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# Diverse associations in the ternary systems of β-cyclodextrin, simple carbohydrates and phenyl derivatives of inorganic oxoacids

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ABSTRACT

Complex formation reactions of phenylboronic, phenylphosphonic, phenylarsonic and 4-aminophenyl arsonic acids with β-cyclodextrin (cycloheptaamylose, β-CD) and some simple carbohydrates (mannitol, sorbitol, glucose) have been studied using spectrophotometric, potentiometric methods and solubility measurements, supplemented with HPLC and IR analyses of the solid samples. Equilibrium constants have been determined at ionic strength of 0.2 M (NaCl) and 25 °C. β-CD forms the most stable complexes with the neutral, undissociated forms of the acids, the stability constants are as follows: phenylboronic acid: 320 ± 36, phenylphosphonic acid: 108 ± 25, phenylarsonic acid: 97 ± 4 and 4-aminophenyl arsonic acid: 107 ± 10. The stability constants for the β-CD-complexes of the ionic forms are much lower. Ternary complexes of low stability could be detected in the case of phenylphosphonic acid and sorbitol with the undissociated form and with glucose and the dianion. In more concentrated solutions phenylboronic acid forms insoluble complexes with mannitol, sorbitol and β-CD. The solid phases obtained in the ternary systems are predominantly mixtures of ester type 3:1 complexes with the carbohydrate and 1:1 inclusion complex with the β-CD. No significant interaction has been found with glucose. The phenomena can be explained by the differences in the structures of the components and by the changes in the H-bonding network of β-CD on the complex formation.



## 1. Introduction

The recognition and separation of carbohydrates and related compounds is important both from practical and theoretical points of view. Work in this field is mainly done on empirical basis, relatively few reliable data on the equilibria are available.

Cyclodextrins (CD-s) are known to form stable inclusion complexes with a large variety of guests bearing sufficiently hydrophobic moieties of appropriate size.[1] Strongly hydrophilic compounds, however, cannot be included, so no significant interaction could be detected between β-cyclodextrin (cycloheptaamylose, β-CD) and sugars, for example, glucose.[2–4] On the other hand, the primary and secondary alcoholic OH groups on the outer rims of the CD-ring can take part in H-bonds, contributing either to the stabilization of the inclusion complex,[5] or to the interaction with further molecules, thus resulting in the formation of ternary complexes. Owing to the modification of the hydrogen bonding system, smaller, hydrophilic molecules like acetic acid or even perchlorate ion can be attached to the complex containing an appropriate guest.[6,7] This can be the explanation of the observation that in the case of some insoluble drugs much higher increase of the solubility could be achieved when CD-derivatives and hydroxy acids (tartaric acid, citric acid) were applied together, than with the cyclodextrins by themselves.[8]

Carbohydrates themselves do not form complexes of significant stability with cyclodextrins, as it has been proved by several authors.[2–4] The interaction of boric acid or its derivatives with carbohydrates, however, is a well known and thoroughly studied fact.[9–11] It has been shown that the reaction is preferred in alkaline solutions, and results in an ester-like product containing C–O–B bonds and tetrahedrally coordinated boron.[11–13] Sugar alcohols like mannitol or sorbitol and phenylboronic acid may form poorly soluble 1:3 associates,[11] while cyclic sugars can not bind more than one or two borate or boronate ions because of the unfavourable steric position of the OH-groups.[13] The topic has gained a renewed interest in recent years as the basis of sugar sensors,[13–18] nevertheless, the association constants given for the same system spread over a wide range (e.g., for phenylboronic acid–D-glucose between $K = 200$ and $K = 11$ dm$^3$ mol$^{-1}$).[11,14–16]

The interaction of boric acid or borate ion itself with cyclodextrins is also negligible,[2] but the phenyl derivative [phenylboronic

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acid (PhBA) or phenylboronate ion] is expected to give a complex via inclusion of the phenyl group. Thus the phenylboronate–cyclodextrin and phenylboronate–carbohydrate interactions may mutually influence each other and result in the formation of ternary complexes, as it is proved—though with low stability—in the case of benzoic acid.[6]

As phenylboronic acid takes part in the interaction with polyols mainly as a tetrahedral boronate anion, this structure can be related to those of phenylphosphonic and phenylarsonic acids.

Phenylboronic acid is shown to have bacteriostatic activity[11] and both phenylboronic and phenylarsonic acids are subjects of pharmacological research as inhibitors of carbonic anhydrase enzymes.[19,20] A further derivative, 4-aminophenyl-arsonic acid may be used in the synthesis of antileukemic drugs.[21]

In the present paper investigations on the interactions of the above mentioned acids with β-cyclodextrin and some simple carbohydrates are reported—with special regard to the possible formation of ternary complexes. Two open chain sugar alcohols: D-mannitol and D-sorbitol and the cyclic sugar D-glucose have been chosen as model carbohydrates.

The complex formation equilibria were studied using pH-potentiometric titrations, spectrophotometric method based on the competing reaction with an appropriate indicator (phenolphthalein in alkaline and methyl orange in acidic medium),[22,23] and solubility measurements. The solid phases were analysed by means of infrared spectroscopy and HPLC.

## 2. Experimental

### 2.1. Materials

β-Cyclodextrin was obtained from Cyclolab Ltd (Hungary) as a gift within the frames of research collaboration, and was used after recrystallization from hot water.

Phenylboronic acid, phenylphosphonic acid and 4-aminophenyl-arsonic acid (Sigma–Aldrich) and phenylarsonic acid (Merck) were of analytical grade and used without further purification. The concentrations of the solutions were checked by the potentiometric titration.

D-Sorbitol and D-glucose were of pharmacopoeial grade (Ph. Hg. VII), all the other materials were commercial products of analytical grade (Reanal, Hungary) and used without purification, except phenolphthalein which was recrystallized twice from an ethanol–water solution.

Carbonate free NaOH-solution was prepared by the Sorensen method. All the solutions were made in double distilled water.

### 2.2. Spectrophotometry

Spectrophotometry measurements were carried out with Spectromom 195D and Camspec M330 instruments, in 10 mm cells.

In alkaline medium phenolphthalein was used as indicator in a concentration of $3 \times 10^{-3}$ M. The pH was adjusted with 0.02 M $Na_2CO_3$. The concentration of β-CD varied stepwise from 0 to $2.4 \times 10^{-4}$ M and that of the salts of the acids (prepared in solution from weighed amounts of the acids by the addition of calculated amounts of NaOH) from $1 \times 10^{-2}$ M to $6 \times 10^{-2}$ M. The absorbances were measured at $\lambda = 550$ nm.

The complex formation of the acidic forms was studied in 0.1 M HCl solutions with methyl orange as indicatior, at wavelengths of 506 nm and 319 nm. The concentrations were as follows: methyl orange: $2 \times 10^{-5}$ M, β-CD: $0–9 \times 10^{-3}$ M and the acids: $0–2 \times 10^{-2}$ M. Further details of the spectrophotometric methods and the method of the evaluation can be found in Refs. [24,25].

### 2.3. Potentiometric measurements

Aliquots of $5 \times 10^{-3}–2 \times 10^{-2}$ M solutions of the acids were titrated with 0.2 M carbonate free NaOH solution under stirring with nitrogen, in the absence and in the presence of $5 \times 10^{-3}–1.4 \times 10^{-2}$ M β-CD or/and 0.1–0.2 M carbohydrate. The ionic strength was adjusted to 0.2 M by the addition of NaCl. The e.m.f. was recorded with a Radelkis OP 208/1 type pH-meter fitted with a Radelkis OP 0808 P combined glass electrode and a Schott-Gerδte T80/20 automatic burette. The system was calibrated by the titration of 20.00 mL 0.02 M HCl (0.18 M in NaCl) solution with 0.2 M NaOH.

The temperature was kept constant at 25 ± 0.5 °C in all types of measurements.

### 2.4. Solubility measurements and analysis of the solid products

Solubility measurements were applied for phenylboronic acid (PhBA) as it is a too weak acid and is the most sparingly soluble among the acids investigated. Solid PhBA in a small excess with respect to the expected solubility was equilibrated with pure water and with solutions of β-CD or/and the chosen carbohydrates. The concentration of the β-CD varied from 0 to $1.3 \times 10^{-2}$ M (near the solubility limit) and those of the carbohydrates from 0 to 0.25 M. The solubility of PhBA has been found higher (0.145 M) than the values given in the manuals, merely the dissolution process is extremely slow, because the molecules in the solid phase are connected not only by hydrogen bridges but by the stacking of the aromatic rings, which hinder hydration. Sonication of the system can not be recommended since an unfilterable colloidal solution is produced. Equilibration at 25 ± 0.1 °C lasted at least 2–3 days. The concentration of the dissolved PhBA was determined spectrophotometrically after appropriate dilution at $\lambda = 261$ nm. (It was checked in separate experiments that the absorbance was not influenced by the other components present in the actual concentrations.) As in the presence of sorbitol and mannitol and of higher concentrations of P-CD a decrease in the solubility and perceptibly the formation of a new phase was observed, the solid phase was filtered, washed quickly with a small amount of water and dried at 105 °C to constant mass. The composition was analysed by HPLC, and for PhBA spectrophotometrically, too, after dissolution.

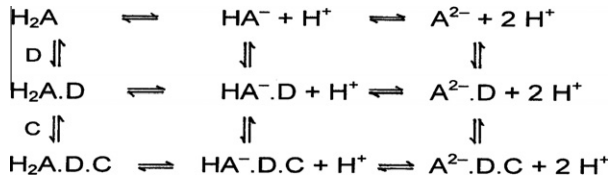
*Infrared spectra* were recorded with a Bruker PM-37 instrument in nujol, with optical elements made of barium-fluoride. In spite of the high absorption of nujol in the frequency range of 2800–3000 $cm^{-1}$, and at 1460 $cm^{-1}$ and 1380 $cm^{-1}$, the KBr pellet technique could not be applied because of the definite interaction between bromide and CD.[8]

The*HPLC analysis* was performed in Cyclolab Ltd, on an Agilent 1100 HPLC system equipped with an Alltech 3300 Evaporative Light Scattering Detector (temperature: 70 °C, gas flow rate: 1.3 L/min). The further experimental conditions were as follows: column: CD-Screen, 5 μm, 4.0 × 250 nm (ChiroQuest Ltd, Budapest, Hungary), eluents: A: water, B: acetonitrile, gradient program: 0–5 min: 0% B, 5–20 min: B increasing linearly 0–50%, flow rate: 1 mL/min, temperature of the column: 30 °C. Sample preparation: 10 mg of the samples was dissolved in 10 mL of 20% acetonitrile–water mixture and 20 μL was injected at once.

## 3. Results and discussion

### 3.1. Evaluation of the experimental data

The simplest equilibria for a divalent acid (supposing 1:1 complexes only with the β-CD) can be symbolized as in Scheme 1. where $H_2A$ stands for the undissociated acid, and β-CD and the carbohydrate are symbolized by D and C, respectively.




**Scheme 1.** Dissociation and complex formation equilibria of a divalent acid in solutions contaning β-CD (D) and a simple carbohydrate (C).

In the case of phenylboronic acid the scheme is simplified, as PhBA can behave as a monovalent acid only, but the acidic dissociation must be described as

$$H_2A + H_2O \Longleftrightarrow H_2A(OH)^- + H^+$$

rather than that in Scheme 1. Concerning the further interactions either with the carbohydrate or with the CD, pH-potentiometric measurements can not distinguish between the simple association like that symbolized in Scheme 1 and the formation of an ester type complex by the loss of $H_2O$.

Under the conditions of the spectrophotometric measurements only one species of acids is present predominantly, so the equilibria are simplified to the association of that species with the CD and the carbohydrate (and the competing reaction between CD and the indicator). The method of the evaluation is described in details in Refs. 24,25.

In most general, the equilibria can be characterised as follows (charges omitted):

$$pH^+ + qA + r\ D + s\ C \Longleftrightarrow H_pA_qD_rC_s \quad (1)$$

and the equilibrium constant:

$$\beta_{pqrs} = \frac{[H_pA_qD_rC_s]}{[H^+]^p[A]^q[D]^r[C]^s} \quad (2)$$

where square brackets denote equilibrium concentrations in mol $dm^{-3}$ (M) units.

When $H_2A$ is chosen as the basic species, q means the number of $H_2A$ units in the given particle and p is the number of additional protons. E.g. for the $A^{2-}$ anion p = −2, and the formation of the $A^{2-}$. D complex can be described as:

$$H_2A - 2H^+ + D \Longleftrightarrow A^{2-} \cdot D$$

$$\beta_{-2110} = \frac{[A^{2-} \cdot D][H^+]^2}{[H_2A][D]}. \quad (3)$$

For the dissociation of the acid:

$$H_2A \Longleftrightarrow A^{2-} + 2H^+$$

$$\beta_{-2100} = \frac{[A^{2-}][H^+]^2}{[H_2A]}, \quad (4)$$

consequently the stepwise formation constant of the $A^{2-}$·D complex can be obtained as:

$$A^{2-} + D \Longleftrightarrow A^{2-} \cdot D$$

$$K_{-2110} = \frac{[A^{2-} \cdot D]}{[A^{2-}] \cdot [D]} = \frac{\beta_{-2110}}{\beta_{-2100}}. \quad (5)$$

The equations of the mass balances:

$$c_A = \sum q\beta_{pqrs}[H^+]^p[H_2A]^q[D]^r[C]^s \quad (6)$$

$$c_D = \sum r\beta_{pqrs}[H^+]^p[H_2A]^q[D]^r[C]^s \quad (7)$$

A similar equation can be given for $c_C$ if a third component is also present, and

$$c_H = \sum (p + 2q)\beta_{pqrs}[H^+]^p[H_2A]^q[D]^r[C]^s \quad (8)$$

The values of $\beta_{0100}$ $\beta_{0010}$ etc. are 1.0 by definition, and the corresponding products give the equilibrium concentrations of the free components.

Since $[H^+]$, $c_A$ and $c_D$ (and $c_C$) are known, the values of $\beta_{pqrs}$ can be computed using Eqs. 3–8 by an iterative computer program with assumed starting values, searching for the best fit between experimental data and the calulated values. (A more detailed explanation of the method is described in Ref. 23.) In our case the calculations justified the assumption applied in Scheme 1, that is, q = r = s = 1.

As it is seen in Scheme 1, the equilibria are not independent of each other, and the effects observed in the pH-potentiometric titrations depend largely on the ratio of the equilibrium constants for two consecutive processes. Therefore potentiometry itself can not give definite values for the individual constants, it is advisable to determine some of them by some independent method, too.

### 3.2. Stability constants from spectrophotometry and potentiometry

The stability constants obtained from the spectrophotometric measurements for some of the complex formation processes are summarized in Table 1.

These data were used as starting values in the evaluation of the potentiometric measurements. Dissociation constants of the acids were taken from the literature[26] and refined in the evaluation process for the titration of the pure acids, to fit the applied conditions. The results are summarized in Table 2.

It must be noted that the uncertainty of the equilibrium constants for the maximally protonated forms of phenylphosphonic acid ($K_{a1}$ or $\beta_{-1100}$) is unusually high because of the uncertainty of potentiometric pH-measurements in the appropriate range (1 < pH < 2), and this involves similar uncertainty in the corresponding complex stability constants.. However, the equilibrium constant for the second dissociation step ($K_{a2} = \beta_{-2100}/\beta_{-1100}$) proved to be fairly constant in the calculations. On the other hand, because of the successive complex formation steps, the effect of the change in one equilibrium constant may be compensated partly by the change in that of the next step. So the uncertainty of the successive complex formation constants ($K$) is often lower than those of the directly calculated overall values.

Binary interactions between the acids (except PhBA) and the small carbohydrate molecules proved to be negligible: the calculations were insensitive to the supposed values and the corresponding successive stability constants were <1.

The stepwise equilibrium constants ($K$) derived from the overall values of Table 2 for the formation of the different binary and ternary associates are collected in Table 3.

**Table 1**
Stability constants ($K$/$dm^3$ $mol^{-1}$) of the β-CD-complexes obtained from the spectrophotometric measurements

| | $H_3A^+$ | $H_2A$ | $HA^-$ ($H_2AOH^-$) | $A^{2-}$ |
|---|---|---|---|---|
| Phenylboronic acid | | 320 ± 36 | 23.7 ± 2.7 | |
| Phenylphosphonic acid | | 58 ± 5[a] | | 29 ± 5 |
| Phenylarsonic acid | | 110 ± 30 | | 12.5 ± 1.6 |
| 4-Aminophenyl arsonic acid | 36 ± 7[b] | | | 16 ± 5 |

[a] cca 79% $H_2A$ and 21% $HA^-$.
[b] cca 90% $H_3A^+$ and 10% $H_2A$.

**Table 2**
Overall stability constants [β, for the definition see Eqs. 2–4 and footnote] obtained from the potentiometric measurements for the different possible associates in the acid-β-CD-carbohydrate systems

| Acid ($H_2A$) | X | Y | 11XY[a] | 01XY[a] | −11XY[a] | −21XY[a] |
|---|---|---|---|---|---|---|
| Phenyl phosphonic acid | 0 | 0 | – | (1.0) | $(2.6 \pm 0.5) \times 10^{-2}$ | $(2.85 \pm 0.6) \times 10^{-9}$ |
| | β-CD | 0 | – | 108 ± 25 | 1.06 ± 0.2 | $(8.5 \pm 1.0) \times 10^{-8}$ |
| | β-CD | Glucose | – | nd | 4.6 ± 1.0 | $(5.8 \pm 1.2) \times 10^{-7}$ |
| | β-CD | Sorbitol | – | 250 ± 50 | 1.7 ± 0.5 | $(2.3 \pm 0.5) \times 10^{-8}$ |
| | β-CD | Mannitol | – | nd | nd | nd |
| Phenylarsonic acid | 0 | 0 | – | | $(3.95 \pm 0.04) \times 10^{-4}$ | $(1.63 \pm 0.02) \times 10^{-12}$ |
| | β-CD | 0 | – | 97 ± 4 | $(2.5 \pm 0.6) \times 10^{-3}$ | $(5.4 \pm 0.4) \times 10^{-11}$ |
| 4-Amino-phenylarsonic acid | 0 | 0 | 86 ± 6 | | $(1.11 \pm 0.04) \times 10^{-4}$ | $(2.08 \pm 0.14) \times 10^{-13}$ |
| | β-CD | 0 | $(2.7 \pm 0.6) \times 10^{3}$ | 107 ± 10 | $(1.8 \pm 0.3) \times 10^{-3}$ | $(1.8 \pm 0.4) \times 10^{-12}$ |

nd: not detectable.
[a] The equilibrium processes corresponding to the different items: 11XY: $H^+ + H_2A + X + Y \Leftrightarrow H_3A^+ \cdot X.Y$, for example, when X = 0 and Y = 0, $H^+ + H_2A \Leftrightarrow H_3A^+$, when X = β-CD and Y = 0, $H^+ + H_2A + D \Leftrightarrow H_3A^+ \cdot D$ 01XY: $H_2A + X + Y \Leftrightarrow H_2A \cdot X \cdot Y$, for example, when X = β-CD and Y = 0, $H_2A + D \Leftrightarrow H_2A \cdot D$, when X = β-CD and Y = carbohydrate, $H_2A + D + C \Leftrightarrow H_2A \cdot D \cdot C$ −21XY: $H_2A + X + Y \Leftrightarrow A^{2-} \cdot Y + 2H^+$, for example, when X = 0 and Y = 0, it means the overall dissociation constant of the acid, when X = β-CD and Y = carbohydrate, $H_2A + D + C \Leftrightarrow A^{2-} \cdot D \cdot C + 2\,H^+$.

From the results it is clear that the stability constants of the binary complexes with β-CD are always the highest with the neutral, undissociated forms, and the values are similar for the three acids. This fact shows that the complex formation is mainly governed by the inclusion of the aromatic ring, while it is hindered by the strong hydration of polar groups. The comparison with the data for 4-aminophenyl-arsonic acid suggests that for the additional H-bonding interactions with the primary and secondary alcoholic OH-groups of the CD, the presence of some proton donating group(s) is more favourable than the completely unprotonated form which could function as proton acceptor only, while the hydration of the amino group causes further hindrance against the inclusion.

The stability constant for the complex of the much weaker phenylboronic acid (PhBA, see Table 1) is substantially higher relative to the other three acids and is closer to that of other weak aromatic acids, for example, benzoic acid.[27] This can be explained again by the inhibiting role of the increased hydration of the stronger acids, in agreement with the observation that the stability constants of β-CD-complexes are often antiparallel to the solubility of the guest.[28]

Concerning the ternary interactions with phenylphosphonic acid and carbohydrates it is very interesting that no ternary complex could be proved with mannitol, while an opposite trend was found in the interactions with differently protonated forms of the primary guest in the case of sorbitol and glucose. This can be understood considering that glucose is more acidic,[16,26] so it can take part in H-bonds as proton donor, while sorbitol is rather a proton acceptor.

In the case of PhBA pH-potentiometry was not used because of the very low acidity and because spectrophotometry was appropriate for the investigation of the two possible forms. Concerning the ternary complexes, some qualitative indications in spectrophotometry experiments with methyl red showed a definite increase of the stability of the PhBA–β-CD complex in the presence of sorbitol (suggesting the formation of ternary complexes), and to a lesser extent with mannitol, while no effect was caused by glucose. Similar conclusion can be drawn from the absorption spectra of PhBA recorded in pure water and in the presence of β-CD ($6 \times 10^{-3}$ M) or/and the carbohydrates (0.1 M). β-CD causes a small increase in the absorbances and a small bathochromic shift near 272 and 266 nm, and the fine structure of the spectrum becomes more expressed, which can be attributed to the less polar environment in the CD-cavity. Glucose has no influence on the spectra at all, neither in itself nor in the presence of β-CD. Mannitol and sorbitol causes changes in the absorbances similar to the effect of β-CD (mannitol ⟨ sorbitol) but, in contrast to that, accompanied by a slight coalescence of the fine structure. When sorbitol and β-CD are present together, the increase in the absorbances is higher than the sum of the individual effects. However, these changes are small, and the evaluation of spectrophotometric experiments for ternary complexes is very complicated and uncertain because of the large number of interdependent equilibria. Therefore, solubility measurements were chosen to get further information on the phenylboronic acid–β-CD–carbohydrate systems.

### 3.3. Solubility experiments, composition of the precipitates

The results of the solubility experiments could not be evaluated to obtain complex stability constants. Glucose had no influence on the solubility of PhBA at all. In the presence of mannitol and sorbitol, an insoluble new phase appeared at rather low concentrations already ($5 \times 10^{-2}$ M and $1 \times 10^{-3}$ M, respectively) and, following a short increasing range ($c_{CD} < 1 \times 10^{-3}$ M), in the presence of β-CD, too. In ternary systems the effects of β-CD and the carbohydrate seemed to be summarized.

**Table 3**
Stepwise formation constants ($K$/dm$^3$ mol$^{-1}$) of the differently protonated acid-β-CD-carbohydrate associates [derived from the data of Table 2, according to equations like Eq. (5)]

| Association process | $H_3A^+$ | $H_2A$ | $HA^-$ | $A^{2-}$ |
|---|---|---|---|---|
| | $(H_3A^+ + D \Leftrightarrow H_3A^+ \cdot D)$ | $(H_2A + D \Leftrightarrow H_2A \cdot D)$ | $(HA^- + D \Leftrightarrow HA^- \cdot D)$ | $(A^{2-} + D \Leftrightarrow A^{2-} \cdot D)$ |
| 4-Aminophenyl-arsonic acid + β-CD | 30 ± 5 | 107 ± 10 | 17 ± 4 | 9 ± 2 |
| Phenylarsonic acid + β-CD | | 97 ± 4 | 6.3 ± 1.5 | 33 ± 2.4 |
| Phenylphosphonic acid + β-CD | | 108 ± 25 | 40 ± 10 | 30 ± 8 |
| Phenylphosphonic acid − β-CD complex + carbohydrate | $(H_3A^+ \cdot D + C \Leftrightarrow H_3A^+ \cdot D \cdot C)$ | $(H_2A \cdot D + C \Leftrightarrow H_2A \cdot D \cdot C)$ | $(HA^- \cdot D + C \Leftrightarrow HA^- \cdot D \cdot C)$ | $(A^{2-} \cdot D + C \Leftrightarrow A^{2-} \cdot D \cdot C)$ |
| Mannitol | | nd | nd | nd |
| Sorbitol | | 2.3 ± 0.4 | 1.6 ± 0.5 | <1 |
| Glucose | | nd | 4.3 ± 1.0 | 6.8 ± 1.0 |

nd: not detectable.

The composition of the precipitates was determined by HPLC and spectrophotometry. The PhBA-content from the HPLC-results was in good agreement with those of the photometric determination. The sum of the total amounts of the components [calculated with $C_6H_5B(OH)_2$] was always >100%, but the excess correlates fairly well with the PhBA-content, so it can be attributed to the loss of $H_2O$ during the ester type complex formation (see IR spectra later). The results in molar units are collected in Table 4.

The molar ratios $n_{PhBA}/n_{\beta\text{-CD}} = 1.18 \pm 0.02$ for both the pure PhBA–β–CD and the PhBA–β-CD–glucose systems (no glucose could be detected in the latter sample). It might mean the presence of 1:1 complex dominantly, accompanied by some undissolved free acid, but the reproducibility of the value >1 suggests rather the formation of some (probably outer sphere type, H-bonded or ester-like) 2:1 complex, too, as it was proved earlier with benzoic acid.[27]

The further data of Table 4 show that in the ternary systems the molar ratios of any two of the components do not have any stoichiometrical meaning, the precipitates can not be regarded as stoichiometrically uniform ternary complexes. Supposing the presence of 1:1 PhBA–β-CD complex and subtracting the corresponding amount of the acid from the total, however, the rest gives $3.13 \pm 0.08$ for the $n_{PhBA}/n_{carbohydrate}$ ratio with sorbitol and $3.22 \pm 0.08$ with mannitol. (Though the homogeneity of the latter sample was poorer, the amounts of the acid and mannitol changed parallel to each other and antiparallel with the CD-content.)

From the reproducibility of the $(n_A - n_D)/n_C$ ratio and its good agreement with the $n_A/n_C$ obtained for the binary PhBA-sorbitol system it can be concluded that the solid phases obtained in the ternary systems with mannitol or sorbitol are mainly the mixtures of 1:1 PhBA–β-CD and 3:1 PhBA–carbohydrate complexes. The solubility of the mannitol-complex is higher than that of the sorbitol-complex, therefore, the ratio of the β-CD complex is higher in the former system. The excess PhBA may be present as a 2:1 PhBA–β-CD complex or interact with two monomeric units forming bridges between them, attached by stacking of the aromatic rings to the carbohydrate complex or simply accommodated in interstitial positions.

Glucose does not form any insoluble complex either with PhBA itself or in the ternary system with β-CD.

### 3.4. Infrared spectra

In the IR spectrum of the precipitate obtained in the reaction of PhBA and sorbitol (Fig. 1c), the OH-bands completely disappeared [not only the characteristic, broad stretching band between 3400 and 3180 $cm^{-1}$ but the deformation vibrations (1000–700 $cm^{-1}$) as well], and significant changes can be observed in the region of the C–O stretching (1200–1000 $cm^{-1}$), proving the formation of ester-like C–O–B bonds. Accordingly, the strong absorption bands of PhBA at 1348 $cm^{-1}$ and 1306 $cm^{-1}$ (B–O vibrations) are shifted to 1342 $cm^{-1}$ and 1317 $cm^{-1}$, respectively, while the $\nu_{C-B}$ (1442 $cm^{-1}$) and the vibration characteristic for the aromatic C–C bonds (~1608 $cm^{-1}$) remain practically unchanged.

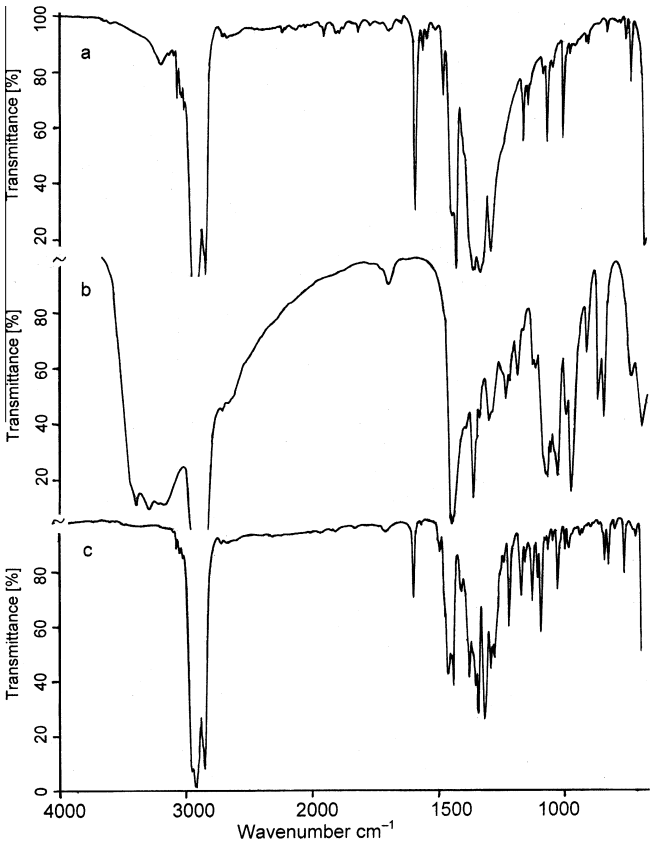



**Fig. 1.** Infrared spectra recorded in nujol: (a) phenylboronic acid, (b) sorbitol, (c) the precipitate obtained on the dissolution of phenylboronic acid in 0.1 M solution of sorbitol.

The spectrum of the PhBA–β-CD system (Fig. 2b) is dominated by β-CD. The stretching vibrations of the C–H bonds of the aromatic ring (3100–3000 $cm^{-1}$) are very suppressed (even if the relatively smaller PhBA-content of the sample is taken into account), while the C–H deformation and the aromatic ring vibrations are somewhat shifted (from 703 $cm^{-1}$ to below 700 $cm^{-1}$ and 1608 $cm^{-1} \rightarrow 1601$ $cm^{-1}$, respectively). The OH-band in the region >3000 $cm^{-1}$ remains almost unchanged, though it becomes somewhat narrower, that may be an indication of the modification in the H-bond system of the β-CD. The same effect (in addition to the decreased moisture content of the complex) can be the source of some minor shifts and significant decreases in the absorptions at 1648 $cm^{-1}$ and in the region <900 $cm^{-1}$. (β-CD samples, even if dried, retake water very fast, while in the case of inclusion complexes, water is excluded from the cavity.) Summarizing the observations, the differences compared to the spectra of the pure components indicate the formation of an inclusion complex, without any ester formation. The decreased solubility of the complex relative to that of the pure β-CD can

**Table 4**
Compositions of the precipitates obtained on the dissolution of phenylboronic acid in the solutions of other components ($n$, mol/100 g dry sample)

| Solution (concn) | Phenyl-boronic acid (A) | β-CD(D) | Carbohydrate (C) | $n_A/n_D$ | $n_A/n_C$ | $n_C/n_D$ | $\frac{n_A-n_D}{n_C}$ |
|---|---|---|---|---|---|---|---|
| 1. β-CD($6.4 \times 10^{-3}$ M) | 0.100 ± 0.002 | 0.0848 ± 0.0006 | | **1.18 ± 0.02** | | | |
| 2. Sorbitol (0.1 M) | 0.627 ± 0.031 | | 0.209 ± 0.029 | | **3.0 ± 0.6** | | |
| 3. β-CD + sorbitol | 0.458 ± 0.004 | 0.0245 ± 0.0010 | 0.138 ± 0.004 | 18.7 ± 0.9 | 3.3 ± 0.1 | 5.6 ± 0.4 | **3.13 ± 0.08** |
| 4. β-CD + mannitol | 0.29 ± 0.06 | 0.055 ± 0.009 | 0.072 ± 0.023 | 5.4 ± 1.9 | 4.1 ± 0.5 | 1.4 ± 0.6 | **3.22 ± 0.08** |
| 5. β-CD + glucose | 0.0985 ± 0.0005 | 0.0831 ± 0.002 | <LOD[a] | **1.19 ± 0.02** | | | |

[a] LOD: limit of detection.

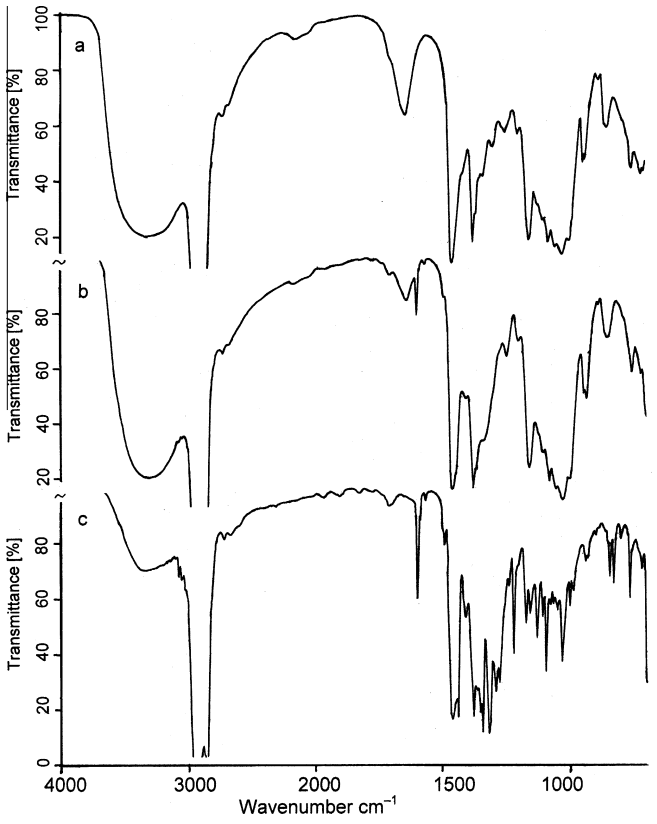



**Fig. 2.** Infrared spectra recorded in nujol: (a) β-CD, (b) β-CD-phenylboronic acid complex, (c) the precipitate obtained in the β-CD-phenylboronic acid-sorbitol system.

be attributed to the modified hydrogen bonding ability caused by the β-CD–PhBA interaction.

In the IR spectrum of the PhBA–sorbitol–β-CD ternary system (Fig. 2c) the characteristic features of both the PhBA-sorbitol and PhBA–β-CD systems can be observed, as if a somewhat decreased PhBA-sorbitol spectrum were superimposed on a decreased PhBA–β-CD spectrum.

Similar observations can be made in comparison of the IR spectra of the PhBA–mannitol–β-CD system and those of the pure components and binary systems: the characteristic absorptions of PhBA appear somewhat shifted, with relatively low intensity, the broad OH-band shows the shape of that of the β-CD-complex and the OH-bands of mannitol disappeared.

## 4. Conclusions

Summarizing the results, the IR spectra are in complete agreement with the conclusions drawn from the results of the HPLC analysis: the precipitates formed on the dissolution of PhBA in solutions of open chain carbohydrates and β-CD consist mainly of binary associates: ester type 3:1 complexes with the carbohydrate and 1:1 inclusion complex with the β-CD. The sequence of the solubilities is: PhBA-sorbitol < PhBA-mannitol < PhBA–β-CD. The presence of some minor amounts of outer sphere type (hydrogen bonded) ternary associates can not be excluded.

The cyclic saccharide glucose does not form any insoluble product, and has no effect on the association equilibria of the other components. This fundamental difference can be understood considering that the tetrahedral form of PhBA[16] and the alpha-furanose form of glucose[13] are suitable for the interaction, and the equilibrium concentrations of both are very low in neutral aqueous solution.

Nevertheless, the stoichiometry of the associates dominating in solution and in solid phase is not necessarily the same, owing to the different solubilities, so these findings do not contradict the results obtained by spectrophotometry and potentiometry in more diluted solutions where some low stability ternary complexes could be detected.

In spite of formal similarities, the behaviour of phenylphosphonic acid and phenylboronic acid in the interactions is substantially different. The much stronger acidity and real dissociation of the former prevents direct association with the small carbohydrate molecules, while the inclusion complex with β-CD can take part in further interactions to form ternary complexes of low stability. On the other hand, the structural differences of the small carbohydrate molecules lead to significantly different complex forming behaviour.

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