

ADSORPTION OF PROTEIN ON NANOPARTICLES*

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ABSTRACT

The adsorption of protein on nanoparticles was studied by using dynamic light scattering to measure the hydrodynamic size of both pure protein and nanoparticles adsorbed with different amounts of protein. The thickness of the adsorbed protein layer increases as protein concentration, but decreases as the initial size of nanoparticles. After properly scaling the thickness with the initial diameter, we are able to fit all experimental data with a single master curve. Our experimental results suggest that the adsorbed proteins form a monolayer on the nanoparticle surface and the adsorbed protein molecules are attached to the particle surface at many points through a possible hydrogen-bonding. Our results also indicate that as protein concentration increases, the overall shape of the adsorbed protein molecule continuously changes from a flat layer on the particle surface to a stretched coil extended into water. During the change, the hydrodynamic volume of the adsorbed protein increases linearly with protein concentration.

INTRODUCTION

It has been a long time in practice to use polymer chains to produce an efficient interfacial layer for stabilizing colloidal dispersions or suspensions. This polymer layer on the particle surface can be introduced by adsorption or by grafting. In contrast to grafting where only one end group "bites" to the particle surface, adsorption involves all monomer segments which might attach to the particle surface by van der Waals force, hydrogen bond and other physical interactions. Frisch, Simha^[1] and Silberberg^[2] described the certain groups adsorbed along the chain as "train"; the segment between two adsorbed groups as "loop"; and the free end of the chain as "tail". Various experimental methods including dynamic light scattering have been used to study this adsorbed polymer layer on the particle surface^[3,4].

Due to its nontoxic property, natural polyelectrolytes, such as protein, are often used in food and pharmaceutical industry as stabilizers to prevent the aggregation of nanoparticles in dispersion or suspension. In the

formulation of vitamins and carotenoids, gelatin (a kind of protein) is normally used as stabilizer. However, the adsorption of gelatin on the particles has not been fully understood.

It is reasonable to assume that any change in the adsorbed gelatin conformation would be reflected in the hydrodynamic size of the particles. In this study, dynamic light scattering (DLS) as an absolute method was used to monitor the hydrodynamic size change of β -carotin particles as a function of the adsorbed gelatin concentration.

EXPERIMENTAL

Samples

A pharmaceutical grade gelatin (B200, $M_w \sim 3 \times 10^5$ g/mol and $M_w/M_n \sim 3$) was purchased from DGFS AG (Germany) and used without further purification. Three types of β -carotin nanoparticles with different initial diameters were prepared by a modified micronization method. The particles were first stabilized in water only by ascorbylpalmitate which acts as an emulsifier, and then mixed with a certain amount of gelatin. The adsorption of gelatin on the β -carotin particles is almost instantaneous. The hypothesis about this adsorption is that the amino acid groups on gelatin "bite" to the hydrophilic end of ascorbylpalmitate through two hydrogen bond. Fig. 1 shows a schematic presentation of the β -carotin particle and the adsorption hypothesis. It should be noted that the detail of adsorption is not relevant to our present study.

Dynamic light scattering (DLS)

The intensity - intensity time correlation function $G^{(2)}(t)$ in the self-beating mode was measured in our present experiment. If dispersion or suspension is dilute, the particle translational diffusion coefficient (D) can be calculated from $G^{(2)}(t)$. Further, D can be related to the hydrodynamic radius (r_h) by the Stokes-Einstein equation, $r_h = k_B T / (6\pi\eta D_{z,o})$, where k_B , T and η are the Boltzmann constant, the absolute temperature and the solvent viscosity, respectively. The detail of dynamic light scattering principle and instrumentation can be found elsewhere^[5,6].

In this study, a modern laser light scattering spectrometer (ALV/SP-76, Germany) was used. The light source is a He-Ne ion laser (spectra Physics 107s, operated at wavelength 632 nm and 30 mw). The state-of-the-art time correlator (ALV-5000, Germany) was used to measure $G^{(2)}(t)$

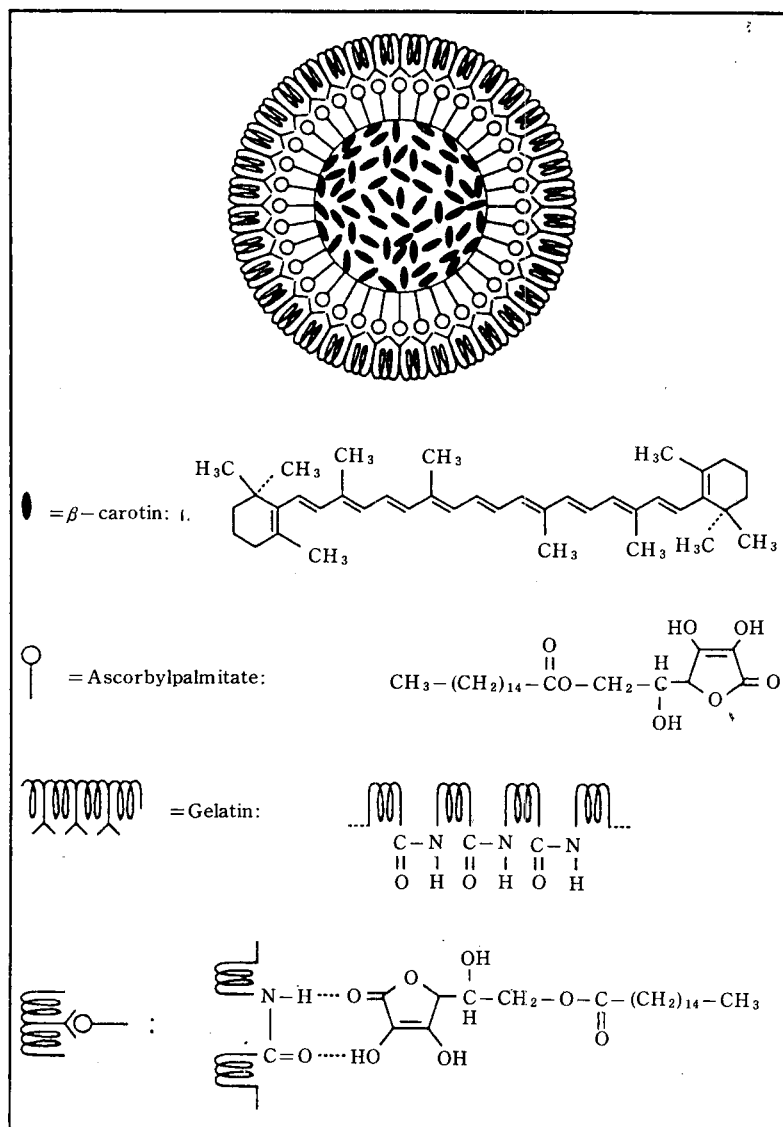


Fig. 1 Schematic presentation of β -carotin particle stabilized with ascorbylpalmitate and gelatin

at $T = 25.0 \pm 0.1^\circ\text{C}$ and different scattering angle.

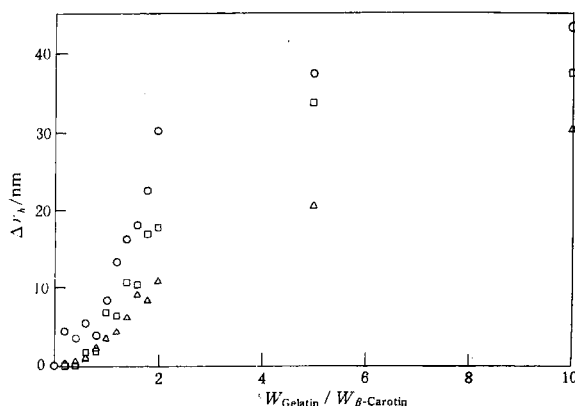
RESULTS AND DISCUSSION

The initial hydrodynamic radii ($r_{h,o}$) of primary β -carotin particles are 26 nm, 35 nm and 61 nm, respectively. The gelatin in water was first studied. It was found that water ($\text{pH} = 7$) is a good solvent for gelatin at $T = 25^\circ\text{C}$ and free (non-adsorbed) gelatin molecules in water at 25°C behave as flexible

coil with a hydrodynamic radius ($r_{h,free}$) of ~ 20 nm. The hydrodynamic thickness of the adsorbed gelatin layer on the particles is defined as the hydrodynamic radius difference (Δr_h) between the β -carotin nanoparticles with and without the adsorbed gelatin molecules.

Fig. 2 shows Δr_h versus the weight ratio of gelatin to β -carotin (W_g/W_β). It can be seen that Δr_h increases as W_g/W_β and, $r_{h,o}$. This is understandable because the surface density of the adsorbed gelatin should be a function of gelatin concentration and the particle surface area. Fig. 2 also shows that the increase of Δr_h slows down when $W_g/W_\beta > \sim 2$, which suggests that the particle surface is saturated with gelatin at this point.

Fig. 2 Typical plot of Δr_h versus W_g/W_β , where Δr_h is the hydrodynamic thickness of the adsorbed gelatin layer on β -carotin nanoparticles; and W_g/W_β , the relative gelatin concentration. "○" represents the primary β -carotin particles with $r_{h,o} = 61$ nm; "□", the primary particles with $r_{h,o} = 35$ nm; and "△", the primary particles with $r_{h,o} = 26$ nm.



The results in Fig. 2 can be quantitatively, or at least semi-quantitatively, represented by our following simplified adsorption model: for a given type of β -carotin nanoparticles with an initial average hydrodynamic radius $r_{h,o}$ the total initial particle surface area,

$$S_{\beta\text{-carotin}} = 4\pi r_{h,o}^2 \cdot n_\beta \quad (1)$$

where $4\pi r_{h,o}^2$ is the surface area of one particle and n_β is the number of β -carotin particles. Further,

$$n_\beta = W_\beta / w_\beta = W_\beta / \left(\frac{4}{3} \pi r_{h,o}^3 \rho \right) \quad (2)$$

where W_β is the total weight of β -carotin, $w_\beta (= \frac{4}{3} \pi r_{h,o}^3 \rho)$ is the average weight of one β -carotin particle, and ρ is the particle density.

A combination of Eqs 1 and 2 leads,

$$S_{\beta\text{-carotin}} = 3W_\beta / (\rho r_{h,o}) \quad (3)$$

For a given W_g , the number of gelatin molecules,

$$n_g = N_A \cdot (W_g / M_g) \quad (4)$$

where M_g is the molecular weight of gelatin and N_A is Avogadro's number. After a combination of Eqs 3 and 4, the average surface area (s_g) occupied by one gelatin molecule adsorbed on β -carotin particle surface is

$$s_g = \frac{s_\beta}{n_g} = \frac{3M_g}{\rho N_A r_{h,o}} (W_\beta / W_g) \quad (5)$$

where we have assumed that all gelatin molecules were adsorbed on the particle surface.

Further, as shown in Fig. 3 if considering each adsorbed gelatin chain occupied an average surface area (s_g) on the particle surface and located in a half corn with an inner radius of $r_{h,o}$ and an outer radius of $(r_{h,o} + \Delta r_h)$, we can calculate the hydrodynamic volume per adsorbed gelatin molecule (v_g) as

$$v_g = \int_{r_{h,o}}^{r_{h,o} + \Delta r_h} s dr = \frac{3M_g}{\rho N_A} \cdot \frac{W_\beta}{W_g} \cdot f\left(\frac{\Delta r_h}{r_{h,o}}\right) \quad (6)$$

where s is the surface occupied by one gelatin molecules at a distance r from the particle center, $s/s_g = (r/r_{h,o})^2$, and

$$f\left(\frac{\Delta r_h}{r_{h,o}}\right) = \left(\frac{\Delta r_h}{r_{h,o}}\right) + \left(\frac{\Delta r_h}{r_{h,o}}\right)^2 + \frac{1}{3} \left(\frac{\Delta r_h}{r_{h,o}}\right)^3 \quad (7)$$

Later we will show that $f(\Delta r_h/r_{h,o})$ is a function of W_β/W_g , too.

Fig. 4 shows a plot of v_g versus W_g/W_β , where we have used the known values of $M_g \sim 3 \times 10^5$ g/mol and $\rho \sim 1$ g/cm³, Δr_h and $r_{h,o}$ to calculate v_g on the basis of Eqs 6 and 7. Fig. 4 shows that v_g versus W_g/W_β can be represented by a straight line

$$v_g (\text{cm}^3) = 2.58 \times 10^{-19} (W_g/W_\beta)^{1 \pm 0.2} \quad (8)$$

which is a least-square fitting result.

One apparent problem is in this empirical equation, i. e., v_g is a linear function of W_g/W_β , instead of W_β/W_g as expected in Eq 6. For solving this mystery, we pictured one adsorbed gelatin chain consists many "loop"s and

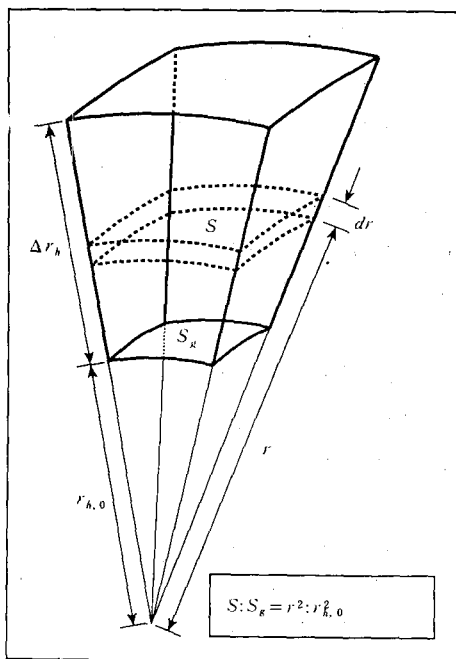


Fig. 3 Schematic presentation of the hydrodynamic volume occupied by each gelatin molecule on the β -carotin nanoparticle surface, where $r_{h,o}$ is the initial hydrodynamic radius of the particle; Δr_h , the hydrodynamic thickness of the adsorbed gelatin layer; and s_g , the surface area occupied by each gelatin molecule

the number of "loop" is n_{loop} . For the first approximation, we assume that every "loop" has the same average hydrodynamic volume (v_{loop}). The two ends of each "loop" are adsorbed on the particle surface and the loop itself is extended into water to form a random coil. According to this picture,

$$v_g = v_{loop} \cdot n_{loop} \quad (9)$$

Further, if assuming that the

"loop" has an average hydrodynamic radius of $r_{h,loop}$, which leads to an average hydrodynamic volume

$$v_{loop} = \frac{4}{3} \pi r_{h,loop}^3 \quad (10)$$

As for a random coil in good solvent, $r_{h,free}$ and $r_{h,loop}$ can be scaled to their weights M_g and M_{loop} respectively, as

$$r_{h,free} = k \cdot M_g^\alpha \quad \text{and} \quad r_{h,loop} = k \cdot M_{loop}^\alpha \quad (11)$$

Where k and α are two scaling constants and $M_{loop} = M_g / n_{loop}$. In theory, $0.5 < \alpha < 0.6$ for polymer coil and $\alpha = 1$ for rigid rod. By respectively replacing $r_{h,loop}$ and M_{loop} with $r_{h,free}$ and M_g ,

$$v_{loop} = \frac{4}{3} \pi r_{h,free}^3 / n_{loop}^{3\alpha} \quad (12)$$

Further, by a combination of Eqs 9 and 12, we have

$$v_g = \frac{4}{3} \pi r_{h,free}^3 n_{loop}^{1-3\alpha} \quad (13)$$

Where n_{loop} should equal to the adsorption points, which can be approximated by the average number of ascorbylpalmitate molecule on each gelatin molecule (see Fig. 1), i. e. ,

$$n_{loop} = (N_A W_a / M_a) / n_g = (W_a / W_g) \cdot (M_g / M_a) \quad (14)$$

where $M_a (= 414 \text{ g/mol})$ and W_a are the molecular weight and the total weight of ascorbylpalmitate, respectively. The adsorption is a dynamic process so that the attaching and releasing of the ends of the "loops" occurs constantly. However, the probability of all attached ends being released at the same time will be extremely small, which means that it is very difficult

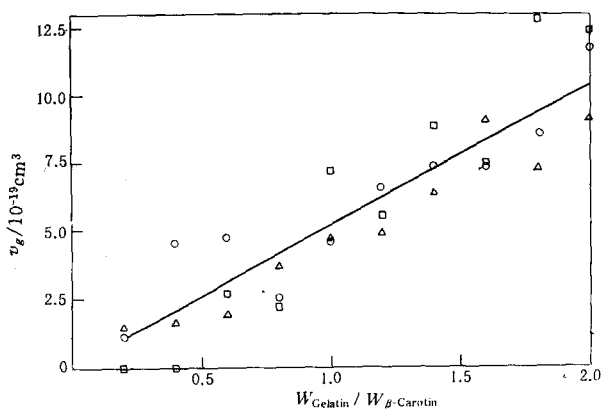


Fig. 4 Typical plot of average hydrodynamic volume (v_g) of each gelatin molecule adsorbed on the β -carotin particle surface versus W_g/W_β , where the symbols are the same as in Fig. 2

for an adsorbed gelatin chain to be completely “ free ” from the particle surface . As gelatin increases , the average attaching points per gelatin chain decreases and the length of the “loops” increases. On the base of Eqs 13 and 14, we have

$$v_g = \frac{4}{3} \pi r_{h,free}^3 (M_a/M_g)^{3\alpha-1} (W_\beta/W_a)^{3\alpha-1} (W_g/W_\beta)^{3\alpha-1} \quad (15)$$

Where $r_{h,free} \sim 20$ nm, $(W_\beta/W_a) \sim 10$, and W_β is introduced as a reference. After comparing Eqs 8 and 15, we found that α should be $(2 \pm 0.2)/3$ which is slightly higher than the predicted value (0.6) for a linear, flexible polymer chain in good solvent. But, this value is reasonable if we consider the two following facts. First, the “molecular weight” of the “loop” segment is lower than 10^4 g/mol so that the chain in the “loop” is short and less flexible. Second, the “loop”s are so closely packed that the interaction among the “loop”s should be much stronger than those in dilute solution. Thus, the segment movement is restricted, which leads to a more rigid chain. It is known that α for a less flexible chain is normally larger than that for a more flexible chain. After putting $\alpha = (2 \pm 0.2)/3$ and the other parameters in Eq 15, we obtain

$$v_g = 4.62 \times 10^{-19} (W_g/W_\beta) \quad (16)$$

It is encouraging to find that on the base of our very simple model the calculated coefficient in Eq 15 and the experimentally obtained coefficient in Eq 8 are in the same order of $\sim 10^{-19}$. It should be noted that in our model, the effect of the two ends of each “loop” attached on the surface has not been taken into the calculation and gelatin might not be completely on the particle surface. These effects will in general reduce the hydrodynamic volume even though we cannot quantitatively predict how much this reduction will be at this moment, which explains, at least partially, why the experimentally determined coefficient in Eq 7 is smaller than the calculated one in Eq 14. A combination of Eqs 6 and 8 leads to

$$f(\Delta r_h/r_{h,o}) = 1.73 \times 10^{-1} (W_g/W_\beta)^{2 \pm 0.2} \quad (17)$$

It shows that if plotting $f(\Delta r_h/r_{h,o})$ instead of Δr_h in Fig. 2 we are able to fit the experimental results with a single master curve.

As expected, Fig. 5 shows that all experimental data follow a single curve within the experimental uncertainties. It enables us to predict the hydrodynamic thickness of the adsorbed gelatin layer for a given gelatin concentration and β -carotin dispersion in practice.

In conclusion, DLS has been successfully used to study the adsorption

of gelatin on β -carotin particles. Our results shows that the thickness of the adsorbed gelatin layer on particles can be properly scaled by the initial radius of β -carotin particle. Further studies should develop an improved model based on statistics, wherein we have to consider the effects of the attached end groups, the difference between different "loop"s, and the chain conformation within the "loop".

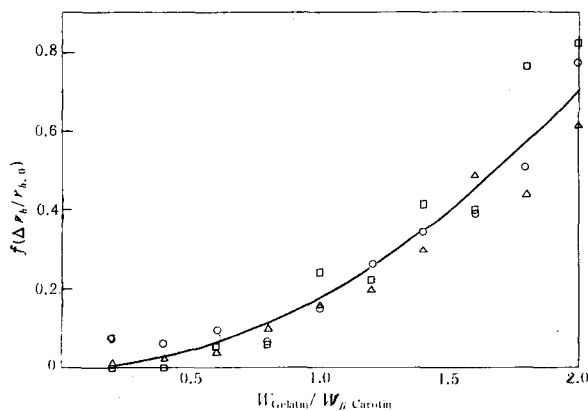


Fig. 5 Typical plot of $f(\Delta r_h/r_{h,0})$ versus W_g/W_β , where and the symbols are identical as in Fig. 2

Keywords:

Adsorption; Dynamic light scattering; Hydrodynamic radius; Gelatin; β -carotin nanoparticles; Conformation on surface

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