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Estimation of association constants between oral malodor components and various native and derivatized cyclodextrins

Andrew W. Lantz [a], Michael A. Rodriguez [a], Sean M. Wetterer [b], Daniel W. Armstrong [a,*]

[a] *Department of Chemistry, Iowa State University, Ames, IA 50011-3111, USA*
[b] *GlaxoSmithKline, Parsippany, NJ 07054, USA*

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Abstract

The association constants of 33 oral malodorous compounds and odor precursors (9 organic acids, 7 amine-containing bases, 11 organic neutral and aromatic compounds, and 6 amino acids) with native and derivatized cyclodextrins were measured using one or more of a variety of techniques including affinity capillary electrophoresis, nuclear magnetic resonance titrations, and head-space gas chromatography. With the exception of formic acid and urea, which had binding constants that were too small to measure, all analytes showed significant binding to at least one of the cyclodextrins studied. In most cases, the native cyclodextrins exhibited the most stable complexes with these analytes. However, with cationic analytes under acidic conditions, the negatively charged sulfated and carboxymethyl cyclodextrins had higher association constants. The six amino acid precursor molecules only bound significantly with the sulfated cyclodextrins. In addition, several analyte–cyclodextrin combinations were observed to form insoluble complexes, indicating that these cyclodextrins are particularly effective at extracting these compounds from aqueous solution.

Keywords: Halitosis; Odor; Oral hygiene; Binding; Cyclodextrins; Complexation

1. Introduction

Halitosis is a common condition that afflicts many individuals, and is generally described as an unpleasant or offensive odor emanating from the oral cavity. While halitosis may be emitted from the gastrointestinal tract or the upper respiratory system, the mouth is the source of 90% of breath odors [1]. Halitosis may be classified as either physiological halitosis, where odors arise from the natural putrefaction of food particulates by saliva or bacteria present in the mouth, or pathologic halitosis that results from a disease or condition such as periodontal disease. Numerous chemicals are responsible for oral malodor and include the volatile sulfur compounds (VSC), as well as a wide variety of short chain fatty acids, polyamines, alcohols, and aromatic compounds [1,2].

The treatment and control of malodorous compounds in the mouth is an important focus of the oral hygiene industry. In 2004, breath freshening products had an estimated retail value of $ 2.4 billion, a significant portion of the $ 7.2 billion oral care industry [3]. Clearly, there is a significant demand for consumer products that effectively extract and eliminate the compounds and precursor compounds responsible for halitosis. Several methods are available for controlling oral malodorous compounds. Antimicrobial agents may be used to limit the growth of bacteria that produce malodorous compounds in the oral cavity [2]. However, while this technique effectively limits the production of new malodorous compounds, it should be coupled with another method to extract or suppress pre-existing odor molecules. The introduction of a pleasant smelling fragrance may mask the perception of odor molecules, although the odor causing chemicals will still be present. Another option is to chemically change (oxidize, reduce, decompose, etc.) the malodorous compounds [4,5], however, in this case the toxicity and chemical strength of both the reactive agents and the product may be an issue. Finally, the use of a complexation agent that effectively binds to the malodorous compounds is an attractive approach. By complexing the odor chemicals with a ligand, it is possible to limit their ability to reach the vapor phase and, therefore, reduce their odor.

* Corresponding author. Fax: +1 515 294 0838.
E-mail address: sec4dwa@iastate.edu (D.W. Armstrong).

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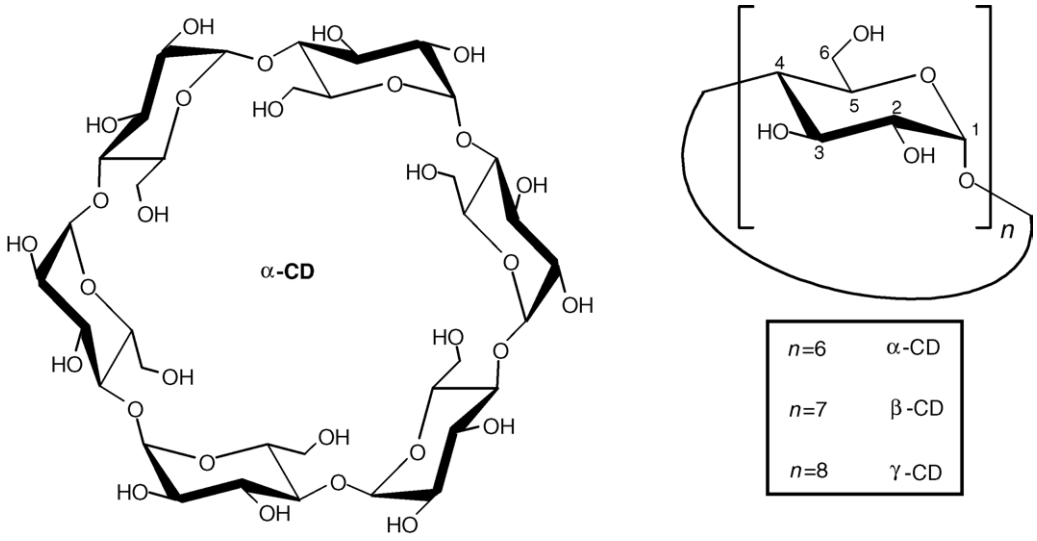

Fig. 1. The structure of native cyclodextrin.

This method could also allow the extraction of odor compounds from the aqueous environment of the mouth altogether.

A promising complexation agent for this purpose is cyclodextrin (CD). Cyclodextrins are cyclic oligosaccharides consisting of glucopyranose units connected by α-(l,4) linkages (Fig. 1) [6]. Three forms of cyclodextrin commonly exist: α-, β-, and γ-cyclodextrin, composing 6, 7, and 8 units, respectively. These macromolecules are produced by the natural breakdown of starch by the cyclodextrin glucosyl transferase (CGTase) enzyme found in *Bacillus macerans* bacteria. Their basic shape is toroidal, with a hydrophilic exterior and more lipophilic interior. This characteristic allows a wide variety of hydrophobic compounds to form inclusion complexes within the cyclodextrin cavity in aqueous environments. Additional solute–cyclodextrin interactions may occur through hydrogen bonding, electrostatic, and dipole–dipole effects. Cyclodextrins may also be derivatized by adding substituents to the hydroxyl groups on the cavity rim in order to change their aqueous solubility, binding properties, and charge [7]. In addition to being relatively inexpensive, native cyclodextrins have negligible toxicity and their bioadaptability has been well documented [8], which make them ideal additives in oral care products. Cyclodextrins have already been used extensively for the protection and solubilization of drugs [8,9] and the extraction of unwanted tastes and odors in foodstuffs [10].

In this study, we measure the binding constants of 33 malodorous compounds and odor precursors commonly present in the mouth with a variety of cyclodextrins and cyclodextrin derivatives. These compounds include 9 organic acids, 7 amine-containing bases, 11 organic neutral and aromatic compounds, and 6 amino acid precursor molecules. Binding constants, also called association constants, between an analyte and a ligand may be obtained by measuring an instrumental system response with varying ligand concentration and constant analyte concentration. This response can then be related back to the concentrations of the free and bound analyte and, therefore, to the extent of binding [11–13]. Here, binding constants were determined using a combination of affinity capillary electrophoresis (ACE), nuclear magnetic resonance (NMR) titrations, and a recently developed head-space GC method [13]. To our knowledge, no previous study has examined the binding of such a wide variety of oral malodorous compounds to cyclodextrins.

2. Materials and methods

2.1. Materials

Native α-, β-, and γ-cyclodextrin were obtained from Advanced Separation Technologies (Whippany, NJ, USA). Sulfated-α-cyclodextrin (S-α-CD), sulfated-β-cyclodextrin (S-β-CD), and hydroxypropyl-β-cyclodextrin (HP-β-CD) were all purchased from Aldrich Chemical Company (Milwaukee, WI, USA), with degrees of substitution (DS) of approximately 1.8, 1.5, and 0.8, respectively. Carboxymethyl-β-cyclodextrin (CM-β-CD, DS ∼ 0.8) was obtained from Cerestar USA Inc. (Hammond, IN, USA). Arginine, lysine, ornithine, tryptophan, citrulline, and cysteine were all received from Sigma (St. Louis, MO, USA). Pyridine was bought from Fisher Scientific (St. Louis, MO, USA), and all other analytes studied were purchased from Aldrich. Sodium phosphate, sodium hydroxide, and 85% phosphoric acid were all purchased from Fisher Scientific. D_2O (99.9%) used in the NMR titrations was also obtained from Aldrich.

2.2. Methods

Due to the wide variety of analytes examined in this study, multiple techniques were needed to determine all the analyte–cyclodextrin association constants (see Table 1). Analytes that had significant water solubility and contained a UV-absorbing chromophore were analyzed by ACE. These compounds include pyridine, 3-methyl pyridine, phenyl acetate, *m*-cresol, phenol, indole, and skatole. Indirect detection was not used for non-UV absorbing analytes, as the addition of a

Table 1
Aqueous binding constants[a] for various malodorous compounds with cyclodextrins at 22 °C[b,c,d]

	α-CD	β-CD	γ-CD	HP-β-CD	S-α-CD	S-β-CD	CM-β-CD	Method
Acids								
Formic acid	–	–	–	–		–	–	NMR titration
Acetic acid	31.4 ± 2.9	–	–	–		–	–	NMR titration
Propionic acid	34.4 ± 4.0[f]	–	–	–		–	–	NMR titration
Butyric acid	117.1 ± 5.9[f]	120.5 ± 57.2	3.3 ± 1.1	45.5 ± 4.1		2.1 ± 1.5	1.5 ± 0.8	NMR titration
Isobutyric acid	14.5 ± 0.8	201.5 ± 2.4[f]	–	33.2 ± 3.9		2.5 ± 1.4	3.1 ± 1.0	NMR titration
Valeric acid	313.5 ± 21.0	190.6 ± 12.6	13.6 ± 1.0	100.1 ± 5.9		13.2 ± 2.7	29.7 ± 2.7	NMR titration
Isovaleric acid	35.4 ± 1.2	203.8 ± 54.2[f]	21.6 ± 3.4	144.8 ± 16.0		5.2 ± 1.2	19.0 ± 1.7	NMR titration
Lactic acid	23.2 ± 5.3	–	–	–		3.6 ± 1.1	327.3 ± 31.3	NMR titration
Succinic acid	64.3 ± 1.1	21.6 ± 16.3	6.6 ± 3.2	4.3 ± 1.3		44.4 ± 7.6	270.4 ± 25.1	NMR titration
Bases								
Ammonia	–	–	–	–		19.6 ± 4.8	2.3 ± 0.8	NMR titration
Methylamine	–	–	–	–		13.7 ± 3.8	4.8 ± 1.0	NMR titration
Cadaverine	28.9 ± 2.8	42.1 ± 2.1	11.2 ± 2.3	16.1 ± 0.2		96.0 ± 9.8	13.9 ± 1.1	NMR titration
Putrescine	32.2 ± 5.1	39.3 ± 11.6	15.2 ± 1.7	6.4 ± 0.9		82.0 ± 6.7	8.8 ± 1.2	NMR titration
Pyridine	12.7 ± 1.6	54.3 ± 1.8	19.1 ± 1.8	25.9 ± 3.1		3.5 ± 0.1	9.5 ± 0.4	NMR titration
					75.6 ± 15.8	189.7 ± 10.6	38.8 ± 3.6	ACE pH 4.5
3-Methyl pyridine	18.6 ± 1.3	32.0 ± 2.6	29.1 ± 3.2	28.0 ± 3.2		3.9 ± 0.3	10.2 ± 0.7	NMR titration
					66.1 ± 9.1	264.1 ± 11.7	27.5 ± 1.4	ACE pH 4.5
Diphenylamine	24.1 ± 2.4	216.7 ± 12.2[f]	57.4 ± 7.7[f]	248.6 ± 16.1		33.2 ± 2.9	82.7 ± 5.8	NMR titration
Neutrals								
Hydrogen sulfide	20.73 ± 0.76[f]	7.38 ± 2.20	4.98 ± 0.83	6.29 ± 0.48	1.12 ± 0.14	0.88 ± 0.07	1.65 ± 0.38	HS-GCMS[e]
Methanethiol	23.05 ± 0.30[f]	1.70 ± 0.47	2.53 ± 0.10	1.90 ± 0.12	0.75 ± 0.13	0.18 ± 0.06	0.35 ± 0.07	HS-GCMS[e]
Dimethyl sulfide	24.84 ± 2.19[f]	17.27 ± 3.19[f]	8.31 ± 0.45[f]	4.34 ± 0.12	0.37 ± 0.06	0.43 ± 0.03	1.57 ± 0.15	HS-GCMS[e]
Urea	–	–	–	–		–	–	NMR titration
Phenyl acetate	6.9 ± 1.2	106.5 ± 5.3	14.0 ± 1.6	111.8 ± 7.2				NMR titration
					6.4 ± 0.6	2.6 ± 0.2	65.6 ± 5.4	ACE pH 8.5
					4.6 ± 1.8	6.0 ± 3.6	24.3 ± 8.3	ACE pH 4.5
m-Cresol	48.4 ± 2.3	124.7 ± 27.5	97.4 ± 8.2[f]	130.5 ± 13.9				NMR titration
					3.2 ± 2.4	5.9 ± 0.8	35.1 ± 2.8	ACE pH 8.5
					4.3 ± 3.7	17.9 ± 8.8	6.5 ± 2.4	ACE pH 4.5
Phenol	18.6 ± 1.3	59.6 ± 5.8	2.6 ± 1.4	52.4 ± 5.6				NMR titration
					4.4 ± 2.5	7.5 ± 0.7	29.4 ± 0.9	ACE pH 8.5
					10.7 ± 4.0	16.8 ± 9.1	23.1 ± 2.1	ACE pH 4.5
Indole	5.5 ± 0.8	166.4 ± 0.4	20.8 ± 2.0[f]	136.8 ± 8.5				NMR titration
					15.1 ± 1.4	15.6 ± 2.9	71.1 ± 1.2	ACE pH 8.5
					24.0 ± 19.8	45.4 ± 21.1	70.7 ± 17.9	ACE pH 4.5
Skatole	4.1 ± 0.6	162.3 ± 1.3	29.7 ± 2.9	171.8 ± 6.9				NMR titration
					15.5 ± 6.1	18.9 ± 1.0	72.1 ± 4.3	ACE pH 8.5
					13.9 ± 5.2	22.6 ± 4.0	86.8 ± 24.6	ACE pH 4.5
Dodecanol	142.0 ± 20.3[f]	100.7 ± 33.6[f]	80.5 ± 14.6[f]	157.6 ± 14.3		8.1 ± 2.2	25.3 ± 3.0	NMR titration
Tetradecanol	425.5 ± 71.1[f]	200.3 ± 25.0[f]	85.6 ± 7.8[f]	104.4 ± 6.9		14.5 ± 3.3	35.6 ± 2.5	NMR titration
Amino acids								
Arginine	–	–	–	–	121.6 ± 18.9	324.9 ± 93.8	–	ACE pH 9.4
	–	–	–	–	87.3 ± 18.3	151.8 ± 10.6–	–	ACE pH 4.5
Lysine	–	–	–	–	167.2 ± 12.4	142.0 ± 13.7	–	ACE pH 9.4
	–	–	–	–	103.3 ± 11.9	173.6 ± 3.1	–	ACE pH 4.5
Ornithine	–	–	–	–	139.1 ± 4.8	192.2 ± 22.0	–	ACE pH 9.4
	–	–	–	–	181.8 ± 14.8	368.8 ± 24.6	–	ACE pH 4.5
Tryptophan	–	–	–	–	–	–	–	ACE pH 9.4
		–	–	–	–	49.5 ± 4.9	–	ACEpH 4.5
Citrulline	–	–	–	–	231.8 ± 24.7	1068.6 ± 57.8	–	ACE pH 9.4
	–	–	–	–	222.1 ± 97.8	231.8 ± 24.7	–	ACE pH 4.5
Cysteine	–	–	–	–	40.3 ± 17.8	719.5 ± 37.8	–	ACE pH 9.4
	–	–	–	–	–	250.5 ± 49.1	–	ACE pH 4.5

α-CD: α-cyclodextrin, β-CD: β-cyclodextrin, γ-CD: γ-cyclodextrin, HP-β-CD: hydroxypropyl-β-cyclodextrin, S-α-CD: sulfated-α-cyclodextrin, S-β-CD: sulfated-β-cyclodextrin, CM-β-CD: carboxymethyl-β-cyclodextrin.

[a] All binding constants in units of M^{-1}.

[b] For method details, see Section 2.2.

[c] Empty cells indicate binding constant determination not performed.

[d] –: Extent of binding too low to be measured by the stated method.

[e] Data from Ref. [13].

[f] Precipitation of CD–analyte complex.

UV absorbing buffer additive may compete with the analytes for cyclodextrin binding. Because ACE requires that the ligand and analyte have differing electrophoretic mobilities, the binding constants of neutral analytes with neutral cyclodextrins could not be performed. These analyte–cyclodextrin combinations were examined by ^{1}H NMR titrations. In addition, the cyclodextrin binding of the six amino acid precursors were also determined with ACE. ACE was performed at various acidic and basic conditions (pH 4.5, 8.5, and 9.4) in most cases. NMR titrations were used to measure the binding of the remaining analytes, such as the organic acids and non-aromatic amines, with cyclodextrin.

ACE was performed on a Beckman PACE 2100 (Fullerton, CA, USA) with a 50 μm i.d. × 358 μm o.d. capillary, 37 cm in length (30 cm to the detector). The amino acid analytes, however, were analyzed using a Beckman PACE MDQ (capillary 30, 20 cm to detector) because of its higher detection sensitivity. Capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). Detection was accomplished by UV absorbance at 214 nm. Data analysis was done with Beckman System Gold software. Buffer solutions of 10 mM sodium phosphate buffer and cyclodextrin were made from deionized filtered water, and brought to the desired pH with 85% phosphoric acid and 1 M sodium hydroxide. Cyclodextrin concentrations of 0, 20, 40, 60, and 80 mg/mL were used for each series of runs. Samples were prepared by diluting the analyte samples directly into the phosphate buffer to a concentration of 2 mM. Mesityl oxide was used as an electro-osmotic flow marker. All buffers and sample solutions were sonicated for 5 min prior to their first run. The capillary was conditioned before its first use by rinsing with 1 M sodium hydroxide for 3 min, water for 3 min, sodium hydroxide for 1 min, and finally water for 1 min. Between each run, 1 min sodium hydroxide, water, and run buffer rinses were performed. Samples were injected for 2 s via hydrodynamic pressure of 0.5 psi. All separations were performed in the normal polarity mode with an applied voltage of 15 kV. The capillary was maintained at a temperature of 25 °C.

The association constant determinations for formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, lactate, succinate, ammonia, methylamine, cadaverine, putrescine, diphenylamine, urea, dodecanol, and tetradecanol were performed by ^{1}H NMR titrations using a 7.05T Varian VXR-300 Spectrometer. All analytes were diluted to 2 mM or lower using 99.9% D_2O as a solvent. Cyclodextrin was added to the series of NMR samples resulting in concentrations of 0, 20, 40, 60, and 80 mg/mL. However, due to its limited solubility native β-cyclodextrin concentrations of 0, 5, 10, 15 and 20 mg/mL were used with an analyte concentration of 1 mM. All ^{1}H spectra were analyzed using MestReC software (MestreLab Research, A Coruña, Spain), and the D_2O (δ 4.80) signal was used as an internal reference.

The complexation of the volatile sulfur compounds (VSCs) with cyclodextrin was measured via a previously described method using head-space gas chromatography mass spectrometry (HS-GCMS) [13]. Briefly, the analyte vapor pressure above an aqueous phase is monitored by HS-GCMS with the addition of cyclodextrin to the solution. Upon analyte–cyclodextrin binding, the analyte vapor pressure decreases. By applying the known Henry's constants for these analytes, one is able to quantitate the number of moles of analyte in the gas phase, aqueous phase, and bound to cyclodextrin. From these data, the association constant between the aqueous analyte and cyclodextrin may be obtained. The full method description can be found in the literature [13].

3. Theory

As stated earlier, binding constants of an analyte with a ligand are commonly measured by tracking an instrumental response while varying the ligand concentration with constant analyte concentration. For example, in ACE the electrophoretic mobility of an analyte plug through a buffer filled capillary is measured as the cyclodextrin concentration in the buffer is increased (Fig. 2, left). Similarly, in an NMR titration it is the chemical shift of the analyte that is measured with increasing concentration of cyclodextrin in solution (Fig. 2, right) [11,14]. The change in these values upon analyte complexation with the ligand can be related to the relative amounts of free and bound analyte. All binding isotherms exhibited appropriate hyperbolic curvature, indicating the formation of a 1:1 complex. Generally, the 1:1 binding of an analyte with a ligand can be described by the following equilibria:

$$S + L \overset{K}{\longleftrightarrow} SL, \qquad K = \frac{[SL]}{[S][L]} \tag{1}$$

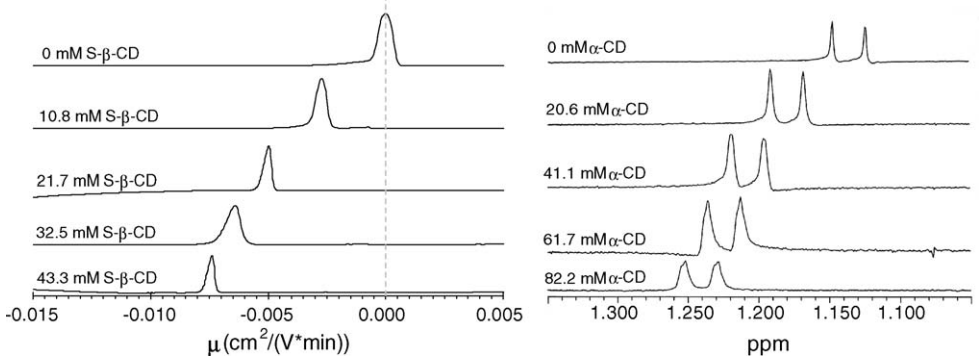

Fig. 2. (Left) Change in the electrophoretic mobility of indole with increasing S-β-CD concentration using ACE at pH 4.5. (Right) Change in the chemical shift of the β-hydrogens of isobutyric acid with increasing α-CD concentration.

where S is the analyte, L the ligand, SL the complex, and K is the association constant. The change in instrumental response can be applied to Eq. (1) to obtain:

$$K[\mathrm{L}] = \left(\frac{r_\mathrm{f} - r_\mathrm{i}}{r_\mathrm{i} - r_\mathrm{c}}\right) \tag{2}$$

where r_f and r_c are the instrumental responses of the free and fully complexed analyte and r_i is the analyte response at a certain concentration of ligand. Isolating the experimentally measured response, $(r_\mathrm{i} - r_\mathrm{f})$, the following equation is produced:

$$(r_\mathrm{i} - r_\mathrm{f}) = \frac{(r_\mathrm{c} - r_\mathrm{f})K[\mathrm{L}]}{1 + K[\mathrm{L}]} \tag{3}$$

Because of the inherent difficulty of measuring r_c, it is advantageous to rearrange Eq. (3) to a form in which the r_c value is not needed to calculate K. One such form is a Scatchard-type (also known as the x-reciprocal) equation:

$$\frac{(r_\mathrm{i} - r_\mathrm{f})}{[\mathrm{L}]} = -K(r_\mathrm{i} - r_\mathrm{f}) + K(r_\mathrm{c} - r_\mathrm{f}) \tag{4}$$

Plotting $(r_\mathrm{i} - r_\mathrm{f})/[\mathrm{L}]$ versus $(r_\mathrm{i} - r_\mathrm{f})$ results in a line with a negative slope of K (Fig. 3A and B) [11,14]. An approximation was made that the equilibrium concentration of the ligand is equal to the initial ligand concentration. This assumption is reasonable when the concentration of the ligand is significantly greater than the concentration of the analyte. These equations were used to determine the association constants reported in this research using ACE and NMR titrations. Data was plotted in Excel, and fit to a line using the least squares method. The error in the reported binding constants were based on the uncertainties of the slopes measured by the software. Briefly, these uncertainties were calculated using propagation of error analysis methods. The formula $\delta m^2 = \sum (\delta m/\delta y_\mathrm{i})^2 \delta y_\mathrm{i}^2$ was used to calculate the uncertainties, where δm is the uncertainty in the slope and δy_i are the uncertainties in the actual y values. The operation $(\delta m/\delta y_\mathrm{i})^2$ is performed on the equation used to determine the slope based on the experimental values. The error values reported here represent 67% confidence limit. HS-CGMS calculations and methods are described in detail in a previous publication [13]. An example plot of the change in the apparent gas–liquid partition coefficient for methanethiol with HP-β-CD concentration is shown in Fig. 3C.

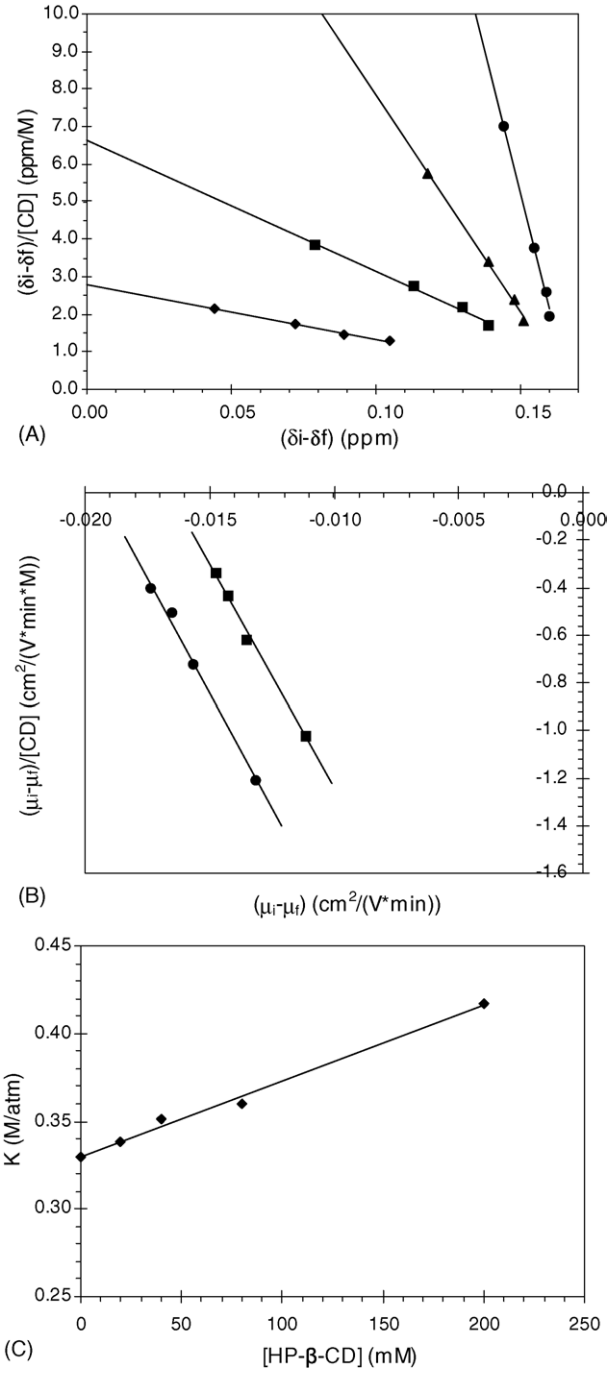

Fig. 3. (A) x-Reciprocal plots (Eq. (4)) for α-CD at 22 °C with: (■) propionic acid, (▲) butyric acid, (♦) isobutyric acid, and (●) valeric acid; (B) x-reciprocal plots (Eq. (4)) for S-β-CD at 22 °C and pH 4.5 with: (■) pyridine, and (●) 3-methyl pyridine; (C) plot of the apparent partition coefficient vs. HP-β-CD concentration for methanethiol (see Ref. [13]).

4. Results and discussion

Table 1 lists the association constants of all the malodorous compounds with various cyclodextrins examined in this study. Dashes in the table indicate that the extent of binding was too low to be measured by the performed method, while empty spaces designate analyte–cyclodextrin combinations or methods that were not performed. Several analytes were observed to form insoluble complexes with certain cyclodextrins when sufficient concentrations of each were present in solution. These combinations are marked with footnote f in Table 1, and include complexes involving propionate, butyrate, isobutyrate, isovalerate, diphenylamine, hydrogen sulfide, methanethiol, dimethyl sulfide, m-cresol, indole, dodecanol, and tetradecanol. Coincidentally, a majority of these compounds are also the most offensive and strongest odor producing components of halitosis. The formation of these insoluble complexes demonstrates that some cyclodextrins are particularly effective at extracting these compounds from the aqueous phase and, therefore, may be promising agents for the suppression of the analytes' volatility/odor. Precipitation of the analyte–cyclodextrin com-

plex invalidates our association constant equation (Eq. (4)) by introducing new equilibria and reducing the actual aqueous concentration of analyte and cyclodextrin in solution. Therefore, for ACE and NMR studies analyte and cyclodextrin concentrations low enough to prevent the formation of the precipitates were used for all association constant determinations. However, some HS-GCMS experiments could not be performed without producing the insoluble complex, and as a result the association constants for the VSCs that precipitated are misleadingly high [13].

The acidic analytes examined generally bound most strongly to native α- and β-CD. Small compounds, such as acetic and propionic acid, only formed stable complexes with α-CD, while formic acid did not bind appreciably to any of the cyclodextrins in this study. Native β-CD, by and large, exhibited higher association constants than the β-CD derivatives with these analytes. This effect is likely due to electrostatic repulsion by the negatively charged analytes and cyclodextrin derivatives. Steric hindrance produced by the substituents attached to the cyclodextrin's rim may also play a role in inhibiting analyte binding [15,16]. However, lactic and succinic acid, both dicarboxylic acids, exhibited significantly higher binding to CM-β-CD and slightly higher binding to S-β-CD than to the native β-CD. The two carboxylate groups on these analytes may interact strongly with the numerous hydrogen-bond donor and acceptor sites provided by the carboxylate and sulfate groups attached to these cyclodextrins. Association constants of the acidic analytes to native γ-CD were lower than with α- and β-CD, likely due to the larger size of the γ-CD cavity. As a result, analyte inclusion into the CD will be a poorer fit and, therefore, weaker [15,16]. Generally, less polar analytes formed more stable associations with the cyclodextrins, supporting the formation of a hydrophobic inclusion complex within the cyclodextrin cavity.

In most cases, similar trends seen for the acidic analytes were observed for the basic compounds examined, where the largest association constants were achieved with native α- and β-CD. Under acidic conditions in which the analyte was positively charged, however, higher association constants were exhibited with the negatively charged cyclodextrins (S-β-CD and CM-β-CD) apparently due to strong electrostatic interactions. Pyridine and 3-methyl pyridine formed notably more stable complexes in acidic conditions (pH 4.5 ACE) with these anionic cyclodextrins than under near neutral conditions (D_2O NMR). The highly hydrophobic diphenylamine bound most strongly to native β- and γ-CD.

The VSCs, a major source of odor in halitosis, formed the most stable complexes with the native cyclodextrins and HP-β-CD, while sulfated- and carboxymethyl-CDs had significantly less binding behavior with these analytes. The neutral aromatic compounds exhibited the highest association constants with native β-CD, and generally exhibited weaker binding to α- and γ-CD. This data may be due to the tightness-of-fit of the analyte within the cyclodextrin cavity, where matching of the size and shape of the host cavity and guest molecule increases the stability of the complex [15,16]. Of the long chain alcohols, tetradecanol complexed with α-CD more strongly than β-CD, again possibly due to a tightness-of-fit within the cyclodextrin cavity. HP-β-CD associated with these analytes to a similar extent as native β-CD, whereas the CM- and S-CDs generally had lower binding constants.

Apparently, only the sulfated derivatives of α- and β-CD showed significant binding behavior to the six amino acids examined in this study with ACE. While amino acids themselves have no odor, these molecules are known to be precursors to a number of malodorous compounds [1,2]. All other cyclodextrins had association constants too low to be measured by ACE. Arginine, lysine, and ornithine, which all contain basic side chains, bound strongly to both the anionic S-α-CD and S-β-CD. The zwitterionic amino acids citrulline and cysteine also complexed to these two cyclodextrins, however, tryptophan only weakly interacted S-β-CD. In addition, pH appeared to have an effect on the magnitude of analyte–cyclodextrin complexation. Arginine, citrulline, and cysteine produced more stable cyclodextrin complexes in slightly basic conditions as compared to acidic solutions, while lysine interacted more strongly at low pH (see Table 1).

It should be noted, however, that the environment in the mouth may be significantly more complex than the aqueous systems in which these binding constant experiments were performed. Saliva contains a wide array of salts, proteins, and bacteria, which may compete with the analyte for cyclodextrin binding. Furthermore, the matrix of the cyclodextrin oral hygiene product may also alter the magnitude of analyte binding. Development of a specific product application of cyclodextrins would eventually require binding determinations be performed under more accurate conditions.

Cyclodextrins and their derivatives bind to a wide variety of malodorous compounds and precursor molecules commonly found in the mouth. In this study, less polar analytes generally exhibited higher association constants with these cyclodextrins, likely due to the formation of a hydrophobic inclusion complex. Size and shape matching between the analyte and the cyclodextrin cavity also increased the stability of the analyte–cyclodextrin complex. Electrostatics may play a large role in binding through either attractive or repulsive interactions. The formation of several analyte–cyclodextrin precipitates further indicates that some cyclodextrins can effectively remove these analytes from solution. Ideally, a mixture of α-, β-, and sulfated-CDs would suppress the volatility of the greatest number of analytes from this study. While the toxicities of many derivatized cyclodextrins are not yet fully evaluated [8], the known negligible toxicity and low cost of native cyclodextrins currently make them attractive complexation agents for malodorous compounds in oral hygiene products.

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