

fectively than C (Figure 1). This is not surprising; the anionic end of B can be readily included within the electrical double layer of surfactant F micelles. Since B is more water soluble than C, it is clear that the affinity of *n*-dodecyltrimethylammonium ion micelles for B is in reality larger than for C. The small substrate A shows a modest rate increase above the CMC, which is consistent with inclusion of the ester within the outer aqueous portion of the micelle interior or else directly on the micelle surface. An interesting feature of Figure 3 is that the hydrolysis rates of the long-chain esters are enhanced *below* the CMC, while *p*-nitrophenyl acetate is not affected in this concentration range. The rate of basic hydrolysis of benzylideneacetophenone also increases in a cationic surfactant solution below the CMC.⁴ This was explained by association of the Schiff base with one or more surfactant molecules to form a positively charged complex which reacts readily with hydroxide ion. Our findings are in agreement with this rationale since the only substrates which display pre-CMC rate perturbations are ones which possess large hydrocarbon moieties that can hydrophobically bind to surfactant. Perhaps the small rate drop below the CMC in Figure 1C is also caused by nonmicellar complexation.

n-Dodecylpyridinium ion (G) has either a small effect

(Figure 4A) or no effect at all (Figures 4B and 4C) on the esters. Therefore, k_1 is nearly equal to k_2 (eq 1) or else there is little substrate adsorption (K is a small number). Some complexation is taking place because the solubilities of esters B and C are increased in the presence of *n*-dodecylpyridinium ion micelles. We conjecture that, because of the bulk and flatness of the surfactant "heads," the micelles are unusually loose structures containing considerable amounts of water. Association constants and rate perturbations are consequently not large. In any event the properties of solutions of *n*-dodecylpyridinium ion and *n*-dodecyltrimethylammonium ion are appreciably different despite the structural similarity of the surfactants.

A great deal remains to be learned about the effect of micellation upon reaction rates. In addition, virtually nothing is known about how surfactants affect the stereochemistry and product distribution of thermal and photochemical reactions. These important aspects of molecular aggregate chemistry are also currently under investigation in these laboratories.

Acknowledgment. The authors are grateful to the McCandless Fund of Emory University and to the Petroleum Research Fund for assistance. National Defense Education Act predoctoral support for Carolyn E. Portnoy is also appreciated.

Aggregation of Metallochlorophylls¹

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Abstract: The self-aggregation of divalent nickel, copper, and zinc methyl pheophorbides a and b has been examined by infrared spectroscopy. Of these, only the zinc compounds show significant coordination aggregation. Nmr measurements on zinc pheophytin a and zinc methyl pheophorbide a and b show that both coordination aggregation and π - π aggregation occur in nonpolar solvents. Nmr and infrared measurements indicate that under comparable conditions in nonpolar solvents the zinc chlorophylls are aggregated to a considerably lesser extent than are the magnesium chlorophylls. Visible and infrared spectra of nickel, copper, and zinc methyl pheophorbides a and b are reported, and tentative band assignments are made. Considerations of absorption position and relative intensities suggest that the extent of metal-ligand interaction follows the order: Ni > Cu > Zn > Mg.

Metal ions play a decisive but little understood role in the photosynthetic unit. For example, the most important compound of photosynthesis, chlorophyll, is a magnesium(II) complex of a dihydroporphyrin (chlorin) ligand. Examination of chlorophyll derivatives in which magnesium is replaced by other metal ions (metallochlorophylls) are valuable in that they provide information about the contribution of the metal to the various properties and functions of chlorophyll. The state of aggregation of chlorophyll is one property in particular that is a subject of keen interest today.³ Association of chlorophyll molecules in solu-

tion can occur in a number of ways. One mechanism, designated coordination aggregation, involves the intermolecular coordination of the ketone oxygen of one chlorophyll molecule to a magnesium atom in another molecule.⁴ An important question then is: is coordination aggregation a unique property of magnesium-containing chlorophylls, or do other metallochlorophylls show similar behavior? The aggregation properties of metallochlorophylls in solution appear not to have been previously studied.

This paper presents some new information derived from infrared and nmr measurements on the aggregation behavior of divalent nickel, copper, and zinc chlorophylls. The structural formulas, nomenclature, and proton numbering of the materials studied are given

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

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(3) See, for example, J. J. Katz, R. C. Dougherty, and L. J. Boucher in "The Chlorophylls," L. P. Vernon and G. R. Seely, Ed., Academic Press Inc., New York, N. Y., 1966, p 186.

(4) J. J. Katz, G. L. Closs, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Am. Chem. Soc.*, **85**, 3801 (1963).

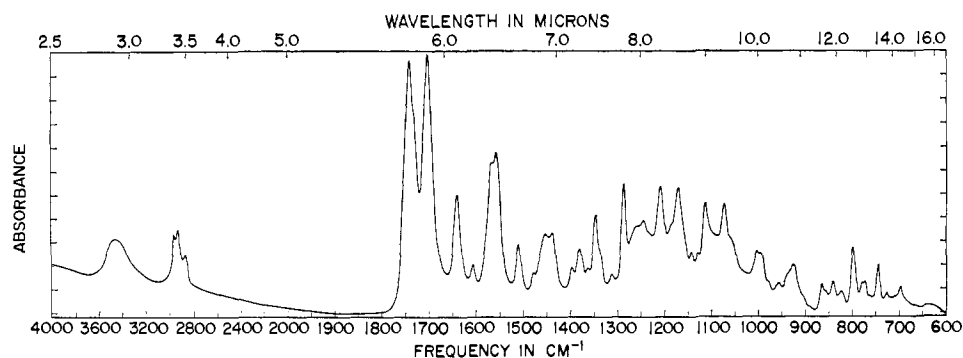


Figure 3. Infrared spectrum of copper methyl pheophorbide a in potassium bromide pellet.

band. The absorption spectra are similar to those of the chlorophylls⁸ and consistent with the proposed structure of the metallochlorophylls.

Table I. Absorption Maxima of Metallochlorophylls

Complex	Peaks, $m\mu$						A_v/A_r
	I	II	III	IV	V	VI	
	Methyl pheophorbide a						
Mg	664	617	574	532	432	415	1.31
Zn	658	608	556	514	426	408	1.30
Cu	654	608	546	505	425	399	1.18
Ni	652	608	539	494	423	394	0.95
	Methyl pheophorbide b						
Mg	647	598	570	545	458	437	2.67
Zn	638	593	564	540	448		2.61
Cu	633	587	556	522	444		2.31
Ni	632	593	553	516	430		1.93

Two pieces of information that can be obtained from the spectra are of immediate interest. One is the position of the maximum, particularly of the red and blue bands, and the other is the relative intensities of these bands. The λ_{\max} of the red and blue bands move to higher energy in the order Ni > Cu > Zn > Mg. The shifts for the methyl pheophorbide a complexes are somewhat less than for the methyl pheophorbide b complexes. Ratios of the absorbances of the blue and red bands are given in the last column of Table I. The ratio varies for the series of compounds with Ni < Cu < Zn ≤ Mg. Finally, the ratio for the methyl pheophorbide a complexes are considerably less than for the methyl pheophorbide b complexes.

A typical infrared spectrum is shown in Figure 3. The frequencies of the prominent absorption peaks for the methyl pheophorbide a and b complexes in the 4000–650- cm^{-1} region are collected in Table II. Data for the methyl chlorophyllides are again listed for comparison. The assignments given in Table II follow those given previously for the chlorophylls³ and metalloporphyrins.⁹ The infrared spectra obtained are entirely consistent with the proposed structures of the metallochlorophylls. Most of the ligand absorption positions are more or less independent of the metal ion. On the other hand, C–C and C–N stretching absorptions of the chlorin macrocycle are sensitive to the particular metal ion present. The complexes show two prominent medium-intensity absorptions in the 1500–1700- cm^{-1} region. The frequency of the bands at 1670–1600 cm^{-1}

and 1585–1535 cm^{-1} is in the order: Ni > Cu > Zn > Mg. The relative intensity of the macrocycle stretching vibration and metal ion dependence of the frequency are greater for the chlorophylls than for the corresponding metalloporphyrins.⁹ Far-infrared spectra of the copper chlorophylls show the strongest absorption below 650 cm^{-1} at 304 cm^{-1} . This band is absent from the spectra of the metal-free ligand and probably arises from a copper–ligand vibration.¹⁰ This absorption band is at a higher frequency for the metallochlorophylls, which may be interpreted to indicate a greater stability for the copper complex than for the magnesium complex.

The electronic and vibration spectra vary considerably in the series of metallochlorophylls. Frequency shifts and relative intensity variation give rise to the order: Ni > Cu > Zn > Mg. Spectroscopic evidence leads to an identical order for the metalloporphyrins.¹¹ The chemical stability of the metal–ligand linkage also varies in a similar way for the metalloporphyrins. It is not unreasonable to suppose that the stability of the metal–ligand band is related in the same way to the spectroscopic order for the metallochlorophylls. Unfortunately, no thorough study of the stability of the metallochlorophylls has as yet been carried out. Bearing on this point is the report that zinc chlorophyll is more stable to acid demetalization than is the magnesium compound^{12a} and that the stability of metallochlorophylls to photochemical decomposition is in the order Cu > Zn > Mg.^{12b}

Aggregation Properties. Examination of the solvent dependence of the carbonyl region (1750–1600 cm^{-1}) infrared absorption can furnish useful information about the coordination aggregation of chlorophyll compounds.⁴ In nonpolar solvents, and in the solid, chlorophyll a exists primarily in the aggregated state. The C-9 ketone absorption appears as two peaks: one is the normal ketone absorption at $\sim 1700 \text{ cm}^{-1}$; the other, originating in the ketone oxygen coordinated to magnesium, occurs at somewhat lower frequency ($\sim 1650 \text{ cm}^{-1}$). The latter may properly be termed an aggregation peak, and its occurrence in an infrared spectrum is a valid diagnosis for the presence of chlorophyll-like aggregates. In polar solvents such as tetra-

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Table II. Infrared Absorption Bands (cm^{-1}) in Metallochlorophylls^a

Methyl pheophorbide a				Methyl pheophorbide b				Assignment	
Mg	Zn	Cu	Ni	Mg	Zn	Cu	Ni		
2955 w	2960 w	2955 w	2955 w	2960 w	2950 w	2960 w	2955 w	ν(C-H)	Aldehyde Ester Ketone Aldehyde
2935 w	2930 w	2930 w	2925 w	2925 w	2925 w	2930 w	2930 w		
2870 w	2865 w	2860 w	2860 w	2860 w	2865 w	2870 w	2870 w		
1736 s	1739 s	1741 s	1742 s	1735 s	1740 s	1743 s	1741 s	ν(C=O)	
1696 s	1693 s	1704 s	1707 s	1696 s	1708 s	1710 s	1712 s		
1607 m	1617 m	1638 m	1661 m	1603 m	1616 m	1642 m	1665 s		
		1607 w	1632 w				1612 w	ν(ring)	
1547 m	1552 w	1556 m	1576 m	1544 s	1556 s	1572 m	1585 m		
1531 w	1539 m	1510 w	1521 w	1517 w		1504 w	1516 w		
1486 w	1498 w	1480 w	1480 w	1474 w	1489 w	1468 w	1484 w		
1457 w	1450 w	1451 w	1452 w	1441 w	1449 w	1455 w	1452 w		
1449 w	1439 w	1437 w	1437 w			1439 w	1438 w		
1373 w	1378 w	1370 w	1382 w	1376 w	1380 w	1382 w	1380 w		
1340 w	1347 w	1345 w	1347 w	1343 w	1349 w	1349 w	1348 w		
1281 m	1288 m	1286 m	1283 w	1284 m	1292 m	1289 m	1287 m		
1252 w	1240 w	1245 w	1244 w		1242 w	1238 w	1239 w		
1194 w	1189 w	1208 w	1214 w	1189 w	1211 w	1218 w			
1162 w	1160 w	1170 w	1169 w	1166 w	1169 w	1170 w	1169 w	ν(C-O)	Ester
1134 w	1134 w			1143 w	1138 w	1132 w	1134 w		
1086 w		1112 w	1108 w		1083 w	1107 w	1108 w		
1064 w	1069 w	1073 w	1076 w	1062 w	1066 w	1070 w	1073 w		
1049 w	1042 w			1038 w	1048 w	1051 w	1052 w		
984 w	991 w	1003 w	1002 w	1002 w	1005 w	1008 w	1014 w		
914 w	920 w	926 w	939 w	921 w	928 w	942 w	946 w		
839 w	846 w	841 w	842 w	839 w	833 w	837 w	843 w		
799 w	797 w	798 w	798 w	799 w	798 w	801 w	798 w	π(C-H)	
780 w	764 w	775 w	764 w	758 w	758 w	759 w	759 w		
748 w	742 w	743 w	747 w	737 w	736 w	740 w	743 w		
693 w	709 w	695 w	697 w	702 w	704 w	704 w	705 w		

^a Potassium bromide pellets; s = strong, m = medium, w = weak.

hydrofuran, chlorophyll exists primarily in monomeric form; the aggregation peak is completely absent from the carbonyl region spectrum.

The infrared spectra of the metallochlorophylls both in solution and in the solid form were examined. Typical spectra for zinc pheophytin a are given in Figure 4. In nonpolar solvents, such as carbon tetrachloride and chloroform, and in the solid, an aggregation peak is easily seen at 1660–1655 cm^{-1} . The spectra in polar solvents on the other hand do not show an aggregation peak. Zinc methyl pheophorbide a exhibits a similar behavior. Here the aggregation peak is much more intense for the carbon tetrachloride solution than for a destabilized chloroform solution or for the substance in the solid state. For chlorophyll b, both the aldehyde and ketone carbonyl oxygens participate in aggregate formation by coordination to the metal, and these provide two sources of aggregation peaks in the infrared at 1660 and 1610 cm^{-1} . Thus, for the aggregated species in nonpolar solvents: (1) the peak near 1660 cm^{-1} is partially due to the aggregation (ketone) peak and partially to the free aldehyde; (2) the peak near 1610 cm^{-1} is partially due to the ring vibration but also contains a contribution from an aldehyde aggregation peak.⁴ The relative intensities of the bands (1700–1600 cm^{-1}) in polar and nonpolar solvents support this interpretation of the chlorophyll b infrared spectra. For zinc methyl pheophorbide b the carbonyl region spectrum shows solvent dependence, which is consistent with aggregation in nonpolar solvents and in the solid. The relative intensity of the free ketone absorption is less, while that of the peak near 1610 cm^{-1} is greater in carbon tetrachloride than in tetrahydrofuran. Molecular weight measurements of zinc methyl pheophorbide

b are informative, as the assessment of the extent of aggregation from the infrared spectra is not entirely straightforward. Solutions of zinc methyl pheophorbide b in destabilized chloroform with a calculated molarity of 0.040 and 0.020 *M* have an observed molarity of 0.025 and 0.012 *M*, and this is clearly indicative of substantial aggregation in these solvents.

There is no evidence from the infrared spectra to indicate coordination aggregation for either nickel or copper methyl pheophorbide a or b, and the carbonyl region spectra of these compounds show no solvent dependence. The spectra, in fact, are identical in nonpolar solvents, in the solid state, and in polar solvents. The far-infrared spectra of copper chlorophylls also show no solvent dependence.

Proton magnetic resonance measurements on solutions of the zinc chlorophylls likewise yield information about the state of aggregation of these substances. In chloroform, the nmr spectra are poorly resolved. In tetrahydrofuran solution, the spectra become sharp, and a number of the proton resonance signals are observed to show changes in chemical shifts. These solvent-dependent shifts are particularly noticeable in the methyl and methine resonance regions and in the behavior of the C-10 proton signal. Nmr data are summarized in Table III. Chemical shift assignments are based on previous assignments of the nmr spectra of the chlorophylls.¹³ The nmr spectra of the zinc chlorophylls in tetrahydrofuran, it may again be noted, are entirely consistent with the proposed structure of the materials.

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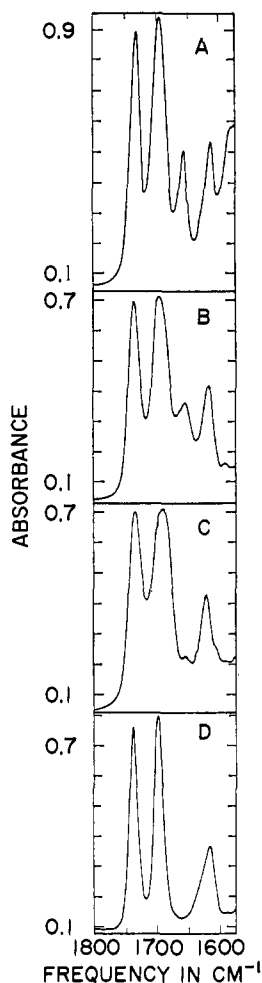


Figure 4. Infrared spectra of zinc pheophytin a: (A) in carbon tetrachloride; (B) in destabilized chloroform; (C) in potassium bromide pellet; (D) in tetrahydrofuran.

The large paramagnetic shifts experienced by some of the proton resonance in nonpolar solvents can be attributed mainly to specific solute-solute interactions.¹⁸ In chloroform the zinc chlorophylls are aggregated and

Table III. Chemical Shifts for Zinc Chlorophylls Dissolved in CDCl_3 (A) and $\text{THF-}d_6$ (B)^b

Proton ^c	δ , ppm ^a					
	Ia		IIa		IIb	
	A	B	A	B	A	B
α	8.46	9.24	8.64	9.17	9.22	10.15
β	8.78	9.51	8.92	9.40	9.07	9.58
δ	8.24	8.46	8.27	8.38	8.22	8.44
10	5.42	6.07	5.64	6.08	4.93	6.12
11	3.66	3.68	3.70	3.75	3.53	3.58
5	3.15	3.54	3.24	3.48	3.19	3.45
1	3.11	3.26	3.15	3.28	3.15	3.18
3a	2.55	3.14	2.69	3.15		
3b					9.12	11.06

^a From hexamethyldisiloxane. ^b Concentrations: Ia, 0.08 M; IIa and IIb, 0.06 M. ^c Proton numbering and compound designation are given in Figure 1.

in tetrahydrofuran the materials are monomeric (disaggregated). The observed solvent dependence of the nmr spectra entirely parallels the solvent dependence of the infrared spectra. In the aggregated form, protons

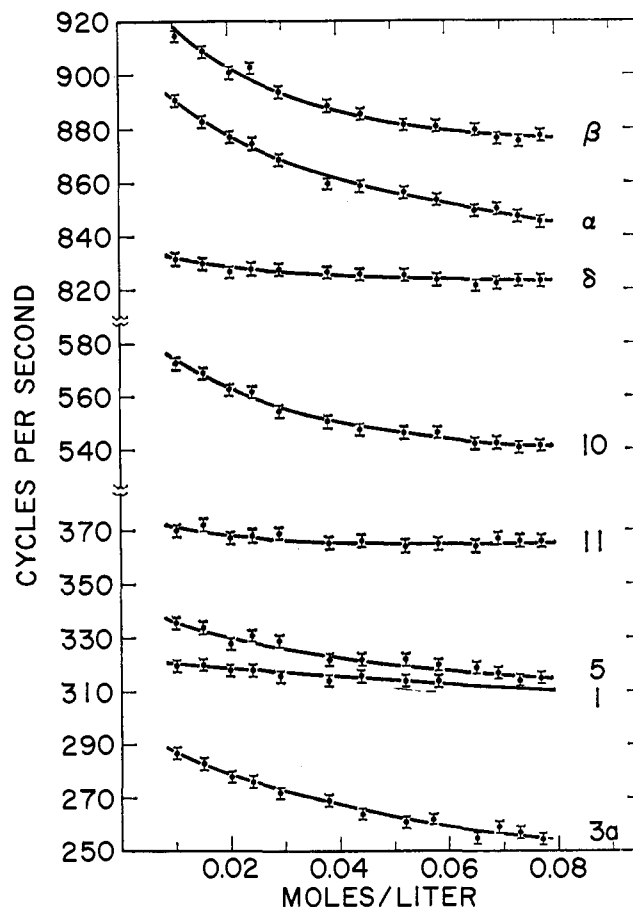


Figure 5. Nmr dilution shifts of zinc pheophytin a in deuteriochloroform.

in regions of the molecule that involve mutual overlap are expected to be highly shielded. In fact, aggregates of the zinc chlorophylls appear to have two highly shielded regions: (1) in the region of ring II for the α , β , and 3a (3b) protons; (2) in the region of ring V and the ketone carbonyl as deduced from the behavior of the 5, 10, and 11 protons. The first region coincides with what is observed for the π - π aggregation of the metal-free methyl pheophorbide. The relatively large shifts of the aldehyde protons may also indicate that the aldehyde oxygen is involved in coordination aggregation. The second region of mutual overlap in the aggregates is consistent with coordination aggregation.³ However, the small change in chemical shift of the C-11 protons as the aggregate is dissociated to monomer may indicate that the extent of coordination aggregation is not large. At any rate, the nmr spectra are consistent with the idea that two kinds of aggregates exist in nonpolar solution of the zinc chlorophylls.

The change of chemical shift values with dilution of chloroform solutions of zinc pheophytin a is shown in Figure 5. The extent to which aggregated species occur in solution is strongly concentration dependent. From the maximum concentration to about 0.05 M the chemical shift values for the 11, 10, and 5 protons appear to be independent of concentration. On the other hand, the α and 3a protons, and to a lesser extent the β proton, show chemical shifts that vary significantly with concentration. The π - π aggregation of the metal-free methyl pheophorbides is also strongly concentration

dependent, with the α , 3a, and β proton chemical shift values varying in a similar way as for the zinc compounds. Coordination aggregation of the chlorophylls, however, is not concentration dependent in this concentration range. Below 0.5 *M* for zinc pheophytin a, the chemical shift values of the α , 3a, β , 10, and 5 protons are all clearly concentration dependent. In the high concentration range only the π - π aggregates dissociate appreciably, while in the lower concentration range both the π - π and the coordination aggregates are dissociating. Since only one set of lines exists for the protons in the aggregated solution, labile equilibrium must exist among the two types of aggregates and the monomeric unit.

The solvent dependence of the infrared spectra show that the only metallochlorophylls that undergo significant coordination aggregation are the zinc complexes. Copper and nickel methyl pheophorbides, mercury and cadmium pheophytin a, and silver pyropheophytin¹⁴ do not undergo aggregation *via* the coordination mechanism. Metallochlorophylls, like metalloporphyrins, undoubtedly experience π - π aggregation in nonpolar solvents. Recent esr spectral evidence¹⁵ indicates that copper and silver methylpheophorbides form π - π aggregates. The nmr dilution shift measurements for zinc pheophytin a also testify to the importance of this type of aggregation in metallochlorophylls.

The relative intensity of the infrared aggregation peak for the zinc chlorophylls in the most favorable solvent is only about one-third that for chlorophyll itself. The concentration dependence of the chemical shift values for zinc pheophytin a is consistent with the substantial dissociation of the coordination aggregates in the concentration range near 0.02 *M*. At this and lower concentrations, chlorophyll aggregates are virtually undissociated. These data thus indicate that the stability

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of the zinc chlorophyll aggregates is much lower than the magnesium-containing chlorophylls under similar conditions. In fact, the zinc chlorophyll coordination aggregates are quite likely not much more stable than their π - π aggregates. Further, the zinc chlorophylls appear to be (from infrared) only slightly aggregated in the solid state, whereas coordination aggregation appears to be an important crystal-forming force in the chlorophylls.¹⁶ Infrared measurements on chlorophylls show a greater extent of aggregation in the solid phase than in solution. It can be concluded from these observations that strength of the zinc-oxygen interaction is not great enough to stabilize a particular structure in the crystal. Relevant is the report that only magnesium, and to a lesser extent zinc, chlorophylls form colloids in dioxane-water mixtures,¹⁷ while cobalt, nickel, and copper chlorophylls do not to any significant extent.

The ability of the magnesium and zinc chlorophylls to form coordination aggregates agrees closely with the ability of these compounds to add a fifth (axial) ligand. Magnesium and zinc chlorophylls (and porphyrins) readily add ligand, while the copper and nickel complexes do not.¹⁸ The differences between the magnesium and zinc chlorophylls may be rationalized by considering the result of a molecular orbital calculation on metalloporphyrins which shows positive charge on the metal to be 0.57 for magnesium and 0.40 for zinc.¹⁹ The higher positive charge makes the electrostatic interaction with the ketone oxygen involved in aggregated formation more favorable for the magnesium compounds than for the zinc compounds, and to a certain extent this difference in charge accounts for the differences in aggregation properties and perhaps for other important properties as well.

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