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Solubilization and deaggregation of cobalt bis(dicarbollide) derivatives in water by biocompatible excipients

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ABSTRACT

In the field of medicinal chemistry, cobalt bis(dicarbollide) derivatives are promising therapeutic agents. The potential pharmaceutical utilization of metallacarboranes is complicated due to spontaneous self-assembling in water. This problem can be solved by using suitable deaggregating agent. We present here the comprehensive screen of substituted cobalt bis(dicarbollide) derivatives with cyclodextrin derivatives, classical surfactants and amphiphilic copolymers to find general biocompatible excipients. Preliminary results are obtained by using UV/Vis spectroscopy as the technique with the best ratio of applicable information to time and source dependence.

In the field of medicinal chemistry, cobalt bis(dicarbollide) derivatives and other boron clusters were studied as promising therapeutic agents for boron neutron capture therapy (BNCT),[1,2] modulators of enzyme activity (HIV-1 protease[3] or protein kinase C[2,4]) or as medical imaging agents (magnetic resonance imaging (MRI), radioimaging).[2,5] Important properties of cobalt bis(dicarbollide) derivatives are extraordinary stability due to delocalized cluster bonding of the transition metal with ligand orbitals, rigid skeleton and totally delocalized negative charge.[6] They are able to form dihydrogen bonds between partial negative charged hydrogens bonded to electropositive boron atoms (B–H) and the partial positive charge located on hydrogens of common organic molecules (NH, OH).

The potential pharmaceutical utilization of metallacarboranes is complicated in particular due to their interaction with serum albumin and the poor cell membrane permeability. The high hydrophobicity of metallacarboranes causes their low solubility in water and spontaneous self-assembling in aqueous media.[7] The possibilities of the solubilization of metallacarboranes to ease of their transport into cells are either the preparation of stable complex with suitable water soluble cavitand or the using of surfactant. More than 60% of future drugs (API) are lipophilic and difficult to formulate. Surprisingly, the research of suitable excipients improving the bioavailability of metallacarboranes lags behind the investigation of new structures and their properties. The goal of this work is the comprehensive screen of substituted cobalt bis(dicarbollide) derivatives with cyclodextrin derivatives, classical surfactants and amphiphilic copolymers to find general suitable excipients and basic rules applicable to explain and predict strength of binding.

In our best knowledge there are only few studies of well-behaved small carboranes with unsubstituted cyclodextrins making simple complexation stoichiometry.[8] In previous work[9] we have shown the complexation of large metallacarborane $Na[Co(B_9C_2H_{11})_2]$ with alfa-, beta- and gamma-cyclodextrin. We have tried to explain obtained curious titration curves but accurate the determination of binding constants of these compounds was complicated beside amount of different complex of higher stoichiometries by metallacarborane aggregates,[7] cyclodextrin aggregates[10] and probably also compound aggregates. These facts allowed us only more or less qualitative description of binding strength as a function of cyclodextrin cavity size. Based on these experiences it's reasonable to expect that in case of metallacarborane derivatives with not well-defined copolymers and substituted cyclodextrins (sometimes randomly substituted) the system will tend to compose the mixtures of various complexes and aggregates. Nevertheless, it should be plausible to acquire at least qualitative information and trends. We tried several analytical techniques for this screening and finally have chosen UV/vis spectroscopy as the technique with the best ratio of applicable information to time and source dependence.

In this study we have prepared 10 representatives of cobalt bis(dicarbollide) derivatives, all of them slightly soluble in water. Schematic structures with used abbreviations are shown in Figure

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1. The synthesis of **1**–**9** is based on dioxane-ring opening reaction of 8-dioxane-3-cobalt bis(dicarbollide) (**11**) with N-, S- or O-nucleophiles. Synthesis description in notes.[11]

The experiment was carried out as follows: solid metallacarborane (approx. 2 mg) was dissolved by shaking and ultrasound for 24 h in 0.5 mL of 0.1% (w/w) solution of excipient (i.e., 0.5 mg of excipient in 0.5 mL of deionized water), dissolved substances was centrifuged (14,000 rpm ≡ 11000*g* for 10 min) and the solution of metallacarborane with excipient after appropriate dilution (usually 1:10) was measured by UV/vis spectroscopy. Spectrum of cobalt bis(dicarbollide) derivative with excipient was corrected for absorbance of the same excipient (in most cases barely measurable) and the absorbance peak was compared with the absorbance peak of alone metallacarborane dissolved in deionized water (10 MΩ). Effect of excipient to metallacarborane solubility was determined as the ratio of absorbance of metallacarborane in presence of excipient versus absorbance of metallacarborane without excipient. One can argue that the spectrum of complexed and free form may be different (the wavelength, the extinction coefficients), but it can be shown that, after dilution of sample before the actual measurement of absorbance, all the balances are shifted to the free form and thus the error is negligible and does not affect the trends.

This kind of experiment does not lead to direct solubility quantification but only to relative effect of excipient to metallacarborane solubility. It is advantageous for drug formulation to use amount of excipient as small as possible. This is the reason why we search for excipient with the strongest effect.

In the first part of the experiment they were used 19 representatives of cyclodextrins and their derivatives, all were purchased from Cyclolab Ltd, Hungary. Cyclodextrins[12] are macrocyclic oligosaccharides shaped like the truncated cone with slightly hydrophobic central cavity and hydrophilic outer surface. The driving forces leading to the formation of an inclusion complex with various guest molecules in water include the release of enthalpy-rich water molecules from the cavity, attractive interactions (electrostatic, hydrophobic, van der Waals, hydrogen bonding) and the release of conformation strain. The most widely used β-cyclodextrin is formed from seven α-1,4 linked α-D-(+)-glucopyranose units. List of all cyclodextrin derivatives are shown in Table 1 and structures in Supplementary data (labels taken from the manufacturer).

The results are summarized in the attached table (Fig. 2). Rating is based on the relative absorbance of the metallacarborane in the solution of cyclodextrin versus the absorbance of the aqueous solution of metallacarborane. Metallacarboranes are marked with numbers, cyclodextrines with letters. Colors are used to visually highlight differences, where green means substandard result compared to other cyclodextrins, average results are orange and higher than average are red. By far the best results achieved cyclodextrin 'N', that is, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB).

The potentiality of metallacarborane complexation with cyclodextrin derivatives have been investigated by theoretical calculations of average structures. In accordance with the experimental results it was found that the DIMEB has the most useful steric arrangement with suitable charge distribution for incorporation of metallacarborane. It was shown that substitution at both 2- and 3-position of all glucose units hinder inclusion of metallacarborane into cyclodextrin cavity (Supplementary data).

The solubilization of metallacarboranes using surfactants and copolymers (excipients) is the extension of the previous experiment on other substances as alternatives to cyclodextrin. There have been studied representatives of Pluronics (nonionic triblock copolymers composed of a central hydrophobic chain of polypropylene glycol flanked by two hydrophilic chains of polyethylene glycol), Eudragit (pH-dependent polymethacrylate copolymers with

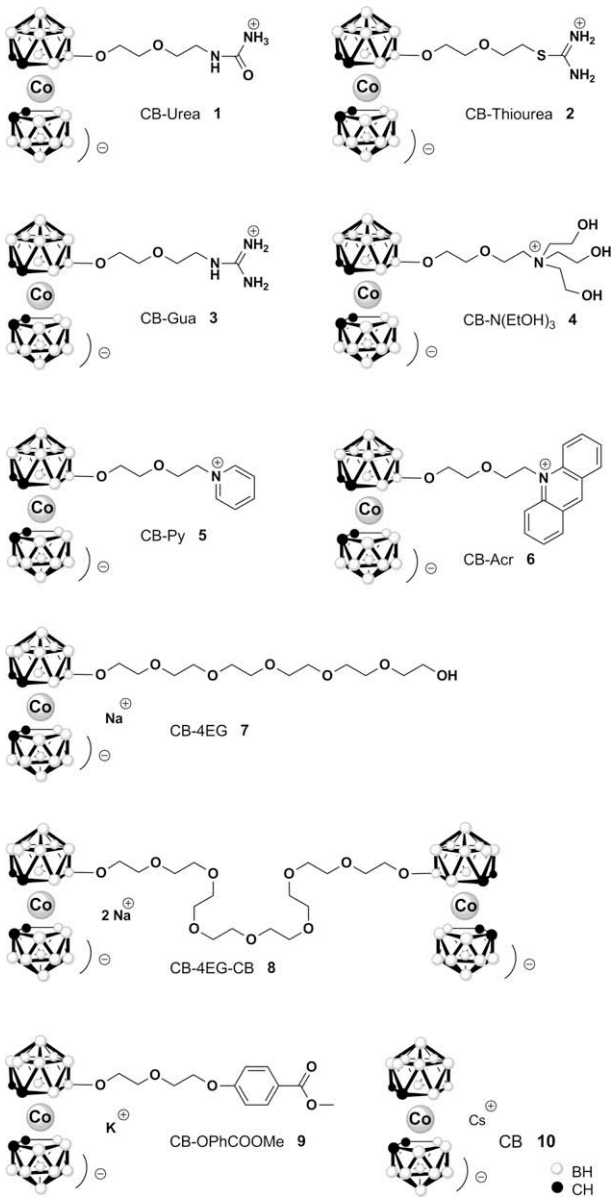

Figure 1. Schematic structures of cobalt bis(dicarbollide) derivatives.

Table 1
List of cyclodextrine derivatives

Abbreviations	Compound name (DS—average number of substituents)
A	α-Cyclodextrin
B	β-Cyclodextrin
C	γ-Cyclodextrin
D	Methylated β-cyclodextrin (DS ~12)
E	(2-Hydroxy)propyl-β-cyclodextrin (DS ~4.5)
F	Sulfopropylated β-cyclodextrin (DS ~2)
G	β-Cyclodextrin phosphate sodium salt (DS ~2–6)
H	Heptakis(2,3,6-tri-*O*-acetyl)-β-cyclodextrin
I	Succinylated β-cyclodextrin (DS ~3.5)
J	Carboxymethylated β-cyclodextrin (DS ~3.5)
K	Carboxyethylated β-cyclodextrin (DS ~3)
L	Soluble β-cyclodextrin polymer
M	Soluble anionic β cyclodextrin polymer
N	Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin
O	Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin
P	Sulfated β-cyclodextrin (DS ~13)
Q	Acetylated β-cyclodextrin (DS ~7)
R	6-*O*-Maltosyl-β-cyclodextrin (DS ~1.5)
S	Succinylated (2-hydroxy)propyl-β-cyclodextrin

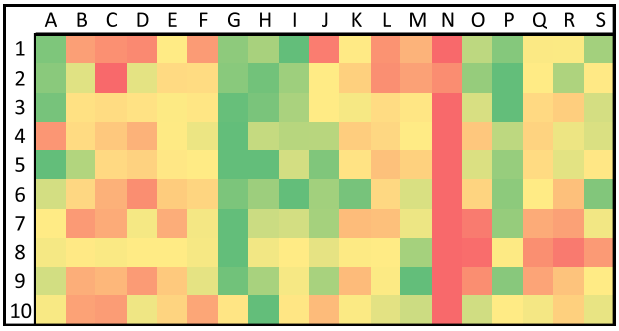

Figure 2. Influence of various cyclodextrins on the relative absorbance of metallacarboranes (inc. from green to red). (1) CB-Urea, (2) CB-Thiourea, (3) CB-Gua, (4) CB-N(EtOH)$_3$, (5) CB-Py, (6) CB-Acr, (7) CB-4EG, (8) CB-4EG-CB, (9) CB-OPhCOOMe, (10) CB; labeling of cyclodextrins in Table 1.

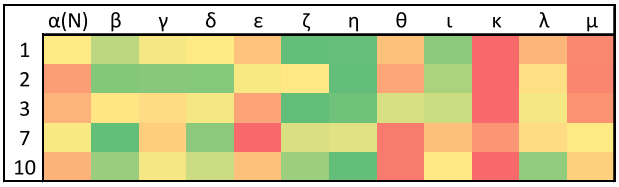

Figure 3. Influence of various surfactants and copolymers on the relative absorbance of metallacarboranes (increasing effect from green to red). (1) CB-Urea, (2) CB-Thiourea, (3) CB-Gua, (7) CB-4EG, (10) CB; labeling of excipients in Table 2.

Table 2
List of surfactants and copolymers

Abbreviations	Compound name
α (N)	Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB)
β	Eudragit FS 30 D (anionic polymethacrylate copolymer)
γ	Eudragit RS 30 D (cationic polymethacrylate copolymer)
δ	Eudragit NE 30 D (neutral polymethacrylate copolymer)
ε	Polyvinylpyrrolidone (PVP)
ζ	PEG 1500
η	Pluronic L-31
θ	Brij 56 (polyethylene glycol hexadecyl ether)
ι	Pluronic F-68
κ	Pluronic F-127
λ	1-Perfluorohexyl-4,7-dioxanonan-2,9-diol
μ	Human serum albumin (HSA)

acidic, alkaline or neutral groups), PVP (polyvinylpyrrolidone), PEG (polyethylene glycol), DIMEB (best of cyclodextrins, for comparison) and HSA (human serum albumin). Complexation ability of HSA may be interesting both as potential alternative to the DIMEB and as the study of the protein that will probably contribute to the transport in the body while rival solubilization agent. Experiments were performed with five metallacarboranes of the previous experiment. The results are summarized in the Figure 3, numerical and color coding as in the previous case, complete list of used compounds are listed in Table 2, structures in Supplementary data. It is noteworthy that in comparison with the best cyclodextrin DIMEB (column α(N)) is very interesting Pluronic F-127 (column κ), Brij 56 (column θ), PVP (column ε) and HSA (column μ).

In conclusion, our preliminary results presented here show, that active pharmaceutical ingredients based on cobalt bis(dicarbollide) derivative can be solubilized in water media by using Pluronic F-127, DIMEB or PVP as suitable excipient. Strong interaction of metallacarorane derivatives with HSA included in blood will rival used excipient. On the other hand, the same HSA could be used as excipinet too.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.038.

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11. *Preparation of compounds* **1**–**9**: Typical procedure: To a stirred slurry of NaH (16 mg, 60% in oil, 0.4 mmol) in THF (2 mL) was added thiourea (30.5 mg, 0.4 mmol). Reaction mixture was stirred under argon at rt for 20 min. Then soln. of 8-dioxane-3-cobalt bis(dicarbollide) (**11**; 82.8 mg, 0.2 mmol) in THF (3 mL) was added and reaction mixture was stirred at 60 °C for 1 h (TLC monitoring using dichloromethane as eluent). When all starting **11** was consumed, acetic acid (0.5 mL) was added to reaction mixture and evaporated, followed by column chromatography (silica; gradient dichloromethane to dichloromethane/acetone 1:1 v/v). Orange solid product **2** was dried in vacuum, 80 mg (82%). CB-Urea (**1**): NaH (24 mg, 60% in oil, 0.6 mmol), urea (36 mg, 0.6 mmol), **11** (124 mg, 0.3 mmol), THF, 126 mg (89%); CB-Gua (**3**): NaH (32 mg, 60% in oil, 0.8 mmol), guanidine HCl (38 mg, 0.4 mmol), **11** (83 mg, 0.2 mmol), THF. 89 mg (95%); CB-N(EtOH)$_3$ (**4**): triethanolamine (156 mg, 1.05 mmol), **11** (144 mg, 0.35 mmol), THF. 188 mg (96%); CB-Py (**5**): pyridine (5 mL), **11** (306 mg, 0.745 mmol). 361 mg (99%); CB-Acr (**6**): acridine (180 mg, 1 mmol), **11** (328 mg, 0.8 mmol), THF. 425 mg (90%). CB-4EG (**7**): NaH (120 mg, 60% in oil, 3 mmol), tetraethylene glycol (388 mg, 2 mmol), **11** (414 mg, 1 mmol), THF. 555 mg (88%); CB-4EG-CB (**8**): NaH (60 mg, 60% in oil, 1.5 mmol), tetraethylene glycol (97 mg, 0.5 mmol), **11** (493 mg, 1.2 mmol), THF. 566 mg (89%); CB-OPhCOOMe (**9**): K_2CO_3 (414 mg, 3 mmol), methyl 4-

hydroxybenzoate (456 mg, 3 mmol), **11** (423 mg, 1.03 mmol), acetone. 612 mg (99%).
All compounds were verified by ^{1}H NMR, ^{11}B NMR and mass spectroscopy.
Compound **10** and **11** were purchased from KatChem Ltd, Czech Rep.

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