

## ITC XXVI - Carbohydrate-Small Molecule

Akimaru K., Auzenne E., Akimaru Y., Leroux M. E., Hayman A. C., Utsumi T., Soma G., and Klostergaard J. (1995) Formulation and antitumor efficacy of liposomal-caprylated-TNF-SAM2. *Cytokines Mol Ther* **1**, 197-210.

**Abstract:** The tumor necrosis factor (TNF) mutant TNF-SAM2 has previously been shown to have a therapeutic profile superior to parental TNF. To initially evaluate the characteristics of liposomal formulations of TNF-SAM2, it was modified with the N-hydroxysuccinimide ester of caprylic acid to increase its hydrophobic binding to multilamellar and small unilamellar vesicles (MLVs and SUVs). Native PAGE and fluorescamine analysis of acetylated parental TNF and TNF-SAM2 indicated that these proteins both displayed trimeric structures based on crosslinking/SDS-PAGE analysis and behaved similarly with respect to reactivity of their amino functions. Limited N-terminal sequencing analysis of partially acetylated (approx 3 acetyl groups per trimer) TNF-SAM2 indicated that the N-terminal Val was not modified; this was also concluded based on HPLC/mass spectrometric (LC-MS) analysis of Glu C digests. LC-MS analysis of tryptic digests of the acetylated TNF-SAM2 indicated that Lys-98 was unreactive. Molecular ions corresponding to acetylated Lys-containing peptides for all five other Lys residues could be detected; none appeared hyperreactive, but Lys-11 appeared hyporeactive. MLVs composed of DMPC/DMPG (7:3) and SUVs composed of DPPC/DSPC (1:1) displayed high capacity for binding to caprylated TNF-SAM2. These formulations of caprylated TNF-SAM2 displayed tumor necrotizing and growth-inhibitory activity in a syngeneic tumor model, and may be candidates for clinical development.

Boonsongrit Y., Mueller B. W. and Mitrevej A. (2008) Characterization of drug-chitosan interaction by <sup>1</sup>H NMR, FTIR and isothermal titration calorimetry. *Eur J Pharm. Biopharm.* **69**, 388-395.

**Abstract:** Electrostatic interaction between opposite charge of drugs (insulin and benzoic acid) and chitosan was studied by <sup>1</sup>H NMR, FTIR and isothermal titration calorimetry (ITC). No ionic interaction between the carboxyl group of benzoic acid and the amine group of chitosan could be detected. There was a minor change in the FTIR spectra of insulin-chitosan microparticles made of different concentrations of insulin. Exothermic heat of reaction between insulin and chitosan was obtained by ITC. However, the measured interaction enthalpy change ( $\Delta H$ ) was possibly due to the conformational changes and the adsorption phenomena of insulin onto the surfaces of the particles but not to a binding interaction. The binding of tripolyphosphate, a widely used cross-linking agent, to pH 3.3 and pH 5 chitosan was also studied by ITC. The interaction enthalpy change of the binding between tripolyphosphate and chitosan indicated that tripolyphosphate provided a stronger interaction to pH 5 chitosan than to pH 3.3 chitosan. However, it can be stated that the electrostatic interaction forces between the tested molecules insulin, benzoic acid, and tripolyphosphate and chitosan are found to be very weak

Brandenburg K., Jurgens G., Muller M., Fukuoka S., and Koch M. H. (2001) Biophysical characterization of lipopolysaccharide and lipid A inactivation by lactoferrin. *Biol Chem* **382**, 1215-1225.

**Abstract:** The interaction of bacterial endotoxins (LPS Re and lipid A, the 'endotoxic principle' of LPS) with the endogenous antibiotic lactoferrin (LF) was investigated using various physical techniques and biological assays. By applying Fourier-transform infrared (FTIR) spectroscopy, we find that LF binds to the phosphate group within the lipid A part and induces a rigidification of the acyl chains of LPS. The secondary structure of the protein - as monitored by the amide I band - is, however, not changed. Concomitant with the IR data, scanning calorimetric data indicate a sharpening of the acyl chain phase transition. From titration calorimetric and zeta potential data, saturation of LF binding to LPS was found to lie at a [LF]:[LPS] ratio of 1:3 to 1:5 M from the former and 1:10 M from the latter technique. X-ray scattering data indicate a change of the lipid A aggregate structure from inverted cubic to multilamellar, and with fluorescence (FRET) spectroscopy, LF is shown to intercalate by itself into phospholipid liposomes and may also block the lipopolysaccharide-binding protein (LBP)-induced intercalation of LPS. The LPS-induced cytokine production of human mononuclear cells exhibits a decrease due to LF binding, whereas the coagulation of amoebocyte lysate in the Limulus test exhibited concentration-dependent changes. Based on these results, a model for the mechanisms of endotoxin inactivation by LF is proposed.

Bautista-Ibanez L., Ramirez-Gualito K., Quiroz-Garcia B., Rojas-Aguilar A. and Cuevas G. (2008) Calorimetric measurement of the CH/ $\pi$  interaction involved in the molecular recognition of saccharides by aromatic compounds. *J Org. Chem* **73**, 849-857.

**Abstract:** Can a benzene molecule differentiate between two isomeric carbohydrates? It is generally accepted that two factors govern molecular recognition: complementarity and preorganization. Preorganization requires the presence of cavities for positioning the host's groups of complementary nature to those of the guest. This study shows that, in fact, groups should be complementary to recognize each other (for the case presented here, it is controlled by the CH/ $\pi$  interaction) but preorganization is not essential. Since weak interactions have their origin in dispersion forces, they also have impact on the enthalpic term of the free energy, so it was considered that their participation can be demonstrated by measuring the energy involved. For recognition to happen, two conditions must be satisfied: specificity and associated stabilizing energy. In this study we evaluated the heat of dissolution of different carbohydrates such as methyl 2,3,4,6-tetra-O-methyl- $\alpha$ -D-mannopyranoside and methyl 2,3,4,6-tetra-O-methyl- $\beta$ -D-galactopyranoside using different aromatic solvents. The solvation enthalpies in benzene were  $-78.8 \pm 3.9$  and  $-88.7 \pm 5.5$  kJ mol<sup>-1</sup> for each carbohydrate, respectively; and these values yielded a CH/ $\pi$  energy of interaction of 9.9 kJ mol<sup>-1</sup>. In addition, NMR studies of the effect of the addition of benzene to chloroform solutions of the two carbohydrates showed that benzene specifically interacts with the hydrogen atoms of the pyranose ring at positions 3, 4, and 5 located on the  $\alpha$  face of the methyl- $\beta$ -galactoside, so it is, in fact, able to recognize it. Thus, the interactions between carbohydrates and the aromatic residues of proteins occur in the absence of the confinement generated by the protein structure. By experimentally measuring the energy associated with this interaction and comparing it to theoretical calculations, it was also possible to unequivocally determine the existence of CH/ $\pi$  interactions between carbohydrates and proteins

Castronuovo G., Elia V., Iannone A., Niccoli M., and Velleca F. (2000) Factors determining the formation of complexes between  $\alpha$ -cyclodextrin and alkylated substances in aqueous solutions: a calorimetric study at 25 degrees C. *Carbohydr Res* **325**, 278-286.

**Abstract:** The formation of complexes of  $\alpha$ -cyclodextrin with cycloalkanediols, monoalkylamines and 1-alkanols has been studied calorimetrically at 25 degrees C in water, in phosphoric acid, pH 1.3, and in phosphate buffer, pH 5.5, respectively. When a complex is formed, calorimetry enables the calculation of both the enthalpy and the association constant, from which the free energy and the entropy of the process can be obtained. A model is proposed to explain the unusual trend of the association parameters for substances having alkyl chains longer than six-seven carbon atoms. The main role played by the different functional groups, and the forces involved in the association process, are discussed in the light of the signs and values of the thermodynamic parameters obtained. The effect of the variation of the aqueous medium on the hydration of the interacting substances and the consequent changes in the association parameters have been investigated. To this end, the thermodynamic parameters for the formation of the complexes between the cyclodextrin and 1-pentanol were determined at increasing concentrations of phosphate buffer. There is an increase in the association constant due to a positive entropy contribution originating from the relaxation of water molecules from the hydrophobic hydration cosphere of the alkanol to an increasingly disordered bulk. Deauration of the interacting substances is the main factor determining the stability of the inclusion complex.

de la Fuente J. M., Eaton P., Barrientos A. G., Menendez M., and Penades S. (2005) Thermodynamic evidence for Ca<sup>2+</sup>-mediated self-aggregation of Lewis X gold glyconanoparticles. A model for cell adhesion via carbohydrate-carbohydrate interaction. *J Am Chem Soc* **127**, 6192-6197.

**Abstract:** Thermodynamic evidence for the selective Ca<sup>2+</sup>-mediated self-aggregation via carbohydrate-carbohydrate interactions of gold glyconanoparticles functionalized with the disaccharides lactose (lacto-Au) and maltose (malto-Au), or the biologically relevant trisaccharide Lewis X (Le(X)-Au), was obtained by isothermal titration calorimetry. The aggregation process was also directly visualized by atomic force microscopy. It was shown in the case of the trisaccharide Lewis X that the Ca<sup>2+</sup>-mediated aggregation is a slow process that takes place with a decrease in enthalpy of  $160 \pm 30$  kcal mol<sup>-1</sup>, while the heat evolved in the case of lactose and maltose glyconanoparticles was very low and thermal equilibrium was quickly achieved. Measurements in the presence of Mg<sup>2+</sup> and Na<sup>+</sup> cations confirm the selectivity for Ca<sup>2+</sup> of Le(X)-Au glyconanoparticles. The relevance of this result to cell-cell adhesion process mediated by carbohydrate-carbohydrate interactions is discussed.

Gouin S. and Winnik F. M. (2001) Quantitative assays of the amount of diethylenetriaminepentaacetic acid conjugated to water-soluble polymers using isothermal titration calorimetry and colorimetry. *Bioconjug Chem* **12**, 372-377.

**Abstract:** The level of conjugation of diethylenetriaminepentaacetic acid (DTPA) to the polysaccharide sodium hyaluronan (HA) has been measured by a colorimetric assay, isothermal titration calorimetry (ITC), and <sup>1</sup>H NMR spectroscopy. The colorimetric assay is based on the red shift, upon complexation with gadolinium ion (Gd<sup>3+</sup>), of the wavelength of maximum absorption of the dye arsenazo III. It can be performed in a few minutes using as little as 10 μg of polymer with a detection limit of approximately 0.03 mmol of DTPA (gram of polymer)<sup>-1</sup>. The ITC measurements yield values of the amount of DTPA linked to HA identical to those obtained by colorimetry. The levels of DTPA conjugation calculated by integration of signals at 3.1-3.2 ppm (DTPA protons) and at 2.0 ppm (HA acetamide protons) in the <sup>1</sup>H NMR spectrum of HA-DTPA are consistently overestimated by a factor of approximately 2, compared to the data obtained by ITC and colorimetry. The longer relaxation times of protons of the polymer backbone, compared to those of protons attached to the freely moving DTPA side-chains may account for the discrepancy.

Jiang Y., Hu M., Li S., Wang J., and Zhuo K. (2005) Thermodynamics of the interaction of RbCl with some monosaccharides (d-glucose, d-galactose, d-xylose, and d-arabinose) in aqueous solutions at 298.15K. *Carbohydr Res.*(e-publication December 1)

**Abstract:** The Gibbs energy interaction parameters of RbCl with some monosaccharides (d-glucose, d-galactose, d-xylose, and d-arabinose) in water, g(ES), were obtained from electromotive force (emf) measurements of the electrochemical cell without liquid junction and containing two ion-selective electrodes (ISE): K-ISEmid R:RbCl(m(E))mid R:ISE-Cl and K-ISEmid R:RbCl(m(E)),saccharide (m(S))mid R:ISE-Cl, at 298.15K. The enthalpy interaction parameters of RbCl with these monosaccharides in water, h(ES), are determined according to the McMillan-Mayer theory from the measurements of the enthalpies of mixing of aqueous RbCl solutions with aqueous monosaccharide solutions, as well as the enthalpies of dilution of RbCl and monosaccharide solutions in pure water at 298.15K by a calorimetric method. Furthermore, the entropy interaction parameters, s(ES), can be evaluated through g(ES) and h(ES). The results suggest that the electrostatic interactions of these monosaccharides with RbCl in water are predominant compared with structural interactions, and these parameters are controlled primarily by the stereochemical structure of the monosaccharides in water.

McClements D. J. (2000) Isothermal titration calorimetry study of pectin-ionic surfactant interactions. *J Agric Food Chem* **48**, 5604-5611.

**Abstract:** Isothermal titration calorimetry (ITC) was used to measure enthalpy changes resulting from injection of anionic (sodium dodecyl sulfate, SDS) or cationic (dodecyl trimethylammonium bromide, DTAB) surfactants into aqueous 1 wt % pectin solutions (30, 60, or 90% methoxylated). In the absence of pectin, the critical micelle concentrations (cmc) determined by ITC were 14.7 mM for DTAB and 7.7 mM for SDS. Binding of DTAB to pectin was endothermic and was attributed to electrostatic attraction between the cationic surfactant and anionic biopolymer. Binding of SDS to pectin was exothermic and was attributed to hydrophobic interactions. Pectin reduced the cmc of SDS, probably because of long-range electrostatic repulsion between the molecules. Above a particular concentration, which depended on pectin and surfactant type, both ionic surfactants promoted pectin aggregation (monitored by turbidity increase). This study demonstrates the potential of ITC for providing valuable information about interactions between polysaccharides and amphiphiles.

Prado A. G., Macedo J. L., Dias S. C., and Dias J. A. (2004) Calorimetric studies of the association of chitin and chitosan with sodium dodecyl sulfate. *Colloids Surf B Biointerfaces* **35**, 23-27.

**Abstract:** The interaction of hydrophobic chitin and chitosan with sodium dodecyl sulfate (SDS) has been studied by titration calorimetry at 298.15K. The nature of interaction of the surfactant and biopolymers was followed by enthalpy interaction profiles. The mixing enthalpy curves were determined by mixing SDS solutions above their critical micelle concentration with chitin and chitosan suspensions in different concentrations. The Gibbs free energy of aggregation values were -23.21, -22.71 and -21.53 kJ mol<sup>-1</sup> for chitin in 0.02, 0.05 and 0.1% concentration, respectively, and 28.30, 24.38 and 24.20 kJ mol<sup>-1</sup> for chitosan in 0.02, 0.05 and 0.1% concentration, respectively. The critical aggregation concentration (cac) obtained by

calorimetric data gave 6.32, 7.07 and 9.14 mmol kg<sup>-1</sup> in 0.02, 0.05 and 0.1% concentration, respectively, for chitin and 2.09, 4.91 and 5.11 mmol kg<sup>-1</sup> for chitosan in 0.02, 0.05 and 0.1% concentration, respectively.

Rodriguez R., Alvarez-Lorenzo C., and Concheiro A. (2003) Influence of cationic cellulose structure on its interactions with sodium dodecylsulfate: implications on the properties of the aqueous dispersions and hydrogels. *Eur J Pharm Biopharm* **56**, 133-142.

**Abstract:** The interactions of sodium dodecylsulfate (SDS) with the aqueous dispersions and the chemically cross-linked hydrogels of two cationic hydroxyethylcelluloses, polyquaternium-4 (PQ-4) and polyquaternium-10 (PQ-10), commonly used in cosmetics and in topical drug delivery devices, were analyzed. This surfactant was chosen not only for its interest as excipient, but also as a model of the amphiphilic behavior shown by many drugs. In aqueous dispersions, the interaction process was studied through transmittance, surface tension, fluorescence, microcalorimetry titration, viscosity and oscillatory rheometry measurements. The ammonium/sulfate groups ratios at the critical aggregation concentration (0.05% SDS) were 2.61 for PQ-4 and 4.02 for PQ-10; while at the saturation concentration (0.25% SDS), these ratios decreased to 0.52 and 0.80, respectively. The binding process, through ionic and hydrophobic interactions, was strongly exothermic in both water and aqueous NaCl 0.9% solution, which indicates that the salt did not modify the interaction. PQ-4/SDS dispersions had, for all SDS concentrations, higher viscous (G'') and, especially, elastic (G') moduli than the polymer solution. The maxima in G' and G'' (four orders of magnitude greater than PQ-4 only solutions) were observed at the SDS concentrations in which the ammonium/sulfate groups ratio is close to 1. PQ-10/SDS dispersions behaved very differently and, near the neutralization point, the precipitation of the system caused G'' to decrease abruptly, and G' to disappear. The contrasting behavior of the two cationic celluloses may be attributed to their structural differences; PQ-4 has less ammonium groups, in small chains grafted to the cellulose backbone, and more free hydroxyethyl substituents than PQ-10. Therefore, although the neutralization of charges causes the formation of a neutral polyampholyte, the presence of the free hydrophilic hydroxyethyl groups in PQ-4 avoids the precipitation of the aggregates and contributes to the establishment of a three-dimensional network. In contrast, in PQ-10, the ammonium groups are directly bonded to the hydroxyethyl substituents and, in the aggregation process, they may be included in the polyampholyte complex, contributing to the precipitation. This different behavior was easily seen in the surfactant-induced shrinking of the hydrogels around the charges neutralization. Although the SDS binding isotherms were very similar, PQ-10 hydrogels decreased their volume up to 20 times at the neutralization point, while PQ-4 hydrogels reduced their initial volume only three times under the same conditions. These results suggest that the phase transitions of the hydrogels may be used as quick predictors of the behavior of the polymer dispersions.

Wangsakan A., Chinachoti P., and McClements D. J. (2001) Maltodextrin-anionic surfactant interactions: isothermal titration calorimetry and surface tension study. *J Agric Food Chem* **49**, 5039-5045.

**Abstract:** Interactions between maltodextrin (DE = 10) and an anionic surfactant (sodium dodecyl sulfate, SDS) were studied in a buffer solution (pH 7.0, 10 mM NaCl, 20 mM Trizma, 30.0 degrees C) using isothermal titration calorimetry (ITC), surface tension, differential scanning calorimetry (DSC), and turbidity techniques. ITC measurements indicated that the binding of SDS to maltodextrin was exothermic and that, on average, one SDS monomer bound per 24 glucose units of maltodextrin at saturation. Surface tension measurements indicated that there was a critical surfactant concentration (approximately 0.05 mM SDS) below which surfactant and maltodextrin did not interact and that the amount of surfactant bound to the maltodextrin above this concentration increased with increasing maltodextrin concentration. Turbidity measurements indicated that the solutions remained transparent at all maltodextrin (0-1 wt %) and SDS (0-20 mM) concentrations studied, which suggested that phase separation did not occur. DSC measurements indicated that no phase transitions occurred between 10 and 110 degrees C for maltodextrin solutions (0.5 wt %) in the presence or absence of surfactant. A phase diagram was developed to describe the interactions between SDS and maltodextrin.

Wangsakan A., Chinachoti P., and McClements D. J. (2003) Effect of different dextrose equivalent of maltodextrin on the interactions with anionic surfactant in an isothermal titration calorimetry study. *J Agric Food Chem* **51**, 7810-7814.

**Abstract:** Isothermal titration calorimetry (ITC) was used to study interactions between an anionic surfactant (sodium dodecyl sulfate, SDS) and maltodextrins with different dextrose equivalents (DE) in a buffer solution (pH 7.0, 10 mM NaCl, 20 mM Trizma, 30.0 degrees C). The interaction between SDS and

maltodextrin was exothermic, which was attributed to incorporation of the hydrocarbon tail of the surfactant into a helical coil formed by the maltodextrin molecules. ITC measurements indicated that the number of SDS molecules bound per gram of maltodextrin increased with decreasing maltodextrin DE, i.e., increasing molecular weight. It was proposed that SDS only binds to maltodextrin molecules that have a DE greater than 10 glucose units.