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Review Article:

The Properties and Potential Uses of Cyclodextrin Derivatives

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Abstract. The hydroxyl groups can be selectively substituted to control the solubility and the complex forming selectivity of the modified cyclodextrins (CDs). Among the methylated CDs only two well-defined compounds can be taken into consideration: dimethyl- and trimethyl-βCD (DIMEB and TRIMEB). In an aqueous solution of DIMEB the solubility of rather insoluble compounds and drugs like steroids, vitamin D_3, lidocaine and hydrocortisone increases. In some cases their stability and bioavailability are also improved. On the other hand, the hydrolysis rate of carmofur, coumarins etc. is retarded by the methylated cyclodextrins with blocked hydroxyl groups. The drug solubilizing capacity of hydroxypropyl-βCD (HPBCD) is in most cases lower than that of DIMEB. The degree of substitution (DS) shows no remarkable effect on the solubilizing properties of HPBCD in the case of indomethacin, Dipiridamole etc., but in the case of Tolnaftate the solubility was enhanced by increasing the DS, other examples are shown.

Key words. cyclodextrin derivatives, solubility, drug carrier characteristics, complex formation.

1. Introduction

Cyclodextrins (CDs) can be modified chemically, mainly by substituting their hydroxyl groups. The hydroxyls are characterized by different reactivities, and can be selectively substituted. By varying the substituents, the solubility and the complex-forming selectivity of the modified CDs can be controlled. The preparation and properties of hundreds of CD-derivatives has been published already, giving ideas on how to make 'tailor-made' derivatives to solve various problems.

Several CD-derivatives already are produced and utilized industrially, others are prepared only on a laboratory scale, and used for analytical or research purposes, with promising results.

2. Methylated CDs

With increasing degree of methylation the solubility of βCD (in cold water) increases until about 2/3 of all hydroxyls are methylated, then decreases again, i.e. the 14-methoxyl group-containing βCD shows the highest solubility, the permethylated (21 methoxyl-containing) has reduced solubility, although it is considerably higher than that of the unsubstituted βCD.

For many practical purposes the heterogeneous, partially methylated βCDs are applicable. For specific areas – e.g. pharmaceuticals – only two well-defined compounds can be taken into consideration: dimethyl and trimethyl βCD. Their correct

names, heptakis-(2,6-di-*O*-methyl)- and heptakis-(2,3,6-tri-*O*-methyl)-βCD, have been abbreviated to DIMEB and TRIMEB.

An absolutely homogeneous heptakis-(2,6-di-*O*-methyl)-βCD would be too expensive for most purposes, the presence of other isomers (2,3- or 3,6-di-*O*-methyl) have to be tolerated. The 13- or 15-methoxyl group-containing methylated βCDs are very similar to DIMEB, both in their physical and chemical properties. Only careful chromatography (HPLC) can discriminate between them. Preparative elimination of the last traces (several percent) of these contaminating, but very similar isomers and homologues is very expensive, and for industrial purposes it is unnecessary. Other impurities, heavy metals, solvents etc., however, must not exceed the low ppm level in the marketed DIMEB.

DIMEB is soluble in organic solvents, and very soluble in cold water. 20–25% solutions of increased viscosity can be readily prepared. An uncommon property of DIMEB is that the homogeneous and clear solution will suddenly crystallize on heating. The temperature of crystallization depends on concentration, but for given conditions crystallization occurs within a 0.5°C range. Redissolution on cooling is similarly abrupt and the whole process is characterized by a hysteresis loop of 7–12°C.

The LD_{50} of DIMEB in mice is 220 mg/kg i.v. and 350 mg/kg s.c. Administration of 50 mg/kg/day DIMEB intramuscularly to rabbits over 12 days, caused renal necrosis. In mice single i.v. doses of DIMEB were non-toxic up to a dose of 150 mg/kg. No histopathological changes were observed in mice when 50 mg/kg/day DIMEB was given for 12 days. No toxic symptoms were found in mice at up to 3000 mg/kg DIMEB given p.o. The toxicity of DIMEB manifests itself through its haemolytic activity, probably by sequestering the cholesterol from the cell-membranes, causing their destruction.

A number of insoluble or poorly soluble compounds and drugs can be dissolved in an aqueous solution of DIMEB. E.g. the solubility of steroids increases by a factor of 40–1200. It is possible to make a stable 10% aqueous DIMEB solution which contains 13 mg/ml progesterone or 20 mg/ml hydrocortisone. Figure 1 illustrates the solubility isotherms of a steroid as a function of the concentration of various CDs including DIMEB. Table I illustrates the solubility enhancing properties of DIMEB. It is noteworthy that only partially methylated βCD shows such a high solubilizing effect, α- or γCD derivatives are much less effective. Unlike the underivatized CDs, both DIMEB and TRIMEB have surfactant activity.

The DIMEB complexes of vitamin D_3, lidocaine, hydrocortisone, prednisolone and phenanthroline derivatives, flurbiprofen, prostaglandins, disulphiram, diazepam, fluorometholon, coenzyme Q_{10}, bethamethason etc., have been studied. In all cases improved solubility, and in some cases improved stability and bioavailability, was reported.

The interactions between drugs and TRIMEB have been less extensively studied, e.g.: the effect of mechanical grinding of mixtures of drugs with TRIMEB, the partition of drugs between water and chloroform in the presence of TRIMEB, the enhancement of the bioavailability of orally administered ketoprofen in rats and flurbiprofen in rabbits, the X-ray structure of the flurbiprofen-TRIMEB complex, have been established.

While in many cases hydrolytic reactions in aqueous solutions are accelerated by CDs, the methylated CDs in which the hydroxyl groups are blocked, may cause

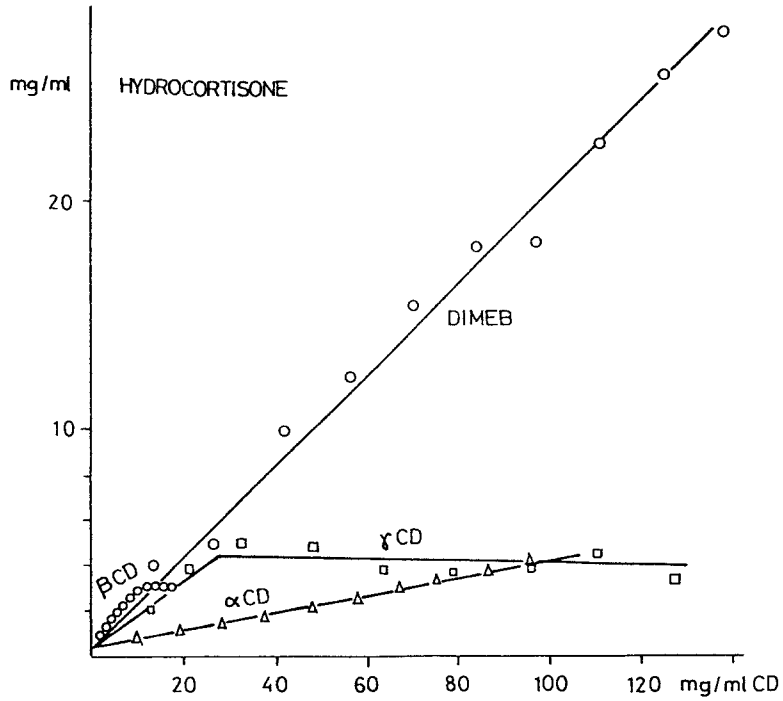

Fig. 1. Solubility isotherm of hydrocortisone as a function of CD-concentration at 25°C.

Table I. Examples of solubility enhancement in a 10 g/100 ml aqueous solution

Substance	Solubility in water mg/ml (S_1)	Solubility in DIMEB sol. mg/ml (S_2)	S_2/S_1
p-aminobenzoic acid	4.05	12	3
p-hydroxybenzoic acid	5.9	25	4
1-naphthol	0.11	1.0	8
2-naphthol	0.62	12.5	20
toluene	0.44	9.6	22
hydrocortisone	0.41	23	56
digoxine	0.27	22.2	81
methyltestosterone	0.071	13.7	193
progesterone	0.016	13.0	812
3β, 17α-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-17,21-dihydroxy-4-pregnen-3,20-dione	0.011	13.70	1245

inhibition of the reaction rather than acceleration. The hydrolysis rate of carmofur, coumarins, digitalis glycosides, ethyl aminobenzoate, indomethacin, epoprostenol and steroid hormones in aqueous solution are significantly retarded by the methylated cyclodextrins. For example the decomposition (dehydration) rate constants of prostaglandin E_2 in aqueous solution (pH 11.0, 60°C) is 62 h^{-1}, 14.7 h^{-1} in the presence of TRIMEB, but in the presence of DIMEB it is only 6.9 h^{-1}.

The less hygrosopic nature of the methylated cyclodextrins compared to the natural CDs is an advantage, as it is moisture sorption that initiates the hydrolytic decomposition of drugs in the solid state.

Oral administration of DIMEB to rats and rabbits fed with large amounts of fats or vegetable oils, strongly improved the digestion and absorption of the fats. Also in bileduct ligated animals nearly normal fat digestion was observed. DIMEB can eventually substitute natural bile.

Adding DIMEB to the culture medium of *Bordatella pertussis*, the synthetized growth inhibiting fatty acids were complexed continuously. This reaction resulted in a 100-fold higher vaccine production. Adding DIMEB to the culture medium of *B. pertussis* has also been published for Leukocytosis promoting factor production.

The survival rate of mice treated with hypervitaminotic doses of vitamin A was improved by intravenous administration of DIMEB to the animals.

The application of DIMEB in the free-radical polymerization of acrylamide in the two-phase system has been reported. DIMEB acted here as a phase-transfer catalyst transporting the water-insoluble initiator (1,1-azobis/1-cyclohexane-carbonitrile) into the aqueous phase.

In analytical chemistry – especially in HPLC – a rapidly developing application of methylated CDs is expected. Another important field of DIMEB utilization will be diagnostic preparations, to enhance the water solubility of poorly soluble substrates.

3. Hydroxypropylated CDs

On treating βCD in alkaline solution with propylene oxide, a 2-hydroxypropyl group will be connected to one or more hydroxyls of the CD, or to the hydroxyls of the 2-hydroxy-propyl groups, already linked to the βCD molecule. The degree of substitution characterizes such a heterogeneous product, it can be expressed in different ways.

S (substitution degree) expresses the number of substituted hydroxyls per glucopyranose unit, it can be 1, 2 or 3.

DS (average substitution degree) expresses the average number of substituted hydroxyls per glucose unit. Within a CD-ring, it can be any number between 0 and 3.

MS (average molar substitution degree) expresses the number of the hydroxypropyl groups per glucose units. Its value can be more than 3.0 because the propylene oxide can react with the hydroxyl group of a hydroxypropyl substituent forming oligo- and even propyleneglycol side chains.

The *MS*/*DS* ratio ($=DP$) defines the degree of polymerization of the polypropyleneglycol side chain.

RS defines the number of the substituents in a CD-ring, which can be 1, 2, 3 . . . 21, but when DP > 1, it can be even >21.

PS defines the average number of the substituents per CD-ring, its value can be any number from 0 to 21, but when DP > 1 it can be even >21.

A product, characterized by a given PS, very probably is a mixture of at least 10 different substances which differ in their RS value. Because the hydroxyls of the CD ring building glucose units can be in C2, C3 or C6 positions, an extremely large number of varieties can be expected. By varying the reaction conditions, not only can the degree of substitution be determined, but even its site (primary or secondary hydroxyls) can be strongly influenced. (e.g. the ratio of substituted primary and secondary hydroxyls can be 2:1 or 1:9).

In addition to this heterogeneity of the hydroxypropyl-βCD, polypropyleneglycol is also formed from the propylene oxide, which is not easy to remove from the product. In some steps of the technology organic solvents are used, they also have to be removed, down to ppm levels. Because this product is for injection it must be pyrogen free, that is to prepare a hydroxypropyl βCD is a very simple task for any organic chemist. To produce an injectable hydroxypropyl-βCD is quite something else – at any rate, not an easy task.

Hydroxypropyl-βCD is an amorphous white powder, but the preparations with higher degrees of substitution (12–14) are semi-solids and neither freeze drying nor treatment with organic solvents can transform them into a powdery form. The HPBCD preparations are very soluble in water, up to 75% w/w solutions can be prepared. They are also 50–60% w/w soluble in ethanol (95%), and samples with degrees of substitution less than 7 have limited solubility in acetone. HPBCD samples with degrees of substitution of 11–14 are soluble in acetone and dichloromethane, but insoluble in cyclohexane.

Because of the heterogeneity and lack of crystallinity there are no problems with the sterilization of solutions, no precipitations can be observed when warming up their aqueous solutions. The hydroxypropyl-CDs with higher degrees of substitution have higher surface activity and thus increased apolar behavior, while a low degree of substitution results in low surface activities.

HPBCD under *in vitro* conditions is even less susceptible to β-amylolytic degradation, than βCD. Orally administered HPBCD is excreted without absorption or any extensive metabolic transformation.

The beginning of the haemolysis of human erthyrocytes can be observed in the presence of 3 mg/ml βCD, 6 mg/ml αCD, or 20 mg/ml γ-CD. In the presence of methylated CDs haemolysis is a serious problem, already 4 mg/ml DIMEB concentration results in a 50% haemolysis (*in vitro*, under standardized conditions).

Hydroxypropyl-βCD behaves similarly to γCD, it shows haemolytic activity only at higher concentrations. Its haemolytic activity at identical concentration is at least 1/8th that of DIMEB.

In mice acute intraperitoneal administration up to 10000 mg/kg and intravenous administration up to 2000 mg/ml did not cause any death.

Preliminary human trials on the intravenous administration of HPBCD showed that this derivative does not cause any toxic symptoms in 1 g per day i.v. dose.

HPBCD exerts only a small effect on human nasal ciliary epithelial function, which is an advantage for chronic nasal administration of this compound.

The drug solubilizing capacity of HPBCD – which depends on the substitution degree and patterns, and of course concentration – in most cases is lower than that

Table II. Solubility enhancement of some drugs in a 10% aqueous solution of the CD derivative, expressed as X-fold solubility enhancement v.s. the uncomplexed drug

	Ibuprofen	Tolnaftate	Indomethacin	Griseofulvin	Hydrocortisone	Dipiridamole
Beta-CD[a]	2.1	70	3.0	2.0	18	7.0
DIMEB	28	4600	22.5	4.6	87	218
TRIMEB	1.9	95	1.5	3.2	17	25
RAMEB	28	2600	16.0	4.0	43	87
SUMEB	27	2100	20.0	2.0	35	146
HPBCD-3,2	23	140	17.0	3.1	67	12
CDPS	17	400	–	–	–	–
CDPSI-3,2	15	180	–	–	–	–

DIMEB = heptakis (2,6-di-*O*-methyl)beta-CD
TRIMEB = heptakis (2,3,6-tri-*O*-methyl)beta-CD
SUMEB = monosuccinyl-DIMEB
HPBCD = hydroxypropyl-beta-CD, 3.2-hydroxypropyl-groups per CD-ring
CDPS = epichlorohydrin-crosslinked, soluble beta-CD-polymer, M_w about 5000
CDPSI = carboxymethyl group containing CDPS, 3.2 or 5.2 carboxymethyl groups per CD-ring

[a]Saturated solution (about 1.3% at 25°C).

of DIMEB (see Table II) but because of its lower haemolytic effect for parenteral administration HPBCD is considered to be superior to DIMEB.

The degree of substitution (*DS*) shows no remarkable effect on the solubilizing properties of the studied HPBCD samples in the case of indomethacin, Dipiradamole, Lidocain base, Griseofulvin and Ibuprofen. However the solubility of Tolnaftate was markedly enhanced with the increasing DS while the solubilizing effect of HPBCD samples showed a decreasing tendency with increasing DS in the case of steroids (Hydrocortisone, Methyl-testosterone and Triamcinolone-acetonide).

The solubilizing property of HPBCD strongly depends on the properties of the guests: it is a weak solubilizer for Dipiridamole and Tolnaftate, but it is effective for Hydrocortisone.

To attain identical solubility levels of Tolnaftate, 1.4% DIMEB or 10% HPBCD-3 is needed in the aqueous solution. In this and similar cases however the haemolytic activities are very similar therefore the HPBCD cannot be considered as the only possibility among the CD-derivatives.

4. CD Derivatives as Drug Carriers

Cyclodextrins and their pure, homogeneous derivatives are easily crystallizable. An even higher tendency to crystallize is a characteristic feature of their inclusion complexes. In many cases the goal is to isolate crystalline complexes, but there are cases, when such crystallization results in noxious effects. Parenterally administered βCD is concentrated in the kidneys, where formation of insoluble inclusion complexes cause nephrotoxic lesions.

The majority of the described CD-derivatives are either insoluble in water, easily crystallizable, or toxic through their surface active or cell membrane damaging effect. Reviewing all important properties, it became clear, that the derivative that fulfils the above requirements, should be found among the statistically substituted, highly heterogeneous and hydrophilic hydroxyalkyl CD-derivatives.

A potential parenteral drug carrier (solubilizer for preparation of injectable solutions of poorly soluble drugs) has to be:

- well soluble in water
- non crystallizable at any temperature
- non surface active
- good complex former, resulting in well soluble complexes
- must not have too high an affinity towards cell membrane components
- industrially producible simply, cheaply, in high pharmaceutical quality.

Table III demonstrates clearly the correlation between the lipophilicity and other important properties (solubilizing capacity, complex stability, tissue irritating effect) of some CD-derivatives. A similar tendency is observed for the haemolytic activity, surface activity etc. The most hydrophobic derivative shows the highest solubilizing power, but simultaneously the highest adverse effects. 2-Hydroxypropyl-βCD with its good, but not unsurmountable solubilizing capacity has to be considered as a temporary compromise, or the first choice from an oncoming series of similar or better CD-derivatives.

Table III. Correlation between hydrophobicity and several characteristic properties of some CD-derivatives

Hydrophilicity ← increasing of βCD derivatives → Hydrophobicity						
Complexed drug	2,3-Dihydroxy propyl	Hydroxy ethyl	βCD	2-hydroxy propyl	3-hydroxypropyl	DIMEB
			Apparent association constants			
Digitoxin	14000	17000	17000	18000	20000	84000
Prednisolone	760	820	1600	1800	2000	7000
Testosterone	5200	5100	7000	12000	–	29000
			Intramuscular irritation on rabbit muscle Score points (Max. irritation = 5.0)			
	0.00	0.20	0.25	0.38	0.25	3.50
			Solubilizing enhancement factor (X-fold) in 1.5% CD deriv. aq. solution			
Flurbiprofen	–	–	2.4	28	–	44
Progesterone	–	–	3.1	88	–	150
Digitoxin	–	–	27	150	–	390

Table IV. Solubility enhancement factor [CD] = 15 mg/ml.

Drug	βCD	HPBCD	DIMEB
Diazepam	3.6	2.8	9.0
Digoxin	90	57	92
Indomethacin	2.5	1.7	4.5
Prednisolone	14	9	13

In some cases 2-hydroxypropyl-βCD seems to be an even weaker solubilizer than natural βCD itself, as it is seen in Table IV.

The first patent [1] dedicated to a pharmaceutical use of a highly soluble CD derivative describes the preparation of an aqueous solution of poorly soluble biologically active substances using heptakis-(2,6-di-*O*-methyl)-βCD (=DIMEB) as solubilizer. It is true that for many highly hydrophobic drugs this solubilizer is the most effective to date (Table II). This CD derivative shows an extremely high surface activity and high affinity to cholesterol, even 1 mg/mL concentration results in haemolysis of the human erthyrocytes.

A second group of beta-CD derivatives intended for parenteral administration is represented by the dihydroxypropyl derivatives, prepared by reaction of epichlorophydrin with CDs in alkaline aqueous solution [2]. These derivatives – particularly those dihydroxypropyl derivatives which also contain some ionic groups (like carboxymethyl or diethylaminoethyl groups) [3] – are excellent solubilizers for basic or acidic type drugs, respectively. They show no surface activity or haemolytic activity. Nevertheless these substances were not studied in detail as potential parenteral drug carriers, because they contain a certain amount of oligomeric crosslinked highly soluble cyclodextrin polymers with average molecular weights in the range of 3–8000 D. These derivatives are used, e.g. in the photochemical industries and certainly can be used in oral or external drug formulations.

A third group is represented by the less heterogeneous (considering the molecular weight distribution) hydroxyalkylated CDs, like hydroxyethyl, 2-hydroxypropyl, 3-hydroxypropyl-CDs etc., derivatives [4–6]. These show only negligible surface activities and also strongly reduced haemolytic properties. Detailed toxicological studies showed that at least one of these compounds, 2-hydroxypropyl-βCD (=HPBCD) is very well tolerated parenterally even in extreme high doses. It is expected that the approval and marketing of 2-hydroxypropyl-βCD solubilized injectable drug preparations will soon be realized. In such cases, when the solubilizing capacity is satisfactory, this derivative gives an optimum solution of solubility problems. (Table II). Regrettably, however, there are plenty of drugs for which the solubilizing capacity of 2-hydroxypropyl-beta-CD is not satisfactory, or it could be used only at extremely high solubilizer doses or the stability of the solubilized drug is inadequate.

Table V summarizes the known possibilities for the choice of the most appropriate CDs for drug formulation. The search for better CD-based solubilizers led to two further groups of βCD derivatives. It has been observed that introducing highly hydrophilic ionic alkyl- or aryl groups into the methylated βCD

Table V. Choice of CDs for drug formulation.

CD	Most recommended formulation	Remarks
alpha	Parenteral limited to intraarterial infusion	e.g. in intraarterial infusion of PGE_1-alpha-CD. Utilization is restricted to small molecules, or to slim side chain containing ones.
beta	oral (in tablets)	e.g. in Prioxicam-Beta-CD to improve bioavailability and garlic-oil beta-CD, to improve stability. Large amounts will be used in solid oral formulations because of its properties and price.
gamma	parenteral	In parenteral formulations in most cases can be substituted by modified CDs, however for some extreme larger drug molecules (e.g. macrolide antibiotics, large substituent bearing steroids) it will be the most promising choice
Methylated CDs (e.g. DIMEB)	parenteral (limited to low doses, or highly diluted infusions). Oral (in programmed release formulations topical)	This is the most hydrophobic CD, the best solubilizer but has the strongest haemolytic activity. Recommended for a highly hydrophobic guest or for extremely humidity sensitive drugs.

Hydroxypropyl cyclodextrin (HPBCD)	parenteral	The choice No. 1. parenteral formulations, approval and marketing is expected within 2 years.
Hydroxyethylated-β-CD HEBCD	parenteral	Could be used as HPBCD, but has no specific advantage over it.
Branched CDs	oral(?)	Enzymatically 1 glucose or maltose is attached to the CD rings: very heterogeneous, amorphous, non-haemolytic CD. Eventually can be used in oral and topical formulations.
Ethyl- and ethyl-carboxy-methyl-CD	oral(?)	Their complexes are poorly soluble therefore sustained release can be attained. Nothing is known yet on their toxicology.
Dihydroxypropyl-CD and soluble neutral and ionic CD-polymers	oral topical	Less haemolytic than HPBCD, but nothing is known about their toxicity. Specific effects: diffusion deceleration by the soluble polymers. In many cases a better solubilizer than HPBCD.

(by esterifying one or more free hydroxyl groups) it retains its excellent solubilizing capacity but loses its strong haemolytic activity. Such βCD derivatives are e.g. succinyl-dimethyl-βCD, or maleinyl-dimethyl-βCD [7]. These mixed ether-ester derivatives are crystalline, and more or less homogeneous compounds.

The other new group of potentially useful CD derivatives consists of the aminoalkyl [8] or mixed (alkyl, hydroxyalkyl, or caboxyalkyl) [8] ether derivatives. These heterogeneous CD derivatives are amorphous, non-crystallizable derivatives. All alkyl, aminoalkyl, or carboxy-alkyl groups are linked directly to the CD ring through ether linkages. The mentioned Patents do not disclose data concerning the drug solubilizing capacity of these aminoalkyl or mixed ethers. It is however a known fact, that with increasing number of bulky substituents on the ring of the CD-torus, the accessibility of the CD-cavity decreases, therefore a relatively low substitution degree is more advantageous in this respect. On the other hand however, increasing substitution degree improves the solubility, the presence of ionic groups reduces the haemolytic activity and through establishing ionic interactions with ionic guest molecules enhances the complex stability and solubility.

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