Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan

In vitro study of the interaction between omeprazole and the metal ions Zn(II), Cu(II), and Co(II)

I. I. HAMDAN

The purpose of this study was to investigate the possibility of a chemical interaction between omeprazole (OM) and the metal ions Zn(II), Cu(II), and Co(II). Using UV absorption spectroscopy and elemental analysis, it was demonstrated that all of the studied metals form complexes with OM. The spectral changes associated with the complexation reaction were used to obtain the stoichiometry and formation constants of the complexes. In all cases complexes were found to form in 1 : 2 metal to OM ratio. In the case of cobalt another complex species which appeared as a green precipitate was also evident. Copper was shown to form the complex with the formula $Cu_3(OM)_2$ in addition to $Cu(OM)_2$. The complexation of cobalt and copper to OM was found to be time dependent and the time required for the completion of the reaction was determined (about 6 h). Apparent binding constants were also determined.

1. Introduction

Omeprazole (OM) is a proton pump inhibitor, which is known chemically as 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridyl)methylsulphinyl]-1*H*-benzimidazole. OM is used for the treatment of peptic ulcer, reflux esophagitis, duodenal ulcers and Zollinger-Ellison syndrome [1–3]. Several reports have demonstrated the superiority of OM over the H_2 receptor antagonists like ranitidine and cimitidine [4, 5]. Similar to the H_2 receptor antagonists OM is believed to work through decreasing the gastric acid secretions albeit through a different mechanism [6]. However the drug is not free of side effects. A part of the minor side effects of OM like diarrhea and headache; it has been demonstrated that long term use of OM results in a reduction of serum cyanocobalamin [7–11]. Our preliminary results suggested that there is a potential chemical interaction (complexation) between OM and some important metal ions, which could explain some of its side effects including the reduction of the level of cyanocobalamin. Complex formation between OM and other metal ions may have an important role in the mechanism of action of the drug. A recent report [12] has demonstrated that OM has a significant effect on the levels of copper/zinc superoxide dismutase enzymes. This effect might involve some form of interaction between the metals in the enzymes and OM. The chemical complexation of drugs to metal ions has been shown to have serious implications on their pharmaceutical utilization. Complex formation may decrease the absorption of drugs as is the case with tetracycline antibiotics. In other cases complexation ability is the basis for drug action e.g. of penicillamine. Yet in many cases complex formation between metal ions and various drugs has been the basis for the selective determination of these drugs in their dosage forms. In this paper we describe an *in vitro* absorption spectroscopic study for the interaction of OM with Zn(II), Co(II) and Cu(II). The aim of this study was to obtain insight into the possible interaction between OM and the cited metals which could be helpful in understanding the mechanism of action or the side effects of OM or even in the chemical analysis of OM. Because of the low aqueous solubility of OM at pH values > 7 and its rapid degradation at pH values < 7.8 [13] it was difficult to study the complexation reaction in aqueous medium and under physiological conditions. Ethanol was chosen as an appropriate solvent for the study because it provides good stability and solubility for OM.

2. Investigations, results and discussion

2.1. Zinc and cobalt

Absorption spectra for solutions containing identical amounts of OM and increasing concentrations of the metal to be studied were recorded against proper blanks. The results of this titration showed consistent and progressive shifts in the absorption spectra of OM as the concentrations of the metals were increased. Both of cobalt and zinc showed similar spectral changes which appeared as hypsochromic shift at the λ_{max} of OM (302 nm) with the

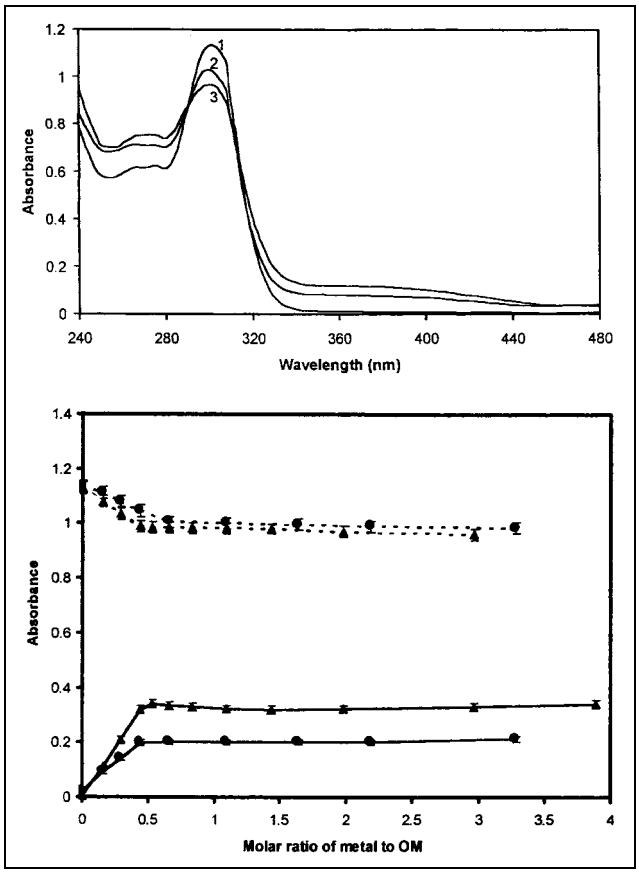

Fig. 1: (A) Overlaid absorption spectra of free OM (1) and OM with increasing molar ratio of metal to OM (m). In (2) m = 0.23 and (3) m = 0.64. (B) Mole ratio plots for the titration of both of cobalt (triangle) and zinc (circle) at 302 nm (dotted lines) and 400 nm (solid lines)

appearance of a very broad and low absorption peak in the visible region (330–440 nm) as shown in Fig. 1A. The decrease in the absorptivity at the λ_{max} of OM was only about 20% but it was consistent and progressive in response to the increase in the ratio of the metal to OM. Spectral shifts were obtainable within 20 min after preparation of the solutions. Measurements of the same set of titration solutions over a 6 h period has shown no significant changes in the obtained spectra. These spectral changes were explained in terms of complex formation between OM and the metals. The absorbance of the formed complex at 302 nm seems to be slightly lower than that of the free OM, consequently as more metal is available more of the complex is formed and the absorbance decreases further until it plateaus at about the stoichiometric ratio. The assumed complexation reaction was studied by the mole ratio method [14]. The absorbance of each solution at λ_{max} of OM (302 nm) and at 400 nm was plotted against the molar ratio (m) of the added metal to OM as shown in Fig. 1B. Fig. 1B shows a typical mole ratio plot for the interaction of both of Co (II) and Zn (II) with OM. The plots at both wavelengths (302 and 400 nm) suggested a stoichiometry of 1 : 2 metal to OM for both of cobalt and zinc. The equilibrium constants for the complexation reaction were measured as explained in the experimental section (at $m = 0.5$) and found to be 9.1×10^7 and 3.3×10^8 for cobalt and zinc respectively. With such high values of binding constants it might be possible that complexes are formed between OM and zinc in zinc-containing metallozymes or cobalt in cyanocobalamin.

Because cobalt exhibits some absorption in the visible region (blue color) in ethanol while OM has no absorption above 400 nm it was possible to titrate the metal with OM i.e. keeping the concentration of cobalt constant and increasing the concentration of OM. However, a higher concentration range of OM ($0 - 5 \times 10^{-3}$ M) and cobalt (1.65×10^{-3} M) was used in this experiment because the absorptivity of cobalt in the visible region was not high enough. Spectral changes were monitored in the range 300–700 nm. As soon as solutions of OM were added to the cobalt solution; clear color changes were noticeable where the solution started to turn into green in a manner dependent on the concentration of OM. Because these color changes appeared to be time dependent, the time required for the completion of the reaction was determined by recording the absorption spectra of the sample (containing 1 : 1 OM to cobalt) over a period of 10 h. Plot of the corrected absorbance (after dilution) at the wavelength of maximum change (528 nm) against time indicates that the reaction needs about 6 h for completion (Fig. 2A). This time dependency which was not observed in the first experiment with cobalt i.e. when OM was titrated with cobalt (concentration of OM was much lower $= 1.5 \times 10^{-4}$ M), seems to be dependent on the overall concentration of both of the metal and the ligand. If the reaction was monitored by eye one could see that sample solutions containing OM change their colors from blue to green to pink. In addition to the spectral and color changes a green precipitate appeared in sample solutions with relatively high concentrations of OM (higher than 0.6). These observations can be explained in terms of complex formation between OM and cobalt where there are two complex species: (1) the green one, which started to form rapidly before turning into the pink complex form or precipitating out of solution; (2) the pink one, which takes longer time to form and stays in solution. The green complex does not precipitate out of solution instead it seems to be converted

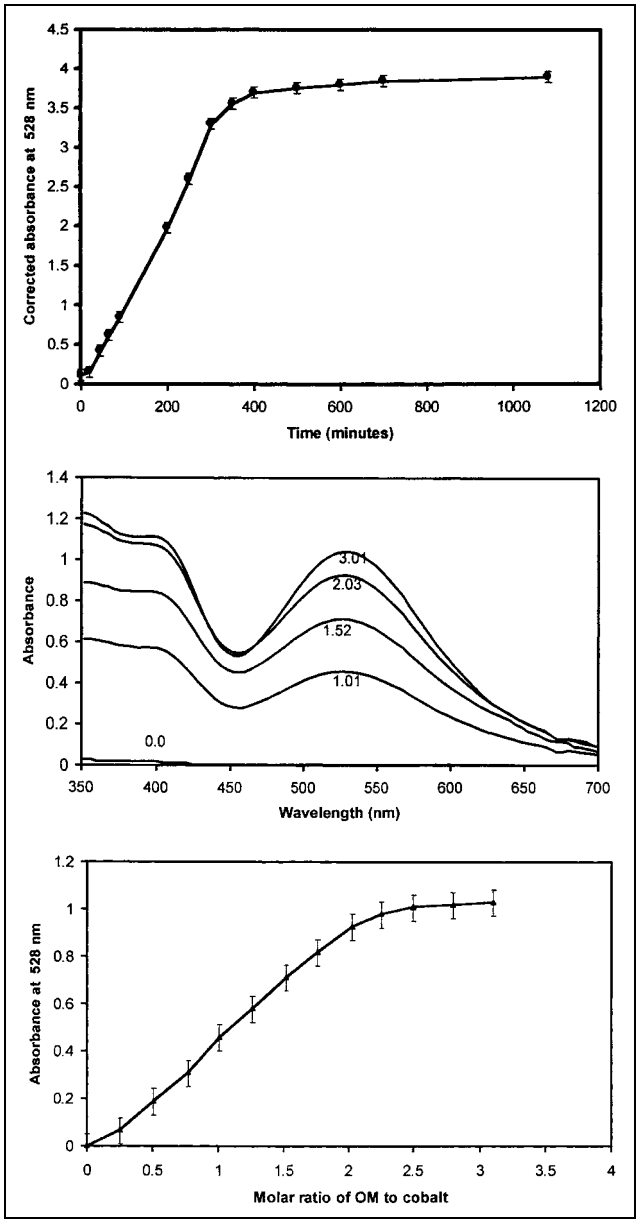

Fig. 2: Plot of the corrected absorbance against time for a sample solution containing cobalt and OM in 1 : 1 molar ratio. (B) Overlaid spectra for cobalt solutions with increasing molar ratios of OM to cobalt (ratio are directly indicated on each spectrum). (C) Mole ratio plot for the titration of cobalt with OM

to the pink form until the maximum amount of the pink complex is formed ($m = 0.5$). When m is larger than 0.5 excess OM leads to the formation of the green precipitate. 7 h after preparation the samples were centrifuged and absorption spectra for the pink supernatant were recorded (Fig. 2B). Fig. 2B shows only the absorption spectra (400–600 nm) of the pink complex form because neither cobalt (at the studied concentration level), OM nor the green complex form (precipitate) has any absorption above 400 nm. Similar to the earlier treatment, mole ratio plots were obtained by plotting the absorbance at 528 nm against the molar ratio m as shown in Fig. 2C. The mole ratio plot suggested that the pink complex has the formula: $Co(OM)_2$ in accordance with the results from the first experiment where OM was titrated with cobalt. These findings support the suggestion that OM forms complexes with cobalt in vitamin B12 leading to a decrease in absorption and its concentration in serum.

2.2. Copper

Experiments with copper suggested that it also forms complexes with OM although its complexation behavior appeared to be slightly different. If spectra were recorded 20 min after preparation of solutions a similar hypsochromic shift to that obtained with cobalt and zinc is observed together with a new peak in the visible region (330–440 nm). The absorbtivity of the newly formed peak appeared to be higher than that seen for cobalt or zinc. If the same set of the titration solutions were measured some time later a significant change in the absorption spectra was noticeable suggesting that longer time is required for the completion of the reaction. Therefore the time required for the completion of the reaction was monitored by obtaining absorption spectra for one of the solutions (at $m = 1:1$) every 30 min over a period of 10 h. The obtained absorption spectra showed a progressive blue shift for the λ_{max} of OM together with a hyperchromic shift for the broad peak appearing at about 400 nm. Plots of absorbance at 400 or 302 nm against time were obtained (Fig. 3A). The plots in Fig. 3A show an initial rapid decrease in absorbance followed by a gradual increase until it plateaus at about 6 h of the time of preparation. Therefore the whole set of copper-OM solutions were measured 7 h after preparation (Fig. 3B). From the overlaid spectra in Fig. 3B there seem to be two complex species of OM with copper. The first one is evident from the increase in the absorptivity of the broad absorption peak (at about 400 nm) that is associated with a blue shift for the λ_{max} of OM. If higher ratios of copper were available then another complex was formed, which was evident from the hypochromic shift for the absorption peak of the first complex species (at about 300 nm). However, the spectra of the two complex species were similar at about 400 nm.

The value of λ_{max} or the absorbance at either 302 or 400 nm against the molar ratio of the metal to OM was used to obtain a mole ratio plot (Fig. 3C). Plots of λ_{max} or absorbance at about 400 nm against m show that copper binds to OM in 1 : 2 ratio similar to the case of cobalt and zinc. However, a plot of absorbance at 302 nm against m suggested two stages of complex formation: the first one which is associated with an increase in the absorbance at 302 nm and has a general formula of $Cu(OM)_2$ and the other one which is characterized by an absorptivity (at 302 nm) similar to that of free OM and has the general formula $Cu_3(OM)_2$. The observed initial rapid change in the absorption spectra of OM when mixed with copper ions may suggest that the complexation reaction starts with one form of the complex which then slowly rearranges itself into a more stable form which has a stoichiometry of 3 : 2. Apparent equilibrium constant was also calculated for the complexation reaction of copper to OM ($m = 1.5$) when samples were measured after 7 h, utilizing spectral changes at 302 nm, and found to be 5.8×10^{15}. Again this high value of a binding constant suggests a possible interaction between OM and copper ions in copper metallozymes in biological systems.

Questions may still be raised about the nature of the reaction between OM and the studied metals because it might be thought of as a metal-catalyzed degradation reaction of OM. This possibility is of particular importance in cases of labile compounds like OM. However; It is less likely that the observed spectral changes were a result of the degradation of OM since these changes were noticeable soon after the preparation of the solution; even faster than the rate of degradation of OM in 10% acetic acid (unpublished data). Such spontaneity of the reaction is typical for complexation reaction.

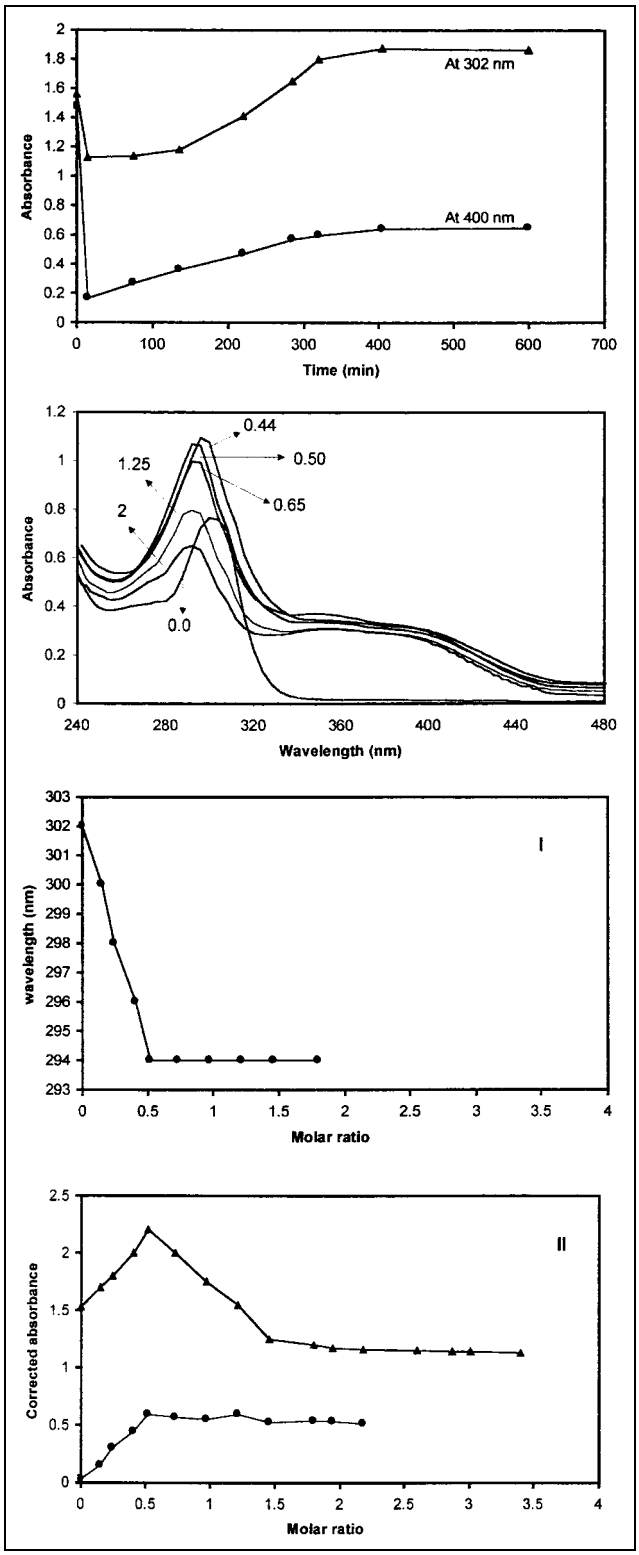

Fig. 3: (A) A plot of the corrected absorbance at 302 nm (triangle) and 400 nm (circle) against time for a sample containing copper and OM in 1 : 1 molar ratio. (B) Overlaid spectra for OM solutions with increasing molar ratios of copper to OM (ratios are indicated on each spectrum). (C) Mole ratio plots for the titration of OM with copper obtained by plotting either λ_{max} (I) or the absorbance (II) at either 400 nm (circle) or 302 nm (triangle).

More conclusive evidence came from the acidification of all solutions by adding 5 ml of 10% acetic acid to each tube. Metal complexes are generally known to be less

Table 1: Summary of the TLC data

	Fresh OM	Degraded OM	Solutions of OM with cobalt	Solutions of OM with copper	Copper solution	Cobalt solution
Number of spots	1	5	3	3	1	1
*R_f values	0.7_{256}	0.07_{365} 0.38_{365} $0.46_{365+256}$ 0.7_{256} 0.89_{365} (Green)	0.0_{256} 0.7_{256} 0.86_{365} (yellow)	0.0_{256} 0.7_{256} 0.85_{365} (white)	0.0_{256}	0.0_{256}

* R_f values are reported as the average values of three experiments. The subscripts indicate the wavelength of light underwhich the spots could be detected. The color of some spots as seen under the indicated wavelength is given between brackets

stable at acidic pH values [15]. Upon acidification the spectra of the native OM were reproduced in all solutions, which suggests the re-dissociation of the complex form to give the free drug. The acidified solutions then undergo (with time) color changes that are characteristic of OM.

Further evidence on complex formation was obtained from TLC experiments. Solutions of freshly prepared OM, solutions of degraded OM (stored for two months), cobalt solutions, copper solutions as well as the set of solutions used for the titration of OM with either cobalt or copper were all spotted on a normal silica TLC plate. The plate was developed in ethanol, visualized under UV light and the results were summarized in Table 1. The results show that there were four degradation products for OM. Solutions that contained OM with various ratios of the metal showed generally three spots each. The spots with R_f equal zero are due to the free metal (cobalt or copper). The spot with $R_f = 0.7$ is due to some of the OM being dissociated from the complex during migration on the plate. The dissociation of the complex is also evident from the strong fronting and tailing seen for the spots of the free metal (was not observed in spots of the metal alone) and that for the complex ($R_f = 0.86$) respectively. The third spot in the chromatograms of the titration solutions ($R_f = 0.86 + 0.85$), which is believed to be caused by the complex, may be mixed up with one of the degradation products of OM ($R_f = 0.89$) due to the close similarities for their R_f values. However, it can be unequivocally confirmed that they were different species by virtue of their clearly-different colors. The degradent with a R_f value of 0.89 appeared as a green substance 365 nm while the cobalt complex appeared as an intense yellow spot. The copper complex appeared as a white spot under the same light. Therefore the combination of the separation ability of the TLC together with different spectral properties between the degredants and the complex species has confirmed what has been described is a complexation reaction rather than a chemical degradation of OM.

Moreover, elemental analysis was used to prove that the metals comprised part of the products under investigation i.e. complex formation. In order to do that complexes were isolated using CC and the collected fractions were subjected (after being matched with the R_f value of the corresponding complexes on TLC) to Kjeldahl analysis as well as metal determination using atomic absorption spectroscopy. The results were expressed as weight percentage of nitrogen or metal from the weight of the taken sample. Then the ratio of the estimated metal to nitrogen was compared to its theoretical value assuming complex formation as discussed earlier. The results summarized in Table 2 indicate again that metals were still part of the produced compound which supports that the product was a metal complex. Moreover the practically found ratios of metals to nitrogen match the expected theoretical values of the assumed complexes, which further supports the idea of complex formation.

Table 2: Summary of the metal and nitrogen content analysis of the isolated complexes

Isolated complex of:	R_f value on TLC	Theoretical percentage of metal to nitrogen in the sample	Practical percentage of metal to nitrogen in the sample
Copper	0.85	0.88	0.86
Cobalt	0.86	0.82	0.80
Zinc	0.87	0.91	0.93

In conclusion, OM was found to form complexes with zinc, cobalt and copper in ethanol. In all cases a stoichiometry of 1 : 2 metal to OM was indicated and in the case of copper and cobalt other forms of complex have been suggested. The complexation reaction in the case of cobalt and copper may require up to 6 h for completion of the reaction. In the case of cobalt the time and the resulting complex species appeared to be dependent on the overall concentrations of both the metal and the ligand. We suggest that the observed ability of OM to form complexes with the studied metal in ethanol may imply important rule of the complexation process in the mechanism of action as will the side effects of the OM. Of immediate concern is the previously reported reduction of the serum level of vitamin B12 as a result of long term use of OM [7–11].

3. Experimental

3.1. Chemicals and apparatus

Ethanol (HPLC) grade was obtained from Labscan Ltd. (Stillorgan Ind. Park, Co. Dublin, Ireland). OM was a kind gift from Dar Al Dawa (Amman, Jordan). Cobalt chloride, copper bromide and zinc sulfate were obtained from BDH chemicals (Poole, England Ltd). Normal silica TLC plates (60 F_{254}) were obtained from Merck (Darmstadt, Germany). All UV-spectroscopic measurements were made on a Cary E-1 UV/visible spectrophotometer with 1 cm quartz cells. A ultraviolet fluorescence analysis cabinet (Spectroline model CX-20) was used for visualizing TLC plates. A Perkin Elmer 4000 atomic absorption spectrometer was used for the determination of the percentage of the metals in the isolated complexes.

3.2. Spectroscopic titration

3.2.1. Titration of OM with metals

A stock solution of OM was prepared to contain about 1.5×10^{-4} M in ethanol. From that solution, 2 ml aliquots were transferred to each of fifteen tubes which would make the titration series. Increasing amounts of the metal solution to be studied were added to each of the tubes, so that the molar ratio of the metal to OM (m) ranges from zero to about 4. The volume was completed volumetrically to 12 ml with ethanol. Proper blank was prepared for each solution in the titration series by adding the same amount of metal in the sample solution but without the presence of OM. The total volume for all tubes was kept constant to 12 ml.

3.2.2. Titration of cobalt with OM

In this experiment the concentration of cobalt was kept constant and that of OM was increased gradually to make a ratio of OM : cobalt in the

range: 0 to 4. A stock solution of cobalt was prepared in ethanol from which 2 ml were transferred to each of the 15 tubes used for the titration. Before completing the volume to 12 ml, an appropriate volume of OM (stock solutions) was added to each of the tubes so that the final OM to cobalt ratio in the series of the test tubes ranges from 0 to 4.

3.2.3. Calculation of binding constants

Apparent binding constants were calculated manually according to the mole ratio methods described in [14]. Some modifications were made so as to account for the possible contribution of the free metal and ligand to the absorption at the peak of the complex. Therefore the calculations were based on the formula:

$$A = \varepsilon_c C_c + \varepsilon_{fo} C_{fo} + \varepsilon_{fm} C_{fm} - \varepsilon_m C_{tm}$$

Where A, ε, and C indicate absorbance, molar absorptivity and molar concentration respectively. The subscripts c, fo, fm, and tm represent the the complex, free OM, free metal, and the total added metal respectively. The contribution of the total metal concentration ($\varepsilon_m C_{tm}$) to the absorbance was deducted because it was used as the blank at each titration point. In the case of 1 : 2 stoichiometry; C_{fm} is equivalent to $C_{tm} - C_c$ and $C_{fo} = C_{to} - 2C_c$. Thus by substituting these values into the above formula it can be rearranged to:

$$A = Cc[\varepsilon_c - \varepsilon_m] + \varepsilon_f [C_{to} - 2C_c]$$

Since A is measured experimentally at the required m and the molar absorptivities were determined under the experimental conditions it was possible to solve the equation for C_c and consequently the concentration of the free OM as well as the free metal could be determined.

3.3. TLC experiment

Normal phase silica TLC plates were used to resolve the complex species from OM and free metal as well as to compare the behavior of the complex species with that of the degradation products of OM. About 10 µl of each of the solutions used in the spectroscopic titration as well as solutions of freshly prepared OM and solution of OM which was stored in ethanol for two months. The plate was developed in ethanol in a way that the degradation rate will not be altered. This system was able to resolve five components for the degraded OM solution. The plates were visualized under UV light (256 and 365 nm).
All experiments were performed three times or more and the average values were used for obtaining the various plots. For all spectroscopic measurements the percentage relative standard deviations were less than 2.5%.

3.4. Isolation and elemental analysis of the complexes

Complexes were isolated using standard columns (2 cm i.d. × 50 cm) filled with silica gel 60 (0.06–0.2 mm). Samples were eluted using ethanol a lone as was done for TLC. Fractions were collected and correlated with Rf values observed on the TLC. The process was repeated several times until about 200 mg of each assumed complex were obtained. The complexes were then analysed using atomic absorption spectroscopy for their metal contents (copper, zinc, cobalt). In order to determine the percentage of nitrogen in the isolated complexes kjeldahl method was employed as outlined in Ref. [16].

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Imad I. Hamdan
Pharmaceutical Sciences
Faculty of Pharmacy
University of Jordan
Queen Rania St.
Amman
Jordan
iimad@hotmail.com