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Dimethyl-β-cyclodextrin as Parenteral Drug Carrier★

J. SZEJTLI
Biochemical Research Laboratory of CHINOIN Pharmaceutical Chemical Works, 1026 Budapest, Endrödi S. u. 38–40, Hungary

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Abstract. The following advantages are expected from the application of a soluble non-toxic inclusion-complexing drug carrier: (a) aqueous injectable solutions could be prepared from insoluble or poorly soluble drugs; (b) the stability of the dissolved drug could be improved; (c) by decelerating the elimination (by diffusion, circulation, metabolism) the duration of the biological effect could be prolonged; (d) by reducing the plasma level of the active drug (= free, non-complexed drug) through complexation, the toxic effects could also be reduced; (e) in oral administration the bioavailability will be strongly enhanced.

The heptakis-2,6-di-*O*-methyl-β-cyclodextrin (abbreviated as dimethyl-β-cyclodextrin, or DIMEB) is very soluble in cold water. In such aqueous solutions many insoluble (poorly soluble) compounds, drugs, fat-soluble vitamins, etc. can be dissolved. E.g. the solubility of steroids in water increases by a factor of 40–1200; 13 mg/ml progesterone or 20 mg/ml hydrocortisone can be dissolved in a 100 mg/ml DIMEB solution.

NMR and circular dichroic spectra indicate the formation of inclusion complexes. Chemical stability and duration of the biological effects of drugs are enhanced, diffusion and biological elimination are also decreased on complexation with DIMEB.

Key words: Dimethyl-beta-cyclodextrin, injectable solutions, enhancement of solubility, inclusion complexation of drugs.

1. Introduction

A great number of papers and patents have been dedicated to the application of cyclodextrins for the complexation of drugs. Through complexation with β-cyclodextrin (= host molecule) stability, bioavailability and formulation of numerous drugs (= guest molecules) can be favourably modified [1, 2]. Various factors set limits to the application of this kind of molecular encapsulation, but nevertheless a slow but steady invasion of cyclodextrins is expected in oral drug preparations in the forthcoming years.

Parenteral application of β-cyclodextrin-complexed drugs remains however very limited because their solubility is not satisfactory for injectables, and mainly because parenterally administered β-cyclodextrin has resulted in serious renal toxicity [3].

The following advantages are expected from the application of a soluble non-toxic inclusion-complexing drug carrier:

(a) aqueous injectable solutions could be prepared from insoluble or poorly soluble drugs
(b) stability of the dissolved drug could be improved
(c) decelerating the elimination (diffusion, circulation, metabolism) the duration of biological effect could be elongated

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(d) reducing the plasma level of the active drug (= free non-complexed drug) through complexation, toxic effects could also be reduced
(e) in oral administration the bioavailability will be strongly enhanced.

Among appropriately designed cyclodextrin derivatives probably one or more compounds can be found that meet the requirements, and consequences mentioned above.

Heptakis-(2,6-di-*O*-methyl)-*β*-cyclodextrin (Figure 1) is such a compound that has been studied as a potential parenteral drug-carrier.

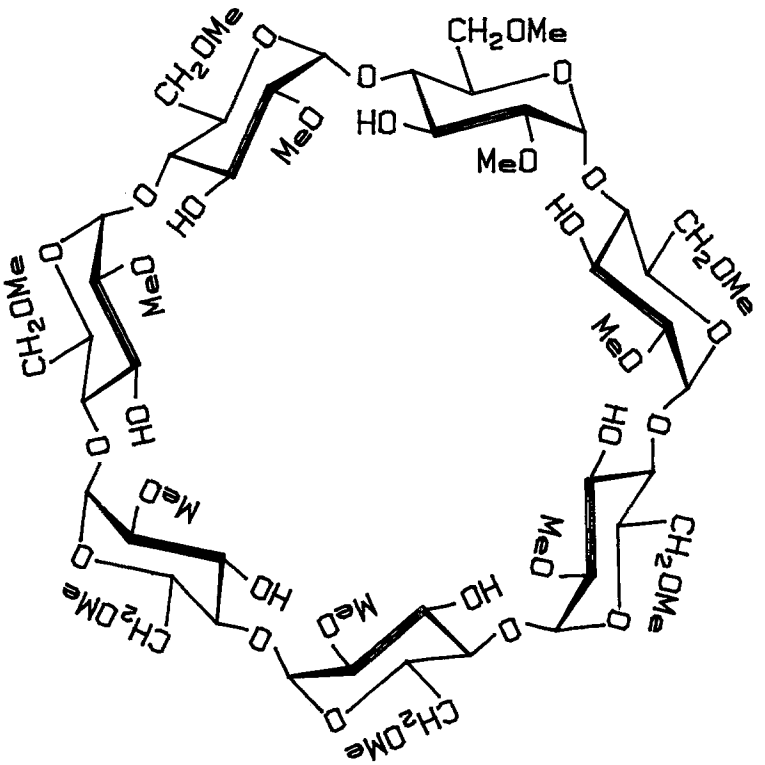


Fig. 1. Chemical structure of DIMEB (Me = CH_3).

2. Preparation and Properties of the Host-Molecule

Heptakis-(2,6-di-*O*-methyl)-*β*-cyclodextrin is also called tetradecakis-methyl-, or more simply dimethyl-*β*-cyclodextrin. This long name is abbreviated to DIMEB.

DIMEB is prepared by selective methylation of all C(2) secondary and all C(6) primary hydroxyls of *β*-cyclodextrin while C(3) hydroxyls remain unsubstituted. The solubility and the solvent effect of the permethylated heptakis-tri-*O*-methyl derivative product is lower, while a non-defined, heterogeneous, partially methylated product cannot be seriously considered for pharmaceutical application. Only a well-defined, homogeneous, highly water-soluble pure substance, with good complexing abilities – i.e. with high solvent effect – seems to be acceptable as a parenteral drug carrier. Until now DIMEB is the only candidate for this purpose among CD-derivatives.

The methods for the alkylation of cyclodextrins have been recently surveyed by Lipták *et al.* [4]. An essential feature of all former published methods was the methylation of β-cyclodextrin in absolute DMFA and DMSO solutions, in presence of BaO and $Ba(OH)_2$. These methods are appropriate for the preparation of DIMEB on laboratory scale, but are not suitable for scaling-up, because of low yield, the amount of expensive solvent required, and the environment polluting by-products (barium salts).

A somewhat improved method (repeated, controlled methylation in aqueous system) has already been elaborated [5], nevertheless we do not yet possess an adequate technology for the production of DIMEB on an industrial scale. Efforts are continuing toward such a technology.

The DIMEB used in our studies was prepared by a slightly modified [6] version of the original Casu method [7]. The substance is a white, crystalline powder, its melting point (crystallized from water by warming(!) up to 80 °C) is 298–300 °C.

$[\alpha]_D = +150\,°C$ (c = 1, water)

$[\alpha]_D = +121\,°C$ (c = 1, chloroform)

$R_f = 0.54$ (benzene : methanol = 8 : 2)

$R_f = 0.65$ (benzene : methanol = 7 : 3)

Characteristic proton shifts in deuterochloroform: 4.96, 5.06, 3.64, 3.39 ppm.

DIMEB is soluble in organic solvents, and very soluble in cold water, too. 25–30% solutions of increased viscosity can be readily prepared, while a syrupy 50% solution can be prepared by prolonged stirring and shaking. The highly enhanced solubility of substituted cyclodextrins can be rationalized by assuming that the dissolution of cyclodextrin in water

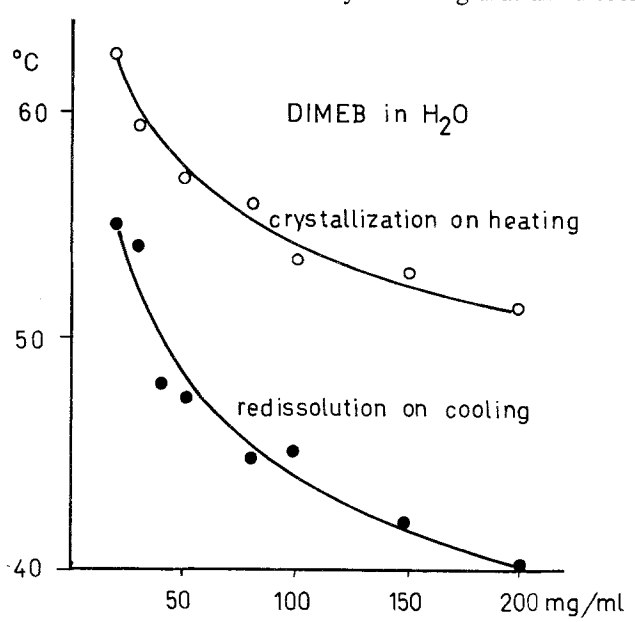


Fig. 2. Crystallization (on heating!) and redissolution (on cooling!) temperatures in function of DIMEB concentration.

itself is anomalous. The low solubility of cyclodextrins (1,8 g β-cyclodextrin in 100 ml water at 20 °C) may be a consequence of intermolecular hydrogen bonding between hydroxyl groups along the edges of the ring preventing adequate hydration by water molecules. Partial methylation precludes the formation of intermolecular hydrogen bonding and therefore unsubstituted C(3)-OH groups become highly hydrated.

An uncommon property of DIMEB solutions is that the homogeneous and clear solutions suddenly crystallize on heating. The temperature of crystallization depends on concentration but, for given conditions, occurs within a 0.5 °C range. On cooling, redissolution is similarly abrupt and the whole process is characterized by a hysteresis loop of 7–12 °C. Crystallization and redissolution temperatures are compiled in Figure 2 as a function of temperature.

Separation of the crystalline DIMEB (or its complexes) from warm solutions has to be performed on a heated filter, otherwise the substance gets redissolved on cooling.

3. Preliminary Studies on Excretion and Toxicity of Parenterally Administered DIMEB

Determination of DIMEB in blood and urine is a relatively simple procedure: DIMEB is the only carbohydrate which can be extracted by chloroform from deproteinized biological liquids. Following oral administration of DIMEB, rabbits excreted only 2.7% of the administered dose with the urine within 24 h. Being a highly water soluble metabolite-like compound, DIMEB is probably not absorbed. According to Szabó *et al.* [8], intramuscularly administered DIMEB is fully excreted with the urine within 24 h. Six hours after intravenous administration only traces of DIMEB can be detected in the blood – the majority of the administered substance is removed from the circulation within the first 2 hours, (Figure 3) and can be found in the urine.

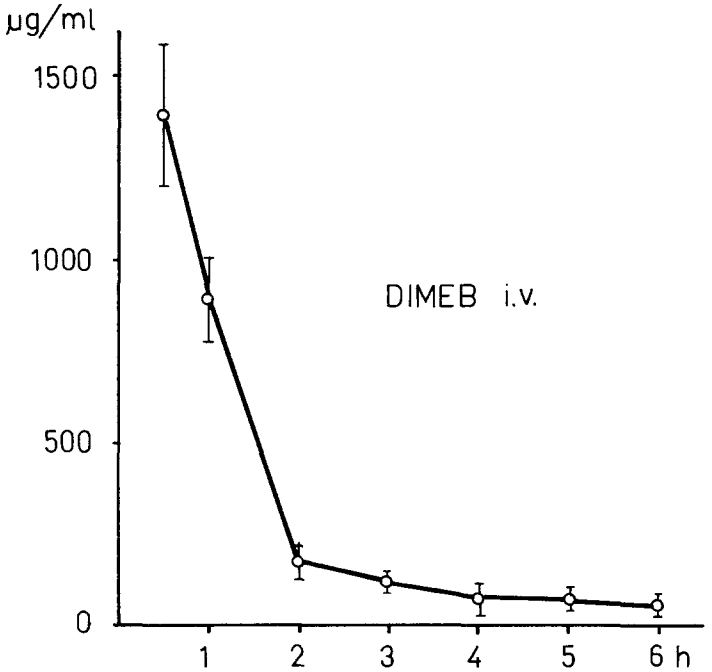


Fig. 3. DIMEB blood level in rabbits after intravenous administration (150 mg/kg).

Considering the nephrotoxic effect [3] of parenterally administered unsubstituted β-cyclodextrin the crucial point is the effect of DIMEB on the kidneys. Intramuscularly administering 50 mg/kg/day dose DIMEB for 12 days to rabbits, Serfözö *et al.* [9] found no fundamental difference in the noxious renal effects of β-cyclodextrin, and its mono-, di- and trimethylated derivatives, but DIMED showed to be the less toxic. At any rate, 50 mg/kg/day in chronic treatment seems to be a provocative dose.

Intravenous treatment of mice (CLFP, male, 25–30 g body weight) with a single dose of DIMEB up to 150 mg/kg appeared to be non-toxic [10] (50, 100, 150 and 300 mg/kg doses with 100 mg/ml DIMEB solution, pH 7, 3–5 animals sacrificed in 4th, 24th and 48th hours following intravenous administration, kidneys studied under light- or electron microscope). Four hours after the injection, electron microscopic investigations showed a significant increase in the number of autophage vacuoles and secondary ribosomes, but the regeneration was complete within 48 hours. Reversible histological alterations of this type are generally observed following intravenous injection of sugars, e.g. sucrose or mannitol.

At a 50 mg/kg DIMEB dose no histopathological difference was found between treated and control animals, while a 50 mg/kg intravenous β-cyclodextrin dose already led to irreversible changes (at 25 mg/kg changes were still reversible). In rats the intravenous LD_{50} was 220 mg/kg, and 350 mg/kg on subcutaneous application.

Oral administration of DIMEB (in aqueous solution) to male and female mice (10 animals of both sexes for each studied dose) resulted in no toxic symptoms up to 3000 mg/kg. No effect of DIMEB on active and passive transport on ^{42}K and ^{86}Rb ions, or on hemolysis of human erythrocytes was observed up to 10^{-2} mole/litre concentration (about 13 mg/ml), however at 1.7×10^{-2} mole/litre, DIMEB resulted in hemolysis. Therefore on parenteral administration, a local concentration higher than 20 mg/ml DIMEB should be avoided (e.g. intravenously it should be injected slowly).

Table I. Examples for enhancement of solubility in a 10 g/100 ml aqueous DIMEB solution (25 °C)

Substance	Solubility in water mg/ml (S_1)	Solubility in DIMEB sol. mg/ml (S_2)	$\frac{S_2}{S_1}$
p-aminobenzoic acid	4.05	12	3
p-hydroxybenzoic acid	5.9	25	4
1-naphthol	11	10	8
2-naphthol	6.2	12	20
toluene	0.44	0.6	22
hydrocortisone	0.33	23	56
digoxine	0.27	22.2	81
methyltestosterone	0.071	13.7	193
progesterone	0.016	13.0	812
nortestosterone	0.31	14.7	47
3β, 17α, 21-triacetoxy-5-pregnen-20-one	0.01	10.2	1025
3β, 17α, 21-trihydroxy-5-pregnen-20-one-21-acetate	0.008	9.1	1137
16α-methyl-Reichstein *S*	0.011	13.7	1245

4. Inclusion complexes of DIMEB

In an aqueous solution of DIMEB a number of insoluble (poorly soluble) compounds, drugs can be dissolved. E.g. the solubility of steroids increases by a factor of 40–1200; it is possible to prepare a stable aqueous 10% DIMEB solution which contains 13 mg/ml progesterone or 20 mg/ml hydrocortisone [11]. Table I illustrates some examples of this enhancement of solubility.

It is very interesting that only partially methylated β-cyclodextrin shows such a high solubilizing effect, α- or γ-cyclodextrin derivatives are much more inferior [12].

The increase of solubility depends on the concentration of DIMEB, as is illustrated in Figure 4. It is conspicuous that an increase of 2 mol in DIMEB concentration results in an increase of 1 mol in the concentration of dissolved lidocaine base.

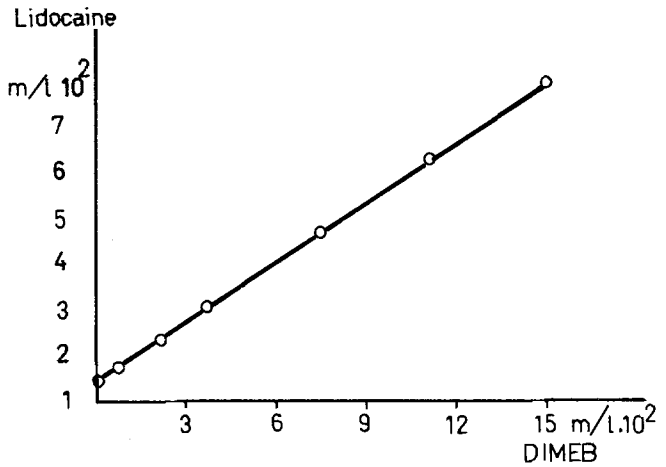
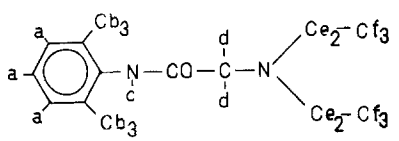


Fig. 4. Solubility of lidocaine base in aqueous DIMEB solution at 25 °C, agitation 3h, 30 mg lidocaine base/ml, grain size 100–200 μ. Determination at 263 nm, after dilution with 0.004N HCl.

Table II. Evaluation of ^{1}H-NMR spectra



Δ = +0,13 -0,04 +0,14 +0,05

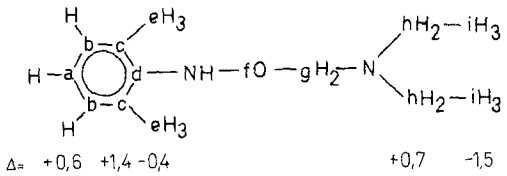
Proton	Chemical shift values (ppm)		
	Free	Complexed	Δ
a	7.06	7.19	+0.13
b	2.24	2.20	−0.04
c	8.86	−	
d	3.21	+	
e	2.65	2.79	+0.14
	2.72	2.86	+0.14
f	1.13	1.18	+0.05

− = does not appear
+ = covered by DIMEB

NMR spectra delivered direct proof for the inclusion of lidocaine in the DIMEB cavity.

Table II contains the ^{1}H-NMR chemical shift data, compared to the data registered in a $CDCl_3$ solution of lidocaine. The same solutions were used for registration of ^{13}C-NMR spectra. (Table III.)

Table III. Evaluation of ^{13}C-NMR spectra



Carbon atom	Chemical shift values (ppm)		
	Free	Complexed	Δ
a	126.9		
b	128.2	128.8	+0.6
c	130.8	–	
d	135.1	136.5	+1.4
e	18.5	18.1	−0.4
f	169.9	–	
g	57.7	+	
h	49.0	49.7	+0.7
i	12.6	11.1	−1.5

– = does not appear
+ = covered by DIMEB

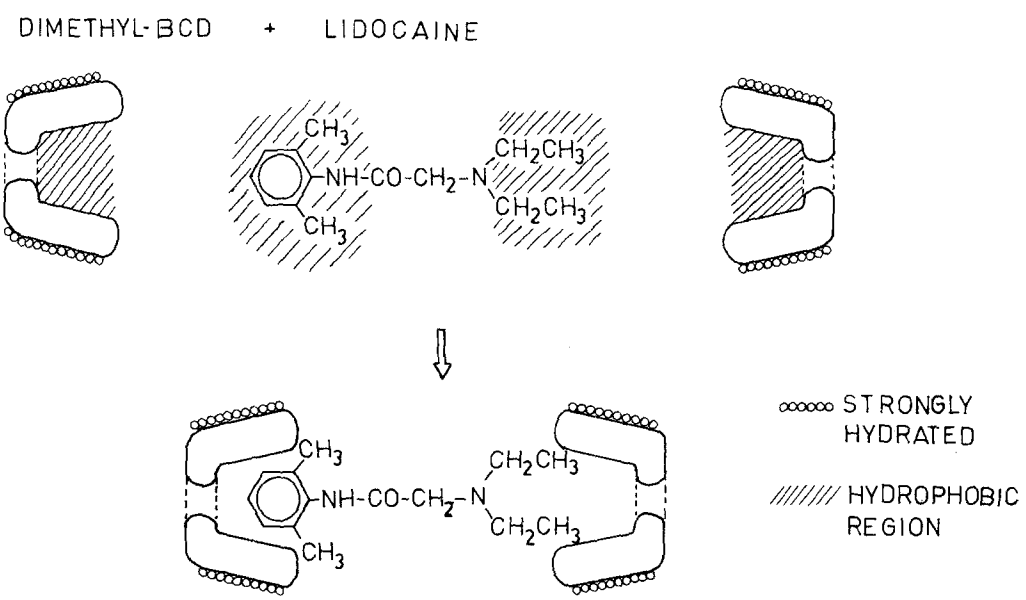


Fig. 5. Schematic illustration of the probable interaction between lidocaine and DIMEB.

Chemical shifts could not be observed for all proton or carbon atoms, because a part of them were disguised by the DIMEB signals. Nevertheless the shifts observed indicate strong interaction between the dimethylamino-end group of lidocaine and DIMEB as well as the aromatic ring and DIMEB. It seems to be likely, that two DIMEB molecules form a 'capsule' for one lidocaine base molecule (Figure 5). The weight ratio (445 mg DIMEB + 45 mg lidocaine, molecular weights are 1330 and 234, respectively) approximates to a 2 : 1 stoichiometry.

A very similar structure was observed with ^{13}C-NMR for vitamin D_3 (cholecalciferol) dissolved in DIMEB D_2O (Figure 6) [13]. The two opposing moieties of the molecule of vitamin D_3 (the ring bearing a methylene group, and the isopropyl end group) penetrate into two facing molecules of DIMEB, as can be seen from the signals of C-atoms in the methylene group, and the vicinal C-atom of the ring, as well as that of the two terminal methyl groups of the alkyl-chain, which are considerably shifted, whereas the rest of the signals remain unchanged. (For details see [13]).

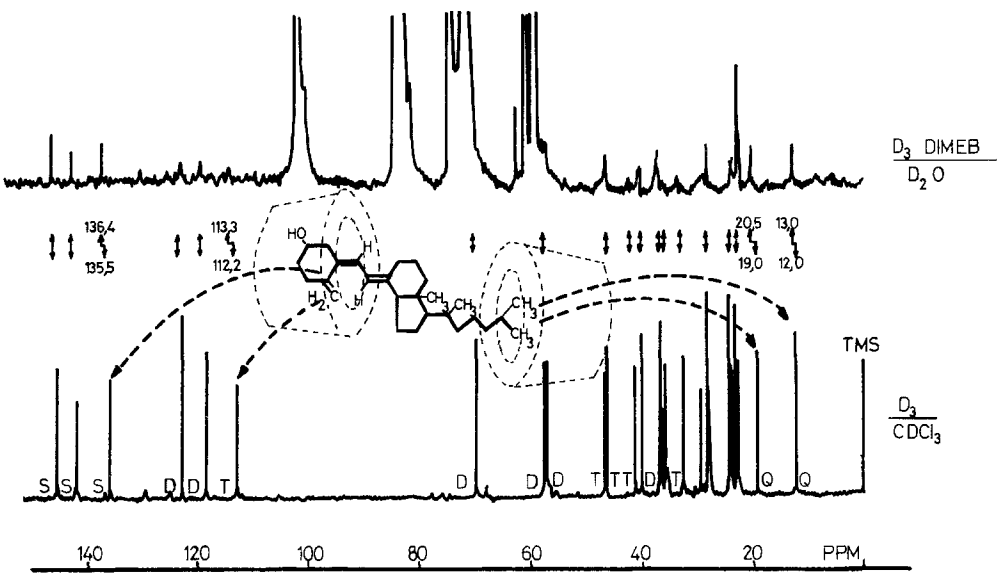


Fig. 6. ^{13}C-NMR spectrum of vitamin D_3 (cholecalciferol) in aqueous DIMEB solution.

For smaller molecules – e.g. menadione (vitamin K_3) – a single DIMEB molecule is enough for inclusion complexation [14]. ^{1}H-NMR spectra prove that the aromatic ring is situated inside the cyclodextrin cavity while C(2)-CH_3 and C(3)-H are located outside the cyclodextrin ring.

Achiral molecules, when incorporated into the cavity of cyclodextrin become, as a part of a chiral system, optically active. Therefore, provided that the guest has a chromophore absorbing in the measurable spectral range (above 190 nm), the induced Cotton effects around the absorption bands of the guest are diagnostic of complex formation [1]. By varying the molar ratio of guest and cyclodextrin, as well as the concentration, the stability constant of the complex can be calculated [15].

An inherent drawback of this technique is that with guest molecules having an asymmetric carbon atom the recorded spectrum becomes too complicated. Qualitatively a change of the

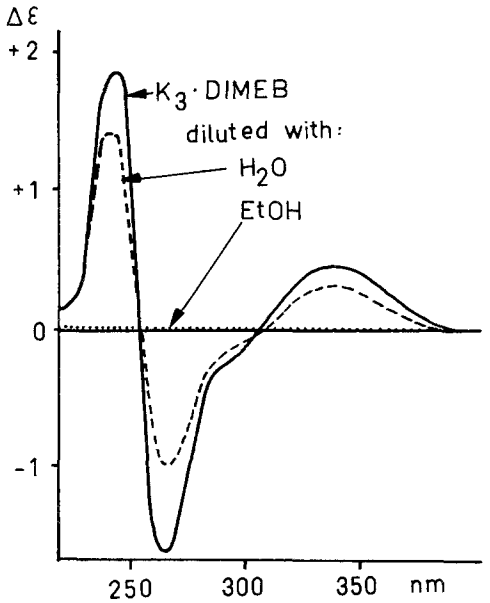


Fig. 7. Circular dichroism spectrum of (a) menadione (5×10^{-4} M) in aqueous DIMEB (3.6×10^{-2} M) solution. (b) the same after a tenfold dilution with water; (c) tenfold dilution with ethanol.

optical activity indicates an inclusion of the potential guest molecule – e.g. cholecalciferol – however the possibility of correct interpretation is restricted to optically inactive guest molecules. A very simple evidence is illustrated in Figure 7 for the correlation between inclusion and induction of Cotton-effects. Diluting the complex solution (Menadione,

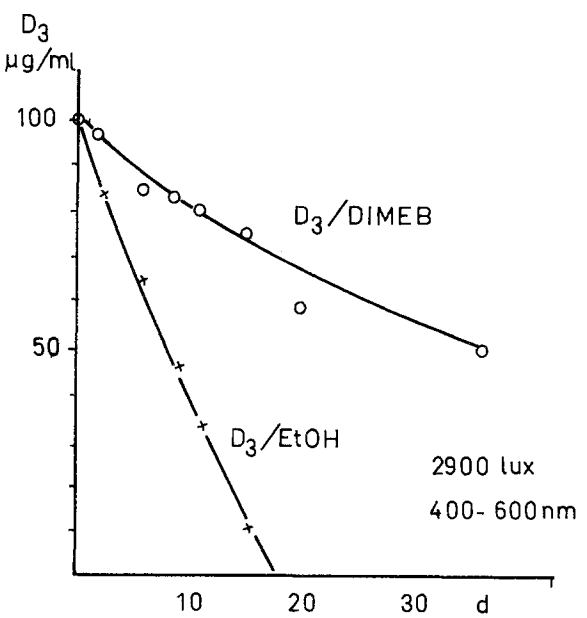


Fig. 8. Effect of light on the destruction of vitamin D_3 in ethanolic (96 vol.%), and in DIMEB (2 mg/ml) containing aqueous solutions (2900 lux, 400–600 nm, 100 μg/ml vitamin D_3).

DIMEB) with water, the induced optical activity decreases corresponding only to the complex equilibrium. If however, the solution is diluted with ethanol, the induced optical activity disappears (menadione is expelled from the DIMEB-cavity by ethanol molecules).

DIMEB stabilizes unstable compounds in aqueous solutions. Figure 8 illustrates the protection of vitamin D_3 against light-induced degradation. 1 mg vitamin D_3 + 20 mg DIMEB in 10 ml water (equivalent to 4000 I.U. vitamin D_3 per ml) can be stored betweed 0 and 45 °C for a long time without any appearance of turbidity [11].

5. Drug – DIMEB Interactions

5.1. ELONGATION OF LOCAL EFFECTS

The main factor in the elimination of locally administered drugs – e.g. elimination of a local anaesthetic drug from the site of injection – is the diffusion process. Diminishing the rate of diffusion results in a longer-lasting effect.

Complexation of a drug with DIMEB means a considerable increase in molecular mass of the drug – without establishing any covalent bonds, i.e. without a chemical modification of the drug. The consequence is a reduced diffusion rate, as is illustrated in Figure 9.

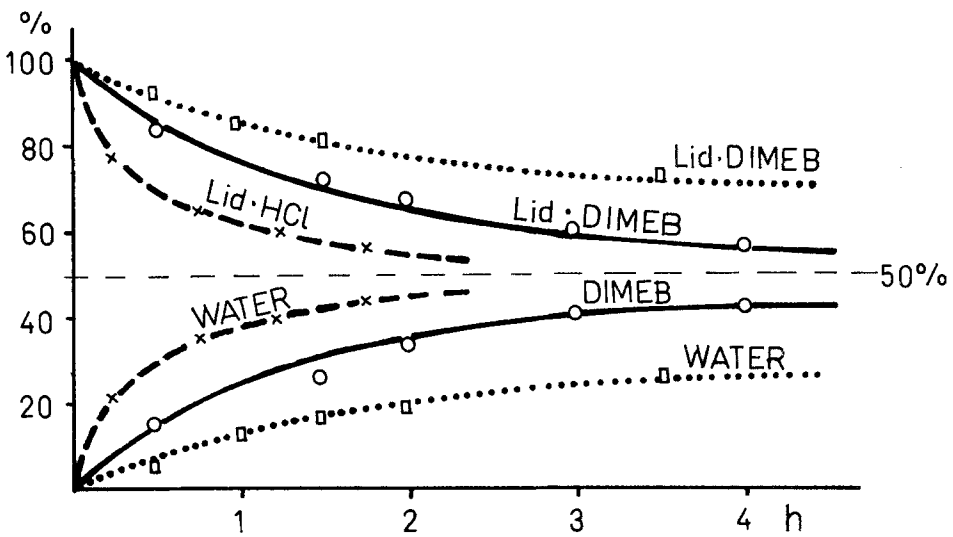


Fig. 9. Diffusion of lidocaine (approximating a distribution equilibrium in a two-compartment diffusion cell) through a cellophane membrane.

In the case of lidocaine · HCl the equilibrium approximated to 90% in about 2 hours, while with lidocaine · DIMEB in 4 hours equilibrium was only to about 50%.

To transform this observation into *in vivo* conditions lidocaine · DIMEB was compared with lidocaine · HCl in anaesthetic tests on rabbits, guinea pigs and rats [16].

In the first experiment surface anaesthesia was studied by applying two drops of one of the appropriately diluted solutions upon the upper margin of the cornea of rabbits. Any irritation of the cornea – e.g. with a wild boar bristle – elicits the reflex closure of the eyelid. This reflex is relieved by a locally acting anaesthetic. By systematically irritating the cornea, and plotting the number of the elicited reflexes as a function of time, the half life of the

Table IV. The $t_{eff\,50}$ values of Lid·HCl and Lid·DIMEB in rabbit cornea

Lidocaine mg/ml	$t_{eff\,50}$ value in minutes		Increase (in %)
	lid·HCl	lid·DIMEB	
2.5	4′54″	6′36″	+ 34.69
5.0	11′45″	18′40″	+ 58.86
7.5	12′10″	24′40″	+102.73

anaesthetic effect ($t_{eff\,50}$) was determined (Table IV). Using DIMEB complex, a 35–102% longer effect was established – depending on the lidocaine concentration.

In a second experiment, lidocaine-containing solutions were injected intracutaneously to guinea pigs, and the pain sensation was monitored by pin-pricking. As Table V illustrates, the duration of the anaesthesia was 55–64% longer when applying the DIMEB complex.

Table V. The $t_{eff\,50}$ values of wheal test on guinea pig

Lidocaine mg/ml	$t_{eff\,50}$ value in minutes		Increase (in %)
	lid·HCl	lid·DIMEB	
5.0	19′55″	31′00″	+55.64
7.5	28′10″	46′45″	+64.28
10.0	38′00″	60′00″	+58.79

In the third experiment, conduction anaesthesia was practised on rats. The solutions were injected to the tail base, and pain reflexes elicited by electric shocks (electrode connected to the tail) were monitored as a function of time. As Table VI illustrates, the duration of anaesthesia was nearly doubled at all concentrations.

Table VI. Duration of conduction anaesthesia in rat tail test

Lidocaine mg/ml	Duration of anaesthesia in minutes		Increase (in %)
	lid·HCl	lid·DIMEB	
5.0	76 ± 15	146 ± 40	92.10
7.5	117 ± 30	215 ± 56	83.76
10.0	222 ± 39	464 ± 42	109.00

A similar experiment was performed with another anaesthetic, marcaine (bupivacaine). Equivalent doses of marcaine·HCl (adrenalin-free) and marcaine·DIMEB were administered subcutaneously to the lower arm of 5 human volunteers. At 30 minutes intervals the skin was stimulated by pin-pricking, and pain sensation was registered. (In 4.5 hours 5 persons gave 45 answers). The number of 'no pain' answers was 9 when physiological saline (placebo) was injected, 17 when marcaine·HCl and 30 when marcaine·DIMEB were used (Figure 10). Within the observed 270-min period the duration of the anaesthesia was nearly doubled on the effect of DIMEB.

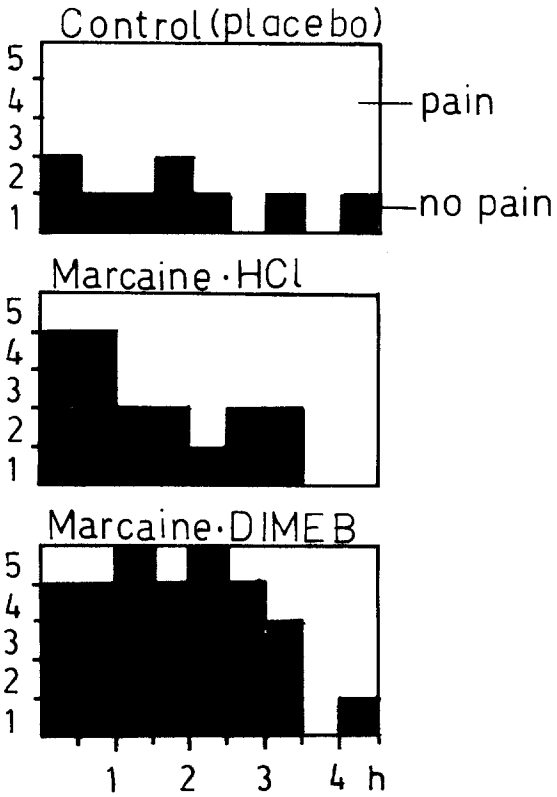


Fig. 10. 'No pain' or pain responses of human volunteers on pin-pricking after subcutaneous injection of 0.2 ml physiological saline, marcaine · HCl or marcaine · DIMEB solution (150 mg DIMEB and/or 4.5 mg marcaine base in 1 ml water).

5.2. ENHANCEMENT OF SYSTEMIC ACTIVITY

The analgesic effect of morphine – studied on rats by the hot plate method – is significantly enhanced by DIMEB [17]. On account of the low solubility of morphine base, it could be

Table VII. ED_{50} values (mg/kg) of various forms of morphine on rats, in hot plate test

	ED_{50} mg/kg		
	i.v.	i.p.	s.c.
Morphine base	–	12.0	–
Morphine · HCl	3.6	9.1	4.6
Morphine base 4 mg/ml DIMEB 100 mg/ml	0.9	5.5	5.0
Morphine · HCl 10 mg/ml DIMEB 100 mg/ml	1.7	–	2.7

injected in suspension only intraperitoneally. Table VII illustrates the ED_{50} values, in mg/kg. Application of DIMEB reduced the ED_{50} value to 46–58% of morphine, i.e. the analgesic effect is doubled. It is particularly important that morphine base can be administered i.v. and s.c. with DIMEB. In this form the activity is fourfold – as compared to morphine · HCl.

For studying the narcosis potentiating effect 35 mg/kg venobarbital was administered i.v. to rats (120–150 g), which resulted in a 526 ± 34 sec sleeping time. Administering 1 mg/kg morphine (as the HCl salt) intravenously increased the sleeping time to 1852 sec (407%), and the same in DIMEB complexed form (10 mg/kg) increased it to 3480 sec (853%). The narcosis potentiating effect was even more pronounced in the case of morphine base. 10 mg/kg base administered i.p. resulted in a 3000 sec (722%) sleeping time, while only 1 mg/kg base + 23 mg/kg DIMEB resulted in a sleeping time of 4104 sec (1024%). DIMEB itself had no significant effect on barbiturate narcosis.

5.3. DETOXIFICATION

Drug levels – exceeding the toxic level – could be successfully reduced by the method of Perrin *et al.* [18]; the extraction of barbiturates from rats by intraperitoneal dialysis was about 3 times faster when the dialysing solution contained β · CD. Pitha and Szente [19] recently studied the relievement of A-hypervitaminosis, by parenteral application of DIMEB. About 50 μg/kg/day of the retinoid proved to be sufficient to prevent avitaminosis. At a hypervitaminotic level of about thousand times higher dose, retinoids inhibit the growth of carcinogen-induced cancers in epithelial cells. At such high concentrations, however, retinoids are toxic – the binding capacity of the retinoid–carrier proteins is not high enough. Administering 100 mg/kg retinoic acid intraperitoneally to mice, only 37% survived, while 69% survived and recovered from hypervitaminosis when DIMEB was also injected. Probably, the level of the free vitamin was decreased by complexation with DIMEB, resulting in a reduced toxicity.

6. Design of a Parenteral Drug Carrier

The majority of drugs are hydrophobic, and can therefore circulate only as water-soluble protein complexes in the body. Plasma and tissue proteins have hydrophobic regions, cavities, where hydrophobic drugs reside – and this equilibrium process is governed by the corresponding complex stability constants. There are specific binding proteins – e.g. for transport of steroids – and non-specific carrier proteins. Plasma albumin can bind not only hydrophobic drugs, but also azo dyes, as, e.g., methyl orange.

A serious problem arises, when the binding capacity of the plasma is not sufficient, as, e.g., in the case of tumor treatment with vitamin A. Too high a level of free, noncomplexed vitamin A is toxic. In such cases an artificial, non-toxic parenteral drug carrier probably could reduce toxicity. Using such a carrier, detoxification would be faster, and more effective.

The design of such a carrier is a complicated task, because strong complexing carriers may deprive vital compounds from the circulation, therefore highly specific carriers are needed. Only a limited amount of an artificial complexing agent can be introduced into the circulation, and it must compete with plasma proteins to bind the minute quantities of the drug, or toxic substance, effectively.

The possibility of the design of an artificial drug carrier may be estimated as follows [20]. Blood plasma represents about 55% of the blood and an adult body contains about 3 liters of plasma. Human serum albumin represents about 65% of the total of plasma proteins and

is also one of the main carriers of synthetic drugs. Plasma contains 4.5% of this protein, its molecular mass is 67000, i.e. 3 liter plasma contains 2×10^{-3} mol carrier protein. This is equivalent to about 2.7 g DIMEB. For an adult this is less than 40 mg/kg. Such a dose seems to be acceptable; the problem lies, however, in the complex's stability. The stability constants of the complex of the anticoagulant warfarin with serum protein is 24×10^4 mol^{-1}, of the tranquilizer diazepam 18×10^4 mol^{-1}, both having 1 : 1 stoichiometry [21]. Stability constants of DIMEB complexes are of around 10^2–10^3 order of magnitude only, therefore DIMEB cannot be considered as a final solution for a drug carrier, but as a first step it seems to be quite a reasonable approximation.

It is noteworthy, that while the K value for Methyl orange · Cyclodextrin is 5×10^3, the value for Methyl orange · DIMEB is nearly ten-fold higher, 4.6×10^4. Therefore a detailed study of stability of drug-DIMEB complexes seems to be a rewarding task.

7. Summary

Cyclodextrins are adequate 'molecular encapsulating agents' for many compounds (drugs). Stability and solubility of unstable, poorly soluble drugs are enhanced by inclusion complexation, nevertheless, only in a few cases does the solubility reach that level which is necessary for preparation of injectable solutions. Moreover the most thoroughly studied inclusion complexing agent, the β-cyclodextrin, is nephrotoxic on parenteral application.

The heptakis-2,6-O-dimethyl-β-cyclodextrin (DIMEB for short) seems to be an acceptable potential parenteral drug carrier. Its solubility is very good in cold water, and its solubilizing effect is excellent for a number of poorly soluble drugs. Stable clear aqueous solutions of several mg/ml concentration can be prepared from steroids, fatsoluble vitamins and drugs of similar solubility. The partial methylation of β-cyclodextrin apparently not only enhances the solubility and complex stability but simultaneously reduces the parenteral toxicity. According to preliminary toxicity tests the provocative intramuscular dose is about 50 mg/kg. Haemolytic concentration is over 10^{-2} mole/litre (about 13 mg/ml) concentration, i.e. an intravenous injection should be administered slowly to avoid local haemolytic concentration.

Injecting two ml of a 100 mg/ml DIMEB-containing solution means only 3 mg/kg for an adult. Thus, for drugs applied in low doses in treatments of short duration, DIMEB can be taken into consideration as a potential parenteral drug carrier.

A preponderant number of drugs in circulation are bound to carrier proteins, and unbound drugs are often toxic. Specific parenteral drug carriers for reduction of toxicity, to decelerate the elimination and to enhance the biological activity should be sought among DIMEB-like non-toxic, soluble inclusion complex-forming agents.

8. Experimental

Preparation of DIMEB: see in [6].

Excretion and toxicity studies: see [8] and [9].

Determination of DIMEB in blood and urine as well as results of detailed toxicity studies will be published later.

NMR spectrum: 445 mg DIMEB in 3 ml D_2O + 45.3 mg lidocaine spectrum registered at 40 °C on a Varian XL–100 FT–15 spectrometer.

Preparation of DIMEB complexes:

DIMEB complex solutions are prepared simply by dissolving the guest substance in aqueous DIMEB solution [11]. Elevated temperature (40 °C), intensive agitation, nitrogen atmosphere (for easily oxidizable substances), but first of all, appropriate host : guest ratio are the preconditions required. Two molecules of DIMEB are needed to maintain one lidocaine base molecule in solution, but 6 molecules DIMEB are needed for one molecule cholecalciferol. (5 w%, vitamin D_3 for DIMEB.) Solutions of higher cholecalciferol ratio (up to 12%) and concentrations up to 20 mg/ml can be prepared, but such solutions are not stable; a certain amount of the dissolved substance precipitates within hours, and only about 5 mg vitamin D_3 + 100 mg DIMEB remain dissolved in 1 ml water.

On heating such a solution above 60 °C, the complex precipitates out immediately. It has to be filtered on heated filters (on cooling it redissolves instantly). After drying, the crystalline complex is stable. The X-ray diffraction pattern of this substance differs significantly from the corresponding patterns of crystalline DIMEB and vitamin D_3.

Prevention of photodegradation:

1 mg vitamin D_3 and 20 mg DIMEB were dissolved in 10 ml water and compared with 1 mg vitamin D_3 dissolved in 10 ml ethanol. The solutions were sealed under nitrogen in glass vials, and kept under 2900 lux irradiation (400–600 nm) for 34 days. (Figure 8.)

Diffusion test:

The two chambers of a stainless steel diffusion cell (2 + 2 ml) were separated by a membrane (Visking Tubing, average pore diameter 24 Å, surface 4.15 cm^2). Lidocaine content was followed as a function of diffusion time (25 °C, 55 stroke/min), the cell contained lidocaine · HCl solution *versus* water, or lidocaine · DIMEB solution *versus* water.

Lidocaine · DIMEB solutions (eye drops and injections):

15 g DIMEB and 1,5 g lidocaine base were dissolved in 100 ml physiological saline (pH adjusted to 6.4–6.7). As control, adrenalin-free lidocaine · HCl solution of identical lidocaine concentration was used in physiological saline.

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