



**HAL**  
open science

## Lipase hydration state in the gas phase: Sorption isotherm measurements and inverse gas chromatography

Zsuzsanna Marton, Ludovic Chaput, Guillaume Pierre, Marianne Graber

### ► To cite this version:

Zsuzsanna Marton, Ludovic Chaput, Guillaume Pierre, Marianne Graber. Lipase hydration state in the gas phase: Sorption isotherm measurements and inverse gas chromatography. *Biotechnology Journal*, 2010, 5 (11), pp.1216. 10.1002/biot.201000272 . hal-00590750

**HAL Id: hal-00590750**

**<https://hal.science/hal-00590750>**

Submitted on 5 May 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## Lipase hydration state in the gas phase: Sorption isotherm measurements and inverse gas chromatography

Journal:	<i>Biotechnology Journal</i>
Manuscript ID:	biot.201000272.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	15-Sep-2010
Complete List of Authors:	Marton, Zsuzsanna; Université de la Rochelle, UMR LIENSs Chaput, Ludovic; Université de La Rochelle, UMR LIENSs Pierre, Guillaume; Université de la Rochelle, UMR LIENSs Graber, Marianne; Université de la Rochelle, UMR LIENSs
Primary Keywords:	Biocatalysis
Secondary Keywords:	Methods
Keywords:	Water, Lipase, Adsorption, Inverse Gas Chromatography, Solid/Gas Biocatalysis

SCHOLARONE™  
Manuscripts

1  
2  
3  
4  
5  
6 **Evaluation of lipase hydration state in the gas phase, by sorption isotherm**  
7  
8 **measurements and inverse gas chromatography; Relation with activity of**  
9  
10 **the lipase in solid/gas reactor.**  
11  
12

13  
14  
15  
16 *Zsuzsanna Marton<sup>1</sup>, Ludovic Chaput<sup>1</sup>, Guillaume Pierre<sup>1</sup> and Marianne Graber<sup>1</sup>*  
17

18  
19 <sup>1</sup>Université de la Rochelle, Pôle Sciences, Bât. Marie Curie, UMR 6250 LIENSs CNRS-ULR,  
20 Avenue Michel Crépeau, 17042 La Rochelle, Cedex 01, France.  
21

22  
23  
24  
25  
26 Keywords: Water, Lipase, Adsorption, Inverse Gas Chromatography, Solid/Gas Biocatalysis  
27

28  
29  
30 Correspondence: Prof M. Graber, Université de la Rochelle, Pôle Sciences, Bât. Marie Curie,  
31  
32 UMR 6250 LIENSs CNRS-ULR, Avenue Michel Crépeau, 17042 La Rochelle, Cedex 01,  
33  
34 France.  
35

36  
37 E-mail : [mgraber@univ-lr.fr](mailto:mgraber@univ-lr.fr)  
38

39  
40 Fax : +33 5 46 45 82 65  
41

42  
43  
44 Abbreviations: **IGC**, Inverse Gas Chromatography **a<sub>w</sub>**, water thermodynamic activity, **CALB**,  
45  
46 *Candida antarctica* Lipase B, **BET**, Brunauer-Emmet-Teller.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Abstract**

The adsorption of water and substrate on immobilized *Candida antarctica* lipase B was studied by performing adsorption isotherm measurements and using Inverse Gas Chromatography (IGC). Water adsorption isotherm of the immobilized enzyme showed singular profile absorption incompatible with the BET model, probably due to the hydrophobic nature of the support, leading to very low interactions with water. IGC allowed determining the evolution with  $a_w$  of both dispersive surface energies and acidity and basicity constants of immobilized enzyme. These results showed that water molecules progressively covered immobilized enzyme, when increasing  $a_w$ , leading to a saturation of polar groups above  $a_w$  0.1 and full coverage of the surface above  $a_w$  0.25. IGC also enabled relevant experiments to be performed to investigate the behavior of substrates under  $a_w$  that they will experience, in a competitive situation with water. Results indicated that substrates had to displace water molecules in order to adsorb on the enzyme from  $a_w$  values between 0.1 to 0.2, depending on the substrate. As the conditions used for these adsorption studies resemble the ones of the continuous enzymatic solid/gas reactor, in which activity and selectivity of the lipase were extensively studied, it was possible to link adsorption results with particular effects of water on enzyme properties.

## 1 Introduction

Contrary to expectations that enzymes are only active in aqueous solutions, enzyme activity in almost anhydrous media was demonstrated in the 1930s and has been extensively explored for the last decades [1, 2]. These media include among others organic liquid, supercritical fluids, solvent-free, ionic liquids and gas phase systems.

These non-conventional almost anhydrous reaction media possess great advantages over classical aqueous ones, such as shifting of thermodynamic equilibrium in favor of synthesis over hydrolysis and allowing easier recovery of products. However a small amount of water is required for catalytic action of enzyme, as some residual bound water are absolutely essential for maintaining the enzyme active conformational state and for promoting flexibility of enzyme, by acting as a lubricant [3, 4].

The quantity of required water varies depending on each individual case. The optimal amount depends on several parameters, including, polarity of enzyme active site, solvent hydrophobicity, substrate and reaction conditions. In any case, the activity, stability and specificity of enzymes in non-conventional media are greatly affected by the level of water present in the medium.

The best way to quantify the availability of water to the biocatalyst in non-conventional media is to use the parameter “thermodynamic water activity” ( $a_w$ ), which describes the effect of mass action of water on the equilibrium and is therefore a better measure of the effect of water than the water content [5].

Many studies were performed in organic solvents at varying  $a_w$ . Nevertheless, the influence of water on biocatalysis, remains unclear and needs to be further elucidated. In particular, no general trend has been found for the dependence of selectivity, especially enantioselectivity, on  $a_w$  [6, 7].

1  
2  
3  
4  
5 For several years, we have been working with continuous gas phase reactors for performing  
6 enzymatic reactions. In this type of reaction medium, a solid packed enzymatic sample is  
7 percolated by a carrier gas, which simultaneously carries gaseous substrates to the enzyme  
8 and removes gaseous products. In this system the  $a_w$  in the reaction medium can be perfectly  
9 controlled and fixed independently of the other reaction medium components, by  
10 incorporating water in the inlet gas at the correct molar fraction. If the gas phase is assumed  
11 as ideal,  $a_w$  is then obtained by dividing the partial pressure of water by its saturation pressure  
12 at the operating temperature. In case of organic media however, the calculation of  $a_w$  requires  
13 the knowledge of activity coefficients, whose estimation is far from being straightforward.  
14  
15 The solid/gas reactor is therefore a powerful tool for studying accurately the sole influence of  
16  $a_w$  on biocatalysis [8, 9].

17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

The effect of  $a_w$  on different types of reactions catalyzed by lipase B from *Candida antarctica* has been studied extensively in our laboratory, by using solid/gas reactor. It was observed that water had a very complex role, and could simultaneously act as a lubricant, by change in enzyme flexibility and increase of enzyme activity, as a competitive and enantioselective inhibitor, as a competitive substrate, and by interacting with all reactants, free enzyme, transition state and products, like solvent molecules do in liquid media [10-13].

In our different studies performed in solid/gas reactors, all these different effects of water have been assessed as a function of  $a_w$  in the reaction medium, but could not be relied to real hydration state of the enzyme. This is unfortunate as such knowledge is essential to gain a fundamental understanding on the effect of water on enzyme catalysis. At which  $a_w$  protein charged groups are they likely to be surrounded by clusters of water molecules? Above which  $a_w$  is a water monolayer retained on the enzyme surface, surrounding both charged and polar groups of the enzyme? Above which  $a_w$  has the substrate to displace water molecules adsorbed to the enzyme to interact with it? The answer to this last question is not only

1  
2  
3 dependent on  $a_w$  level allowing water monolayer formation, but also on the ability of  
4  
5 substrate to compete with water for adsorption on the immobilized enzyme.  
6  
7

8 In the following study, we are trying to answer all these questions, by studying adsorption of  
9  
10 both gaseous water and substrates on enzyme, thanks to adsorption isotherm measurements  
11  
12 and the use of Inverse Gas Chromatography (IGC), which is a dynamic and very accurate  
13  
14 vapor adsorption technique, allowing accurate characterization of surface properties [14-16].  
15  
16

17 In IGC the roles of stationary (solid) and mobile (carrier gas and vapor probe molecule)  
18  
19 phases are inverted from traditional analytical Gas Chromatography (GC). Indeed, in GC, a  
20  
21 standard column is used to separate and characterize several vapor molecules, whereas in  
22  
23 IGC, known vapor probe molecules are injected into a column packed with the solid sample  
24  
25 under investigation, which is immobilized CALB in our case, and whose surface  
26  
27 characterization is performed.  
28  
29  
30

31 There have been very few experimental studies addressing both water and substrates  
32  
33 adsorption on enzymes [17, 18], although these adsorption phenomena might be a way to  
34  
35 better elucidate enzyme kinetics and specificity. Recently we have studied molecular  
36  
37 mechanism of hydration of free, deglycosylated, and desalted pure CALB in the gas phase.  
38  
39 We showed that hydration occurs in two phases. For water activities below 0.5, water initially  
40  
41 binds to well-defined water-binding sites at the protein surface and then above 0.5, gradually  
42  
43 a spanning water network is formed [19].  
44  
45  
46  
47

48 Nevertheless these results cannot be directly used to explain the reaction kinetics we  
49  
50 measured in solid/gas reactor, for which we had to use an immobilized CALB preparation on  
51  
52 silanized Chromosorb. Indeed, this porous diatomite support allows a uniform carrier gas and  
53  
54 probe vapour flow through the enzymatic fix bed, and a better access of gas to the enzyme  
55  
56 surface. Moreover, it avoids large pressure drop across the column containing the enzyme  
57  
58 sample.  
59  
60

1  
2  
3 In the present paper, we show adsorption studies performed with the same immobilized  
4 enzymatic sample, in order to be able to overlay these adsorption phenomena and CALB-  
5 catalyzed reaction kinetics in continuous solid/gas reactors.  
6  
7  
8  
9

## 10 11 12 **2 Materials and methods**

### 13 **2.1 Enzyme and chemicals**

14  
15 *Candida antarctica* lipase B was a lyophilized preparation (Chirazyme L2) kindly supplied by  
16 Roche Industrie (Penzberg, Germany). All probes used in IGC experiments were of the  
17 highest purity (99% minimum) and checked by gas chromatography before use.  
18  
19  
20  
21  
22  
23  
24  
25  
26

### 27 **2.2 Adsorption of lipase onto a solid support**

28  
29 CALB adsorption was performed onto 60-80 mesh Chromosorb P AW DMCS, (Acid Washed  
30 DiMethylChloroSilanized; Varian, France), as previously described [20], by using 30 mg of  
31 lyophilized Chirazyme L2 per 6 g of support.  
32  
33  
34  
35  
36  
37

### 38 **2.3 Measurement of biocatalyst water adsorption isotherm**

39  
40 Water adsorption isotherm of Chromosorb alone and with immobilized lipase were measured  
41 at 25°C by using a DVS Advantage automated moisture sorption analyzer (Surface  
42 Measurement Systems Ltd., London, UK) between 0 and 95% relative humidity. The samples  
43 were initially dried for 10 h at 25°C under totally dried flowing air in the DVS moisture  
44 sorption analyzer to obtain the starting dry sample mass. The required humidity is generated  
45 by mixing dry and water saturated vapor gas flows in the correct proportions using two mass  
46 flow controllers and one vapor humidifier. The instrument was run with a  $dm/dt$  ( $m$ , mass;  $t$ ,  
47 time) set at  $0.002\% \text{ min}^{-1}$  to reach the equilibrium. This means that the relative humidity will  
48 remain constant until the mass variation falls consistently below this threshold. Once this  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



condition has been reached, the relative humidity of the system will be raised or lowered to the next level.

The water adsorption curve was modeled using the Brunauer-Emmet-Teller (BET) [21] gas adsorption equation (Eq 1).

$$\frac{a_w}{(1-a_w) \times m} = \frac{1}{m_0 \times C} + \frac{a_w \times (C-1)}{m_0 \times C} \quad (1)$$

where  $m$  is the mass of bound water (in mg per mg of dry enzyme),  $m_0$  is the monolayer water coverage,  $a_w$  the water activity and  $C$  a constant. From the linear regression of the plot of

$\frac{a_w}{(1-a_w) \times m}$  versus  $a_w$ , the theoretical monolayer water coverage can be calculated:

$$m_0 = \frac{1}{S + I}, \text{ where } S \text{ is the slope and } I \text{ the y-intercept [22].}$$

## 2.4 Inverse Gas Chromatography (IGC) measurements

### *Theory of IGC*

IGC consists in measuring the retention times of vapor probes of known properties in a column packed with the solid material of interest, which is immobilized CALB in our case. The retention time of these probes is related to the affinity of the probes for the support. Measuring how this retention time changes as a function of probe molecule chemistry, column temperature and ambient relative humidity, allows obtaining a wide range of physical properties of the solid under investigation.

In the present study, IGC was operated in the infinite dilution regime, which means that a very small amount of vapor probe was injected into the carrier gas stream through the column, leading to a very low surface coverage by the adsorbates. The retention volume of the probe is thus independent of its concentration and the result is a linear adsorption isotherm, which can be described by Henry's Law.

The specific retention volume  $V_G$  of vapor probes, normalized to a temperature of 273.15K and 1g of supported enzyme was calculated using Eq 2, where  $t$  (min) is the retention time of the probe,  $t_0$  (min) is the dead-time obtained with methane,  $F$  (mL.min<sup>-1</sup>) the carrier gas flow rate,  $m$  (g) the mass of immobilized CALB and  $T$  (K) the temperature of the column. The James and Martin compressibility factor,  $j$ , was calculated using Eq 3, where  $P_i$  and  $P_0$  are the inlet and outlet pressures, respectively.

$$V_G = j \cdot \frac{(t - t_0)}{m} \cdot \frac{F \times 273.15}{T} \quad (2)$$

$$j = \frac{3}{2} \cdot \frac{(P_i / P_0)^2 - 1}{(P_i / P_0)^3 - 1} \quad (3)$$

The specific retention volume  $V_G$  of vapor probes is related to the variation of the free energy of adsorption  $\Delta G_{ads}$  in the following way:

$$\Delta G_{ads} = -(R \times T \times \ln V_G) + C$$

where  $C$  is a constant depending on the choice of a reference state of the adsorbed probe and also on the total area of the solid accessible to the probe.

$\Delta G_{ads}$  takes into account two kinds of interactions: dispersive interactions (corresponding to London forces) and specific interactions, which consists mostly out of Lewis acid-base contributions.

$$\Delta G_{ads} = \Delta G_{ads}^d + \Delta G_{ads}^{sp}$$

To obtain the dispersive as well as the specific parts of  $\Delta G_{ads}$ , different apolar and polar vapor phase probe molecules are respectively injected and their specific retention volume  $V_G$  determined.

In case of apolar probes, like alkanes, which can only interact by dispersive interactions,  $V_G$  is related to  $\Delta G_{ads}^d$  by Eq 4 :

$$\Delta G_{ads}^d = -RT \ln V_G + C = -2N(\gamma_S^d)^{\frac{1}{2}} a(\gamma_L^d)^{\frac{1}{2}} + C' \quad (4)$$

Where  $N$  is the Avogadro constant,  $R$  the gas constant,  $a$  the probe surface area,  $\gamma_L^d$  the dispersive component of the probe surface energy,  $\gamma_S^d$  the dispersive component of the solid, and  $C'$  a constant. If a series of alkanes is injected,  $\gamma_S^d$  can be derived from the slope of the alkane line in a plot of  $RT \ln V_G$  versus  $a(\gamma_L^d)^{\frac{1}{2}}$ .

If amphoteric polar probe molecules are also injected, then specific interactions can be measured. The experimental points for the polar probe molecules are located above the alkane line in the surface energy plot. The vertical distance between each point and the alkane straight line represents the specific contribution of the interaction, which is expressed as the specific free energy  $\Delta G_{ads}^{sp}$  (Figure 1). In the present study, the specific free enthalpy of adsorption and the dispersive component of the surface energy of supported enzyme were calculated using the constants given in Table 1.

The enthalpic part of  $\Delta G_{ads}^{sp}$  ( $\Delta H_{ads}^{sp}$ ) can be calculated by performing the experiment at different temperatures, and calculating the slope of the plot  $\Delta G_{ads}^{sp}/T$  versus  $1/T$ , according to Eq 5, derived from the Gibbs equation:

$$\Delta G_{ads}^{sp}/T = \Delta H_{ads}^{sp}/T - \Delta S_{ads}^{sp} \quad (5)$$

These  $\Delta H_{ads}^{sp}$  values can then be converted into acid-base parameters if acid-base concepts are applied, which were developed by several authors, including Gutmann [23]. The Gutmann approach involves a simple four-parameter equation, with an acid and base term for each amphoteric polar probe according to Eq 6:

$$\Delta H_{ads}^{sp} = K_A \times DN + K_B \times AN^* \quad (6)$$

$K_A$  is the acceptor (acid) number and  $K_B$  is the donor (base) number of the solid surface.  $DN$  and  $AN^*$  are the donor and acceptor numbers of the amphoteric vapor probe molecules, which are available from literature [24] and given in Table 1.

$K_A$  and  $K_B$  can then be obtained by plotting  $\Delta H_{ads}^{sp} / AN^*$  versus  $DN / AN^*$ , with  $\Delta H_{ads}^{sp}$  obtained for several amphoteric probes.

For the determination of  $\Delta H_{ads}$  of pentan-2-ol and hexan-3-ol, it was assumed that the adsorption enthalpy was independent on temperature on the range studied.  $\Delta H_{ads}$  was then obtained from a plot of  $\ln(V_G/T)$  versus  $1/T$ , according to Eq 7, derived from the Clausius-

Clapeyron equation  $\left( \frac{\partial \ln p}{\partial (1/T)} \right)_v = -\frac{\Delta H_{st}}{R}$ , giving the differential isosteric heat of adsorption

$\Delta H_{st}$ , by plotting  $\ln p$  versus  $1/T$ , applied to chromatographic data at infinite dilution, which are necessarily isosteric (constant adsorbate coverage of zero) [25, 26].

$$\ln(V_G / T) = -\Delta H_{ads} / RT + C \quad (7)$$

#### *Conditions for IGC measurements*

Experiments were performed using an SMS-IGC2000 apparatus (Surface Measurement Systems Ltd., London, U.K.), allowing fully automated determination of interactions of ten different gas probe molecules with solid column packing material under investigation (Chromosorb alone or with immobilized CALB in our case), under a wide range of humidity and temperature conditions. A complete explanation of the design of this apparatus can be found in reference [27].

For each IGC experiment, DMCS treated 4 mm ID glass column packed with 500 mg of support alone or immobilized CALB was used. Before measurements, column packed was pre-conditioned for 10 hours at the initial temperature and  $a_w$  studied.

To obtain the dispersive as well as the specific surface energies, different polar and non-polar vapor phase probe molecules were injected and their specific retention volume  $V_G$  determined. 250  $\mu\text{L}$  of each probe were injected with  $p/p^\circ$  was fixed to 0.025, where  $p$  and  $p^\circ$  being respectively equal to partial pressure of probe in the gas and saturated vapour pressure of probe, calculated by assuming an ideal gas type behavior by  $p^\circ(T)=e^{(-\Delta H_{\text{vap}}/RT)}$ , at temperatures comprised between 313 and 338K and  $a_w$  ranging from 0 to 0.4. The carrier gas (helium) flow rate was 10 mL/min. All measures of retention time were triplicated.

To determine the enthalpies of adsorption ( $\Delta H_{\text{ads}}$ ) of pentan-2-ol and hexan-3-ol on immobilized CALB, 250  $\mu\text{L}$  of pentan-2-ol or hexan-3-ol at  $p/p^\circ = 0.05$  were injected at varying temperature: between 308 and 324K for  $0 < a_w < 0.15$  and between 314 and 322K for higher  $a_w$  values with pentan-2-ol; between 314 and 322 for all  $a_w$  values with hexan-3-ol.

### 3 Results

#### 3.1 Water adsorption by immobilized CALB, studied by water adsorption isotherm

Water adsorption experiments were carried out with both silanized Chromosorb support alone and the same support with immobilized CALB (Figure 2). Although the ratio of enzyme CALB to support was only 1/200 (m/m), the presence of enzyme on the support induced significant changes: Support alone presented a slight uptake of water in the total range of  $a_w$ , with an overall uptake of around 0.15% mass increase at  $a_w = 0.9$ . Support with immobilized enzyme adsorption isotherm showed an uptake of water between 2 and 3 higher than support alone, in the first part of the curve ( $a_w$  0 to 0.35) and a water sorption exponential and much higher for  $a_w$  values above 0.35, reaching 0.92% mass increase at  $a_w = 0.9$ .

The water adsorption isotherm shape of Chromosorb with immobilized CALB differs from the sigmoid shape of curves obtained with CALB alone [19], and more generally with isolated proteins [28], which can be modeled by the BET equation.

1  
2  
3 The BET model assumes the gradual recovery of surface by homogenous layers, characterized  
4 by a constant  $C$ , associated to the energy of hydration of the surface. It allows in particular  
5 revealing the formation of a water monolayer and its weight, and the occurrence of free water  
6 for higher  $a_w$  [17]. Yet, BET model is considered as a good mathematical tool to characterize  
7 the water adsorption isotherm and to determine the size of the monolayer for proteins, and this  
8 for  $a_w$  up to 0.4. Beyond this value, the BET model is known to gradually sweep from  
9 experimental observations [21].

10  
11 In our case, the curve which presents  $a_w/(1+a_w)m$  as a function of  $a_w$  (Figure 3) does not fit  
12 the BET model. This assumption is highlighted at  $a_w = 0.2$ , where the curve displays a break  
13 point whereas a linear curve is expected for enzymes following the BET model [19].

14  
15 These results suggest that Chromosorb support prevents the possibility to evaluate the CALB  
16 hydration with BET model. The water adsorption isotherm we obtained, resembles type III  
17 isotherm, according to IUPAC classification proposed by Brunauer, Deming and Teller [29].  
18 This uncommon isotherm suggests that interactions between water and solid are very low,  
19 which is actually the case with silanized Chromosorb of hydrophobic nature.

20  
21 Thus, we used an alternative method, i. e. IGC, to analyze the feature of CALB adsorbed on  
22 support. IGC allows determining many parameters of solid surface, such as dispersive and  
23 specific surface energy. By following the evolution of these parameters with varying  $a_w$ , the  
24 hydration state of CALB can be estimated indirectly.

### 25 26 27 **3.2 Determination of $a_w$ allowing saturation of water binding sites on immobilized** 28 **CALB, by studying surface properties of immobilized lipase by IGC**

29  
30 The saturation of water binding sites at the surface of immobilized enzyme CALB, when  
31 increasing  $a_w$ , can be estimated by determining the surface properties, like propensity to  
32

1  
2  
3 adsorb apolar molecules or surface Lewis acid-base properties, as a function of  $a_w$ . These  
4  
5 investigations can be performed by IGC, which has become a widely used technique to  
6  
7 characterize the surface properties of solid organic compounds like natural fibers [30] and  
8  
9 food materials [31].  
10  
11

#### 12 13 14 15 *Influence of $a_w$ on the adsorption of apolar molecules on immobilized CALB*

16  
17 Figure 4 presents the evolution of the specific retention volumes  $V_G$  of five apolar probes *n*-  
18  
19 undecane (C11), *n*-decane (C10), *n*-nonane (C9), *n*-octane (C8) and *n*-heptane (C7) with the  
20  
21  $a_w$  of the carrier gas, through the column filled with immobilized CALB, at 313K. For the  
22  
23 longest alkanes, *n*-undecane and *n*-decane, it appears that the retention volumes decrease and  
24  
25 then reach a plateau near  $a_w = 0.2$ . This same observation has been reported by Comte *et al.*  
26  
27 on glass beads for  $a_w \geq 0.15$ , and was interpreted as coverage of the surface of the support by  
28  
29 water molecules [32].  
30  
31  
32

33  
34 Indeed water molecules progressively hide interaction sites from alkane probes and their  
35  
36 retention time decrease. When the specific retention volume becomes constant it may be  
37  
38 assumed that the surface of the immobilized CALB is covered by a layer of water molecules.  
39  
40 Then the alkane probes adsorb on a film of water rather than on supported CALB.  
41  
42

43  
44 The value of  $\gamma_S^d$  for the immobilized enzyme followed the same trend with increasing  
45  
46 humidity (Table 2), thus confirming the hypothesis of water coverage of the surface at this  
47  
48 water activity level.  
49  
50

#### 51 52 53 *Influence of $a_w$ on the adsorption of polar molecules*

54  
55 The use of polar probes allows evaluation of Lewis acid-base interactions between these  
56  
57 probes and a solid support. First, the acid-base character of the immobilization support alone,  
58  
59 silanized Chromosorb, was determined by IGC at  $a_w = 0$ . Monopolar probes were used:  
60

1  
2  
3 chloroform and 1,4-dioxane, being respectively Lewis acid and basic probes, in order to  
4  
5 obtain respectively a measure of the Lewis basicity and acidity of the support. It needs to be  
6  
7 emphasized that  $\Delta G_{ads}^{sp}$  values measured are not intrinsic properties of the support but depend  
8  
9 on the properties of the adsorbate as well. Nevertheless results allowed to conclude that the  
10  
11 support presented predominantly an acidic or electron-accepting character, as it presented a  
12  
13 much higher affinity for 1,4-dioxane ( $\Delta G_{ads}^{sp} = 9.5$  kJ/mol/mg) compared to chloroform  
14  
15 ( $\Delta G_{ads}^{sp} = 5$  kJ/mol/mg). This could be attributed to the presence of residual silanol groups at  
16  
17 the surface of the support after silanization treatment.  
18

19  
20  
21 Moreover, the Lewis basicity and acidity of the support with the immobilized CALB were  
22  
23 determined in the same conditions with both monopolar probes. It was found that support with  
24  
25 the enzyme and buffer salts has an acidic character even more pronounced than the  
26  
27 Chromosorb alone: the immobilized CALB affinity for 1,4-dioxane is higher ( $\Delta G_{ads}^{sp} = 12.4$   
28  
29 kJ/mol.mg) and its affinity for chloroform even lower ( $\Delta G_{ads}^{sp} = 2.1$  kJ/mol.mg). These  
30  
31 variations cannot be attributed to the immobilization buffer salts (pH 7.5), they are probably  
32  
33 due to the presence of the enzyme. We assume that amino acids rather basic nature, such as  
34  
35 arginine, lysine and histidine, are in contact with the support (including residual silanol  
36  
37 groups) and become thus hidden. As a result, only the amino acid with acidic side chain may  
38  
39 interact with the polar probes, resulting in a decrease in basicity.  
40  
41  
42  
43  
44  
45  
46  
47  
48

49 To determine the effect of  $a_w$  on Lewis constants for the immobilized CALB,  $\Delta G_{ads}^{sp}$  of  
50  
51 different polar amphoteric probes (ethanol, acetonitrile and ethyl acetate) were determined at  
52  
53 different humidity. Results obtained at 313K are shown on Figure 5. It appears that values of  
54  
55  $\Delta G_{ads}^{sp}$  for the different probes strongly decrease when increasing  $a_w$  from 0 to 0.1 and then  
56  
57 stabilize. Such a drop in the specific free energy of sorption with increasing  $a_w$  at low  
58  
59 humidity level, followed by a stabilisation at higher  $a_w$  level, has been observed by Comte *et*  
60



1  
2  
3 *al.* for the adsorption of gaseous probes on mineral solids [32]. This evolution was interpreted  
4  
5 as a progressive coverage of the surface by water molecules until polar sites were saturated.  
6  
7

8  
9 The enthalpic part of  $\Delta G_{ads}^{sp}$ ,  $\Delta H_{ads}^{sp}$ , was calculated for each of these probes by performing  
10  
11 these experiments at different temperatures for each  $a_w$ . Figure 6 shows an example of the  
12  
13 curves obtained for  $a_w = 0.2$ , between 313 and 338K.  
14

15  
16 Then, from the donor or acceptor character of electron of injected probes and from  
17  
18  $\Delta H_{ads}^{sp}$  values,  $K_A$  acceptor (acidity) and  $K_B$  the donor (basicity) properties of the immobilized  
19  
20 CALB, were evaluated. The Lewis acidity ( $K_A$ ) and basicity ( $K_B$ ) constants decrease when  $a_w$   
21  
22 increase (Table 2), following the same trend as  $\Delta G_{ads}^{sp}$  values. These results indicate that water  
23  
24 molecules are able to progressively mask polar surface groups of immobilized enzyme and  
25  
26 that these groups are completely covered by water at  $a_w$  equal to 0.1.  
27  
28  
29

30  
31 In accordance with the result obtained with monopolar acidic and basic probes, the supported  
32  
33 enzyme presented a higher Lewis acidity constant  $K_A$  than Lewis basicity constant  $K_B$ .  
34  
35  
36  
37

### 38 **3.3 Determination of competitive adsorption of substrates and water at the surface of** 39 40 **immobilized lipase by IGC**

41  
42 In our experiments with enzymes in gas phase, different polar substrates were used, including  
43  
44 primary or secondary alcohols. These compounds may compete with water for adsorption  
45  
46 sites on the immobilized enzyme. A way to investigate this competition is to measure the  
47  
48 enthalpy of adsorption ( $\Delta H_{ads}$ ) of polar substrates, on immobilized enzyme at different  $a_w$ .  
49  
50  
51 This parameter is a direct measure of the strength of interaction between substrate and  
52  
53 supported enzyme and the evolution of this parameter with  $a_w$  can be ascribed as site blocking  
54  
55 by water in a competitive adsorption situation.  
56  
57  
58  
59  
60

1  
2  
3 The influence of  $a_w$  on  $\Delta H_{ads}$  of pentan-2-ol and hexan-3-ol on immobilized CALB is shown  
4  
5 on figure 7.  
6

7  
8 First, it can be observed that on the total range of  $a_w$  studied, for both pentan-2-ol and hexan-  
9  
10 3-ol adsorption,  $|\Delta H_{ads}|$  values are higher than the enthalpy of condensation, being  
11  
12 respectively equal to -52.6 kJ/mol and -43.4 kJ/mol. This implies that the enthalpies of  
13  
14 adsorption are not only due to the heat of condensation of the compound onto the solid, but  
15  
16 also to physic-chemical interactions between compound and immobilized enzyme.  
17

18  
19 Secondly, it appears that the enthalpy of adsorption of pentan-2-ol and hexan-3-ol vary in two  
20  
21 stages with  $a_w$ . In the first stage,  $|\Delta H_{ads}|$  decreases rapidly with increasing  $a_w$ . In the second  
22  
23 stage, it continues to decrease but with a very low slope. This stabilisation of the  $|\Delta H_{ads}|$   
24  
25 values corresponds to a decrease of alcohol retention times, and to their lower variation with  
26  
27 temperature. This could be attributed to the coverage of the immobilized lipase by adsorbed  
28  
29 water molecules, which hides binding sites from alcohols, in a competitive adsorption  
30  
31 situation. The  $a_w$  corresponding to the breaking point between these two parts of the  
32  
33 adsorption curve decreases from 0.2 to 0.1, when changing from pentan-2-ol to hexan-3-ol.  
34  
35  
36  
37  
38  
39  
40  
41

### 42 **3.3 Relation between hydration state and CALB activity and selectivity**

43  
44 The IGC study allows finding some correlation between CALB catalytic activity at different  
45  
46  $a_w$  in gas phase, and water status (strongly or weakly bound). In our solid/gas enzymatic  
47  
48 reactor experiments, the optimal  $a_w$  with respect to alcoholysis reaction was found to be in  
49  
50 range 0.05 to 0.1 [33]. This corresponds to a water activity level at which only stronger  
51  
52 adsorptive interactions with water occurs, and polar groups of immobilized enzyme are not  
53  
54 totally covered by water. This high catalytic activity of CALB at very low  $a_w$  was also  
55  
56 observed by other authors in liquid media [34]. It could be assigned to the ability of the  
57  
58 dehydrated enzyme to keep essential water molecules in its structure by tight bonding. For  
59  
60

1  
2  
3 many other enzymes, it was shown that they begin to regain their catalytic activity at  
4 hydration levels comprised between 0.12 and 0.2 mg H<sub>2</sub>O/mg protein, corresponding to  $a_w$   
5 values much higher than 0.1 [35].  
6  
7  
8  
9

10 In our solid/gas experiments, we have extensively studied the ability of CALB to resolve  
11 racemic mixtures of secondary alcohols, and found that the influence of  $a_w$  on CALB  
12 enantioselectivity was very significant. For example in case of the CALB-catalyzed resolution  
13 of pentan-2-ol or hexan-3-ol, through the esterification with methyl propanoate, the  
14 enantiomeric ratio (E value) varied respectively from 101 and 67 at  $a_w$  close to 0, to a  
15 maximum of 320 around  $a_w = 0.2$  and 120 at  $a_w = 0.1$  [12, 13]. Above these optimal  $a_w$  values  
16 for enantioselectivity, E values decreased for both secondary alcohols.  
17  
18  
19  
20  
21  
22  
23  
24  
25

26 As far as pentan-2-ol is concerned, the point  $a_w = 0.2$  appears as a “turning point” where the  
27 CALB enantioselectivity towards this alcohol starts to decrease with increasing  $a_w$ . We have  
28 shown that the point  $a_w = 0.2$  corresponds also to a breaking point for the enthalpy of  
29 adsorption of pentan-2-ol on immobilized CALB. Then above  $a_w 0.2$ , water molecules that  
30 were trapped at the enzyme have to leave in order to make room for the adsorption of alcohol,  
31 and then gain motional freedom. It can be suggested that above this point, water acts more as  
32 a “solvent”, with high entropic effects, than as an enantioselective inhibitor, through very  
33 specific steric effects, as it was shown for very low  $a_w$  values [12]. This can also be connected  
34 to the large entropic term seen for enantioselectivity at high water activity. As CALB  
35 enantioselectivity is disfavoured by entropy, the result is a decrease of E with  $a_w$ , for  $a_w >$   
36 0.2, and an increase of E at low  $a_w$  where water predominantly acts through steric effects, by  
37 binding in the stereospecificity pocket.  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53

54 Concerning hexan-3-ol, enantioselectivity starts to decrease at a lower  $a_w$  value equal to 0.1,  
55 than for pentan-2-ol [13]. This gap between pentan-2-ol and hexan-3-ol for “ $a_w$  turning point”  
56 may be explained by the bigger size and less polar character of hexan-ol, for which difficulty  
57  
58  
59  
60

1  
2  
3 in gaining access to non-wetted part of the surface of the immobilized biocatalyst is higher.  
4  
5 Indeed the change in  $a_w$  dependence for enantioselectivity coincides with that of the  
6  
7 stabilization for the enthalpy of adsorption of hexan-3-ol on immobilized CALB.  
8  
9

#### 10 11 12 **4 Concluding remarks**

13  
14 We have shown that water adsorption and IGC studies can be used successfully to monitor  
15  
16 hydration state of immobilized CALB, and also to investigate the adsorption of substrates in a  
17  
18 competitive situation with water. As the conditions used for these studies resemble the ones of  
19  
20 the continuous enzymatic solid/gas reactor, in which we have extensively studied activity and  
21  
22 selectivity of CALB, it was possible to link adsorption results with particular effects of water  
23  
24 on enzyme properties. As a result, this study allows a further insight into the complex and as  
25  
26 yet not fully understood role of water on biocatalysis.  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

*This work was supported by the French Ministry of National Education, Higher Education and Research and the French National Agency for Research ANR (Program “Chimie et Prcédés pour le Développement Durable”). Dr Majid Naderi (Surface Measurement Systems Ltd., London, U.K.) is thanked for water adsorption isotherm measurements of Chromosorb alone and with immobilized lipase.*

## 5 References

[1] Bell, G., Halling, P.J., Moore, B.D., Partridge, J., Rees, D.G., Biocatalyst behaviour in low-water systems. *Trends Biotechnol.* 1995, 13, 468-473.

[2] Dordick, J.S., Principles and selected applications of non-aqueous enzymology, in: Blanch H.W., Clark D.S. (Eds.), *Applied Biocatalysis*, Marcel Dekker, New York, 1991, pp. 1-51.

[3] Helms, V., Protein Dynamics Tightly Connected to the Dynamics of Surrounding and Internal Water Molecules. *ChemPhysChem* 2007, 8, 23-33.

[4] Trodler, P., Pleiss, J., Modeling structure and flexibility of *Candida antarctica* lipase B in organic solvents. *BMC Struct. Biol.*, 2008, 8, art. n° 9.

[5] Halling, P.J., Biocatalysis in low-water media: understanding effects of reaction conditions. *Curr. Opin. Chem. Biol.*, 2000, 4, 74-80.

[6] Jonsson, A., Wehtje, E., Adlercreutz, P., Mattiasson, B., Thermodynamic and kinetic aspects on water vs. organic solvent as reaction media in the enzyme-catalysed reduction of ketones. *Biochim. Biophys. Acta*, 1999, 1430, 313-322.

[7] Persson, M., Wehtje, E., Adlercreutz, P., Factors Governing the Activity of Lyophilised and Immobilised Lipase Preparations in Organic Solvents. *ChemBioChem*, 2002, 3, 566-571.

1  
2  
3 [8] Lamare, S., Legoy, M.D., Graber, M. Solid/gas bioreactors: powerful tools for  
4 fundamental research and efficient technology for industrial applications. *Green Chem.*, 2004,  
5 6, 445-458.  
6  
7

8  
9  
10  
11  
12 [9] Létisse, F., Lamare, S., Legoy, M.D., Graber, M. Solid/gas biocatalysis : an appropriate  
13 tool to study the influence of organic compounds on kinetics of lipase catalyzed alcoholysis.  
14  
15 *Biochim. Biophys. Acta - Proteins & Proteomics*, 2003, 1652, 27-34.  
16  
17

18  
19  
20  
21  
22 [10] Graber, M., Bousquet-Dubouch, M.P., Sousa, N., Lamare, S., Legoy, M.D., Water plays  
23 a different role on activation thermodynamic parameters of alcoholysis reaction catalyzed by  
24 lipase in gaseous and organic media. *Biochim. Biophys. Acta - Proteins & Proteomics*, 2003,  
25 1645, 56-62.  
26  
27  
28

29  
30  
31  
32  
33 [11] Graber, M., Bousquet-Dubouch, M.P., Lamare, S., Legoy, M.D., Alcoholysis catalyzed  
34 by *Candida antarctica* lipase B in a gas/solid system: effects of water on kinetic parameters.  
35  
36 *Biochim. Biophys. Acta - Proteins & Proteomics*, 2003, 1648, 24-32.  
37  
38

39  
40  
41  
42  
43 [12] Léonard, V., Fransson, L., Lamare, S., Hult, K., Graber, M. A water molecule in the  
44 stereospecificity pocket of *Candida antarctica* lipase B enhances the enantioselectivity  
45 towards 2-pentanol. *ChemBiochem*, 2007, 8, 662-667.  
46  
47  
48

49  
50  
51  
52  
53 [13] Léonard-Nevers, V., Marton, Z., Lamare, S. Hult, K., Graber, M. Understanding water  
54 effect on *Candida antarctica* lipase B activity and enantioselectivity towards secondary  
55 alcohols. *J. Mol. Catal. B: Enzym.*, 2009, 59, 90-95.  
56  
57  
58  
59  
60

1  
2  
3 [14] Greene, S., Pust, H., The determination of heats of adsorption by gas-solid  
4 chromatography. *J. Phys. Chem.*, 1958, 62, 55-58.  
5  
6  
7

8  
9  
10 [15] Kiselev, A., Yashin, Y. Gas-chromatographic determination of adsorption and specific  
11 surface for solids. In: Kiselev A., Yashin Y., (Eds.), *Gas Adsorption Chromatography*,  
12 Plenum Press, New York, 1969, pp. 104-145.  
13  
14  
15

16  
17  
18 [16] Gray, D., Guillet, J., A gas chromatographic method for the study of sorption on  
19 polymers. *Macromolecules*, 1972, 5, 316-211.  
20  
21  
22

23  
24  
25 [17] Dimoula, K, Pohl, M, Büchs, J, Spiess, A. C., Substrates and water adsorption  
26 phenomena in a gas/solid enzymatic reactor. *Biotechnol. J.*, 2009, 4, 1-10.  
27  
28  
29

30  
31  
32 [18] Hidaka, N., Matsumoto, T., Gaseous ethanol oxidation by immobilized enzyme in a  
33 packed bed reactor. *Ind. Eng. Chem. Res.*, 2000, 39, 909-915.  
34  
35  
36

37  
38  
39 [19] Branco, J. F., Graber, M., Denis, V., Pleiss, J., Molecular mechanism of the hydration of  
40 *Candida antarctica* lipase B in gas phase: water adsorption isotherms and molecular  
41 dynamics simulations. *ChemBioChem*, 2009, 10, 2913-2919.  
42  
43  
44

45  
46  
47 [20] Léonard, V., Lamare, S., Legoy, M.D., Graber, M., Enantioselective acylation of R-2-  
48 pentanol in a solid/gas reactor catalysed by lipase B from *Candida antarctica*. *J. Mol. Catal.*  
49 *B: Enzym.*, 2004, 32, 53-59.  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 [21] Brunauer, S., Emmett, P. H., Teller, E., Adsorption of gases in multimolecular layers. *J.*  
4  
5  
6 *Am. Chem. Soc.* 1938, *60*, 309-319.

7  
8  
9  
10 [22] Costantino, H.R., Curley, J.G., Hsu, C.C., Determining the water sorption monolayer of  
11  
12 lyophilized pharmaceutical proteins. *J. Pharm. Sci.* 1997, *86*, 1390-1393.

13  
14  
15  
16 [23] Gutman, V., *Donor-Acceptor approach to Molecular Interactions*, Plenum Press, New  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
York, 1978.

[24] Mukhopadhyay, P., Schreiber, H.P., Review: Aspects of acid-base interactions and use of  
inverse gas chromatography. *Colloids Surfaces A: Physicochem. Eng. Aspects*, 100, 1995, 47-  
71.

[25] Condor, J.R., Young, C.L., *Physicochemical Measurement by Gas Chromatography*,  
John Wiley and Sons, Chichester, 1979.

[26] Kiselev, A.V., Yashin, I., *Gas Adsorption Chromatography*, Plenum Press, New York,  
1969.

[27] Newell, H.E., Buckton, G., Butler, D.A., Thielman, F., Williams, D.R., The use of  
inverse phase gas chromatography to study the change of surface energy of amorphous lactose  
as a function of relative humidity and the processes of collapse and crystallisation. *Int. J.*  
*Pharm.*, 2001, *217*, 45-56.



1  
2  
3 [28] Lee, S.B., Kim, K.-J., Effect of water activity on enzyme hydration and enzyme  
4 reaction rate in organic solvents. *J. Ferment. Bioeng.*, 1995, 79, 473-478.  
5  
6  
7

8  
9  
10 [29] Brunauer, S., Deming, L.S., Deming, W.E., Teller, E., On a theory on the Van der Waals  
11 adsorption of gases. *J. Amer. Chem. Soc.*, 1940, 62, 1723-1732.  
12  
13  
14

15  
16  
17 [30] Heng, J.Y.Y., Pearse, D.F., Thielmann, F., Lampke, T., Bismarck, A., Methods to  
18 Determine Surface Energies of Natural Fibres: A Review. *Compos. Interface*, 2007, 14, 581-  
19 604.  
20  
21  
22

23  
24  
25 [31] Zhou, Q., Cadwallader, K.R., Effects of flavor compound chemical structure and  
26 environmental relative humidity on the binding of volatile flavor compounds to dehydrated  
27 soy protein isolates. *J. Agr. Food Chem.*, 2006, 54, 1838-1843.  
28  
29  
30  
31

32  
33  
34 [32] Comte, S., Calvet, R., Dodds, J.A., Balard, H., Surface properties of low specific surface  
35 powders using inverse gas chromatography, *Powder Technol.*, 2005, 157, 39-47.  
36  
37  
38  
39

40  
41  
42 [33] Bousquet-Dubouch, M.P., Graber, M., Sousa, N., Lamare, S., Legoy, M.D., Alcoholysis  
43 catalyzed by *Candida antarctica* lipase B in a gas/solid system obeys a Ping Pong Bi Bi  
44 mechanism with competitive inhibition by the alcohol substrate and water. *Biochim. Biophys.*  
45 *Acta*, 2001, 1550, 90-99.  
46  
47  
48  
49

50  
51  
52 [34] Valivety, R.H., Halling, P.J., Peilow, A.D., Macrae, A.R., Lipases from different sources  
53 vary widely in dependence of catalytic activity on water activity. *Biochim. Biophys. Acta.*  
54 *Protein structure and molecular enzymology*. 1992, 1122, 143-146.  
55  
56  
57  
58  
59  
60

1  
2  
3 [35] Roger, B.G. (Ed.), *Protein-Solvent Interaction*, Marcel Dekker, New York, 1994.  
4  
5  
6

7  
8 [36] Riddle, F.L., Fowkes, F.M., Spectral shifts in acid-base chemistry. 1. Van der Waals  
9 contributions to acceptor numbers. *J. Amer. Chem. Soc.*, 1990, *112*, 3259-3264.  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

Table 1: Characteristics of *n*-alkane and polar probes used in IGC: molecule surface area (*a*), surface tension ( $\gamma_L^d$ ) at 20°C, Fowke's acceptor number (*AN*\*) and Gutman's electron donor numbers (*DN*).

The two parameters *AN* (acceptor number) and *DN* (donor number) define the Lewis acidic and basic nature of the probes. *DN* (kcal/mol) measures the energy of a coordinate bond between a donor atom and the Lewis reference acid  $\text{SbCl}_5$ , in a solution of 1,2-dichloromethane [23]. *AN* (dimensionless number) is derived from relative  $^{31}\text{P}$  NMR chemical shifts of the basic reference  $\text{Et}_3\text{PO}_4$  dissolved in the working acid. *AN*\* (kcal/mol) corresponds to *AN* corrected values, considering *AN* value of  $\text{Et}_3\text{PO}_4$  equal to 40 kcal/mol [36].

	<i>a</i> (m <sup>2</sup> )	$\gamma_L^d$ (J/m <sup>2</sup> )	<i>AN</i> * (kcal/mol)	<i>DN</i> (kcal/mol)
acetonitrile	2.15 E-19	0.0275	4.7	14.1
1,4-dioxane	3.14 E-19	0.0250	0	14.8
ethyl acetate	3.3 E-19	0.0196	1.5	17.1
ethanol	3.53 E-19	0.0211	10.3	19
chloroform	4.4 E-19	0.0332	5.4	0
<i>n</i> -heptane	5.73 E-19	0.0203	-	-
<i>n</i> -octane	6.3 E-19	0.0213	-	-
<i>n</i> -nonane	6.9 E-19	0.0227	-	-
<i>n</i> -decane	7.5 E-19	0.0234	-	-
<i>n</i> -undecane	8.1 E-19	0.0246	-	-

Table 2: Evolution of the dispersive component of surface energy,  $\gamma_S^d$ , at 313K and acid-base properties of immobilized CALB with  $a_w$ . (n.d. for not determined)

$a_w$	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4
$\gamma_S^d$	26.5 ±0.3	25.5 ±0.4	25.3 ±0.6	25.3 ±0.5	25.0 ±0.6	24.5 ±0.6	24.4 ±0.4	24.7 ±0.5	24.1 ±0.5
$K_A$	1.46	1.02	0.86	0.88	0.84	0.78	1.00	n.d.	n.d.
$K_B$	0.70	0.89	0.25	0.10	0	0.22	0.37	n.d.	n.d.

## Figure legends

Figure 1: Determination by IGC of the dispersive component of the surface energy of the solid (immobilized CALB)  $\gamma_S^d$  and the specific free energy of adsorption  $\Delta G_{ads}^{sp}$  of polar probes with the solid.

Figure 2: Water adsorption isotherm at 298K of Chromosorb alone ( $\Delta$ ) and with immobilized *Candida antarctica* lipase B ( $\bullet$ ) (5mg of lyophilized Chirazyme L2 per gram of Chromosorb) (results from [13]).

Figure 3: BET plot for water adsorption at 298K on immobilized *Candida antarctica* lipase B (5mg of lyophilized Chirazyme L2 per gram of Chromosorb).

Figure 4: Evolution of specific retention volume  $V_G$  of apolar molecules with  $a_w$  on immobilized CALB at 313K : undecane ( $\blacklozenge$ ), decane ( $\square$ ), nonane ( $\blacktriangle$ ), octane ( $\diamond$ ) and heptane ( $\circ$ ).

Figure 5: Variation of specific free energy of adsorption of ethanol ( $\diamond$ ), acetonitrile ( $\Delta$ ) and ethyl acetate ( $\blacksquare$ ) with  $a_w$  on immobilized CALB at 313K. Standard deviations were too small to be represented on the figure.

Figure 6: Determination of enthalpy of adsorption of ethanol ( $\diamond$ ), acetonitrile ( $\Delta$ ) and ethyl acetate ( $\blacksquare$ ) at  $a_w$  equal to 0.2, by plotting  $\Delta G_{ads}^{sp}/T$  versus  $1/T$  for T comprised between 313 and 338 K.

Standard deviations were too small to be represented on the figure.

1  
2  
3 Figure 7: Evolution of enthalpy of adsorption of pentan-2-ol ( $\Delta$ ) (results from [13]) and  
4  
5 hexan-3-ol ( $\blacklozenge$ ) on immobilized CALB as a function of water activity.  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

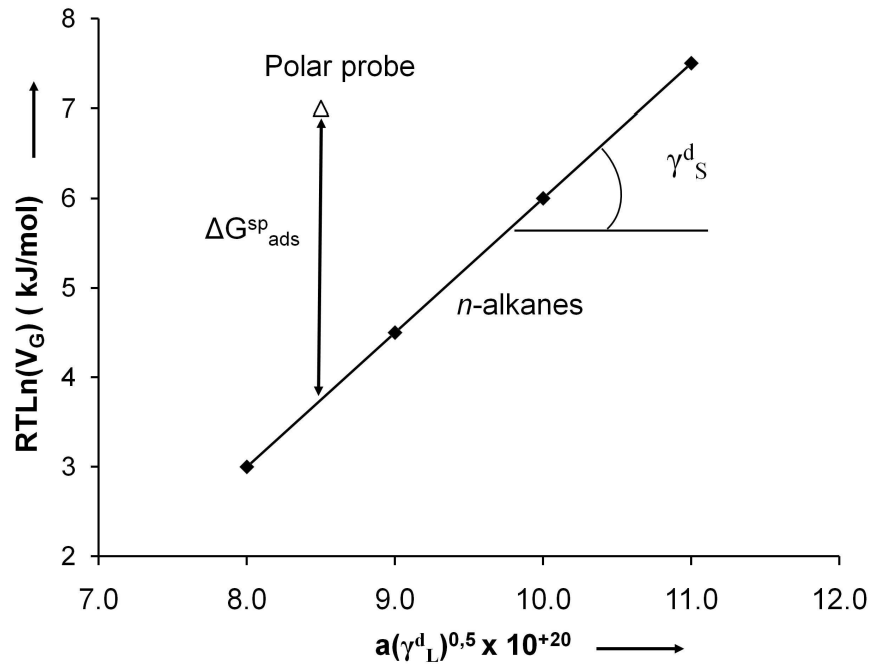


Figure 1: Determination by IGC of the dispersive component of the surface energy of the solid (immobilized CALB) and the specific free energy of adsorption of polar probes with the solid.  
120x80mm (600 x 600 DPI)

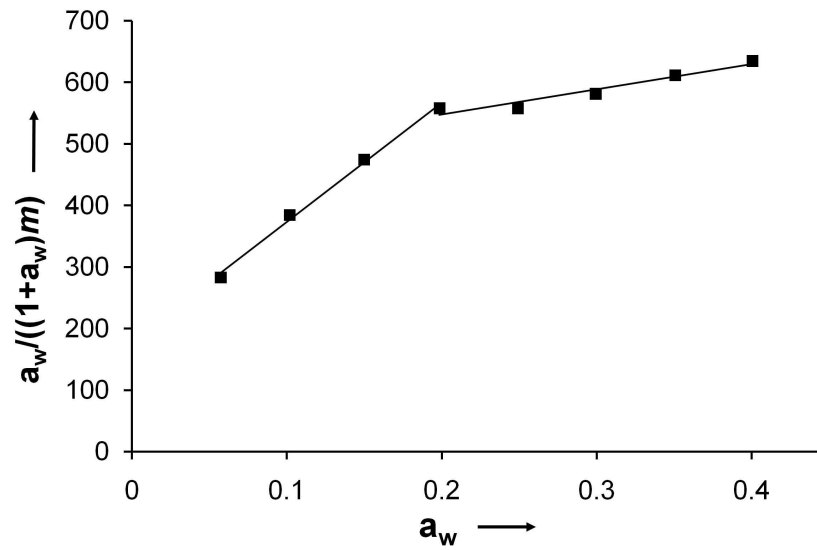


Figure 2: Water adsorption isotherm at 298K of Chromosorb alone ( $\Delta$ ) and with immobilized *Candida antarctica* lipase B ( $\bullet$ ) (5mg of lyophilized Chirazyme L2 per gram of Chromosorb) (results from [13]).  
120x80mm (600 x 600 DPI)



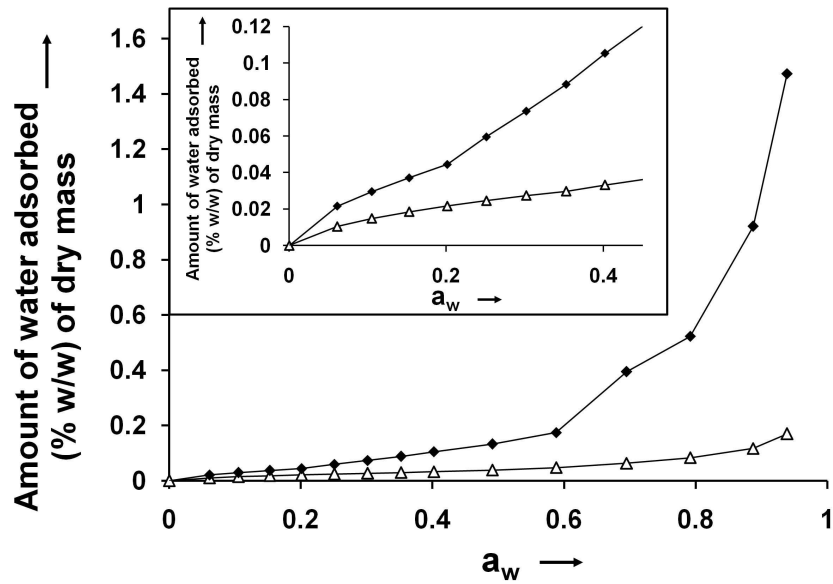


Figure 3: BET plot for water adsorption at 298K on immobilized *Candida antarctica* lipase B (5mg of lyophilized Chirazyme L2 per gram of Chromosorb).  
120x80mm (600 x 600 DPI)

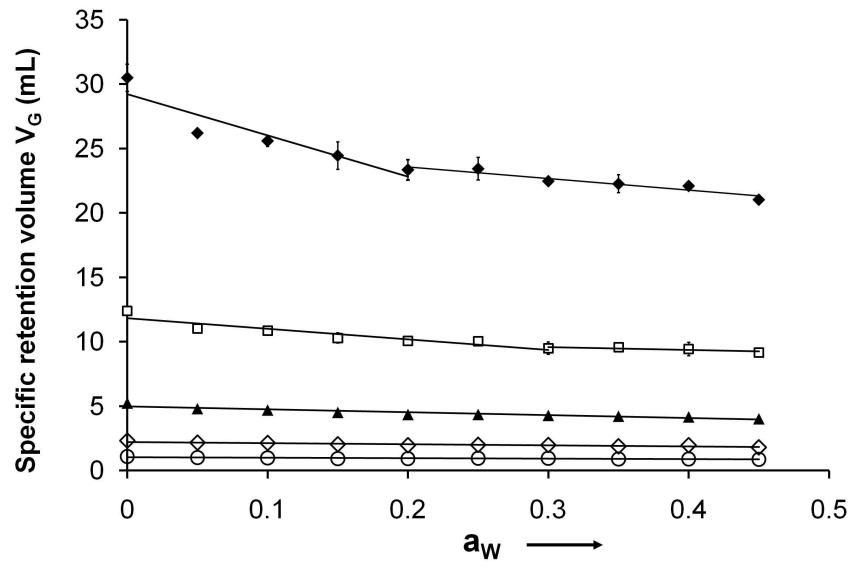


Figure 4: Evolution of specific retention volume  $V_G$  of apolar molecules with  $a_w$  on immobilized CALB at 313K : undecane (◆), decane (□), nonane (▲), octane (◇) and heptane (○).  
120x80mm (600 x 600 DPI)

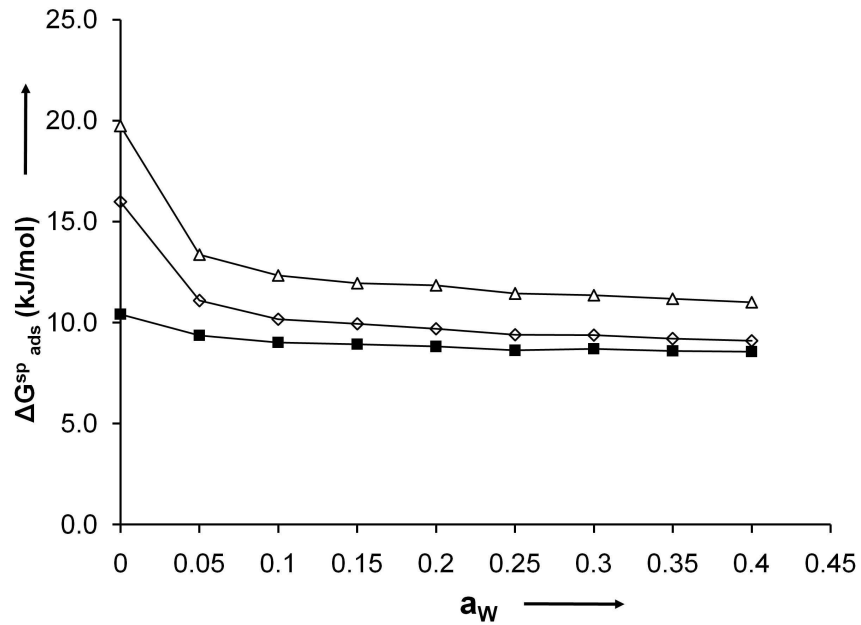


Figure 5: Variation of specific free energy of adsorption of ethanol ( $\diamond$ ), acetonitrile ( $\Delta$ ) and ethyl acetate ( $\blacksquare$ ) with  $a_w$  on immobilized CALB at 313K. Standard deviations were too small to be represented on the figure.  
120x80mm (600 x 600 DPI)

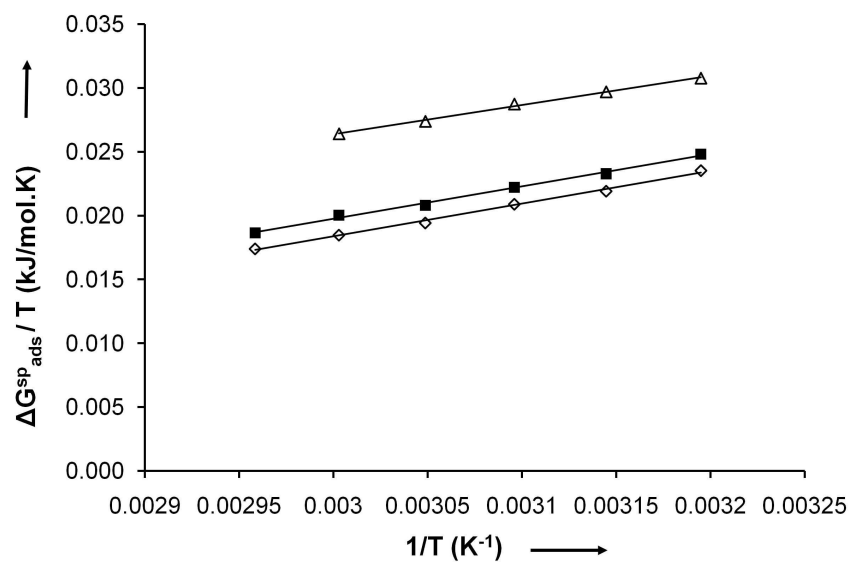


Figure 6: Determination of enthalpy of adsorption of ethanol ( $\diamond$ ), acetonitrile ( $\Delta$ ) and ethyl acetate ( $\blacksquare$ ) at aW equal to 0.2, by plotting  $\Delta G^{\text{SP}}$  of adsorption/T versus  $1/T$  for T comprised between 313 and 338 K.

Standard deviations were too small to be represented on the figure.

120x80mm (600 x 600 DPI)

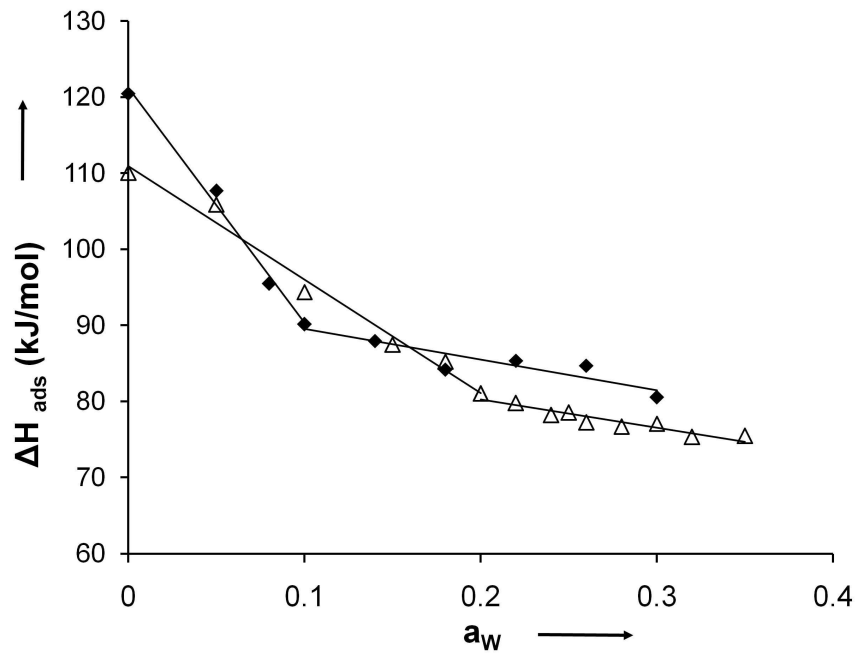


Figure 7: Evolution of enthalpy of adsorption of pentan-2-ol ( $\Delta$ ) (results from [13]) and hexan-3-ol ( $\blacklozenge$ ) on immobilized CALB as a function of water activity.  
120x80mm (600 x 600 DPI)