Determination of Redox Potential and Stability Constant of Iron(III)-3-Hydroxy-2-Methyl-4-Pyrone Complexes

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Summary: Maltol (3-HYDROXY-2-METHYL-4-PYRONE) is a monobasic bidentate ligand for a number of metal ions. It forms a stable 5-membered chelate ring with the metal ions. Having a very low toxicity index it has found extensive use as a flavouring agent. Depending on the oxidation state of the metal ion and the metal to ligand stoichiometry, the complex ion can be uncharged or it may have a +1 or +2 charge. This study describes the results of a potentiometric study for the determination of stability constants and a spectrophotometric determination of pH dependent redox potential at pH 3.0, 4.0, 5.0 and 7.0 using ascorbate as a reducing agent. At 25° C, the results were: $\log \beta_1 = 11.20$, $\log \beta_2 = 21.90$ & $\log \beta_3 = 28.20$ and $E^\circ_{complex}$ (vs NHE) at pH 3.0 = 0.229; pH 4.0 = 0.144; pH 5.0 = 0.107; pH 7.00 = 0.0442. These results are interpreted in terms of efficacy of maltol for its proposed usage for different medical applications.

Introduction

Maltol, 3-hydroxy-2-methyl-4-pyrone (Fig. 1) has a very favourable toxicity profile [1]. It is approved as a food additive by the US FDA and is used extensively in various bakery products. It is also a strong metal ion chelator, particularly for trivalent ions (Fe^{3+}, Al^{3+}, In^{3+}, *etc.*) as well as for some divalent ions, *i.e.*, $[VO]^{2+}$, $[MoO_2]^{2+}$, Zn^{2+}, Ru^{2+}, *etc.* [2-5]. Chelation takes place after ionization of the proton of 3-hydroxyl group located ortho to the 4-one position giving rise to a very stable 5-membered ring [6-8]. Thus each ligand takes on a single negative charge prior to chelation making 1:3 complexes with trivalent ions and 1:2 complexes with divalent ions neutral. Bioavailability of such complexes is very high (Fig. 2). In view of these properties, it has been suggested that the maltol complexes could be used for mineral nutrition (Fe) [9], therapeutic agents (for V based antidiabetic drugs [10]) and as imaging agents. However, as a ligand, maltol which was once considered a good candidate for chelation therapy in cases of iron overload and aluminum toxicity is now known to be actually a promoter of Al accumulation [11, 12]. As far as the treatment of iron overload is concerned, pyridinones are much stronger Fe(III) chelators ($\log \beta_3 \sim 37$) [13] and hence new drugs of choice.

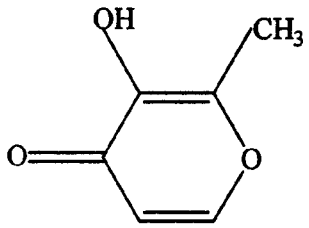

Fig. 1: Maltol (3-hydroxy-2-methyl-4H-pyran-4-one).

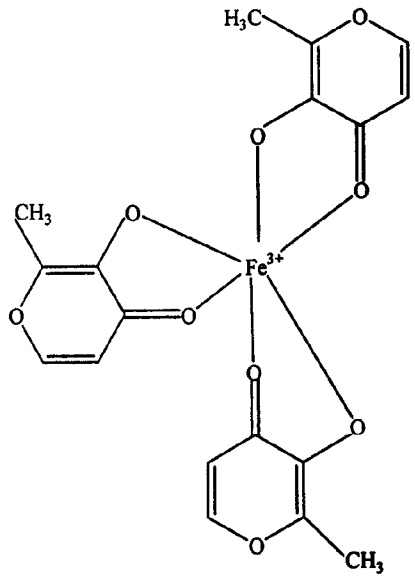

Fig-2: 1:3 Fe(III)-Maltol complex.

Some of these metal ions are redox active and the formation constants for metal ion-maltol complexes are oxidation state dependent. Since the ligand itself is involved in proton dissociation

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equilibrium, the extent of complex formation is also pH dependent. Therefore, in order to use this very useful ligand in any of the above mentioned applications as a supplement for mineral nutrition, for removal of toxic metal ions, as an agent for delivery of potentially therapeutic or diagnostic metal ion, a careful measurement of stability constants, rates of complex formation and redox equilibria should be made. In the present study we have undertaken a potentiometric measurement of stability constants of stepwise complex formation between Fe(III) and maltol and a spectrophotometric evaluation of pH dependent redox potential of the Fe(III)-maltol system. Computer program BEST was used for evaluation of stability constants [14]. This program is based on measurement of pH values as an aqueous solution of the metal and a known excess of the ligand is titrated with base. The measured pH values are compared to the values calculated from mass balance equations and assumed stability constants of the different species likely to be present in the system. The differences between these calculated and observed pH values are minimized by varying the assumed stability constants. A spectrophotometric procedure was used to determine the equilibrium concentrations of the reduced and oxidized forms of the Fe(III)-maltol complex at different pH. A recent report has validated such a procedure for determination of redox potential of cytochrome c [15]. After allowing the complex to react with a known quantity of a reducing agent (ascorbate), the absorbance is noted to determine the ratio of oxidized to reduced product. From these ratios, the equilibrium constant of the reaction at a given pH is calculated from the relationship:

$$N\ F\ E^{o} = RT\ ln\ K$$

This value of E^{o} is the redox potential ($E^{o}_{reaction}$) of the overall reaction. E^{o} of the complex was then calculated from the relationship:

$$E^{o}_{reaction} = E^{o}_{oxidant} - E^{o}_{reductant}$$

The Fe(III)-maltol complex is the oxidant while ascorbate is the reductant. Literature values of E^{o} for ascorbate were used to calculate the E^{o} of the Fe(III)-maltol complex. Finally log β_{III} for Fe(II) complexation maltol is evaluated from the expression:

$$E^{o}_{complex} - E^{o}_{aq} = -59.15 \log (\beta_{III}/\beta_{II})$$

where $E^{o}_{complex}$ and E^{o}_{aq} are redox potentials of the tris maltol complex of iron and of the uncoordinated iron (+3/+2) ions respectively in millivolts versus the normal hydrogen electrode (NHE).

Results and Discussion

The Data for pH titrations of maltol and Fe(III)-maltol complex is given in Table-1. The Fig. 3 shows the corresponding plot. Table-2 shows the ML_n species distribution as a function of pH as calculated by the program BEST. Fig. 4 shows the corresponding distribution curve. Table-3 shows the Log β values of Fe(III)-maltol complex species as calculated by the program BEST from the experimental data of this study. When these values are viewed in comparison to the corresponding values of stability constants of hydroxamic acids (aceto, benzo *etc.*) [16-18] the log β_3 are slightly higher although not as high as those of natural trihydroxamate siderophores, desferrioxamine B or E [19]. However a more pertinent comparison is that

Table-1: pH titration of maltol and Fe(III)-maltol complex.

$Fe^{3+} = 2.5 \times 10^{-3}$ mmoles $H^{+} = 0.4$ mmoles Temperature = 25 °C			Maltol = 1.25×10^{-2} mmoles NaOH = 0.195 M	
S. No.	Volume of NaOH added ml	pH of Maltol	Volume of NaOH added ml	pH of Fe-maltol Complex
01	0.00	6.70	0.00	2.15
02	0.05	8.60	0.10	2.16
03	0.10	9.51	0.50	2.25
04	0.15	9.95	1.00	2.36
05	0.20	10.15	1.50	2.52
06	0.25	10.30	1.55	2.56
07	0.30	10.46	1.60	2.58
08	0.35	10.51	1.65	2.59
09	0.40	10.58	1.70	2.61
10	0.45	10.65	1.75	2.62
11	0.50	10.70	1.80	2.64
12	0.55	10.72	1.85	2.65
13	0.60	10.74	1.90	2.71
14	0.65	10.76	1.95	2.73
15	0.70	10.84	2.00	3.05
16	0.75	10.88	2.05	5.98
17	0.80	10.90	2.10	6.99
18	0.85	10.93	2.15	8.45
19	0.90	10.95	2.20	9.19
20	0.95	10.97	2.25	9.44
21	1.00	11.00	2.30	9.66
22	1.05	11.03	2.40	10.00
23	1.10	11.05	2.50	10.21
24	1.15	11.07	2.60	10.37
25	1.20	11.09	2.70	10.49
26	1.25	11.10	2.90	10.66
27	1.30	11.11	3.20	10.82
28	1.35	11.13	3.40	10.88
29	1.40	11.15	3.60	10.94

Table-2: Species distribution of Fe(II)-maltol complex at different pH calculated by "best".

pH	ML	ML_2	ML_3
0.1	98.1	1.9	-
0.3	97.0	3.0	-
0.5	95.3	4.7	-
0.7	92.9	7.2	-
1.0	86.6	13.3	0.1
1.2	80.4	19.5	0.1
1.4	72.3	27.5	0.2
1.6	62.4	37.0	0.6
1.8	51.2	47.6	1.1
2.0	39.9	58.0	2.1
2.2	29.4	66.8	3.8
2.5	16.9	74.8	8.3
2.8	8.7	75.0	16.3
3.0	5.2	70.7	24.1
3.2	3.0	63.4	33.6
3.5	1.2	48.6	50.2
3.8	0.4	33.0	66.6
4.0	0.2	24.0	75.8
4.2	0.1	16.8	83.1
4.5	-	9.3	90.7
4.7	-	6.1	93.9
5.0	-	3.2	96.8
5.5	-	1.0	99.0
6.0	-	0.3	99.7

Table-3: Log β values of Fe(iii)-maltol complex calculated by "best".

Sigma Fit = 0.0486

Fe(III)-maltol complex	log β
$\log \beta_{101}$	8.310
$\log \beta_{10-1}$	-3.564
$\log \beta_{111}$	-2.456
$\log \beta_{22-2}$	-6.236
$\log \beta_{110}$	11.200
$\log \beta_{210}$	21.900
$\log \beta_{310}$	28.200

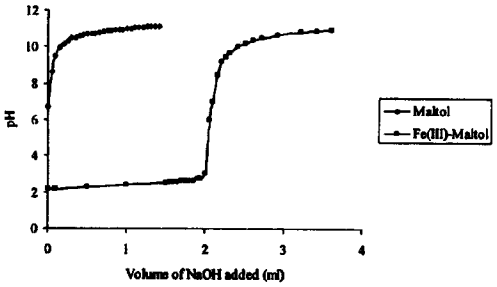

Fig. 3: Titration of Fe(III)-maltol system with NaOH at 25 °C (conditions listed in Table-1).

Table-4: Reduction potentials of Fe(III)-maltol complex at different pH.

pH	log K	ε°_{cell}	$\varepsilon^\circ_{DHA/ASC}$	$\varepsilon^\circ Fe^{3+/2+}$
3.0	-0.065	-0.0019	0.2315	0.2295
4.0	-0.587	-0.0174	0.1610	0.1436
5.0	-0.529	-0.0156	0.1223	0.1066
7.0	-0.592	-0.0175	0.0617	0.0442

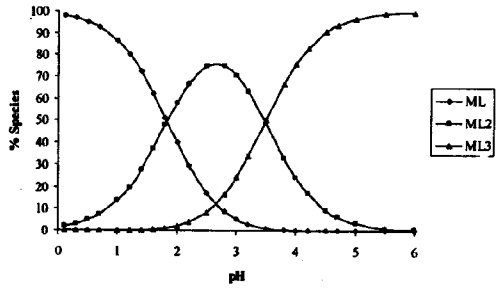

Fig. 4: Fe(III)-maltol species distribution as a function of pH.

the maltol remains coordinated up to very low pH value. This is because the ligand (maltol) has a lower pK_a value [20] as compared to the hydroxamic acid analogues. On the other hand, deferiprone (1,2 dimethyl-3-hydroxy-4-pyridinone) has a much higher log β_3 [21] than maltol or even the trihydroxamate siderophores. As such, it (deferiprone) is rapidly replacing desferrioxamine B (desferral) as a drug of choice for treatment of iron overload. Thus Fe-maltol complex would be a very good choice for an iron supplement while the deferiprone complex would not be. The reason is that the supplement should be such that it should release its iron readily when needed. The relatively low reduction potential of the Fe-maltol complex (this study) and its relatively facile reduction by biological reductants would ensure its availability to ferrochelatase (the enzyme which catalyses incorporation of iron into porphyrin as a first step for heme biosynthesis). A summary of log K and the redox potentials of the Fe(III) complexes at different pH is shown in Table-4. It is seen that as the pH is increased the maltol complex of Fe(III) becomes more difficult to reduce.

In conclusion, maltol is a very suitable agent for an iron supplementation for the following reasons: (i) sufficiently high formation constant for Fe(III) complex so as to prevent catalytic formation of reactive oxidative species (ROS); (ii) high aqueous solubility of the iron complex; (iii) Trismaltolato iron complex is neutral so that passage through cell membrane is possible; (iv) no toxic effects of the ligand are known and it has been in use as a food additive for flavour enhancement; (v) The ferric complex is readily reducible for transfer to other biomolecules.

Experimental

All chemicals used were of reagent grades and were used without further purification. $Fe(NO_3)_3.9H_2O$ was obtained from E. Merck. Stock solutions of Fe(III) (0.010 M) were maintained in dilute (0.1 M) nitric acid and the solution was standardized by measuring the absorbance of $Fe(o\text{-phen})_3^{2+}$ (λ=510nm, ε=1.011x10^4 L cm^{-1} mol^{-1}) prepared from taking an aliquot of the standard solution and diluting to mark in a volumetric flask with a solution containing *o*-phenanthroline and a reducing agent (hydroxylamine hydrochloride) in a pH 5.0 acetate buffer. 3-hydroxy-2-methyl-4-pyrone (maltol) was purchased from Aldrich and its stock standard solution was made by dissolving a weighed quantity in appropriate buffer in a volumetric flask. Working standards were prepared from dilution of this stock standard.

pH was measured on an Orion 720 Digital pH meter and the spectral measurements were made on a Shimadzu 160A spectrophotometer.

For the study of reduction equilibria in order to calculate redox potential of Fe(III)-maltol complex, the solutions of the complex were prepared in appropriate buffers (pH 3.00 and 4.00, formate buffer, pH 4.00 and 5.00, acetate buffer, pH 7.00, *Tris*-HCl buffer) and were deoxygenated by bubbling oxygen free nitrogen. Absorbance at the appropriate λ_{max} of the complex at the given pH was measured after incremental addition of ascorbate in the same buffer as the Fe(III) complex and which was similarly deoxygenated. The equilibrium concentration of the Fe(III) complex was then calculated from the previously determined molar absorption coefficient at the given pH. The difference between the total (starting) Fe(III) complex concentration and its equilibrium concentration (after making dilution corrections) gave us the Fe(II) concentration at equilibrium. The dehydroascorbate (the oxidation product of ascorbic acid) concentration at equilibrium was assumed to be one half that of equilibrium concentration of Fe(II) since ascorbic acid is a 2 electron reductant. Likewise, from difference between the total ascorbate and the DHA concentration at equilibrium, the equilibrium concentration of ascorbate was determined. With all the equilibrium concentrations now available, the equilibrium constants at different pH were determined. This permitted us to calculate the values of $E^o_{reaction}$. $E^o_{oxidant}$ values were then determined by adding the pH dependent literature values of $E^o_{reductant}$ to the experimental values (this study) of $E^o_{reaction}$.

$$E^o_{oxidant} = E^o_{reaction} + E^o_{reductant}$$

References

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