A Spectrophotometric Study of the Complexation of Olsalazine and Salicylic Acid with Calcium and Magnesium

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Olsalazine sodium, the disodium salt of 3,3'-azo-bis(6-hydroxybenzoic acid), is currently being introduced in the treatment of ulcerative colitis. The complexation of the two ligands 3,3'-azo-bis(6-hydroxybenzoate) and hydrogen salicylate with Ca^{2+} and Mg^{2+} has been studied by spectrophotometry. The latter ligand is intended to be used as a model substance in the assessment of the complexing strength of the former. Stability constants are reported for the chelate complexes formed in each system and the carboxylate complex of hydrogen salicylate with Ca^{2+}. The results are combined with those from earlier measurements on Cu^{2+}, and the data suggest a linear relationship between the free energies of complex formation of the two ligands with different metal ions.

Olsalazine sodium, the disodium salt of 3-3'-azo-bis(6-hydroxybenzoic acid), is the active component in Dipentum®, a new drug against ulcerative colitis. For simplicity, olsalazine will be denoted as H_4A. Previously we have studied the protolytic reactions of H_4A and the complexation between Cu^{2+} and H_2A^{2-}.[1,2] These studies have now been extended to the physiologically important ions Ca^{2+} and Mg^{2+}. Investigations of olsalazine complexes are made difficult by the limited solubility of the uncharged complexes. Therefore, measurements on the corresponding complexes with the hydrogen salicylate ion ($HSal^-$) were made in order to establish any relationship between the complexing ability of the two ligands, whose formulas are shown in Fig. 1. The salicylate ion has been extensively studied as a ligand,[3–10] and its formation constants could then be used to estimate the values for the corresponding olsalazine complexes.[11]

When Cu^{2+} is chelated by olsalazine large spectral changes take place in the UV/VIS region.[2] For Ca^{2+} and Mg^{2+} similar effects are found which have been used for the determination of the stability constants. Formation of the corresponding salicylate complexes gives rise to considerably smaller spectral changes. Nevertheless, these chang-

es are large enough to allow calculation of stability constants from spectrophotometric measurements.

Experimental

Chemicals and stock solutions. Olsalazine sodium was provided by Pharmacia AB and specified as being of chromatographic purity, >99.5 % (UV detection, 360 nm). Sodium salicylate was Merck p.a. Both substances were used without further purification, and stock solutions were prepared by dissolving them in water. $CaCl_2$ standard solutions were prepared by dissolving $CaCO_3$ in concentrated HCl and diluting this to the desired volume with water. An $MgCl_2$ standard solution was prepared from magnesium metal (Johnson-Matthey, 99.99 %). The metal was washed with 1 M HCl, water and ethanol, dried and weighed. It was then dissolved in 1 M HCl and diluted to volume. The NaOH used was EKA p.a. All other chemicals used were Merck p.a. unless stated otherwise.

Instruments and electrodes. The experiments were carried out as combined potentiometric and spectrophotometric titrations. The spectrophotometer used was a Zeiss DM4 equipped with a 10 mm quartz flow-through cell. For the ph (= $-\log$ [H^+]) measurements a Radiometer PHM64 pH meter was used in conjunction with a glass electrode and a calomel reference electrode with a salt bridge. The salt bridge was filled with 3 M NaCl to keep the liquid junction potentials small. The cell was calibrated against a 1.000 mM solution of HCl (with NaCl added to an ionic strength of 0.5 M). For the measurements on olsalazine an Ingold glass electrode (type 102013064) was used. This electrode was found to have an alkaline error affecting measurements above ph 9, and was replaced by a Metrohm 6.0102.000
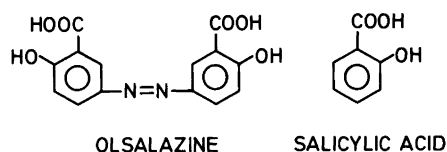

Fig. 1. The structural formulas of olsalazine (H_4A) and salicylic acid (H_2Sal).

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electrode for the measurements on salicylate. The latter electrode is claimed by the manufacturer to have negligible alkaline error below pH 12. The electrode was tested for $ph < 11$ by titration of 2 mM NaOH with HCl, and was found to have theoretical slope. The alkaline error of the Ingold electrode was estimated by titration of a proline buffer with NaOH, measuring ph with both glass electrodes, and assuming the ph values determined with the Metrohm electrode to be correct. All ph values previously determined with the Ingold electrode were then corrected. The correction term was at most 0.03 ph units (at ph 10.3). These results are in accord with earlier measurements using Ingold electrodes of the same type against the hydrogen electrode.[12]

Start solutions. The ionic strength of all start solutions was adjusted to 0.5 M by the addition of NaCl. The calculated amount of NaCl was weighed into a 100 ml volumetric flask. Appropriate volumes of ligand and NaOH stock solutions were added whereupon water was added to about 80 ml. Finally, stock solution of the metal ion was added under vigorous stirring. After the volume had been adjusted the solution was filtered into a Metrohm titration vessel. The purpose of the filtration was to remove dust and calcium or magnesium hydroxide that may have precipitated during mixing. It also served to detect precipitation of the olsalazine salts. If a coloured precipitate was visible on the filter (Millipore HA, 0.45 μm) the solution was discarded. Some filters used for the solutions with salicylate were leached with 1 M HCl overnight and the resulting solutions were analysed by atomic absorption spectrophotometry for calcium and magnesium. In none of the cases examined were more than 0.1 % of the metal ions retained on the filter. The examined filters were those used for filtering the start solutions with the highest ph values.

The total concentration of olsalazine used was 10^{-5} M. The total concentrations of Ca^{2+} ion in the experiments were 5, 10 and 20 mM, and those of Mg^{2+} ion were 5 and 10 mM. In the experiments with salicylate the concentrations were 10^{-4} M of the ligand, 50 and 100 mM of Ca^{2+} and 5 and 10 mM of Mg^{2+}.

Set-up and experimental procedure. The bulk of the test solution was kept in the titration vessel, which was immersed in a water bath thermostatted at 25 ± 0.1 °C. The solution was pumped to the cuvette and back to the vessel through PTFE tubings (0.5 mm i.d.) by a peristaltic pump (Pharmacia P-3). The total volume of the loop, including pump, tubings and cuvette, was 2 ml and the flow rate 10 ml min⁻¹. For the measurements on the calcium–salicylate system the PTFE tubes were encapsulated in thicker rubber tubes with a nitrogen flow in the space between the tubings, in order to minimize uptake of CO_2 from the surrounding air. The bulk solution was always kept under nitrogen and was stirred constantly using a magnetic bar.

The ph was changed by the addition of small volumes of 0.1–1 M solutions of HCl. The total change in volume

caused by these additions was never larger than 0.1 %. Thus, in every titration the total concentrations of metal ion and ligand were kept virtually constant and only the hydrogen ion concentration was changed. After each addition of HCl stable absorbance readings were obtained within 3 min. The time for stable ph readings to be reached was sometimes a few minutes longer for solutions of very low buffer capacity. The titrations comprised 5–15 points each. At each point in a titration the absorbance was read from the digital display of the photometer at a number of chosen wavelengths. The wavelengths used were, for salicylate 315, 320, 325 and 330 nm, and for olsalazine 400, 410, ..., 470 and 480 nm. An integration time of 3.5 s was used because of the relatively low absorbances measured.

Equilibrium models and calculations

Except at extreme pH values (above ph 11 for olsalazine and ph 13 for salicylic acid) the dominating ligand species are H_2A^{2-} and $HSal^-$. The most important complexes present in the solutions studied are the chelate complexes whose formation involves the carboxylate and phenolate groups. For olsalazine the principal scheme is hence reaction (I), where Me^{2+} is a divalent metal ion.
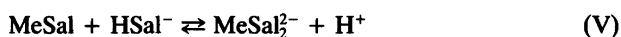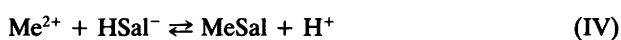
$$Me^{2+} + H_2A^{2-} \rightleftarrows MeHA^- + H^+ \qquad (I)$$
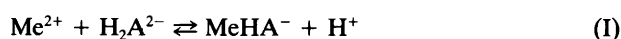
There is also the possibility of addition of a second metal ion to the symmetric olsalazine molecule.

$$MeHA^- + Me^{2+} \rightleftarrows Me_2A + H^+ \qquad (II)$$
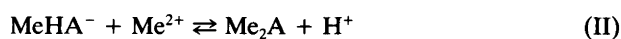
At high ph the complex MeA^{2-} is also expected to form, according to reaction (III).

$$Me^{2+} + H_2A^{2-} \rightleftarrows MeA^{2-} + 2H^+ \qquad (III)$$
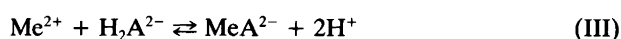
In the case of salicylate equilibria (IV) and (V) can be envisaged.

$$Me^{2+} + HSal^- \rightleftarrows MeSal + H^+ \qquad (IV)$$

$$MeSal + HSal^- \rightleftarrows MeSal_2^{2-} + H^+ \qquad (V)$$

However, since the metal ion is present in a large excess the second step can be neglected.

Also conceivable is the formation of carboxylate complexes according to schemes (VI) and (VII).

$$Me^{2+} + H_2A^{2-} \rightleftarrows MeH_2A \qquad (VI)$$

$$Me^{2+} + HSal^- \rightleftarrows MeHSal^+ \qquad (VII)$$

It would be advantageous if the extent of reactions (VI) and (VII) could be estimated beforehand, since they are expected to cause only minor spectral changes. No previous data are available for olsalazine, and in the case of salicylic

acid only calcium complexes have been studied. Some conclusions can be drawn, though. Four different authors using different methods, report stability constants for the complex CaHSal⁺. [13-16] Their values of the quotient [CaHSal⁺]/[Ca²⁺][HSal⁻], when extrapolated to an ionic strength of 0.5 M, all fall in the range 0.7–1.2 M⁻¹ (see Table 2 later). In the extrapolation we used Davies' equation. [17]

$$\log \gamma = -0.5z^2 \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \right)$$

Considering the variety of methods used it seems reasonable to assume that the constant lies within this range, or close to it.

A survey of the literature reveals that the stability constants of carboxylate complexes of Ca^{2+} and Mg^{2+} are of approximately the same magnitude. [3-5] If this is true also for the carboxylate complexes of hydrogen salicylate, the magnesium complex need not be considered at the Mg^{2+} concentrations used in this work.

H_2A^{2-} is a slightly stronger base than $HSal^-$. For olsalazine $\log k_3{}^H$ is 2.80 at an ionic strength of 0.5 M, whereas $\log k_2{}^H$ for salicylic acid is 2.68. [1,10] The stability constants of the proton complexes thus differ by a factor of 1.3. Hence one would expect the carboxylate complexes of H_2A^{2-} with Ca^{2+} and Mg^{2+} to be somewhat more stable than the corresponding hydrogen salicylate complexes. Even so, only small amounts of these complexes can be expected to be formed under the prevailing experimental conditions.

The following notation will be used: B and L are the total concentrations of metal ion and ligand, respectively, whereas b and l are the corresponding free concentrations. Thus, l is $[H_2A^{2-}]$ or $[HSal^-]$. The free hydrogen ion concentration is denoted by h. Charges will be omitted from here on in equations.

For olsalazine the stability constants sought are given by eqns. (1)–(4).

$$\beta_{1-11} = \frac{[MeHA]h}{bl} \tag{1}$$

$$\beta_{2-21} = \frac{[Me_2A]h^2}{b^2 l} \tag{2}$$

$$\beta_{1-21} = \frac{[MeA]h^2}{bl} \tag{3}$$

$$\beta_{101} = \frac{[MeH_2A]}{bl} \tag{4}$$

Since some of the measurements were carried out at high ph, the protolysis of H_2A^{2-} also needs to be taken into account, while the protonation of the ligand can be neglected. The relevant stepwise stability constants are given by eqns. (5) and (6). (The values given are taken from Ref. 1 and extrapolated from 0.25 to 0.5 M ionic strength by Davies' equation. [17])

$$k_1^H = \frac{[HA]}{[A]h} = 10^{11.98} \tag{5}$$

$$k_2^H = \frac{[H_2A]}{[HA]h} = 10^{10.99} \tag{6}$$

The total concentration of olsalazine is given by eqn. (7).

$$L = [H_2A] + [HA] + [A] + [MeHA] + [Me_2A] + [MeA] + [MeH_2A] \tag{7}$$

The absorbance of a solution containing these species is given by eqn. (8).

$$A = [H_2A]\varepsilon_{H_2A} + [HA]\varepsilon_{HA} + [A]\varepsilon_A + [MeHA]\varepsilon_{MeHA} + [Me_2A]\varepsilon_{Me_2A} + [MeA]\varepsilon_{MeA} + [MeH_2A]\varepsilon_{MeH_2A} \tag{8}$$

Combining eqns. (1)–(8) yields the experimental variable Y, defined in eqn. (9).

k_1^H and k_2^H were determined in an earlier study, along with ε_{H_2A}, ε_{HA} and ε_A. [1] This leaves the parameters β_{1-11}, β_{2-21}, β_{1-21}, β_{101}, ε_{MeHA}, ε_{Me_2A}, ε_{MeA} and ε_{MeH_2A} to be determined by fitting eqn. (9) to experimental Y-values. However, this is too large a number of parameters to be evaluated from the restricted data range available. It was necessary to reduce the number of parameters by neglecting the presence of some complexes.

As discussed above, the carboxylate complexes MgH_2A and CaH_2A are expected to be present only in small amounts and were therefore not included in the calculations. In the Mg^{2+}–H_2A^{2-} system, where the measurements were carried out at p$h < 9.3$, the complex MgA^{2-} cannot be present in significant amounts unless the complex $MgHA^-$ is a considerably stronger acid than H_2A^{2-}. When only the complexes $MgHA^-$ and Mg_2A are included in the calculations, eqn. (9) reduces to eqn. (10), which contains a manageable number of parameters.

$$Y = \frac{A}{L} = \frac{\varepsilon_{H_2A} + (k_2^H h)^{-1}\varepsilon_{HA} + (k_1^H k_2^H h^2)^{-1}\varepsilon_A + \beta_{1-11}bh^{-1}\varepsilon_{MeHA} + \beta_{2-21}b^2 h^{-2}\varepsilon_{Me_2A} + \beta_{1-21}bh^{-2}\varepsilon_{MeA} + \beta_{101}b\varepsilon_{MeH_2A}}{1 + (k_2^H h)^{-1} + (k_1^H k_2^H h^2)^{-1} + \beta_{1-11}bh^{-1} + \beta_{2-21}b^2 h^{-2} + \beta_{1-21}bh^{-2} + \beta_{101}b} \tag{9}$$

$$Y = \frac{A}{L} = \frac{\varepsilon_{H_2A} + (k_2^H h)^{-1}\varepsilon_{HA} + (k_1^H k_2^H h^2)^{-1}\varepsilon_A + \beta_{1-11}bh^{-1}\varepsilon_{MgHA} + \beta_{2-21}b^2h^{-2}\varepsilon_{Mg_2A}}{1 + (k_2^H h)^{-1} + (k_1^H k_2^H h^2)^{-1} + \beta_{1-11}bh^{-1} + \beta_{2-21}b^2h^{-2}} \tag{10}$$

In the Ca^{2+}-H_2A^{2-} system it was harder to make predictions about the relative importance of the complexes Ca_2A and CaA^{2-}. Different models were tried.

For the regressions the Simplex method[18] was used and the error function $U = \Sigma(Y_{exp} - Y_{calc})^2$ was minimized. Calculations were somewhat simplified by the large excess of metal ion over ligand, allowing the approximation $b = B$.

For hydrogen salicylate the corresponding stability constants are given by eqns. (11) and (12).

$$\beta_{1-11} = \frac{[MeSal]h}{bl} \tag{11}$$

$$\beta_{101} = \frac{[MeHSal]}{bl} \tag{12}$$

The protolysis of $HSal^-$ can be neglected, since $k_1^H > 10^{13}$ M^{-1}; thus the total concentration of salicylate is given by eqn. (13) and the absorbance of the solution by eqn. (14).

$$L = [HSal] + [MeSal] + [MeHSal] \tag{13}$$

$$A = [HSal]\varepsilon_{HSal} + [MeSal]\varepsilon_{MeSal} + [MeHSal]\varepsilon_{MeHSal} \tag{14}$$

A combination of eqns. (11)–(14) gives eqn. (15).

$$Y = \frac{A}{L} = \frac{\varepsilon_{HSal} + \beta_{1-11}bh^{-1}\varepsilon_{MeSal} + \beta_{101}b\varepsilon_{MeHSal}}{1 + \beta_{1-11}bh^{-1} + \beta_{101}b} \tag{15}$$

Again, this equation was fitted to the experimental data by the Simplex method to obtain values of the parameters ε_{MeSal}, ε_{MeHSal}, β_{1-11} and β_{101}. ε_{HSal} was determined separately. Since in the experiments with salicylate and Mg^{2+} the quotient B/L was as low as 50:1 the approximation $b = B$ was no longer justified. Therefore, at each vertex of the Simplex, b had to be calculated from the relevant total concentrations and the current value of β_{1-11} (the carboxylate complex could be neglected). From eqns. (11), (13) and the expression for the total concentration of Mg^{2+}, eqn. (16), eqn. (17) can be derived.

$$B = b + [MgSal] \tag{16}$$

$$b^2 + \frac{\beta_{1-11}L - \beta_{1-11}B + h}{\beta_{1-11}}b - \frac{Bh}{\beta_{1-11}} = 0 \tag{17}$$

This equation is easily solved for b.

Results and discussion

Representative plots of Y against ph are shown in Fig. 2 for all combinations of metal ion and ligand. Each combination will be discussed separately.

Mg^{2+} *and olsalazine.* Eqn. (10) was fitted to experimental data, Y, b and ph, as described above. To begin with, this was done at one wavelength at a time, for a number of fixed values of the quotient $k=\beta_{1-11}^2/\beta_{2-21}$, i.e. the quotient between the stepwise stability constants. Plots of U and β_{1-11} as a function of wavelength for a number of values of k indicated that β_{1-11} is in the region of 1×10^{-7} and that k is larger than the statistical value of 4. Then attempts were made to use data from several wavelengths in the same regression. It was found practical to use data from no more than five wavelengths simultaneously. When a regression was performed with data from 400, 420, 440, 460 and 480 nm the following values of the constants were found: $\beta_{1-11}=1.11\times10^{-7}$ and $\beta_{2-21}=2.4\times10^{-15}$, while the use of data from 410, 430, 450 and 470 nm resulted in $\beta_{1-11}=1.04\times10^{-7}$ and $\beta_{2-21}=1.6\times10^{-15}$. Weighted averages of these values are $\beta_{1-11}=1.08\times10^{-7}$ and $\beta_{2-21}=2.0\times10^{-15}$. The standard deviation of the fit of eqn. (10) to all data, s_A, was found to be 5.2×10^{-4} absorbance units (AU).

As is evident from Fig. 2(b) only part of the formation curve could be recorded. This is due to the formation of a coloured precipitate, presumably Mg_2A.

Ca^{2+} *and olsalazine.* As described earlier, various reduced versions of eqn. (9) were fitted to the experimental data by the Simplex method. The best fit was found for a model including the complexes $CaHA^-$ and CaA^{2-}. Attempts to include Ca_2A in the model failed to give physically meaningful values of all parameters.

We found for the wavelengths 400, 420, 440, 460 and 480 nm the following values of the constants: $\beta_{1-11}=3.49\times10^{-9}$ and $\beta_{1-21}=1.3\times10^{-19}$ M, and for 410, 430, 450 and 470 nm: $\beta_{1-11}=3.62\times10^{-9}$ and $\beta_{1-21}=1.2\times10^{-19}$ M. The weighted averages of these values are $\beta_{1-11}=3.55\times10^{-9}$ and $\beta_{1-21}=1.3\times10^{-19}$ M. s_A was 6.8×10^{-4} AU.

The value of β_{1-21} found implies that $CaHA^-$ is a stronger acid than H_2A^{2-}. It is conceivable that the high value is the result of contributions to the recorded spectra from the species Ca_2A, since this complex is expected to be present in the solutions studied, albeit in low concentrations. Because of the limited data range available it was not possible to extract values of the stability constants of all complexes.

When only the complex $CaHA^-$ was included in the model we found $\beta_{1-11}=3.37\times10^{-9}$, with a poor fit of the Y-function to the data, $s_A=2.2\times10^{-3}$ AU. When the model comprised $CaHA^-$ and CaH_2A we found $\beta_{1-11}=3.30\times10^{-9}$ and $\beta_{101}=18$ M^{-1} with $s_A=9.2\times10^{-4}$ AU. In these regressions data from all wavelengths were used simultaneously. In the latter regression ε_{CaH_2A} was assumed to be equal to ε_{H_2A}. It is reassuring to note that the value of the equilibrium constant of the predominating complex, β_{1-11}, does not seem to be very sensitive to which assumptions are made about the presence of other complexes.
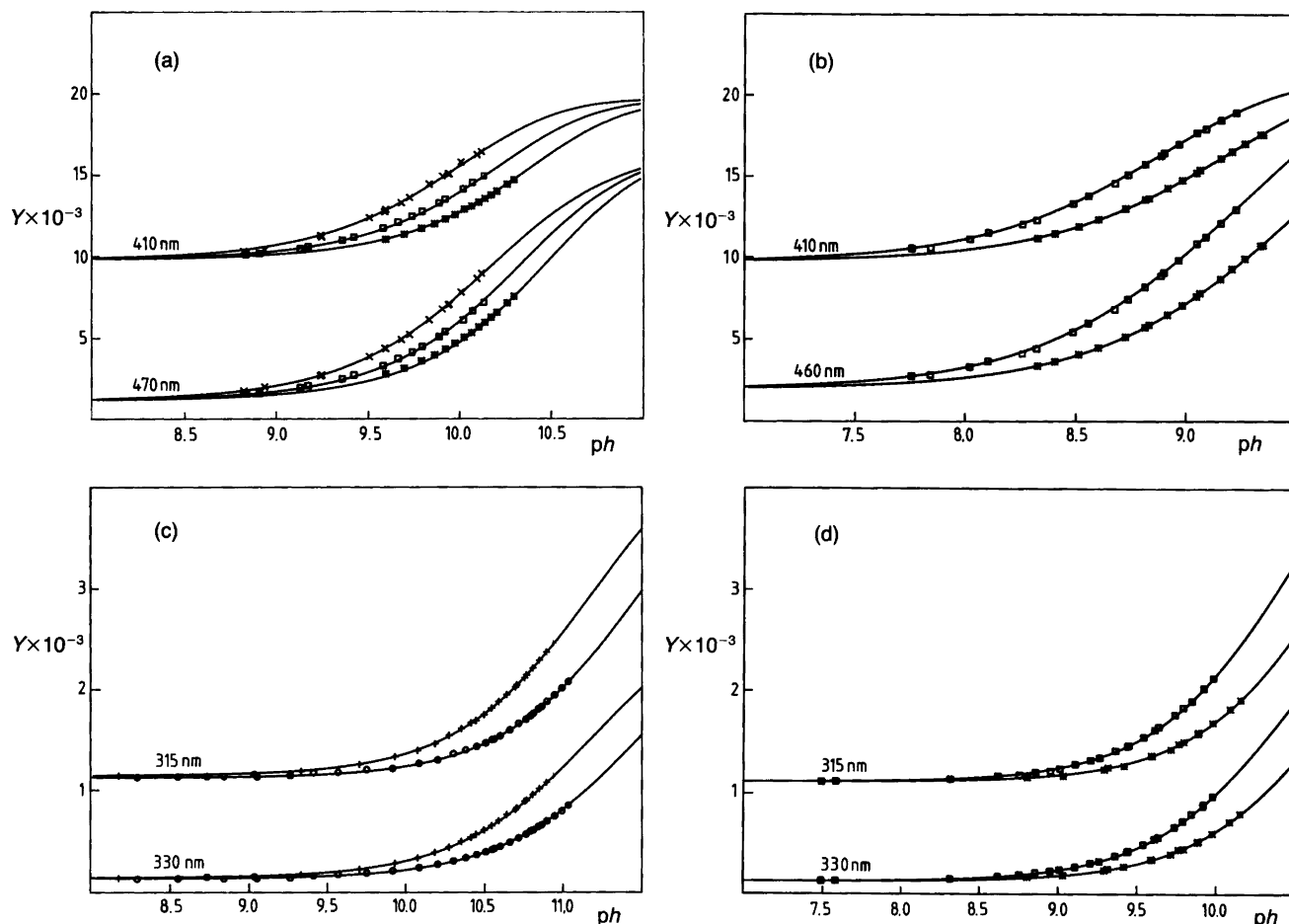
Fig. 2. Plots of Y vs. ph for all four combinations of metal ion and ligand: (a) Ca^{2+} and olsalazine, (b) Mg^{2+} and olsalazine, (c) Ca^{2+} and salicylate, (d) Mg^{2+} and salicylate. Symbols: B/mM: +, 100; ○, 50; ×, 20; □, 10; *, 5. The solid curves are calculated from the reported constants and ε values determined together with the constants.

Analogous to the case of Mg^{2+}, it is the formation of a coloured precipitate that limits the recorded formation curve.

In both the systems investigated, a good fit of the function to data was obtained with only two complexes in the model. It is thus unlikely that any further parameters can be extracted from the data available, even if a more powerful calculation procedure is used.

Ca^{2+} and $HSal^-$. As described earlier, eqn. (15) was fitted to the experimental data. All parameters sought were refined simultaneously at all measured wavelengths. We found $\log \beta_{1-11} = -10.19$ and $\log \beta_{101} = -0.58$. Calculations were also done for a number of fixed values of β_{101}. The results are summarized in Table 1. Although the best fit is found for a value slightly lower than reported by several other workers[13-16] (see the previous section and Table 2) it is evident that the fit deteriorates only slowly with an increase in β_{101}. The value of β_{1-11} varies only little when β_{101} is changed between 0.1 and 1.0 M⁻¹. This is not surprising, considering the small quota of $CaHSal^+$ present. Furthermore, the ε_{CaHSal} values found in the regression are close to the corresponding ε_{HSal} values. Thus, the effect of

formation of the complex CaHSal on the absorbances recorded is only indirect. The conclusion must be that an accurate value of β_{101} cannot be found from the measurements.

The highest ph used was about 11. Above this ph a correction would be needed for the presence of $CaOH^+$.[19]

Table 1. The influence of various fixed values of β_{101} on the value of β_{1-11} and the standard deviation in the fit of eqn. (15) to the experimental data for the combination Ca^{2+} and $HSal^-$.

β_{101}/M⁻¹	$\beta_{1-11} \times 10^{11}$	$s_A \times 10^4$ [a]
0.00	7.41	12.30
0.05	6.58	6.8
0.10	6.54	6.8
0.15	6.51	6.7
0.261	6.44	6.6
0.40	6.35	6.7
0.60	6.23	7.2
0.80	6.11	7.9
1.00	5.99	8.8

[a] The standard deviation of the fit expressed in absorbance units. The resolution of the instrument display is 0.0001 A.U.

Also there would be a risk of excessive precipitation of $Ca(OH)_2$ during mixing. Furthermore, the protolysis of $HSal^-$ would have to be considered in the calculations. So, even if it is desirable to reach a high degree of complexation, it is unlikely that anything is to be gained by increasing ph above 11, in terms of more accurate values of the stability constants. Also the use of higher calcium concentrations would increase the complexation of the ligand, but the concentrations used are already quite high and with further increase there is risk of substantial variation of the activity coefficients and the liquid junction potential.

Mg^{2+} and $HSal^-$. The results from the measurements on the Mg^{2+}-$HSal^-$ system are somewhat simpler to evaluate. The concentration of metal ion used was lower than in the previous case. Thus, very little of the complex $MgHSal^+$ is expected to form. This is confirmed by the results from the regression. Any attempt to include the carboxylate complex in the calculations failed to yield meaningful results as far as β_{101} or ε_{MgHSal} was concerned. The value of $\log \beta_{1-11}$ found was -8.48.

There is also here a limited ph range in which the measurements can be conducted. At p$h > 10$ more than 1% of the total Mg^{2+} will be present as $MgOH^+$, regardless of the magnesium concentration. Furthermore, the limited solubility of $Mg(OH)_2$ sets a ph limit of about 10 for the Mg^{2+} concentrations used.[20]

Summary of results. Table 2 lists our results together with those found by other authors. The uncertainties in the values of the equilibrium constants obtained in this work can only be estimated for the main complexes. These estimates are based on differences in result found between refinements carried out with data from different sets of wavelengths or different equilibrium models. Those who have used potentiometric measurements to study the Ca^{2+} and Mg^{2+} complexes of salicylate report larger values of β_{1-11} than we do. This is most pronounced for Ca^{2+}, which

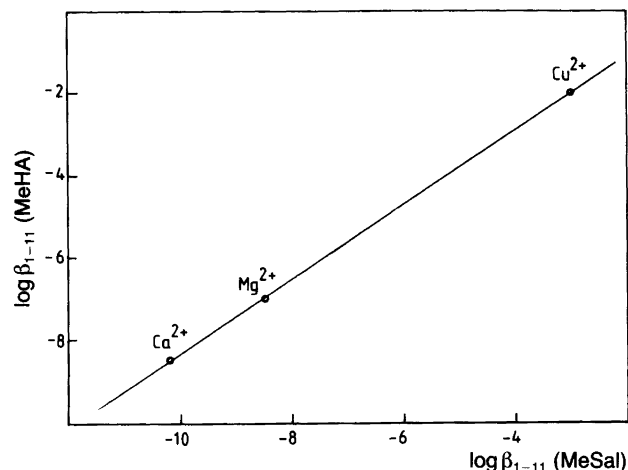Fig. 3. Log β_{1-11} for olsalazine complexes with Ca^{2+}, Mg^{2+} and Cu^{2+} plotted against the corresponding values for salicylate complexes.

forms the weakest complexes. The results of Arena *et al.*[6] or Brun and Schröder[7] are rather incompatible with the Y vs. ph curves we have recorded for Ca^{2+}. It should be noted, though, that the measurements by Arena *et al.* were carried out at 37 °C. Part of the discrepancy in this case may thus be due to the difference in temperature. We believe, however, that results from potentiometric measurements on these systems at relatively low concentrations of the reactants (up to a few mM) are quite susceptible to the presence of acid-base impurities. In fact, potentiometric measurements,[21] carried out after the completion of our work, on the Ca^{2+}-$HSal^-$ system with $B=100$ mM and $L=40$ mM indicate that $\log \beta_{1-11}$ is about -10 rather than -9 or larger. Corresponding measurements on the Mg^{2+}-$HSal^-$ system ($B=10$ mM, $L=40$ mM) were also in agreement with our findings. Potentiometry alone cannot be used to study the complexation of olsalazine owing to the low solubility of some of the species involved.

Table 2. The logarithms of the equilibrium constants of the reactions studied.[a]

Reference: Method:[b]	This work sp.	6 pot.	7 pot.	13 sol.	14 kin.	15 pot.	16 ix.
Reaction							
$Ca^{2+} + HSal- \rightleftharpoons CaHSal^+$	-0.58		2.16	0.02	-0.17	0.08	0.09
$Ca^{2+} + HSal- \rightleftharpoons CaSal + H^+$	-10.19 (0.03)	-8.77	-7.81				
$Mg^{2+} + HSal- \rightleftharpoons MgSal + H^+$	-8.48 (0.03)	-7.90					
$Ca^{2+} + H_2A^{2-} \rightleftharpoons CaHA^- + H^+$	-8.45 (0.04)						
$Ca^{2+} + H_2A^{2-} \rightleftharpoons CaA^{2-} + 2H^+$	-18.9						
$Mg^{2+} + H_2A^{2-} \rightleftharpoons MgHA^- + H^+$	-6.97 (0.04)						
$2Mg^{2+} + H_2A^{2-} \rightleftharpoons Mg_2A + 2H^+$	-14.7						

[a]All values cited are extrapolated to an ionic strength of 0.5 M. The values within parantheses are the estimated uncertainties. [b]sp., spectrophotometry; pot., potentiometry; sol., solubility measurements; kin., kinetic measurements; ix., ion exchange.

One aim of the investigation was to disclose any relationship between the complexing strength of the two ligands. There is often a linear relationship between the free energies of formation of metal ion complexes with two related ligands.[11] To see if this was the case also for the two ligands studied here we plotted the $\log \beta_{1-11}$ values for olsalazine complexes with Ca^{2+}, Mg^{2+} and Cu^{2+} against the corresponding values for salicylate (Fig. 3). The values for Cu^{2+} are taken from earlier investigations.[2,10] Our results suggest a linear relationship. If so, salicylate constants can be used for the prediction of the strength of olsalazine complexes.

Finally, we conclude that olsalazine is essentially uncomplexed by Mg^{2+} and Ca^{2+} at physiological pH.

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