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Influence of Surface-Active Stabilizers on Porphyrin – Gold Nanoparticles Absorption and Fluorescence

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Systems consisting of (HP) hematoporphyrin- and (PVP) polyvinylpyrrolidone-coated gold nanoparticles (GNP) that proved their efficiency as photosensitizer in in vitro experiments were studied by spectral-photometric and spectral-fluorescent methods. The spectral manifestation of the interaction between HP- and PVP-coated nanoparticles mainly corresponds to the PVP-HP interaction. The optical absorption and fluorescence spectra of the HP-PVP solutions in acidic, neutral, and basic media were investigated. The spectral shifts and changes in the luminescence intensity corresponding to the binding of HP to PVP were measured. PVP was found to reduce the aggregation of HP, and the efficiency of this process increases with pH.

Keywords Aggregation; gold nanoparticles; hematoporphyrin; polyvinylpyrrolidone

1. Introduction

The therapeutic use of photosensitizing drugs in photodynamic therapy (PDT) is based on the photoexcitation of a drug that induces the generation of reactive singlet oxygen (SO) which damages, in turn, surrounding biological structures. But the practical realization of this concept meets several obstacles. Among them, we mention a low transparency of tissue at excitation light wavelengths, which requires extremely sensitive drugs, and the insufficient accumulation of a photosensitizer in tumor. An improvement of the photodynamic efficiency of drugs can be achieved by adding polymers to photosensitizing molecules [1,2]. Particularly, a combination of chlorin e6 with a water-soluble non-toxic polymer polyvinylpyrrolidone (PVP) [3] was shown to be successfully used in PDT [1,2,4]. At the same time, PVP-coated gold nanoparticles (GNP) proved their efficiency in complex

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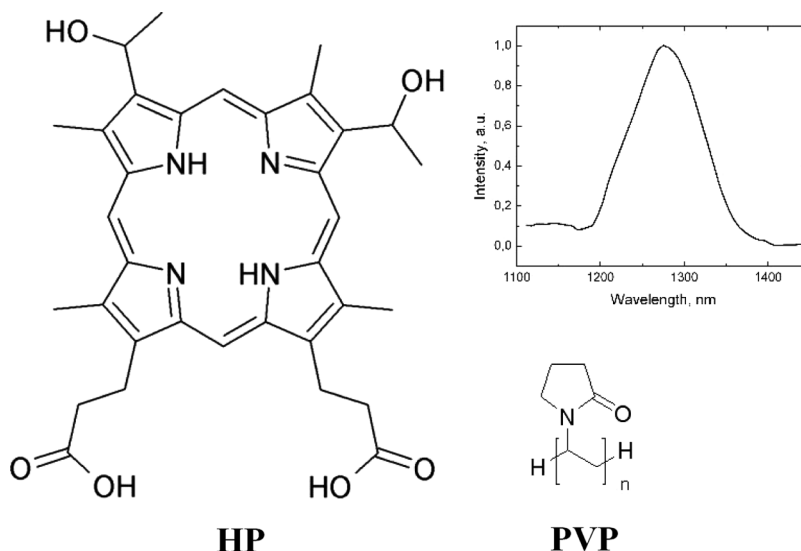


Figure 1. Structure of PVP and HP. The insert shows SO emission spectra from HP dissolved (5×10^{-5} M) in ethanol under excitation at 405 nm.

with hematoporphyrin *in vitro* [5]. Thus, the study of interactions in the porphyrin-PVP and porphyrin-PVP-GNP systems is important for understanding the processes occurring in PDT, as well as for the development of new photosensitizing systems.

Hematoporphyrin (HP) (Fig. 1) as one of the most studied and widely used photosensitizers for PDT was chosen for the current investigation [6,7]. The aggregation and the ionization of HP are pH-dependent and strongly determine the photoactivity of HP [8–10]. At the same time, a pH value of tumor tissue is lower compared to that of normal tissue. In the present work, the qualitative analysis of the HP-PVP interaction is made by spectral-photometric and spectral-fluorescent methods.

2. Experimental

Hematoporphyrin (powder), PVP (100 mg dissolved in 7 g of ethanol), and PVP-coated GNP (15 nm in diameter; 36 mg GNP and 100 mg PVP dissolved in 7 g of ethanol) were received from R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology (Kyiv, Ukraine). A stock solution of HP (concentration 2 mM) was prepared in DMFA. Absorption spectra were measured on a Specord M 40 spectrophotometer (Carl Zeiss, Germany). Fluorescence spectra were measured on a Cary Eclipse fluorescence spectrophotometer (Varian, Australia). To check up the ability of the used HP dissolved in ethanol to generate SO, its characteristic luminescence band at $1.27 \mu\text{m}$ (see the insert in Fig. 1) was recorded on a home-made IR spectrometer equipped with an InGaAs photodiode (EOS Inc.) from a 5×10^{-5} M HP ethanol solution under the laser diode (EOC Inc.) 60-mW excitation at 405 nm. All measurements were carried out at room temperature.

3. Results and Discussion

3.1. HP Aggregation and PVP-Coated GNP-HP Interaction

The study of the absorption spectra of HP water solutions (Fig. 2) reveals the variation of the spectrum with increase in the HP concentration, depending on the pH value. Namely, for pH 11.4, the spectrum shape and the maximum position remain essentially the same. For pH 7.8, an increase of the HP concentration leads to the appearance of a shoulder on the short-wavelength side of the Soret band (Fig. 2b). For pH 3.1, the short-wavelength band is clearly distinguished at high concentrations, while it dominates in the spectrum for pH 5.75. Since this short-wavelength band appears at increasing the dye concentration, it obviously corresponds to HP aggregates, which is also supported by literature [9]. We believe that, due to the fact that HP has carboxylic groups ionized at high pH values [11], HP is mainly in the monomeric state in basic water.

The fluorescence emission spectra of HP water solution, as well as those of HP, in the presence of the PVP and PVP-GNP systems are presented in Figure 3. It is seen that the addition of PVP results in noticeable changes in the HP fluorescence spectrum, which is related to the HP-PVP interaction. At the same time, at the addition of GNP stabilized by PVP, the same position of fluorescence maxima is observed as that for HP in the presence of PVP. We can suppose that HP binds with PVP-coated gold nanoparticles mainly through the interaction with the stabilizer, PVP. Meanwhile, the fluorescence intensity of HP in the presence of GNP-PVP is lower than that of HP with only PVP added (Fig. 3). This seems to be due to 1) the inner filter effect caused by the GNP absorption and 2) a lower concentration of PVP groups accessible to HP molecules, since PVP is also bound to GNP.

To shed light on the interaction of HP with PVP-coated GNP, we studied the spectral properties of HP in the presence of PVP in more details.

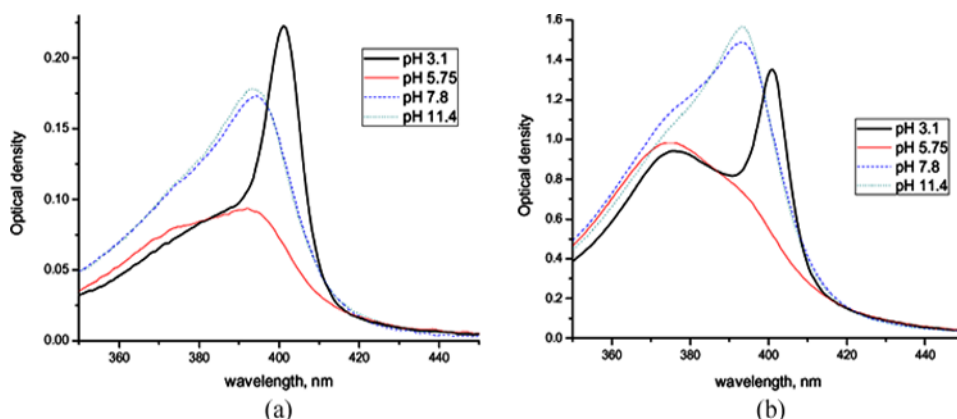


Figure 2. Absorption spectra of HP at various pH values. HP concentrations are 10^{-6} M (a) and 10^{-5} M (b).

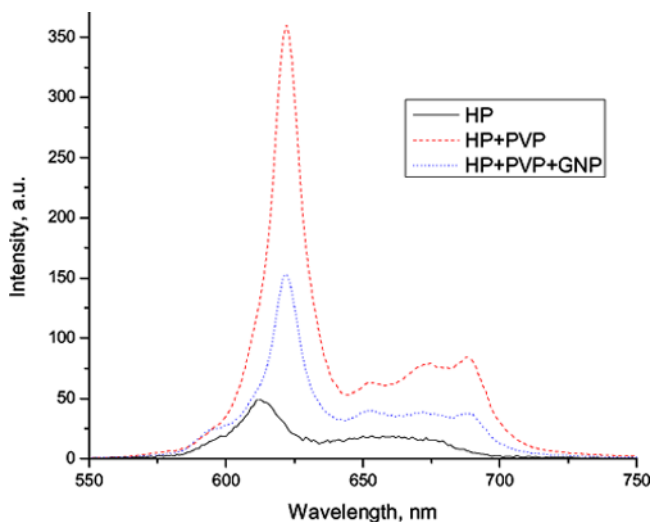


Figure 3. Fluorescence spectra of HP (10^{-5} M) (solid line), HP (10^{-5} M) + PVP (0.25 mM) (dashed line), and HP (10^{-5} M) + PVP (0.25 mM)-GNP(13 μ g/ml) (dotted line).

3.2. Changes in Absorption Spectra

All HP – PVP absorption spectra were measured at an HP concentration of 10^{-5} M.

At pH 11.4, the addition of PVP to a HP solution causes the following changes in HP absorption spectrum: the red shift of the Soret band (from 393 to 396 nm), Q II (from 560 to 566 nm), and Q I (from 610 to 620 nm), as well as the blue shift for Q IV (from 503 to 499 nm) and Q III (from 537 to 534 nm) bands (Fig. 4a). Since HP molecules do not aggregate in basic media, and the shapes of HP spectra in the presence of PVP are close to these of free HP, we believe that these changes correspond to the binding of monomer HP molecules to PVP.

The study of HP absorption spectra at various concentrations (Fig. 2) demonstrated that, at pH 5.75, the Soret band of free HP consists of two bands: the band with a maximum at 375 nm corresponding to aggregated HP molecules and another one at 393 nm corresponding to monomers (at the current concentration, these bands could not be distinguished). At the same time, the absorption spectrum of HP in the presence of PVP at pH 5.75 is close to that at pH 11.4 (Fig. 4b). Thus, the addition of PVP to a HP solution at pH 5.75 causes the destruction of HP aggregates and the binding of HP monomers to PVP.

At pH 3.1, nitrogen atoms at the center of a molecule are protonated [12], which causes changes in the HP absorption spectrum. Namely, the Soret band corresponding to the absorption of aggregates has its maximum at 375 nm, while that of monomers is at 401 nm. In addition, there is only one Q band at 549 nm (its position does not depend on the HP concentration). The addition of PVP causes the strong disaggregation effect that is manifested in a decrease of the 375-nm band. In addition, the Q band extinction decreases, and two new bands which are close to Q II and Q III bands seem to appear on its sides. At the same time, a new Q' band appears at 502 nm that corresponds to the Q IV band (Fig. 4d). It could be supposed that the appearance of the Q II, Q III, and Q IV bands corresponds to the deprotonation

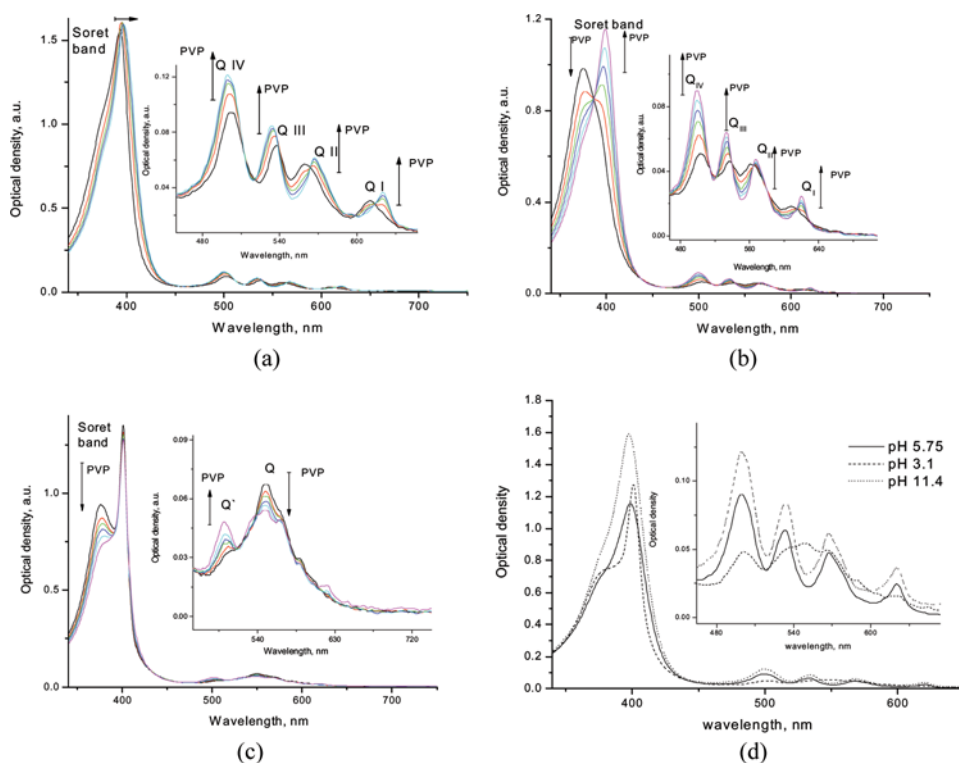


Figure 4. Absorption spectra of HP (10^{-5} M) in the non-bound state and at various PVP concentrations at (a) pH 11.4, (b) pH 5.75, (c) pH 3.1; and (d) the HP spectra at the maximum PVP concentration (2.25 μ M). Arrows indicate an increase in the PVP concentration.

of an HP molecule bound with PVP (it seems that the Q I band also appears but with a too low intensity to be detected surely). It should be mentioned that the binding to PVP in acidic water is less efficient, since, even at the highest PVP concentration used (2.25 μ M), there is still a shoulder corresponding to the Soret band of HP aggregates, and the Q-band is not completely decreased. Hence, at such PVP concentration, a significant part of HP molecules are not bound to PVP (Fig. 4c, d).

Thus, for all pH values, the studied HP is bound to PVP as monomers in aqueous solutions. In addition, the binding to PVP seems to cause the deprotonation of HP molecules at low pH values.

3.3. Changes in Fluorescence Spectra

The tendencies similar to those described above are observed in fluorescence spectra.

At pH 3.1, two bands in the emission spectrum of HP without PVP at 594 nm and 652 nm are observed. They correspond to the HP protonated form (Fig. 5c). At the same time, for other pH values, two bands at 613 nm and 672 nm related to the HP non-protonated form are manifested (Fig. 5a, b).

At the addition of PVP to a HP solution, two bands at 623 nm and 689 nm appear in the HP fluorescence spectrum (Fig. 5a–c). These bands are similar for all the pH values studied and obviously correspond to HP molecules bound to

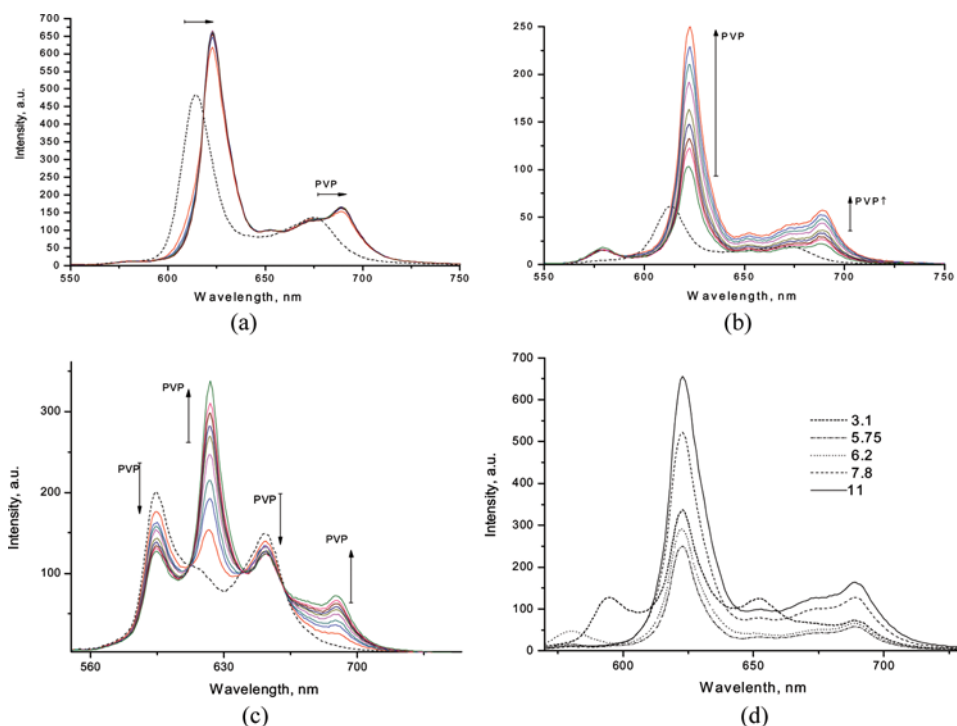


Figure 5. Fluorescence spectra of HP (1.5×10^{-6} M) in the non-bound state (dashed line) and at various PVP concentrations (solid line) at (a) pH 11.4, (b) pH 5.75, (c) pH 3.1; and (d) the HP spectra at the maximum PVP concentration ($2.25 \mu\text{M}$). Arrows indicate an increase in the PVP concentration.

PVP (Fig. 5d). This fact supports our assumption that the binding with PVP causes the deprotonation of bounded HP at low pH. At the same time, in acidic water, the bands corresponding to non-bounded HP are manifested as well (Fig. 5c). This is in agreement with the above-made conclusion about a less efficient HP-PVP binding at low pH values. The fluorescence spectra also were measured for pH values corresponding to those of living cells (pH = 7.8 that is close to 7.5 in normal tissues, and pH = 6.2 that corresponds to lower pH in malignant tissues), but the transformation of spectra under the PVP addition was the same (Fig. 5d).

Fluorescence spectra give us a good idea concerning the disaggregation effect. At high pH values where HP is mainly in the monomeric state, the integral luminescence signal at the PVP addition increases only by 1.15 times. At the same time, at pH 5.75, the integral luminescence signal increases by 2.83 times, and the peak intensity increases by 4.15 times, which indicates the disruption of non-fluorescent aggregates. At pH 3.1, the integral luminescence signal increases by 1.21 times. There is also the disruption of HP aggregates, but this process is not so intense in acidic media.

4. Conclusions

1. The spectral manifestations of the interaction between HP and PVP coated gold nanoparticles correspond mainly to the PVP-HP interaction.

2. In aqueous media, HP molecules interact with PVP as monomers, destructing the HP aggregates present in a solution. This fact is positive for using PVP-HP as a drug.
3. At low pH values where HP molecules are protonated, the binding with PVP leads to the HP deprotonation.

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