SHORT COMMUNICATIONS

ESR Analysis of Free Mn(II) Content in Aqueous Solution of Poly(methacrylate)–Mn(II)

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It has been well established[1,2] that poly(methacrylic acid) (PMA) forms complexes with divalent metal ions in aqueous solution. Spectrophotometric, potentiometric, titration, and equilibrium dialysis methods have been used to study the complexes. Complex formation in aqueous solution can be evaluated from the amount of free metal ions not bound to the polymer. However, this amount is not easy to be estimated, and several methods have been proposed. In this paper, electron spin resonance (ESR) was applied to estimate the amount of free Mn-(II) ions in aqueous PMA containing Mn(II).

The PMA used was prepared by polymerization of methacrylic acid in aqueous solution using hydrogen peroxide as an initiator. Sodium polymethacrylate was used to ensure good solubility. For pH measurement, aqueous solutions of acid form PMA were used. PH of the solution was changed by adding NaOH, and measured by a Hitachi-Horiba M-7 pH meter. All the solutions contained 0.1 M NaCl. The concentrated aqueous solution of $MnCl_2$ was added to a PMA solution to produce the complex.

ESR spectra of Mn(II) ions in aqueous solution were taken on a JEOL JEX-X/K at a frequency of 9.2 GHz at room temperature. The solution was placed in a 0.75 mm i.d. quartz capillary tube. The peak-heights of the six-line spectra of Mn(II) in derivative mode were averaged and expressed in units of the spectral intensity of diphenylpicrylhydrazyl (DPPH) as the external reference.

Figure 1 shows the ESR spectra of aqueous Mn(II) solutions at pH 6.0 without and with added PMA. The upper spectrum is for a 10 mM aqueous solution, and the lower spectrum for the same solution containing PMA at a concentration of 100 mM. The central peak
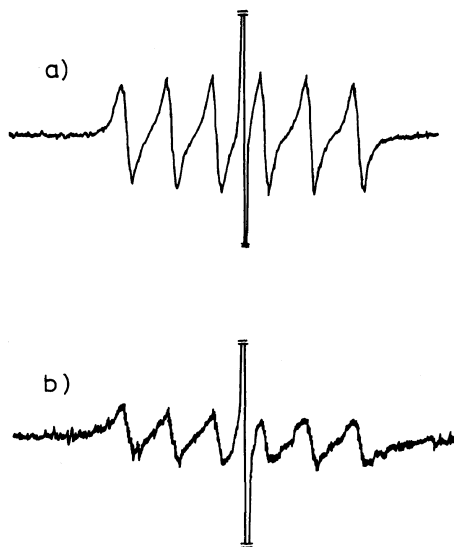

Figure 1. ESR spectra for 10 mM Mn(II) aqueous solution containing 0.1 M NaCl at pH 6.0, a) without PMA and b) with 100 mM PMA at the same receiver gain.
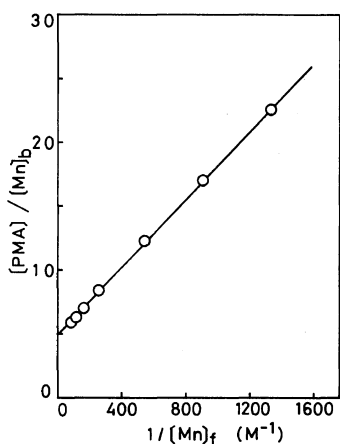
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Figure 2. Plots of $[PMA]/[Mn]_b$ *vs.* $1/[Mn]_f$ at a PMA concentration of 100 mM and at pH 6.0.

Figure 3. Fraction of free manganese ions $[Mn]_f/[Mn]$ *vs.* pH at a PMA concentration of 100 mM and at various Mn(II) concentrations: ○, 5 mM; ×, 10 mM; △, 15 mM; ●, 20 mM; □, 25 mM.

is due to concentrated DPPH in tetrahydrofuran as the reference. The addition of PMA clearly reduced the intensity of the ESR signal for Mn(II). The reduction in intensity of the ESR signal can be interpreted in terms of the binding of Mn(II) to PMA, because Mn(II) bound to a macromolecule gives no ESR signal.[3] By using this reduction in intensity the amount of free Mn(II) ions in the solution can be estimated.

The parameters for Mn(II) binding to PMA were determined by the graphic method of Hughes and Klots.[4,5] The dissociation constant for the Mn(II)–PMA complex is defined by

$$Kd = \frac{[Mn]_f n[PMA]_f}{[Mn]_b} \quad (1)$$

where $[Mn]_f$ and $[Mn]_b$ are the molar concentrations of free and bound manganese ions, respectively, $[PMA]_f$, the free residual molar concentration of PMA, and n, the number of binding sites per residue. Equation 1 can be rewritten as

$$\frac{Kd}{n}\frac{1}{[Mn]_f} + \frac{1}{n} = \frac{[PMA]}{[Mn]_b} \quad (2)$$

where $[PMA]$ is the total PMA concentration. $[Mn]_f$ was estimated from the ESR data ob-
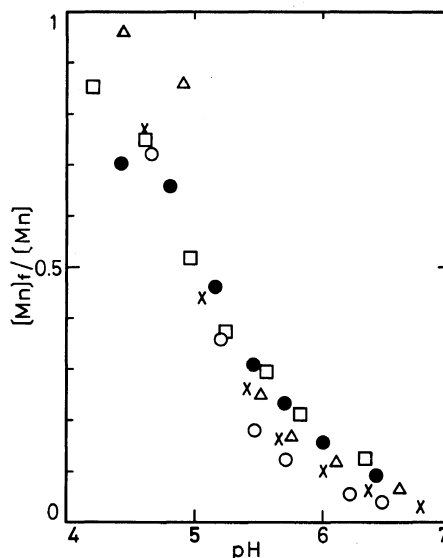
tained above, and $[Mn]_b = [Mn] - [Mn]_f$, where $[Mn]$ is the total Mn(II) concentration. Figure 2 shows a plot of $[PMA]/[Mn]_b$ against $1/[Mn]_f$ at a PMA concentration of 100 mM and pH 6.0. The slope and intercept give $n = 0.2$ and $Kd = 4 \times 10^{-3}$ M. These values indicate that there is one Mn(II) binding site per five methacrylic residues, and that the binding of Mn(II) to PMA is fairly strong.

Figure 3 shows the fraction $[Mn]_f/[Mn]$ of free manganese ions as a function of pH at a PMA concentration of 100 mM and at various Mn(II) concentrations. It can be seen that the fraction of free Mn(II) was found to increase with decreasing pH. We conclude that no binding of Mn(II) to PMA occurs below pH 4 and an almost complete binding occurs above pH 7. Thus, the PMA–Mn(II) complex formation is largely determined by electrostatic interaction.

REFERENCES

1. M. Mandel and J. C. Leyte, *J. Polym. Sci., A*, **2**, 2883 (1964).

2. M. Sunahara, M. Muto, T. Komatsu, and T. Nakagawa, *Nippon Kagaku Kaishi*, 2414 (1974).

3. G. H. Reed and M. Cohn, *J. Biol. Chem.*, **245**, 662 (1970).

4. T. R. Hughes and I. M. Klotz, "Methods of Biochemical Analysis," Vol. 3, D. Glick, Ed., Interscience Publisher, New York, N.Y., 1956, pp 265—299.

5. H. Jouve, H. Jouve, E. Melgar, and B. Lizarranga, *J. Biol. Chem.*, **250**, 6631 (1975).