

# The Complexation of Glycoside Surfactants with Divalent Metal Ions: An Electrospray Ionization Mass Spectrometric Study

Khaled Edbey<sup>1</sup>, Grainne Moran<sup>2</sup>, Gary Willett<sup>2</sup>

<sup>1</sup>*Department of Chemistry, Faculty of Science, University of Garyounis, Benghazi-Libya*

<sup>2</sup>*Department of Chemistry, Faculty of Science, University of New South Wales, Sydney, Australia*

**Abstract:** -The analytical potential of the complexation of non ionic surfactants such as 1-octyl- $\beta$ -D-glucopyranoside and Tween-20 with Zn(II), Cd(II), and Cu(II) was investigated by positive-ion electrospray ionization Fourier transform ion cyclotron resonance (ESI-FTICR) mass spectrometry. The most favorable M(II)/ 1-octyl- $\beta$ -D-glucopyranoside association involves the metal attached to the deprotonated hydroxymethyl group and the hemiacetal oxygen. The binding efficiencies for 1-octyl- $\beta$ -D-glucopyranoside and Tween-20 with Zn(II), Cd(II), and Cu(II) are studied. The metal binding order for 1-octyl- $\beta$ -1-octyl- $\beta$ -D-glucopyranoside is Cu(II) > Zn(II) > Cd(II).

**Keywords:** glycosides, surfactants, heavy metals, mass spectrometry, environment.

## I. INTRODUCTION

In the environment, the toxicity and bioavailability of metals are governed by the ability of natural organic molecules to bind metal ions. Among these molecules carbohydrates and carbohydrate derivatives are the most abundant biomolecules in nature. These macromolecules may be easily converted to a large variety of oligo and monosaccharides, which in turn, may complex metal ions due to their numerous hydroxyl groups.<sup>1</sup>

Glycoside Surfactants, in particular polysorbates, are commonly utilized excipients in the pharmaceutical industry. Polysorbates are classified as nonionic surfactants and have wide range of applications in formulations. They may be utilized as wetting, emulsifiers, or solubilizers.<sup>2-5</sup> Mass spectrometry has become an important analytical technique for Glycoside Surfactants, particularly when small quantities are involved. 1-octyl- $\beta$ -D-glucopyranoside (OGP) can be used as a model system and tween as a surfactant. The reactivity between glycoside and metal ions has been widely studied in solution because of the involvement of metal ion-glycopyranosides interactions in key biological processes. Several groups have also reported the gas phase mass spectrometric study of the complexation by glycosides of alkali, alkaline earth and transition metal ions.<sup>6-10</sup> All these studies showed that gas-phase metal ion chemistry is a powerful tool to determine the binding strengths of metal ion-glycopyranosides.

ESI-FTICR mass spectrometry has been used to examine how divalent metal ions chelate to the oxygen. FTICR mass spectrometry is used to identify the reaction products of the complexation of OGP and the commercial surfactant Tween-20 with divalent metal ions. The selectivity of OGP binding with Zn(II), Cd(II), and Cu(II) is also studied. CID on the complexes of OGP with the Zn (II) and Cu (II) reveals structural information and is used to investigate the binding strengths of the metal ions with OGP. The reactions of divalent metal ions Cd(II), Zn(II) and Cu(II) with two different glycoside surfactants OGP and Tween-20 have been studied. As OGP and Tween-20 both contain oxygen and hydroxyl groups in sugar units, it is of interest to study how the divalent metal ions complex to these organic groups.

## II. EXPERIMENTAL

### 2.1 Raw Material

The surfactants 1-O-octyl- $\beta$ -D-glucopyranoside and Tween 20 were purchased from Sigma (St.Louis, Mo, USA). Cadmium nitrate, copper nitrate, and zinc nitrate were obtained from Sigma-Aldrich and were used without any further purification.

### 2.2 Sample Preparation

The metal complexes were produced by mixing solutions of the appropriate metal salt [MX<sub>2</sub> where M= Cu(II), Zn(II), Cd(II)], and X= (NO<sub>3</sub>)<sub>2</sub>, the surfactants in a methanol (HPLC grade). The mole ratio is 1:1 for the surfactant-metal salt mixture and the concentration of each component before mixing is 1.5 x 10<sup>-4</sup> M. The metal ions were mixed with the surfactants as nitrate, acetate or chloride salts. Data acquisition and processing together with the comparison of the experimental isotope patterns and mass values observed and the theoretical patterns for all major product ions, are performed using Xmass version 6.2 Bruker software.

A Bruker BioApex-II 7T FTICR mass spectrometer with an on- and off-axis Analytica ESI spray source was used in this study. Stronger signals were observed using the off-axis configuration.

### III. RESULTS AND DISCUSSIONS

#### 3.1 Binding of selected glycoside surfactants with M(II) ions.

The reactions of divalent metal ions Cd(II), Zn(II) and Cu(II) with two different glycoside surfactants OGP and Tween-20 have been studied, as OGP and Tween-20 both contain oxygen and hydroxyl groups.

##### 3.1.1 1-octyl- $\beta$ -D-glucopyranoside

Figure 1 shows the positive-ion FTICR mass spectrum of the reaction products of OGP with Zn(II) at capillary skimmer potential difference (CSPD) 30 V. In order to investigate the influence of the CSPD on the nature and concentration of the metal ion complexes, the reaction of OGP with Zn(NO<sub>3</sub>)<sub>2</sub> was studied. While the CSPD was tuned in the range ~30 to 300 V, the ions were stored in the hexapole for 1 s and the other parameters were held constant.

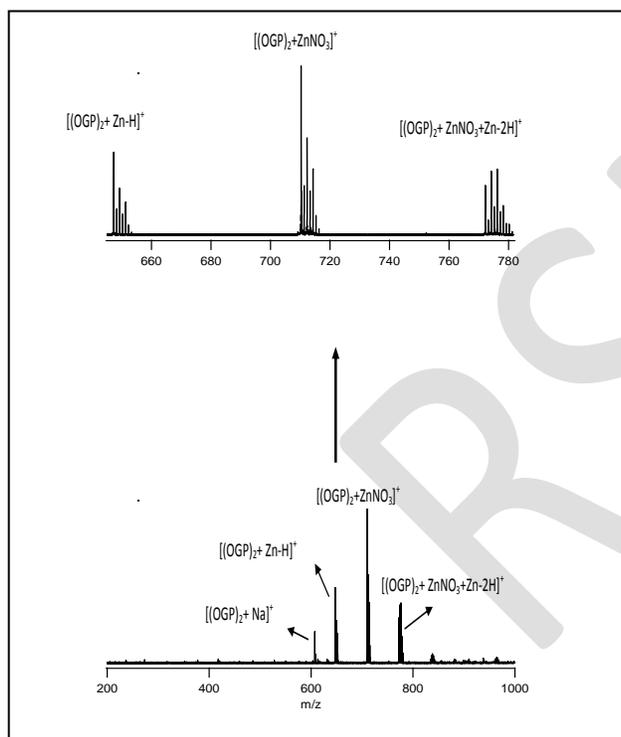


Fig.1: Positive-ion FTICR mass spectrum of the reaction products of OGP showing singly charged ions with Zn(II). (CSPD = 30V, hexapole delay = 1 s).

When the CSPD was set at 30 V singly charged monomer and dimer complex ions were obtained (Figure 2a). These complexes were assigned to peaks at  $m/z$  302.998748, 347.984433, 365.066424, 507.056928, and 570.052540 corresponding to the ions [OGP+ZnNO<sub>3</sub>]<sup>+</sup>, [(OGP)<sub>2</sub>+Zn-H]<sup>+</sup>, [(OGP)<sub>2</sub>+ZnNO<sub>3</sub>]<sup>+</sup> and [(OGP)<sub>2</sub>+ZnNO<sub>3</sub>+Zn-2H]<sup>+</sup> respectively. Experimental and calculated isotope patterns for the complex ions mentioned above are shown in Table 1.

Table 1 Comparison of observed and calculated masses for the most abundant isotopes of assigned ions in Figure 2.

Assigned ion	Observed mass (m/z)	Calculated mass (m/z)	Mass deviation (ppm)
[OGP+Zn-C <sub>2</sub> H <sub>4</sub> O <sub>2</sub> -H] <sup>+</sup>	295.088707	295.088175	1.8
[OGP+Zn-H] <sup>+</sup>	355.109302	355.109295	0.02
[OGP+ZnNO <sub>3</sub> ] <sup>+</sup>	418.105889	418.105004	2.1
[(OGP) <sub>2</sub> +Zn-H] <sup>+</sup>	647.299654	647.297936	2.6
[(OGP) <sub>2</sub> +ZnNO <sub>3</sub> ] <sup>+</sup>	710.294755	710.293604	1.6
[(OGP) <sub>2</sub> +ZnNO <sub>3</sub> +Zn-2H] <sup>+</sup>	772.209857	772.207007	3.6

As shown in Figure 2a the base peak observed at CSPD 30 V corresponds to the complex ion [(OGP)<sub>2</sub>+ZnNO<sub>3</sub>]<sup>+</sup>.

Intense peaks corresponding to [(OGP)<sub>2</sub>+Zn-H]<sup>+</sup> and [(OGP)<sub>2</sub>+ZnNO<sub>3</sub>+Zn-2H]<sup>+</sup> are also observed. A very small peak assigned to the ion [OGP+ZnNO<sub>3</sub>]<sup>+</sup> is also observed. This peak could result from fragmentation of the base peak, [(OGP)<sub>2</sub>+ZnNO<sub>3</sub>]<sup>+</sup>. By increasing the capillary skimmer potential difference to 200 V (Figure 2b), the intensity of the complex ion [OGP+ZnNO<sub>3</sub>]<sup>+</sup> increases and it becomes the base peak. The intensities of the complex ions [(OGP)<sub>2</sub>+Zn-H]<sup>+</sup>, [(OGP)<sub>2</sub>+ZnNO<sub>3</sub>]<sup>+</sup> and [(OGP)<sub>2</sub>+ZnNO<sub>3</sub>+Zn-2H]<sup>+</sup> all decrease.

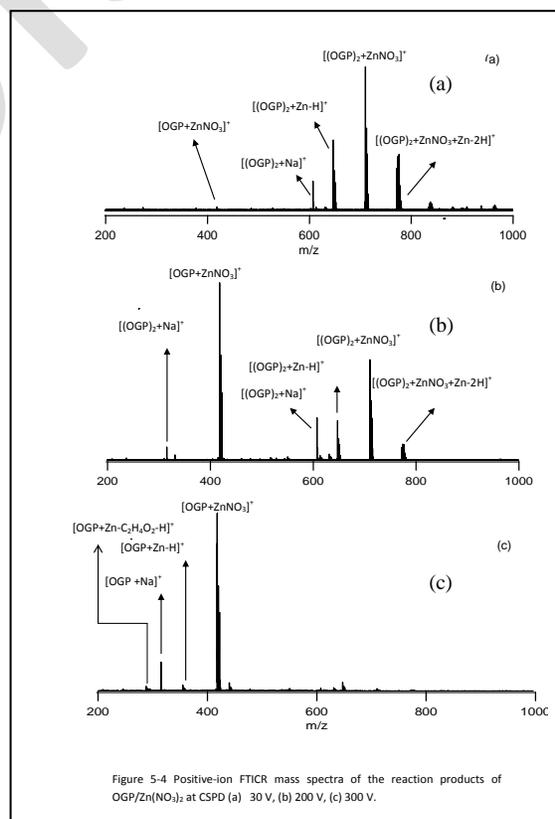


Figure 2: Positive-ion FTICR mass spectra of the reaction products of OGP/Zn(NO<sub>3</sub>)<sub>2</sub> at CSPD (a) 30 V, (b) 200 V, (c) 300 V.

This suggests that loss of OGP is favored at higher CSPD. When the interface conditions were set to very high CSPD 300 V (Figure 2c), the complex ion  $[\text{OGP}+\text{ZnNO}_3]^+$  is still the base peak, while the complex ions  $[(\text{OGP})_2+\text{Zn-H}]^+$ ,  $[(\text{OGP})_2+\text{ZnNO}_3]^+$  and  $[(\text{OGP})_2+\text{ZnNO}_3+\text{Zn-2H}]^+$  disappear. Very small peaks of the fragment ions  $[\text{OGP}+\text{Zn-H}]^+$  and  $[\text{OGP}+\text{Zn-C}_2\text{H}_4\text{O}_2\text{-H}]^+$  are observed. The increase in the intensity of  $[\text{OGP}+\text{ZnNO}_3]^+$  could be due to the increasing of the kinetic energy of the ions so that the collisions increase resulting in the fragmentation of the dimer ion  $[(\text{OGP})_2+\text{ZnNO}_3]^+$  to the monomer ions  $[\text{OGP}+\text{ZnNO}_3]^+$  and  $[\text{OGP}+\text{Zn-H}]^+$ . The fragment ion  $[\text{OGP}+\text{Zn-H}]^+$  represents the loss of  $\text{HNO}_3$ . This could then followed by the loss of a two-carbon oligomer ( $\text{C}_2\text{H}_4\text{O}_2$ ) to produce  $[\text{OGP}+\text{Zn-C}_2\text{H}_4\text{O}_2\text{-H}]^+$ . Results for the Zn(II)-coordinated OGP at high CSPD show that cleavage ions are similar to those produced from CID experiments and will be discussed below.

Figure 3 shows the base peaks assigned to the selected complex ions  $[\text{OGP}+\text{CuNO}_3]^+$ ,  $[\text{OGP}+\text{ZnNO}_3]^+$  and  $[\text{OGP}+\text{CdNO}_3]^+$  at  $m/z$  417.106874, 418.105889 and 466.082068 respectively which are produced through the reaction of OGP with  $\text{Cu}(\text{NO}_3)_2$ ,  $\text{Zn}(\text{NO}_3)_2$  and  $\text{Cd}(\text{NO}_3)_2$  respectively at CSPD 200 V.

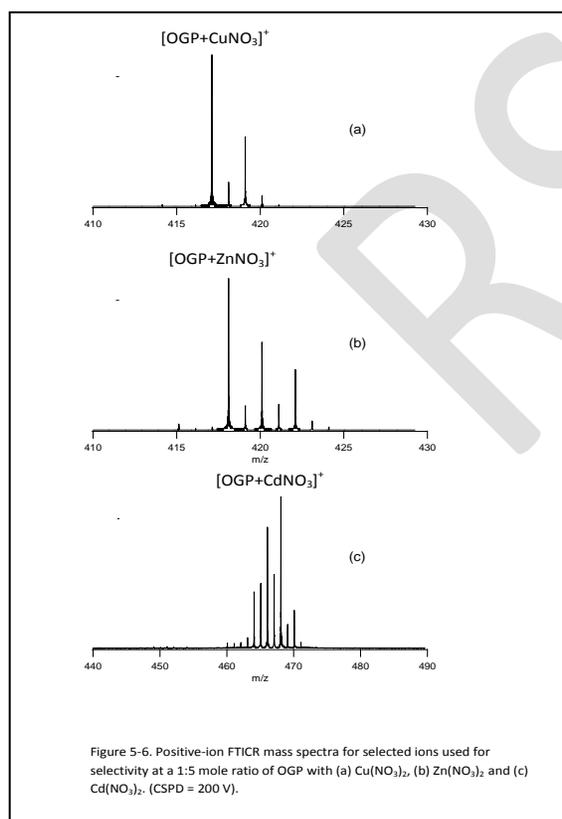


Fig. 3: Positive-ion FTICR mass spectra for selected ions used for selectivity at a 1:5 mole ratio of OGP with (a)  $\text{Cu}(\text{NO}_3)_2$ , (b)  $\text{Zn}(\text{NO}_3)_2$  and (c)  $\text{Cd}(\text{NO}_3)_2$ . (CSPD = 200 V).

Variation of the CSPD for the Cu(II)-coordinated OGP shows similar trends to those observed for Zn(II). However, no ring

cleavage was observed for Cu(II) and the main difference was the reduction of Cu(II) to Cu(I) to form ions such as  $[\text{OGP}+\text{Cu(I)}]^+$ . The variation of the CSPD and its effect on the ions produced from the reaction of Cu(II) with OGP is shown in Figure 4. Figure 4a shows that  $[(\text{OGP})_2+\text{Cu-H}]^+$  is the base peak when the system is operated under low CSPD conditions, eg. 30 V. Intense peaks assigned to the singly charged  $[\text{OGP}+\text{CuNO}_3]^+$  and  $[(\text{OGP})_2+\text{CuNO}_3\text{-Cu-2H}]^+$  are observed as well.

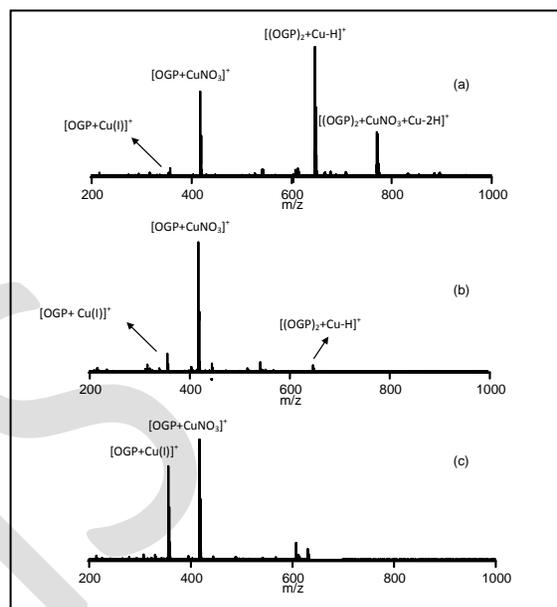


Fig. 4: Positive-ion FTICR mass spectra of the reaction products of Cu(II) with OGP at CSPD (a) 30 V, (b) 200 V, (c) 300 V

As the CSPD is increased to 200 V (Figure 4b), the complex ion  $[\text{OGP}+\text{CuNO}_3]^+$  is now assigned to the base peak. The peak assigned to  $[(\text{OGP})_2+\text{Cu-H}]^+$  almost disappears while  $[(\text{OGP})_2+\text{CuNO}_3\text{-Cu-2H}]^+$  disappears completely. It is also observed that the intensity of the complex ion  $[\text{OGP}+\text{Cu(I)}]^+$  increases under these conditions. When the CSPD was increased to 300 V, (Figure 4c) the intensity of the peak assigned to  $[\text{OGP}+\text{CuNO}_3]^+$  is seen to decrease as the ion fragments to form the singly charged complex ion  $[\text{OGP}+\text{Cu(I)}]^+$ . No ring cleavage was observed at high CSPD in this case.

### 3.1.2 Tween-20

Figure 5 shows the positive-ion FTICR mass spectrum of the products of the reaction of Tween-20 with  $\text{Zn}(\text{NO}_3)_2$ . The FTICR mass spectrum of Tween-20 in the presence of  $\text{Zn}(\text{NO}_3)_2$  is complex and is characterized by two distributions each with a bell shape. The mass spectra of Tween-20 with  $\text{Zn}(\text{NO}_3)_2$  reveals the existence of many oligomeric series of ions containing  $\text{Zn}(\text{NO}_3)^+$  including the polyethylene glycol, isosorbide polyethoxylates, polysorbate and polysorbate monolaurates series. The major signals can be attributed to the presence of polyethylene glycol and sorbitan polyethoxylates.

The Figure shows two different regions dominated by the  $\text{ZnNO}_3^+$  containing molecular ions. The region between  $m/z$  600 and 1000 is assigned to the isosorbide polyethoxylates and polyethylene glycol series and assignments are shown in the Figure. The ions at  $m/z$  712.237035, 756.231951 and 800.238893 are attributed to the isosorbide polyethoxylates, and the ions at  $m/z$  716.268192, 760.264109 and 804.263531 are assigned to the PEG series. The region between  $m/z$  1220 and 1800 in Figure 5 is characterized by the  $\text{ZnNO}_3^+$  adducts of the sorbitanpolyethoxylate and polysorbate monolaurate series. The ions at  $m/z$  1478.697665, 1522.696124 and 1566.69754971 are attributed to the sorbitanpolyethoxylates and those at  $m/z$  1484.765375, 1528.789181 and 1572.796018 assigned to polysorbate monolaurates respectively. Due to the wide isotopic distributions of sorbitanpolyethoxylates, a definite assignment for polysorbate monostearate was not possible. For example, the ion at  $m/z$  1478 would have an isotopic contribution to the signal at  $m/z$  1480, thus making it difficult to assign such ions to polysorbate monostearate.

FTICR mass spectrometry can give information on the composition of Tween-20 which contains a mixture of ethoxylated sugars. By addition of  $1.5 \times 10^{-4}$  M zinc nitrate, species were resolved exclusively as zinc adducts in the positive-ion mode. The addition of zinc nitrate assisted in sample ionization and simplified the spectra by enhancing the zinc adduct peaks to the exclusion of other adduct peaks (particularly  $[\text{polysorbate} + \text{Na}]^+$ ). The advantages of adding a zinc or cadmium salt over alkali metal salts is that the isotope patterns of the former cation can help in peak assignment. For example if sodium salt is added to the surfactants instead of zinc or cadmium it is difficult to recognize whether the observed adduct peaks come from the target surfactants or from the background (normally methanol). So ions such as zinc or cadmium are preferred as they have isotope patterns that facilitate the peak assignments. In this study FTICR mass spectrometry shows that Tween-20 is composed of polysorbate monolaurates, polysorbate myristate, and polysorbate monopalmitates.

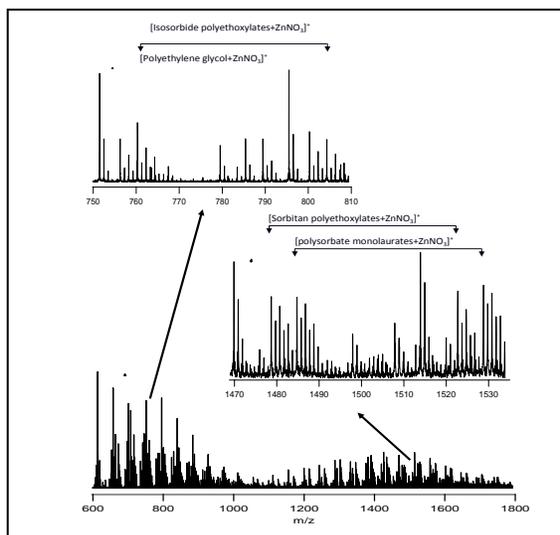


Fig. 5: Positive-ion FTICR mass spectrum of the reaction products of Tween-20 and  $\text{Zn}(\text{NO}_3)_2$ . (CSPD = 30 V).

For the estimation of the binding selectivities, solutions containing OGP and a single divalent metal ion were sprayed individually and then a solution containing the multi-component mixture (OGP with three divalent metal ions) was examined.

### 3.2 ESI for estimation of binding selectivities: 1-octyl- $\beta$ -D-glucopyranoside competing for three divalent metal ions.

Figure 6 shows the positive-ion FTICR mass spectrum of the reaction products of OGP with  $\text{Cu}(\text{NO}_3)_2$ ,  $\text{Zn}(\text{NO}_3)_2$  and  $\text{Cd}(\text{NO}_3)_2$  at CSPD 120 V. The ratio of the products obtained in the ESI spectrum of the multi-component mixture is corrected based on the relative intensities measured from the single component solutions. The base peak for the Cu complex does not overlap with the isotope pattern for the Zn complex and this was used to scale the contributions of each complex in this Figure.

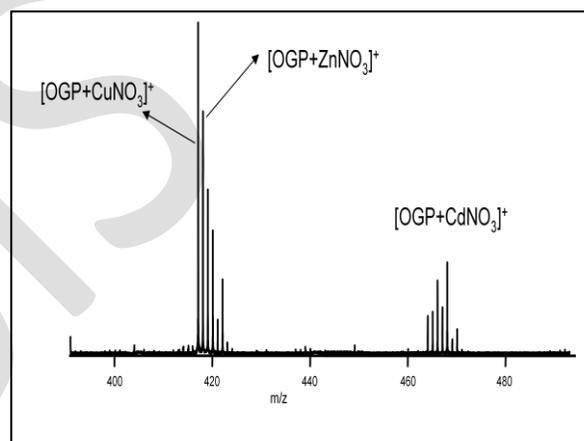


Fig. 6 Positive-ion FTICR mass spectrum of the competitive reaction products of OGP with  $\text{Cu}(\text{NO}_3)_2$ ,  $\text{Zn}(\text{NO}_3)_2$  and  $\text{Cd}(\text{NO}_3)_2$  at a 1:5:5:5 mole ratio of OGP : Cu(II) : Zn(II) : Cd(II) at a CSPD of 120 V.

The absolute peak intensities for  $[\text{OGP} + \text{CdNO}_3]^+$  :  $[\text{OGP} + \text{ZnNO}_3]^+$  :  $[\text{OGP} + \text{CuNO}_3]^+$  are  $3.2 \times 10^8$  :  $3.4 \times 10^8$  :  $1.5 \times 10^8$  and therefore the “ESI intensity coefficients” for  $[\text{OGP} + \text{CdNO}_3]^+$  :  $[\text{OGP} + \text{ZnNO}_3]^+$  :  $[\text{OGP} + \text{CuNO}_3]^+$  are 2.10 : 2.30 : 1.0. The corrected relative binding affinities of OGP (the data are listed in Table 1) for Cu(II) : Zn(II) : Cd(II) in the multi-component solution are then evaluated as 1.7 : 1.2 : 1.0.

The calculations reveal that, relative to binding with Cd(II), OGP shows at least a 1.7-times preference for Cu(II) and a 1.2-times preference for Zn(II). It is concluded that Cu(II) possesses the best complexation ability with OGP. Therefore, the selectivity shown in Table 2 indicates that the order of relative binding affinity for divalent metal ions with OGP in methanol is Cu(II) > Zn(II) > Cd(II).

Table 2: Details for the calculation of binding selectivities of OGP with M(II) ions.

Assigned ion product	Absolute intensity ( $\times 10^8$ ) (Fig. 6)	Relative intensity (Uncorrected) (Fig. 6)	Relative binding affinity (Corrected) $\pm$ Standard deviation
[OPG+CdNO <sub>3</sub> ] <sup>+</sup>	0.22	1.0	1.0 ( $\pm 0.12$ )
[OPG+ZnNO <sub>3</sub> ] <sup>+</sup>	0.60	2.7	1.2 ( $\pm 0.15$ )
[OPG+CuNO <sub>3</sub> ] <sup>+</sup>	0.80	3.6	1.7 ( $\pm 0.19$ )

The general selectivity preference of OGP towards Cu(II) rather than Cd(II) can be understood in part due to the difference in ionic radii of the cations. Cu(II) at 0.072 nm is smaller than Zn(II) (0.88 nm) and Cd(II) (0.97 nm) could allow Cu(II) to more strongly interact with the –OH groups of OGP.

#### IV. CONCLUSION

##### 4.1 Complexation of OGP and Tween-20 components with divalent metal ions

Tween-20 components (polysorbate monolaurates, polyethoxylates, and polysorbate monostearate) react with M(II) to form the complex series [Tween-20+MNO<sub>3</sub>]<sup>+</sup>. The capability for dimer formation by Tween 20, and OGP is dependent on the number of available coordination sites of the surfactant and its ability to fully coordinate the metal ion. For example, Tween-20 components were not observed to form dimer complexes but OGP readily forms dimers. This is because Tween-20 components have sufficient flexibility to wrap around the metal ion and prevent attachment of a second ligand. In contrast a second OGP attaches to [OGP+MNO<sub>3</sub>]<sup>+</sup> because OGP can only provide two donor oxygen groups, leaving vacant coordination sites.

##### 4.2 Binding selectivity of OGP with Cu(II), Zn(II) and Cd(II)

The results for the OGP system with Cu(II), Zn(II) and Cd(II) suggest that estimation of binding selectivities of OGP with divalent metal ions by FTICR mass spectrometric methods may yield semi-quantitative or at least qualitative values. The results indicate that OGP favors binding to Cu(II) over Zn(II) and Cd(II) and that the order of relative binding affinity for the divalent metal ions with OGP is Cu(II) > Zn(II) > Cd(II). This can be attributed primarily to the difference in ionic radii of the cations.

#### ACKNOWLEDGEMENT

The authors would like to thank The Advanced Centre For Technological Skills Development and the University of New South Wales for their support of this work.

#### REFERENCES

- [1]. König, S.; Leary, J. A. J. Am. Chem. Soc. pp.1125-1134, 1998.
- [2]. Cerda, B. A.; Wesdemiotis, C. Int. J. Mass Spectrom., Vol.189, pp.189-204, 1999.
- [3]. Blair, S. M.; Brodbelt, J. S.; Marchand, A. P.; Chong, H. S.; Alihodzic, S. J. Am. Chem. Soc., Vol.11, pp.884-891, 2000.
- [4]. Gaucher, S. P.; Leary, J. A. Anal. Chem., 70, pp.3009-3014, 1998.
- [5]. Asam, M. R.; Glish, G. L. J. Am. Chem. Soc., Vol.8, pp.987-995, 1997.
- [6]. Sible, E. M.; Brimmer, S. P.; Leary, J. A. J. Am. Chem. Soc., Vol.8, pp.32-42, 1997.
- [7]. Zhou, Z.; Ogden, S.; Leary, J. A. J. Org. Chem., Vol.55, pp.5444-5446, 1990.
- [8]. Dongre, A. R.; Wysocki, V. H. Org. Mass Spectrom., Vol.29, pp.700-702, 1994.
- [9]. Cerda, B. A.; Wesdemiotis, C. Int. J. Mass Spectrom., Vol.185, pp.107-116, 1999.
- [10]. Cooks, R. G.; Patrick, J. S.; Kotiaho, T.; McLuckey, S. A. Mass Spectrom. Rev., Vol.13, pp.287-339, 1994.