effects on solubility of drugs," *Int. J. Pharm.*, **29,** 73 (1986).
35. Pitha, J., and Pitha, J., "Amorphous water-soluble derivatives of cyclodextrins: nontoxic dissolution enhancing excipients," *J. Pharm. Sci.*, **74,** 987 (1985).
36. Irie, T., Fukunaga, K., Yoshida, A., Uekama, K., Fales, H., and Pitha, J., "Amorphous water-soluble cyclodextrin derivatives: 2-hydroxyethyl, 3-hydroxypropyl, 2-hydroxyisobutyl and carboxamidomethyl derivatives of β-cyclodextrin," *Pharm. Res.*, **5,** 713 (1988).
37. Pitha, J., Harman, M., and Michel, M., "Hydrophilic cyclodextrin derivatives enable effective oral administration of steroidal hormones," *J. Pharm. Sci.*, **75,** 165 (1986).
38. Pitha, J., U.S. Patent 4,727,064 (1988).
39. Yoshida, M., Yamamoto, M., Irie, T., Hirayama, F., and Uekama, K., "Some pharmaceutical properties of 3-hydroxypropyl- and 2,3-dihydroxypropyl-β-cyclodextrins and their solubilizing and stabilizing abilities," in press (1988).
40. Bodor, N., and Brewster, M. E., "Problems of delivery of drugs to the brain," *Pharmacol. Ther.*, **19,** 337 (1983).
41. Bodor, N., "Redox drug delivery systems for targeting drugs to the brain," *Ann. N.Y. Acad. Sci.*, **507,** 289 (1988).
42. Bodor, N., Farag, H., and Brewster, M. E., "Site-specific, sustained release of drugs to the brain," *Science*, **214,** 1370 (1981).
43. Bodor, N., "Targeting of Drugs to the Brain," In *Methods in Enzymology, Drug and Enzyme Targeting*, Vol. 112, K. J. Widder, Ed., Academic Press, New York, (1985), pp. 381–396.
44. Brewster, M., Estes, K., Loftsson, T., Perchalski, R., Derendorf, H., Mullersman, G., and Bodor, N., "Improved delivery through biolog-

ical membranes XXXI. Solubilization and stabilization of an estradiol chemical delivery system by modified β-cyclodextrins," *J. Pharm. Sci.*, **77,** 981 (1988).
45. Pitha, J., Irie, T., Sklar, P., and Nye, J., "Drug solubilizers to aid pharmacologists: amorphous cyclodextrin derivatives," *Life Sci.*, **43,** 493 (1988).
46. Brewster, M., Estes, K., and Bodor, N., "Acute and subchronic intravenous toxicity of 2-hydroxypropyl-β-cyclodextrin, a useful drug solubilizer," *Int. J. Pharm.*, in press (1989).
47. Derendorf, H., Rohdewald, P., Hochhaus, G., and Mollmann, H., "HPLC determination of glucocorticoid alcohols, their phosphates and hydrocortisone in aqueous solutions and biological fluids," *J. Pharm. Biomed. Anal.*, **4,** 197 (1986).
48. Derendorf, H., Estes, K., and Dietzel, K. Unpublished results.
49. Howes, J., Bodor, N., Brewster, M., Estes, K., and Eve, M., "A pilot study with PR-63 in post-menopausal volunteers," *J. Clin. Pharmacol.*, **28,** 951 (1988) (Abstract).
50. Carpenter, T., Pettifor, J., Russell, R., Pitha, J., Mobarhan, S., Ossip, M., Wainer, S., and Anast, C., "Severe hypervitaminosis A in siblings: evidence of variable tolerance to retinol intake," *J. Pediatr.*, **111,** 507 (1987).
51. Wang, Y., and Hanson, M., "Parenteral formulations of proteins and peptides: stability and stabilizers," *J. Parenter. Sci. Technol.*, **42,** (Suppl.), S4 (1988).
52. Hora, M. S. Unpublished results.
53. Gillis, S., Ferm, M., Ou, W., and Smith, K., "T cell growth factor: parameters of production and a quantitative microassay for activity," *J. Immunol.*, **120,** 2027 (1978).
54. Ostrove, J., and Gifford, E., "Stimulation of RNA synthesis in L-929 cells by factor rabbit tumor necrosis factor," *Prod. Soc. Exp. Biol. Med.*, **160,** 354 (1979).
55. Ruff, M., and Gifford, E., "Rabbit tumor necrosis factor: Mechanism of action," *Infect. Immun.*, **31,** 380 (1981).
56. Lee, J., Hapel, A., and Ihle, J., "Constitutive production of a unique lymphokine (IL3) by the WEH1-3 cell line," *J. Immunol.*, **128,** 2393 (1982).
57. Tenace, L., Brewster, M., unpublished results.

TECHNOLOGY/APPLICATIONS

Expert System Computer Program for Troubleshooting the Gel-Clot *Limulus* Amebocyte Lysate (LAL) Assay

KIYOSHI TSUJI

Pharmaceutical Quality Control Division, The Upjohn Company, Kalamazoo, Michigan

ABSTRACT: This article describes a knowledge-based or expert system computer program that can assist in troubleshooting the gel-clot Limulus *Amebocyte Lysate (LAL) method for the assay of endotoxin in parenteral products and in-process materials. The program may also be used to train new personnel and/or to assist in the development of an LAL method for a new product or material. The program includes LAL assay information for 31 parenteral products and materials. The program uses rules-of-thumb and intuition to diagnose 155 possible LAL assay problems and logically reduce them to 37 recommended problem solving processes. The program also provides references to support its recommendation.*

Introduction

The gel-clot *Limulus* Amebocyte Lysate (LAL) method for the assay of endotoxin is tedious, subjective, and frequented with numerous assay problems (1). Thus, expert knowledge and experience are needed to effectively troubleshoot LAL assay problems. Because expert knowledge is costly, perishable, and easily lost by retirement, job transfer, or disuse, a system to preserve this knowledge can be extremely valuable. Expert system or knowledge-based programs are intelligent computer programs that simulate human expert reasoning by elucidating many of the relevant criteria and make educated inferences similar to those of the human mind (2). The Expert Troubleshooting LAL (ETLAL) computer program contains the expert knowledge required to troubleshoot the LAL assay problem. The knowledge-based programming technique (3, 4) was utilized to construct two other expert systems (5, 6).

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Correspondence address: 49001.

Materials and Methods

The Operating Environment

The Expert Troubleshooting LAL (ETLAL) program was developed using an IBM PC-AT with M.1 software version 2.1 (Teknowledge Inc., Palo Alto, CA). M.1 is a rule-based expert shell architecture that utilizes inference (*modus ponens*), depth first, and backward chaining search techniques with limited forward-chaining capability (7).

In order to accelerate information gathering steps, a notebook or a spread-sheet style data entry format was constructed using "Windows for Data" software (Vermont Creative Software, Richford, VT). All software were interfaced by a C-language-based program that was developed and compiled using a Microsoft C compiler