PORPHYRIN ADSORPTION BY CLAY MINERALS

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Abstract—A study was undertaken to investigate the adsorption of hemin, protoporphyrin and hematoporphyrin by kaolinite and a Ca-montmorillonite in aqueous solutions buffered at pH 4 and 9.

Although experimental restrictions at pH 4 prevented the complete characterization of the adsorption isotherms, kaolinite did exhibit a saturation of exchange sites by the cationic porphyrins. Both kaolinite and montmorillonite displayed a similar saturation of sites by the porphyrins in their anionic forms at pH 9. The major differences in the adsorption isotherms are attributed to differences in the exchange capacities of the clays.

Adsorption of the porphyrins at pH 9 was inhibited largely by phosphate treatment of the clays; this effect is interpreted as blockage of the anion exchange sites by irreversibly-bound phosphate.

INTRODUCTION

Porphyrins, the compounds originally cited as evidence for a biological source of petroleum (Treibs, 1936, 1938), have been detected in a variety of geologic environments (Hodgson *et al.*, 1967) due, no doubt, to their extensive roles in both plant and animal biochemistry and their relative stability. Their transport, accumulation and diagenesis are still a matter of considerable interest, yet their interactions with mineral phases have received little attention.

Previous experiments on clay–porphyrin interactions are few. Weiss and Roloff (1964), using the cationic form of hemin, reported that the porphyrins were sorbed in the montmorillonite interlayers, which increased the compound's thermal stability. On the other hand, Kaufherr *et al.* (1971) used the anionic chlorin, chlorophyllin, and presented evidence from infrared spectroscopy that the chlorophyllin was sorbed on edge surfaces.

The present study was initiated to measure the adsorption of porphyrins in both cationic and anionic forms by clays in an effort to further elucidate porphyrin adsorption.

The porphyrins, hematoporphyrin, protoporphyrin and hematin (Figure 1) were selected as model compounds on the basis of their commercial availability as well as their structures. Protoporphyrin and hematopoporphyrin allow for the determination of the effect of peripheral substitutions and weak hydrophobic forces on adsorption, while protoporphyrin and hematin can be used to gauge the change due to a complexed metal. Suspensions of kaolinite and a Ca-montmorillonite were each buffered to pHs of 4 and 9 to simplify the analysis of the adsorption experiments by eliminating zwitterion forms of the porphyrins. The pH values were selected to fall within the range of values often quoted for equilibrium pHs of aqueous suspensions of the two clays (Grim, 1968).

MATERIALS AND METHODS

Materials

The clay minerals were obtained from the collection at the Department of Geological Sciences of the State University of New York at Buffalo. A kaolinite from Georgia (SR92511) had been previously acid-treated by the supplier, Anglo-American Clay Corp.; the Ca-montmorillonite (Panther Creek) from Mississippi, which was supplied by American Colloid Co., had not been pretreated. The preparation of the clay minerals used in this study generally followed the procedures outlined by van Olphen (1963).

After dry sieving, the <37-μm fraction was dispersed in 30% hydrogen peroxide to remove traces of organic matter. Since this treatment can degrade the clay by causing the pH to become quite acid and thereby increase the dissolution of the clay surfaces, 1 N sodium acetate was added to the peroxide bath as a buffer (Douglas and Fiessinger, 1971) and the pH monitored periodically throughout the treatment. Hydrogen peroxide treatments of 24 hr were found to be sufficient to remove the organic matter.

After the excess hydrogen peroxide was decomposed by mild heating, contaminating electrolytes were removed from the clays by repeated washings with deionized distilled water. These washes were continued until the clay remained suspended owing to peptization. The 0.25–0.75-μm size fraction was then removed for the experiments using sedimentation and decantation techniques.

Portions of the prepared clays were further subjected to phosphate treatment to coat the anion exchange sites. Ten ml of clay suspension were washed with 90 ml of a 50 g/l sodium hexametaphosphate solution five times, each wash lasting 24 hr. After the final wash, the clay was removed by centrifugation and redispersed in deionized distilled water.

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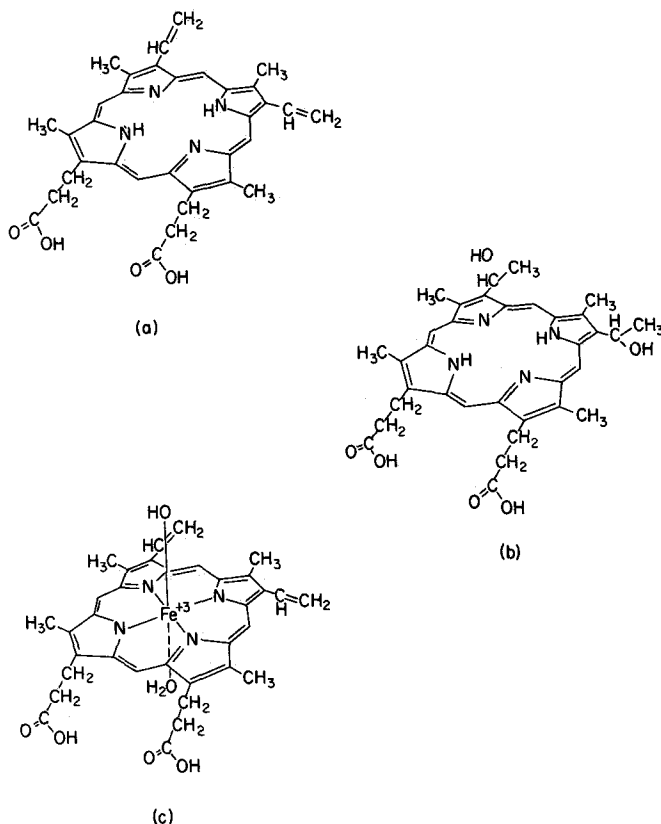
Figure 1. Idealized structures of porphyrins used in study: (a) protoporphyrin IX, (b) hematoporphyrin IX, (c) hematin.

Cation exchange capacities of the selected size fractions at pH 4 and 7 were determined using an ammonia-selective electrode following the procedure of Busenberg and Clemency (1973). The samples were filtered using Millipore® 0.20-μm filters; standard solutions for treatment of the clays at pH 4 were made from the pH 7 standards by titration with acetic acid.

All porphyrins in this study, viz. protoporphyrin IX, hematoporphyrin IX, and hematin, were used as obtained from Sigma Chemicals Co., St. Louis, MO, without further purification.

Buffers

The buffers used in the preparation of samples were prepared according to Bates (1973). In experiments requiring stabilization of the porphyrins, 30% (v/v) ethanol buffers were prepared by titrating an initial mixture with acid or base using a pH meter as an indicator; Bates (1973) has indicated that this approach is valid for water–ethanol mixtures and the measured pH needs no correction.

A problem arises in dealing with protoporphyrin and hematin in aqueous solutions in contact with the atmosphere. The precipitation and concomitant oxidation of these compounds lead to spectral degradation on a time scale of the same magnitude as that of these experiments (Shack and Clark, 1947; Maehly

and Akeson, 1958; Brown *et al.*, 1968). In some experiments using aqueous buffers, it was found that the degradation was sufficient to render any spectral measurements meaningless. Therefore, for both protoporphyrin and hematin, buffers of 30% (v/v) ethanol, as suggested by Maehly and Akeson (1958), were used to stabilize the compounds. To test the effect of these ethanolic buffers on the adsorptive processes, experiments were run with hematoporphyrin, which does not suffer from the above-mentioned stability problems, and compared with similar runs with aqueous buffers. The results indicated that the ethanol had little effect on the adsorption; X-ray investigations of the montmorillonite gave no indication of the formation of detectable amounts of interlamellar ethanol complexes.

Since the pH values employed in this study are in the range in which clays undergo chemical attack of their surfaces, the stability of kaolinite and montmorillonite in the pH buffers was monitored for 96 hr using X-ray powder diffraction. There was no evidence, from broadening of the peaks or reduction in intensities, for the degradation of either of the clays at pH 4 or 9 over the time period studied.

Porphyrin metallation

Although the insertion of metal ions into porphyrins in a laboratory usually involves the use of

higher temperatures, acids, organic solvents, or Grignard reagents (Falk, 1974), the natural conversion of free porphyrins to metalloporphyrins is likely to involve minerals, either as catalytic surfaces or as sources of metal ions (Hodgson and Baker, 1967; Casagrande and Hodgson, 1974). A search was therefore conducted for metalloporphyrins at the conclusion of adsorption experiments involving the free porphyrins.

The ability to detect metalloporphyrins mixed with the parent free porphyrins is increased markedly by forming the mono- or dipyridyl derivatives of the metalloporphyrin (Falk, 1964) simply by adding an excess of pyridine to the solution. This leads to a spectral shift of 2–3 nm and an increase in the extinction coefficient of about $20 \, cm^{-1} \, mM^{-1}$, which permits the detection of the conversion of about 1% of free porphyrin to metalloporphyrin (Vandenbelt and Henrich, 1953).

Measurements performed on the samples run for 24 hr did not indicate the presence of detectable amounts of metalloporphyrins formed from free porphyrins. Therefore, some experiments were allowed to run for 48 hr. Although there were some changes in the spectra, none of these could be attributed to metalloporphyrins, but were due to oxidation and aggregation.

Sample preparation and analysis

Fresh porphyrin stock solutions were prepared immediately prior to each experiment and used within 2 hr. The crystalline porphyrin was weighed directly on a Mettler Model B6 balance and dissolved in 0.01 N NaOH. After occasional shaking over a 15-min period, the solution was diluted to volume with the appropriate buffer, shaken vigorously and filtered through Whatman No. 1. paper to remove insolubles. Concentrations were determined with a Cary Model 14 recording spectrophotometer using the extinction coefficients tabulated by Barrett (1969) and Morell (1969).

Preliminary experiments indicated that the clay–porphyrin suspensions equilibrated within 16–24 hr. After mixing the components of each sample, the suspensions were mechanically shaken in the dark for 24 hr. The spectra of the free and adsorbed porphyrins were found to be identical within the accuracy of the spectrophotometer (0.3 nm). Therefore, in order to determine the amount of adsorbed porphyrin, the total absorption of the clay–porphyrin solution was measured, the sample then centrifuged with a maximum force of 14,500 g for 30 min, and the adsorption of the supernatant determined. Equivalently-diluted porphyrin blanks were run to gauge the alteration of the spectra with time.

Spectral comparison utilized a tandem cell arrangement on the Cary 14 dual beam instrument with ratio recording. The arrangement is similar to that used by Brill and Sandberg (1968) to study hemoprotein complexes, with one major exception: the cells containing the clay suspensions were placed at the end of the sample chamber closest to the detector, thereby increasing the amount of forward-scattered light received by the detector. Briefly, the two cuvettes in the reference beam contained a buffered clay suspension and a buffered porphyrin solution, while one cuvette in the sample beam was filled with clay–porphyrin–buffer, the remaining cuvette containing only buffer to equalize path-lengths. An excellent treatment of the experimental arrangement and its limitations can be found in the paper by Brill and Sandberg (1968).

Scattering baselines were determined by running buffered clay suspensions against each other. Variations less than 0.02 absorbance units were achieved when clay concentrations exceeded 0.4 g/l; at lower concentrations, baselines were irreproducible, presumably due to local variations in solution mixing in the area of the light beam. The maximum error of 0.02 absorbance units occurred in the ultraviolet region; as this is the same region in which the strong Soret band of porphyrins occurs and this band was used for concentration determinations, this difference is the largest source of error in the procedure. Brill and Sandberg (1968) pointed out that the servo-controlled slit of Cary 14-type instruments produces a constant signal-to-noise ratio provided the slit does not open to its maximum extent; care was taken in experiments with large porphyrin concentrations to dilute the sample for measurement so as not to overload the servo mechanism. Also, since the spectral bandwidth received by the detector increase with slit width, shifts in adsorption maxima for adsorbed species were investigated in dilute suspensions.

RESULTS AND DISCUSSION

The adsorption of ions by heterogeneous surfaces such as clays will usually follow a complex isotherm which may represent a combination of adsorptive mechanisms (Bowden et al., 1973). As the complete isotherms obtained here did not conform to a single isotherm type, the adsorption isotherms shown in Figures 2 and 3 have been divided into three distinct regions, similar to that performed by Bache (1964) for gibbsite adsorption of phosphate and Muljadi et al. (1966) for phosphate adsorption by clays. Following Muljadi et al. (1966), the three regions are: (I) at low porphyrin concentration, the adsorption isotherm rises very steeply, remaining close to the y-axis; (II) the region in which the isotherm becomes convex to the y-axis; and, (III) the linear area of the isotherm at medium to high concentrations. Regions I and II are treated by assuming Langmuir isotherms are applicable, while region III is treated with a linear isotherm.

The isotherm of regions I and II is represented by the following equation:

$$U = \frac{K_I V_I [\text{Por}]}{1 + K_I [\text{Por}]} + \frac{K_{II} V_{II} [\text{Por}]}{1 + K_{II} [\text{Por}]},$$
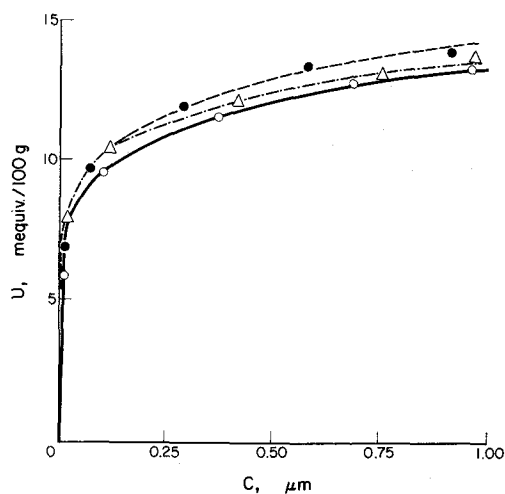
Figure 2. Adsorption of porphyrins by untreated kaolinite at pH 4. —— ● ——, hematoporphyrin; —○—, protoporphyrin; —·—△—·— hematin. Axes are defined as for equation (1).

where U is the total amount of porphyrin adsorbed, V_I and V_{II} are the number of sites capable of adsorbing porphyrin in regions I and II, respectively, K_I and K_{II} are the adsorption constants of porphyrin at a given pH in regions I and II, respectively, and [Por] is the total porphyrin concentration. The analysis for $V_I + V_{II}$, v_{II} ($= V_{II}/V_I + V_{II}$). and K_{II} followed that derived in Muljadi et al. (1966); the values are presented in Table 1.

Adsorption at pH 4

The investigation of adsorptive processes at pH 4 is severely hampered by the limited solubilities of porphyrins at this pH (10–15 μM). Initial experiments with amounts of more than 1 g/l of clay showed complete adsorption of the porphyrins. This would be expected for the low porphyrin concentrations employed, since there is an insufficient amount of porphyrin to saturate the cation exchange sites. At lower clay concentrations (0.4–0.8 g/l), only the kaolinite suspensions exhibited multiple isotherms (Figure 2); due to its larger cation exchange capacity (73.5 ± 2.33 mequiv./100 g), montmorillonite still had unsaturated exchange sites.

At pH 4, both hematoporphyrin and protoporphyrin possess a net charge of $+2$ due to protonation of the two free pyrrole nitrogens (Phillips, 1960). On the other hand, the presence of ferric iron in hematin prevents such protonation; the net charge of hematin
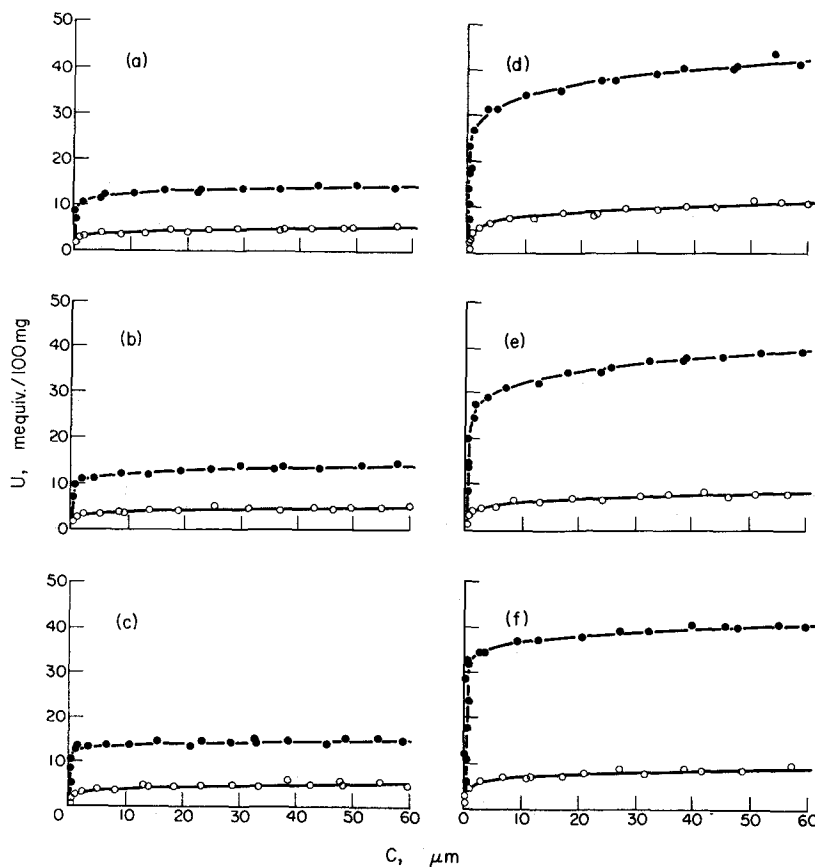

Figure 3. Adsorption of porphyrins by kaolinite and montmorillonite at pH 9. (a) Hematoporphyrin–kaolinite; (b) protoporphyrin–kaolinite; (c) hematin–kaolinite; (d) hematoporphyrin–montmorillonite; (e) protoporphyrin–montmorillonite; (f) hematin–montmorillonite. ○, phosphate-treated clays; ●, untreated clays. Axes are defined as for equation (1).

Table 1. Analysis of adsorption experiments at pH 9

Clay	Porphyrin	$V_I + V_{II}$	V_{II}	K_{II}
Kaolinite	Hematoporphyrin	6.627 ± 0.115	0.808 ± 0.146	1.069 ± 0.053
	Protoporphyrin	6.179 ± 0.860	0.717 ± 0.353	1.216 ± 0.581
	Hemin	14.885 ± 3.952	0.828 ± 0.423	1.448 ± 0.686
Kaolinite-PO$_4$	Hematoporphyrin	2.018 ± 0.143	0.915 ± 0.087	1.181 ± 0.030
	Protoporphyrin	1.965 ± 0.258	0.755 ± 0.217	0.402 ± 0.047
	Hemin	3.762 ± 0.280	0.866 ± 0.311	1.261 ± 0.221
Montmorillonite	Hematoporphyrin	20.695 ± 2.629	0.727 ± 0.211	0.376 ± 0.018
	Protoporphyrin	23.482 ± 4.808	0.824 ± 0.124	0.227 ± 0.004
	Hemin	40.456 ± 7.647	0.638 ± 0.275	0.779 ± 0.239
Montmorillonite-PO$_4$	Hematoporphyrin	4.062 ± 0.825	1.131 ± 0.412	1.472 ± 0.399
	Protoporphyrin	3.487 ± 0.313	0.651 ± 0.154	0.411 ± 0.025
	Hemin	8.047 ± 0.840	0.718 ± 0.186	0.414 ± 0.216

at pH 4 is +1. Thus, the exchange capacity of kaolinite for each porphyrin (derived from the adsorption data) is very similar to that originally measured using the ammonia electrode (3.23 ± 0.17 mequiv./100 g). This suggests that the area of the porphyrin, as viewed by the clay surface, is roughly equal to that of the hydrated ammonium ion (90 Å^2), implying that the porphyrins are adsorbed normal to the clay surface at the exchange sites, since the edge-on area of porphyrins has been measured in monomolecular films to be 85 Å^2 (Alexander, 1937).

Similar experiments with phosphate-treated clays were indistinguishable from those using untreated clays, suggesting that the adsorption of the phosphate anions has no noticeable effect on the adsorption of the cationic porphyrins.

Adsorption at pH 9

A greater range of porphyrin concentrations can be utilized at pH 9 due to the increased aqueous solubility of porphyrins ($>100 \mu$M) at alkaline pH, making a more complete characterization of the adsorption isotherms possible (Figure 3).

The experiments at pH 9 indicated saturation of the exchange sites for both clays. Although the anion exchange capacities were not measured, but were estimated from the literature [10 mequiv./100 g kaolinite, 31 mequiv./100 g montmorillonite; Hofman *et al.* (1956)], analysis of the isotherms again yields reasonably agreeable exchange capacities (6–15 mequiv./100 g for kaolinite, 20–40 mequiv./100 g for montmorillonite). At pH 9, both hematoporphyrin and protoporphyrin have a net charge of -2 due to ionization of their carboxylic acid substituents; hematin has a net charge of -1 at this pH.

Supportive evidence that the porphyrins are adsorbed as anions is provided by the inhibition of the ion-exchange portion of the adsorption isotherms by phosphate treatment of the clays (cf. Figure 3). Experiments by Muljadi *et al.* (1966) have shown that, for many kaolinites, a portion of the phosphate is irreversibly bound by what are considered to be the most reactive sites, i.e. corners, edges and lattic defects. This irreversibly-bound phosphate apparently inhibits the adsorption of porphyrins as anions by occupying the exchange sites. It is assumed that montmorillonite reacts with phosphate in the same manner, as the isotherms are similarly affected.

Comparison with previous work

Previous studies on the adsorption of porphyrins by clays (Hodgson and Hitchon, 1959; Kaufherr *et al.*, 1971) were not primarily concerned with determination of adsorption isotherms; furthermore, their lack of pH buffering makes comparison more difficult. Hodgson and Hitchon (1959) reported that montmorillonite had a much stronger adsorptive capacity for chlorophyllin than did kaolinite; their published isotherms are of the same general form as those presented here, although there are too few points to permit any quantitative comparisons.

Kaufherr *et al.* (1971) reported the formation of a sol of chlorophyllin and montmorillonite in water which resisted removal by centrifugation at 3500 rev/min for 30 min; this sol reportedly remained stable for weeks. No such sol was formed in the experiments reported in this study; the difference may be due to their use of much higher porphyrin concentrations than those of this study, the chlorophyllin salt being much more soluble in water than the porphyrins employed herein.

For those experiments utilizing montmorillonite, samples were examined by X-ray powder diffraction to determine if appreciable amounts of the porphyrins were sorbed in the interlamellar space of the clay. No expansion of the clay due to this form of sorption was detected by changes in the basal *d*-spacings for either the cationic or anionic porphyrins. Whereas the experiments of Kaufherr *et al.* (1971) utilized an anionic chlorin, no interlamellar complexes would be expected and none was reported. On the other hand, Weiss and Roloff (1964) successfully formed an interlamellar complex by using cationic hemin. Apparently, Weiss and Roloff employed a non-aqueous sol-

vent system to increase the porphyrin solubility as well as elevated temperatures (130°C) to form the complexes. As noted previously, our room-temperature experiments failed to saturate the exchange sites of montmorillonite with cations due to a low porphyrin-to-clay ratio; this apparently prevented us from forming any interlamellar complexes (detectable by X-ray powder diffraction) at pH 4 and does not conflict with the results reported by Weiss and Roloff (1964).

Implications for natural systems

In their study of the Mackenzie River drainage basin, the University of Calgary research group (Reeder *et al.*, 1972; Peake *et al.*, 1972) noted that a large fraction of the chlorins detected in the samples was associated with suspended matter. The pH of natural waters, such as those mentioned here, suggests that most, if not all, of the porphyrins would be adsorbed as anions. Although the presence of bentonites and illites was noted, it is difficult to ascertain if they were the principal agents of transport; however, the conclusions of this study suggest that any similar mineral surface can act as an effective adsorbent of porphyrins. The low concentrations of chlorins detected (maximum of 1 ppm) and the geographic distribution of the concentrations indicates that the amount of chlorins transported is a function of the source area and not the transporting agent, even though anionic chlorins are in a competitive situation with other anions.

Interlamellar complexes of porphyrins would not be expected in natural waters, principally because the porphyrins occur as anions or, at best, zwitterions of low solubility. The work of Weiss and Roloff (1964), therefore, is not directly applicable to natural waters and the transport and preservation of porphyrins, but undoubtedly is of importance in considering the higher temperature, organic-rich regime of petroleum source rocks.

SUMMARY

Porphyrins can be adsorbed by clays as either anions or cations, dependent on the pH. In the systems studied, clay adsorption of porphyrins can be represented by a combination of Langmuir and linear isotherms. Both kaolinite and montmorillonite appear to adsorb the three porphyrins studied in a similar fashion, the major differences being due to their different exchange capacities. The presence of irreversibly-bound phosphate reduces the anionic exchange capacity for porphyrins at pH 9.

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