



## Calorimetric Investigation of Chiral Recognition Processes in a Molecularly Imprinted Polymer

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### Abstract

The molecular imprinting of polymers is a promising concept for designing highly selective receptor systems which is now fairly well understood from a structural point of view. However, only limited information is available on the thermodynamics of enantioselective recognition in imprints. In order to study this problem, the interactions of a pair of enantiomers (phenyl- $\alpha$ -L- and phenyl- $\alpha$ -D-mannopyranoside) with a well-characterized imprinted polymer (D-enantiomer acted as template) have been investigated by means of isothermal batch and titration calorimetry. Batch calorimetric measurements with unstirred samples are affected by strong solvent–polymer interactions, such as swelling, causing large changes of the blank effects over long periods of time. Isothermal titration calorimetric measurements proved to be more efficient for studying imprinted polymers. The enthalpies of rebinding for both enantiomers strongly decrease with increasing degree of occupation of the available cavities. Only at low degrees of occupation (< 50%) was a significantly higher endothermic heat effect for the (D)-enantiomer (template) detected which indicates an enantioselective rebinding. The overall heats of rebinding were found to be endothermic and are composed of several different contributions that are discussed. In summary, the reported calorimetric results are generally in accordance with the proposed mechanism of chiral recognition in this type of molecular imprinted polymers.

### Introduction

Chirality and chiral discrimination by molecular recognition are fundamental phenomena of nature and life. Therefore, chiral recognition possesses enormous scientific interest as well as industrial significance. Especially in the life sciences and pharmaceutical industry there is a strong move towards enantiomerically pure compounds linked with a growing need for enantioselective separation, preparation and analysis procedures [1].

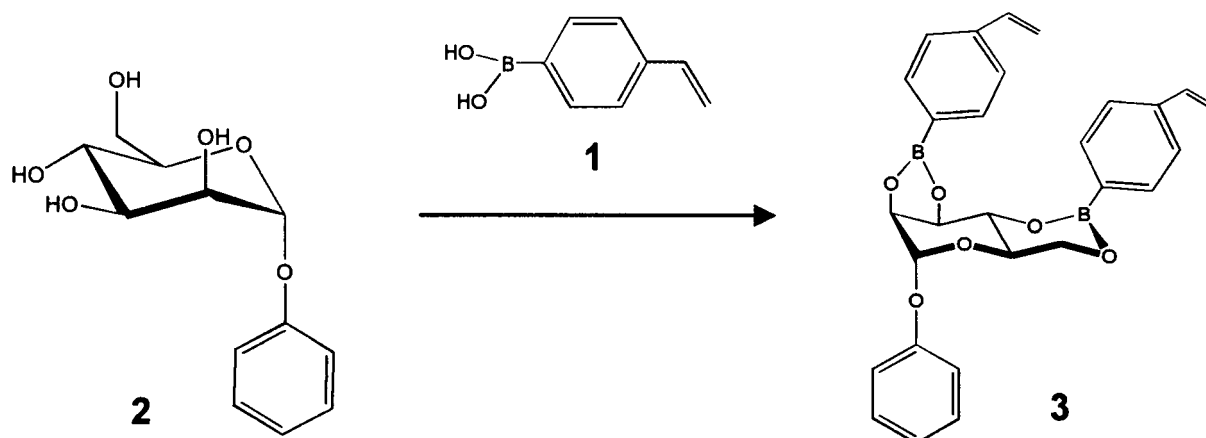
In principle, highly selective receptor materials require, as learned from nature, cavities matching the shape of the molecule to be selectively included and binding sites in a definite three-dimensional arrangement. Despite the usual way to form such cavities by rational synthesis of low molecular weight ring or cage systems [2] such as crown ethers and cryptates [3], or the use of cyclodextrins [4] etc., a promising concept to design selective recognition systems is molecular imprinting in synthetic organic polymers [5]. An aggregate between the target molecule (template) and functional monomers bound either covalently or non-covalently is copolymerized with an excess of cross-linker in the presence of an inert solvent acting as a porogen. This procedure

creates a rigid macroporous polymer matrix exhibiting a large surface area and a permanent pore structure. After removal of the template molecule, polymeric receptors with specific cavities for the template are obtained [5]. The use of a pure enantiomer as template enables the preparation of highly efficient receptor materials for chiral separations [6]. Hence, on the basis of this concept a large number of potential applications are being developed in chromatographic separation [7], catalysis [8], chemosensors [9], as artificial anti-bodies for immunoassays [10] or in bioimprinting [11].

Generally, the mechanism of imprinting and molecular (chiral) recognition seems now fairly well understood from a structural point of view based on extensive experimental studies [5]. However, only limited information is available on the thermodynamics of enantioselective recognition in imprints [12]. Therefore, the aim of this contribution is to address this problem by a thermochemical study of a well characterized model system introduced by Wulff and co-workers [13, 5a].

They used the ability of 4-vinylphenylboronic acid **1** (functional monomer) to form rapidly and reversibly cyclic esters with two OH-groups of monosaccharide-templates, such as enantiomerically pure phenyl- $\alpha$ -D-mannopyranoside **2** (Scheme 1). The binding of two monomers to the template yields a chiral monomer complex

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Scheme 1. Scheme of the template molecule and formation of the monomer.

**3** which is subjected to radical copolymerization forming a macroporous polymer.

After splitting off the template molecules, the remaining cavities possess a shape that is complementary to that of the template and contain boronic acid residues in the exact spatial arrangement to selectively rebind the template (D-form) in non-aqueous media. The efficiency of this approach for racemate resolution has been proved successfully by numerous experimental methods [13, 14] and the following general mechanism for this type of covalent recognition has been derived [5a]: in an ideal case the template molecule (D-enantiomer) is able to rebind to the polymer forming a two-point bond (perfect fit). In contrast, the “wrong” (L)-enantiomer is able to form only one bond because of the non-fitting geometry.

However, in practice the cavities show varying degrees of selectivity due to shrinkage, swelling or surface effects resulting in a selectivity distribution [13, 17].

The consequences of the proposed mechanism for thermochemical investigations with this special model system assuming preferably site separation are: (i) Chiral discrimination of the stereoisomeric target molecules, phenyl- $\alpha$ -D- and phenyl- $\alpha$ -L-mannopyranoside, should be accompanied by different interaction enthalpies, (ii) Assuming the most-selective, ideal case the enthalpy of interaction for the template (phenyl- $\alpha$ -D-mannopyranoside) should be twice the value of phenyl- $\alpha$ -L-mannopyranoside, (iii) The selectivity and interaction energy should decrease with increasing degree of occupation of the cavities.

First batch and liquid flow calorimetric measurements at low recognition site occupation [14, 15] seems to support this hypothesis, however, considerable experimental difficulties such as poor reproducibility, large blank effects and troublesome time effects were also reported. These results gave rise to the more detailed calorimetric investigations presented in this paper. Batch calorimetric measurements have been carried out in order to glean experience by verifying the experiments of Poll [15] and Kirstein [14] and to clarify the reported difficulties. Additional titration calorimetric investigations were focused on the determination of binding enthalpy differences between the stereoisomers of phenyl- $\alpha$ -mannopyranoside and the concentration depend-

ence. The results will be discussed with reference to the proposed recognition mechanism.

## Experimental

### Samples

Three different polymers, synthesized and characterized by Wulff and co-workers [13, 14] were studied:

- **Polymer A:** The polymer was prepared according to literature [13]. 5 wt.-% of template monomer **3** in ethylene dimethylacrylate was used, and 1 mL of acetonitrile/benzene per g of monomer mixture was added as inert solvent. After the polymerization and usual work up [13] 81.7% of template **2** could be split off. Thus 1 g of polymer can take up 82  $\mu$ mol (21 mg) of template **2** in the free cavities.
- **Polymer B:** corresponding to polymer **A** but template remained in place (non-split imprinted polymer).
- **Polymer C:** pure macroporous polymer (without template monomer **3**) made from ethylene dimethacrylate under identical condition

The polymers were imprinted with phenyl- $\alpha$ -D-mannopyranoside as template. Chiral recognition experiments were performed with both the enantiomerically pure stereoisomers, phenyl- $\alpha$ -D-mannopyranoside and phenyl- $\alpha$ -L-mannopyranoside, also prepared by Wulff *et al.* Acetonitrile (p.a. grade from Merck, Germany) dried by standard methods was used as solvent for all measurements. Moisture had to be avoided throughout the experiments because traces of water lead to a noticeable hydrolysis of the specific (covalent) boronic ester bonds between the template and the polymer in the cavities. These experiments are therefore performed using conditions under which either esterification, associated with the formation of water, or transesterification is possible.

### Methods

Batch calorimetric measurements were performed at 25 °C by means of a standard twin heat conduction calorimeter

C 80 made by Setaram (France). The reversal mixing cells (Hastelloy, part number 31/1508) consisting of two separated compartments with volumes of 2.5 mL (inner chamber) and 2 mL, respectively, were used. Usually, the inner chamber was filled with 0.1 g polymer and 2 mL pure acetonitrile and the outer compartment with 0.25 mL of the enantiomer solution ( $c = 0.0156$  mol/l) in acetonitrile or pure solvent for blank experiments. The reaction was started after defined thermal equilibration periods by turning the calorimeter upside down.

Isothermal titration calorimetric (ITC) measurements were carried out by means of a twin heat conduction calorimeter of the Calvet-type (DAK 1A-1, Russia). A home-made titration insertion cell equipped with a calibration heater and a stirrer was used for the experiments. Typical signal noise and drift were  $< 0.5 \mu\text{W}$  (point to point) and  $< 1 \mu\text{W/h}$ , respectively. Reagents were injected by means of a Hamilton syringe (1.0 mL) mounted on a step-motor driven precision syringe pump (SP250i, WPI, USA). A thin stainless steel tube (i.d. 0.2 mm) connected to the syringe reached directly into the calorimetric vessel. The vessel can be filled with 2 up to 6 mL of solution. More details of the titration calorimeter and the experimental procedure are described in [16].

Enantiomer solutions were injected stepwise (0.1 mL per step,  $c = 0.0156$  mol/l and  $c = 0.0162$  mol/l for the D-enantiomer and L-enantiomer, respectively) at a rate of 10 mL/h to a suspension of 0.1 g polymer in 2.5 mL acetonitrile. The next titration step was always started after reaching chemical and thermal equilibrium. The measured heat flow was recorded as a function of time and converted into enthalpies by integration of the appropriate reaction peaks. Dilution effects were corrected by subtracting the results of a blank experiment with pure acetonitrile in place of the enantiomer solutions under the same experimental conditions. Adsorption effects of the enantiomers onto the surface of the calorimetric vessel in contact with the solutions were checked by titrating the enantiomer solutions into pure acetonitrile. The effects can be neglected.

## Results and discussion

A series of batch calorimetric measurements has been performed using a solution of the template ((D)-enantiomer) and polymer **A** by applying different periods of time between filling the chambers of the reaction cell and mixing of the reactants. The ratio between polymer **A** and the (D)-enantiomer corresponds to 50% occupation of available binding sites which is comparable to the conditions used by Poll [15] and Kirstein [14] who worked in a flow cell. Appropriate blank experiments were carried out using pure acetonitrile instead of the enantiomer solution in the outer chamber of the mixing cell. The experimental results (Figure 1) show that both the measuring and blank effects were strongly time-dependent. However, the enthalpy of reaction, calculated from the difference of measured and blank effect at the same time remained nearly constant at  $(6.7 \pm 0.7)$  kJ/mol of enantiomer. The results indicated that

Table 1. Molar enthalpies of reaction of the template with different polymers at 50% of theoretical cavity occupation

	Polymer <b>A</b> (kJ/mol)	Polymer <b>B</b> (kJ/mol)	Polymer <b>C</b> (kJ/mol)
Phenyl- $\alpha$ -D-mannopyranoside	$6.6 \pm 0.7$	$1.1 \pm 0.5$	$< 0.1$

strong solvent–polymer interactions (mainly swelling) occur that are very slow especially in unstirred systems. These processes could also explain the experimental problems reported by Poll and Kirstein for their method because reliable results could only be obtained either by applying extremely long waiting periods (approximately 80 h) before starting the measurement or, with reduced accuracy, by using exactly the same pre-conditioning times in blank and rebinding experiments. For studying imprinted polymers with batch calorimetry an efficient stirring of the polymer suspension is necessary.

Further experiments have been performed by means of isothermal titration calorimetry. In contrast to the batch experiments the stirring of the polymer suspension in the vessel ensured a stable baseline and constant blank effects after 3–4 h of equilibration time. In order to confirm the batch measurements and to check the influence of additional interactions like adsorption at the polymer surface, polymers **A**, **B** and **C** have been investigated by using similar experimental conditions (concentrations and a template to polymer binding sites ratio corresponding finally to 50% site occupation). The results are summarized in Table 1. The interaction energy measured with the non-imprinted polymer **C** is approximately zero confirming that adsorption effects can be neglected in relation to rebinding. ITC measurements with polymer **A** resulted in the same interaction energy as found with the batch calorimeter proving the reliability of the calorimetric methods. Please note that the data reported in Figure 2 are differential values that must be converted into integral values for comparison with the data in Table 1. Polymer **B**, which still contains the template, also showed a small heat effect. This finding might be explained by rebinding to some cavities from which the template has been removed during working up. Injecting a surplus of the template can also cause additional binding of template by one binding site (two templates per cavity) which is detected as a small heat effect.

Furthermore, the chiral discrimination ability of polymer **A** has been studied by ITC in order to check the consistency of the proposed mechanism with measured interaction enthalpies. Calorimetric results for the measurements with the pure (D)- and (L)-enantiomer are shown in Figure 2. The molar enthalpies of reaction for each injection (calculated per mol of enantiomer added) are plotted against the ratio of enantiomer to polymer **A** (degree of site occupation). It is obvious that the interaction enthalpies strongly decrease with the increasing number of injections. Three regions can be distinguished. In the first region up to approximately 50% of theoretical site occupation, the template molecule

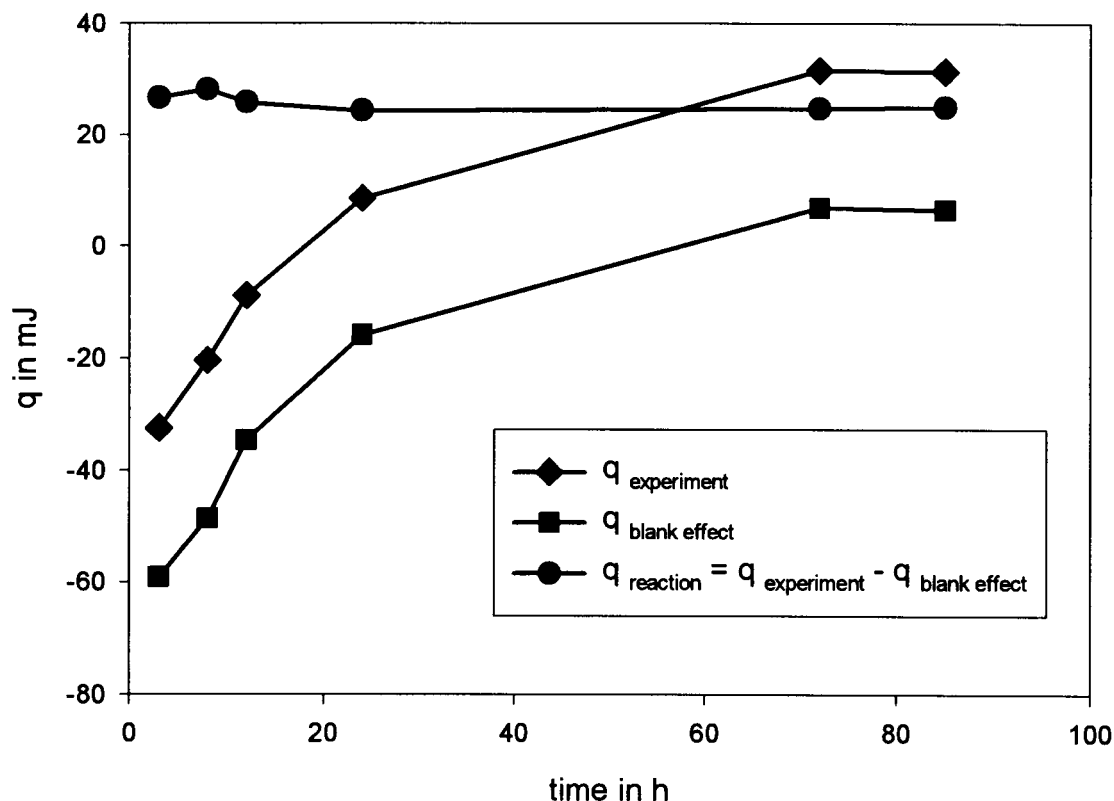


Figure 1. Batch calorimetric results of the dependence on the pre-conditioning time for the interaction of polymer A with phenyl- $\alpha$ -D-mannopyranoside (template) at 50% theoretical site occupation.

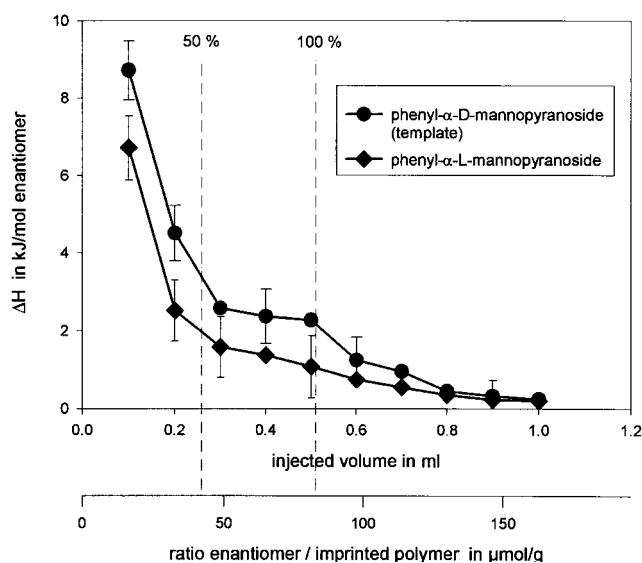


Figure 2. Titration calorimetric results for the interaction of both enantiomers with polymer A in dependence on the injected amount of enantiomer. The broken vertical lines indicate 50 and 100% of theoretical occupation of available binding sites in the imprinted polymer ( $82 \mu\text{mol}$  template per g polymer has been removed). Please note that the presented values are differential enthalpies in contrast to values reported in Table 1. For clarity not all error bars are shown; missing error bars are similar to those shown in the figure.

(D-enantiomer) showed a statistically significant larger enthalpy of reaction suggesting enantioselective interactions. Between approximately 50 up to 100% site occupation, there was still a small but statistically not significant difference

indicating poor chiral discrimination. Above 100% of maximal loading the heat effects quickly approached zero. These results exactly match the proposed mechanism explained above suggesting a selectivity distribution as investigated earlier by other methods [17]. The most perfect cavities are preferably filled first at low enantiomer concentration resulting in a high selectivity. With increasing amount of template injected less perfect cavities are also occupied that show decreasing selectivity (no two-point binding possible). Moreover, the calorimetric results are also in accord with results in chromatographic separations of racemates [17] demonstrating that significant chiral discrimination could only be observed below 50% of maximum cavity loading of the imprinted polymer.

The measured enthalpy values illustrated in Figures 1 and 2 show that the overall heats of interaction for both the enantiomers with the imprinted polymer are endothermic. From batch calorimetric measurements in acetonitrile solution and corrections for heats of mixing of all components an exothermic value of  $-17.5 \text{ kJ/mol}$  for the enthalpy of esterification of phenylboronic acid and propandiol was obtained by Poll [15] at  $25^\circ\text{C}$ . Data for other diols derived from temperature dependent measurements are known to be in the range between  $-11$  to  $-21 \text{ kJ/mol}$  [18]. However, the calorimetric results reported here contain a number of different caloric effects of which the assumed selective esterification (rebinding) reaction is only one part. The experimental value  $\Delta h$  (corrected for blank effects) should be composed of the following contributions:

$$\Delta h = \Delta h_{\text{esterif}} + \Delta h_{\text{solv}} + \Delta h_{\text{surface}} + \Delta h_{\text{polymer}}$$

where  $\Delta h_{\text{esterif}}$  denotes the enthalpy of esterification,  $\Delta h_{\text{solv}}$  the sum of all solvation/desolvation enthalpies of the reactants

$$\Delta h_{\text{solv}} = \Delta h_{\text{desolv}} (\text{enantiomer, boronic acid group}) + \Delta h_{\text{solv}} (\text{ester}) + \Delta h_{\text{solv}} (\text{H}_2\text{O}),$$

$\Delta h_{\text{surface}}$  the sum of interaction enthalpies of the enantiomer and water with the surface of the imprinted polymer and  $\Delta h_{\text{polymer}}$  the contribution to  $\Delta h$  from local movements of polymer chains close to binding sites. Unfortunately, most of the terms cannot be determined or measured because the exact amount of the species involved in the reaction, especially water, is not known. Additional calorimetric experiments have shown that the heat of solvation of traces of water by acetonitrile is strongly endothermic (approx. 7 kJ/mol) and concentration dependent. The presence of imprinted polymer reduced this effect to about one half indicating considerable interactions of water with the imprinted polymer. Therefore, at the present state the reliable separation of the different contributions and a differentiation between the postulated one or two point binding mechanism is not yet possible on the basis of our first calorimetric results.

Further investigations of the extent of rebinding especially the amount of water released and cavity occupation are necessary in order to estimate a reliable enthalpy of esterification within the cavities for comparison with solution data and for specifying the proposed mechanism of chiral discrimination.

## Summary and conclusions

Chiral recognition of the enantiomers phenyl- $\alpha$ -L- and phenyl- $\alpha$ -D-mannopyranoside (template) in a molecularly imprinted polymer have been successfully investigated by isothermal batch and titration calorimetry in acetonitrile. It could be shown that batch measurements with unstirred samples are affected by strong solvent-polymer interactions, such as swelling, causing large changes of the blank effects during approximately 80 hours. Experimental difficulties reported in the literature can probably be attributed to this behavior. Nevertheless, reliable batch calorimetric measurements are possible by applying very long pre-conditioning periods or an exact timing. Isothermal titration calorimetric measurements proved to be more efficient for studying imprinted polymers. The enthalpies of rebinding for both enantiomers strongly decrease with increasing degree of occupation of the available cavities. Only at low degrees of occupation was the difference of the detected heat effects for the (D)-enantiomer (template) and the (L)-enantiomer statistically significant which indicates an enantioselective rebinding.

These results further support the finding that there is a strong selectivity distribution of the binding sites obtained

by molecular imprinting. It has also been shown by other methods [17] that only a smaller part shows a high selectivity. The higher binding enthalpy for the D-form (the former template molecule) is due to a preferred two-point binding in contrast to a preferred one-point binding of the L-enantiomer.

A direct calorimetric determination of the proportion of two-point binding of the D- and L-form using heats of esterification of the two enantiomers in the imprinted polymer has not yet been successful because a reliable separation of the different heat contributions to the calorimetric signal failed due to the complexity of the rebinding process.

In summary, the reported calorimetric results are generally in accordance with the proposed mechanism of chiral recognition in this type of molecular imprinted polymers. Systematic calorimetric investigations seem to be a promising concept in order to gain new insights into the interesting and important field of imprinted polymers.

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