

UV IRRADIATED SiO₂ NANOPARTICLES AS INSULIN AND IMMUNOGLOBULIN MOLECULE CARRIERS

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Insulin and immunoglobulin therapy is commonly administered through injections that are invasive and painful. They cannot be taken orally because digestive enzymes break them down. To protect against enzymes, drug molecules may be “packed”, which decreases interaction of the active surface of the molecule with the environment. Silica nanoparticles (SiO₂) may be used to attach proteins like insulin or immunoglobulin for use in drug delivery. They are stable and biocompatible. Exposing SiO₂ nanoparticles to UV radiation may control molecule–nanoparticle attachment because of their electrostatic interactions. This study demonstrates the influence of UV radiation of SiO₂ nanoparticles on insulin and immunoglobulin molecule attachment. Alteration of the surface electric potential of SiO₂ nanoparticles by UV radiation was determined using electron work function measurements. Influence of radiation on formation of molecule–nanoparticle complexes was assessed by spectrophotometry of their sedimentation rate in solution. Increasing UV exposure time negatively charges the surfaces of SiO₂ nanoparticles. Insulin exhibits better adsorption with SiO₂ nanoparticles compared to immunoglobulin, likely due to favourable conditions promoting attractive electrostatic interactions. Longer UV exposure weakens insulin adsorption but does not significantly affect immunoglobulin adsorption.

Keywords: drug delivery, surface functionalization, electrostatic interactions, sedimentation, protein–nanoparticle adsorption.

INTRODUCTION

Insulin is an important hormone that regulates metabolism and promotes glucose uptake into cells. Insulin therapy for diabetes is currently done through subcutaneous injections, which have drawbacks such as being invasive and causing skin allergies. Oral insulin administration is advantageous as it follows the normal physiological route, reaching the liver first and then peripheral tissues.

Immunoglobulins are used by the immune system to identify and neutralise foreign bodies like bacteria and viruses. Currently there are methods for intravenous and subcutaneous administration of immunoglobulin to treat various immune deficiencies and autoimmune disorders. Orally administered immunoglobulin shows potential for gastrointestinal diseases, but has limitations regarding entry to the sys-

temic circulation, and therefore, oral administration of immunoglobulin is still not available as a full replacement for invasive administration methods (Jasion and Burnett, 2015).

Nanoparticles can adsorb drugs, protecting them from degradation and enabling controlled drug release and targeted delivery (Rangasamy *et al.*, 2010). Silica (SiO₂) nanoparticles have shown stability and biocompatibility in the gastrointestinal tract, making them attractive for biomedical applications and they are a promising option for drug delivery. Surface modifications of nanoparticles can enhance their ability to absorb protein-based drugs, making their delivery to a cell more effective.

One of the main driving forces for protein–nanoparticle adsorption is electrostatic force. Some amino acids of proteins have charged side chains, which contribute to the overall

charge distribution on the protein surface. The electrostatic attraction between positively charged amino acid residues in proteins and the negatively charged surface of a nanoparticle can promote adsorption of proteins onto the nanoparticle surface, resulting in better adsorption.

The pH, temperature, and composition of a solution in which proteins and nanoparticles are dispersed are other important parameters that affect the properties and behaviour of molecules, as well as have an influence on the net charge of the molecules. An isoelectric point has direct influence on the surface charge and its distribution on surfaces of the molecules and particles. If the pH of the solution is higher than the isoelectric point, the molecule will have more negative surface charge and vice versa. The chemical composition and the ionic strength of a solution may play a significant role. For instance, ionic strength influences the magnitude of electrostatic interactions between nanoparticles and proteins. At higher ionic strengths, the screening effect of ions reduces the strength of electrostatic interactions (Bharti *et al.*, 2014).

Adsorption of proteins onto nanoparticles can increase their effective size and density, leading to an increased sedimentation rate. This is because the protein layer adds mass to the nanoparticles, making them settle faster. Increase in sedimentation in turn affects the light absorption properties measured by spectrophotometry. Therefore, examining both sedimentation rates and absorption spectra over time allows to evaluate the degree of protein-nanoparticle adsorption and to gain valuable insights into the nature of their interactions (Dekhtyar *et al.*, 2014; Giorgi *et al.*, 2021).

Exposing SiO₂ nanoparticles to ultraviolet (UV) radiation, affecting the surface charge, may contribute to better protein-nanoparticle adsorption, as one of the main driving forces for it is electrostatic interaction (Bharti *et al.*, 2014). Since SiO₂ nanoparticles are dielectric, UV radiation charges the surface because of electron transitions within its energy gap. Several studies have found that proteins attach more actively to a nanoparticle if their surface charge is negative, compared to an uncharged nanoparticle (Aramesh *et al.*, 2015; Sathyamoorthy *et al.*, 2017). However, it is unknown if SiO₂ charged nanoparticles can attach insulin and immunoglobulin molecules.

The purpose of the present study was to demonstrate effect of UV radiation on ability of SiO₂ nanoparticles to attach insulin and immunoglobulin molecules.

MATERIALS AND METHODS

Insulin and immunoglobulin. Both insulin and immunoglobulin in lyophilised (freeze-fried) form were purchased from Sigma-Aldrich, USA. The prepared solution can be stored in aliquots (in small vials of the prepared medicine) at a temperature of 2–8 °C for up to a month and –20 °C for long-term storage. Both products had data sheets containing

descriptions of the protein characteristics and recommendations for storage and preparation.

The recombinant insulin was expressed in yeast and suitable for human treatment. The jar contained 50 mg of the product. Its formula weight was 5807.57 g/mol and was intended for concentrations up to 20 mg/ml. The recommended buffer solution was 0.01 M HCl. The isoelectric point for insulin was pH value 5.3.

Immunoglobulin was in the form of γ -globulin from bovine blood. The jar contained 5 g of the product. Its molecular weight was 158,000 g/mol and was intended for concentrations up to 50 mg/ml. The recommended buffer solution was 0.9% sodium chloride (NaCl). The isoelectric point for immunoglobulin was pH value 7.2.

The proteins were stored in aliquots at –20 °C or in the refrigerator (+4 – +8 °C) during the 1–2-week experiment period.

SiO₂ nanoparticles. SiO₂ nanoparticles were obtained from HWNanoMaterials, China. They were in the form of a brown powder consisting of spherical nanoparticles with a diameter of 30–50 nm and molar mass 231.5 g/mol.

Solution preparations. Protein dilution in bidistilled water was performed directly in the vial using precise pipettes and the recommended buffer solution. The diluted protein solutions were then transferred to a 50 ml vial with a sealed cap.

The concentration of the solution was adjusted to a level that allows the spectrophotometer to determine light absorption spectra while providing sufficient volume for repeated measurements.

As buffer solution for insulin 0.01 M HCl was used, according to manufacturer recommendations. For this, 37% HCl was taken and dissolved in bidistilled water, 0.83 ml of HCl per 1000 ml of distilled water.

Insulin was briefly centrifuged; 3 ml of buffer was added to the jar, and it was pipetted. The concentration of the solution according to the data sheet was 20 mg/ml, and since the total insulin amount was 50 mg, the first portion of dissolution was not be less than 2.5 ml. The solution was then transferred to a 50 ml test tube and filled with HCl buffer. The resulting concentration was 1 mg/ml. Then the prepared insulin solution was further diluted to obtain a suitable concentration for spectrophotometry measurements. The typical concentration of insulin for therapeutic use is 10 units (U-10), that is, 0.35 mg/ml.

For nanoparticle solutions, the concentrations of components were calculated based on the protein solution's concentration and molar mass to achieve an approximate one-to-one ratio of nanoparticles to protein molecules. To determine the desired concentration of the protein solution, spectrophotometry measurements were conducted. Various concentrations were compared, aiming for a recognisable light absorption spectrum. Knowing the concentration of

protein solution and the molar weight relation to the molar weight of SiO_2 , the concentration of the nanoparticle solution may be calculated as follows form:

$$C_{\text{SiO}_2} = M_{\text{SiO}_2} / M_{\text{prot}} \cdot C_{\text{prot}}, \quad (1),$$

where C – resulting concentration and M – molar weight of the substance.

Preparation of nanoparticles. Because the nanoparticles were delivered as agglomerates, they were crushed and evenly distributed in solution using sonication by mechanical vibration with an ultrasonic probe. Ultrasonic cavitation generates strong shear forces that can disperse particle agglomerates, leading to more stable and uniform nanoparticle suspensions. A Hielscher Ultrasonics UP200St, Germany probe-type ultrasonic sonicator was used. Visual inspection with a flashlight confirmed homogeneous mixing by even light dispersion. The temperature of the solutions was checked before each measurement was set using a thermocouple thermometer. pH values were also recorded for each solution mixture.

Temperature and pH. The temperature of the solutions was checked before each measurement using a thermocouple thermometer Testo 625, Germany. pH values were measured using a PCE Instruments PE-03, Germany.

Both temperature and pH were measured by immersing probes into solutions at each step of the experiment: for nanoparticles — before and after ultrasonication, for proteins — before and after mixing with nanoparticles. As the pH probe was too large to fit inside 2 ml cuvettes for spectrophotometer, measurements were made in the solutions after mixings of proteins and nanoparticles. Proteins solutions were stored in a refrigerator at $+4^\circ - +8^\circ \text{C}$. The buffer solutions were at room temperature, while the nanoparticle solutions were heated due to the sonication process. During the experiments, the temperature in the laboratory was maintained at $+20^\circ - +22^\circ \text{C}$.

Determination of surface charge. To determine changes in surface charge, the electron work function (EWF) of electrons emitting under UV light can be measured. The EWF is the minimum amount of energy required for an electron to escape from a solid. As electrical charge density changes on the surface of the emitter its EWF is affected.

The work function can be determined based on the obtained data from photoemission spectroscopy and calculated as follows (2):

$$\frac{Y}{dY \frac{dY}{dh\nu}} = \frac{1}{m} h\nu - \frac{\phi}{m}, \quad (2)$$

Y – quantum yield, the ratio between the amount of emitted quanta to the amount of absorbed.

ϕ – photoelectric work function.

$h\nu$ – photon energy.

m – constant, dependent on the material structure.

Equation (2) shows that the function $\frac{Y}{dY \frac{dY}{dh\nu}}$ in dependence of $h\nu$ is a linear function, such that the crossing point with the $h\nu$ axis will give the work function value ($h\nu=\phi$).

Monochromatic light in the mid-UV range (200–277 nm, 4.5–6.2 eV) was used to excite photoelectron emission. An exoelectron emission spectrometer, constructed in Riga Technical University, Latvia (Zakaria *et al.*, 2006), was employed to determine the electron work function of irradiated nanoparticles.

UV irradiation. A Hamamatsu Lightning Cure LC8, Japan LC8 UV light source with a L10852 -01A type lamp with maximal irradiance 4500 mW/cm^2 at 365 nm at output of the source was used for irradiation of the nanoparticles. The distance from the source to the samples was maintained at 20 cm. Treatments used were nanoparticles with no exposure, 30-minute and 60-minute exposures before mixing with proteins. Thickness of the nanoparticle layer that was irradiated was around 0.5 mm.

Taking into consideration the irradiance of a L10852-01A type lamp at various wavelengths (only pikes with relative irradiation higher than 5 % are considered), the surface dose on SiO_2 nanoparticle samples was calculated as:

$$\text{surface dose} = \sum_{i=1}^N \frac{E_e}{\sum_{i=1}^N E_{ri}} \cdot E_{ri} \cdot t \quad (3),$$

where E_e is the total irradiance on the sample surface at a distance of 20 cm from the light source (1020 mW/cm^2), E_{ri} – relative irradiance toward the pike at 365 nm, t – exposure time.

Determination of attachment of molecules to nanoparticles. Spectrophotometry was used to identify sedimentation of particles in solution, as it estimate protein-nanoparticle adsorption by tracking changes in light absorption over time. Protein-nanoparticle complexes tend to sediment faster than individual proteins or nanoparticles. A Thermo Spectronic Helios Gamma, United Kingdom spectrometer was used for sedimentation measurements using RATE mode. These measurements were performed in duration of 1 hour with 5-minute steps, immediately after sonication, mixing, and pipetting of the solutions into cuvettes. Spectrophotometry measured the absorption of light in a solution contained in small 2 ml cuvettes. Each set of measurements was preceded by a baseline recording of a reference spectrum of pure buffer without proteins or nanoparticles, for comparison with mixed solutions.

For each protein, spectrophotometric measurements were performed in 5 sets of measurements — protein solution without nanoparticles; protein-free nanoparticles; mixture of proteins and non-irradiated nanoparticles; mixture with nanoparticles that were irradiated.

Mixing, sonication, and pipetting were performed immediately prior to spectrophotometer measurements so that sedi-

mentation could be observed. Mixing of the prepared protein solution with the sonicated nanoparticle solution was performed directly in the cuvettes by the pipetting method.

All measurements were made in the visible spectrum (400–900 nm) with 0.5 nm steps. A new, clean cuvette was used for each measurement.

Sedimentation rates were measured at one specific wavelength for each protein, determined experimentally during test spectrophotometry measurements of protein solutions. Spectrophotometry measurements were conducted both before and after sedimentation, enabling identification of the wavelength where the difference between the two measurements was most pronounced. This method was employed to pinpoint the specific wavelength that exhibited the greatest sensitivity to alterations in protein concentration induced by sedimentation, thus aiming to enhance the precision and reliability of sedimentation rates measurements of each protein.

After data acquisition for sedimentation, spectrophotometric absorption measurements were performed once again to compare the spectra at different times.

RESULTS

Concentrations of protein and nanoparticle solutions.

Samples of insulin solutions with concentrations of 0.35 and 0.5 mg/ml were compared. 1 mg/ml insulin was added to nanoparticle solution in a 1:1 ratio, diluting the insulin to 0.5 mg/ml.

The absorption spectrum for 0.5 mg/ml solution was more pronounced and had a higher absorbance value, which was used for further calculations and measurements. The maximum absorption was 0.0436 a.u. at 405.5 nm. Since the concentration was chosen to be 0.5 mg/ml, calculation for SiO₂ particles required to mix with insulin was performed, according to formula (1). The resulting concentration of SiO₂ solution for insulin was 0.993 mg per 50 ml, as the solutions were stored in 50 ml test tubes. According to the calculations and given that the weight can be measured with an accuracy of 0.1 mg, each set of measurements was made for 1 mg of nanoparticles. This was done on pieces of paper in a plastic container with a closable lid, such that the nanoparticles could be transported to the UV lamp and photoelectron spectrometer without losing a single particle. After photoemission measurements of nanoparticles, the SiO₂ was mixed with buffer solution and sonicated.

As for immunoglobulin, the concentration used was 50 mg/ml and during mixing with nanoparticles solution it was diluted to 25 mg/ml, and compared with 17.5 mg/ml solution. 17.5 mg/ml had a recognisable light absorption spectrum, so thus was chosen for the remaining measurements. The maximum absorption was 0.0352 a.u. at 401.5 nm. According to formula (1), the resulting concentration of nanoparticles needed for mixing was 1.279 mg per 50 ml. 1.3 mg

of nanoparticles were weighed for measurements and mixed with buffer solution.

UV surface and absorbed doses of SiO₂ nanoparticles.

Using formula (3), the UV surface dose for SiO₂ nanoparticles was calculated. For 30-minute exposure the total surface dose was 182.88 mJ/cm², and for 60-minute exposure — 365.76 mJ/cm².

UV light exposure influence on SiO₂ nanoparticles surface charge.

The results showed that the UV exposure caused a decrease in the EWF values of the nanoparticles. Figure 1 shows the change in EWF values, which was calculated by subtraction of EWF value after the exposure from the value acquired before the exposure. The 0-minute bar represents the control measurement — all values were within the error bands, meaning that the charge did not significantly change after time for non-exposed nanoparticles. The samples that were exposed to UV for 30 minutes and 60 minutes exhibited a shift of −0.044 eV (with a standard deviation of 0.031) and −0.097 eV (with a standard deviation of 0.035), respectively. These results indicate that the longer the exposure time to UV light, the greater the reduction in EWF. As a result, the EWF decreased, leading to higher electron yield and increased surface conductivity.

The results suggest that UV exposure leads to a decrease in electron work function values of SiO₂ nanoparticles, which is likely due to an increase in surface charge density, resulting in a more negative surface charge of SiO₂ nanoparticles. The *p*-values, calculated using a t-test comparing 30-minute exposure to non-exposed and 60-minute exposure to 30-minute exposure, were both ~4.8%, meaning that these results were reliable and repeatable.

Spectrophotometry measurements, sedimentation rates.

There were five measurement sets for spectrophotometry: pure insulin, pure SiO₂ nanoparticles, insulin + SiO₂, insulin + SiO₂ (30 minutes UV), and insulin + SiO₂ (60 minutes UV). Each set consisted of six measurements, as there were six free cells for cuvettes in a spectrophotometer to measure simultaneously. Sedimentation measurements were initially made for four hours with measurements every 30 minutes. After these measurements, it was concluded that the time frame for actual measurements may be selected as one hour with five-minute steps, to optimise the experimental process, as there were no significant changes in the linearity of sedimentation rate over a longer time.

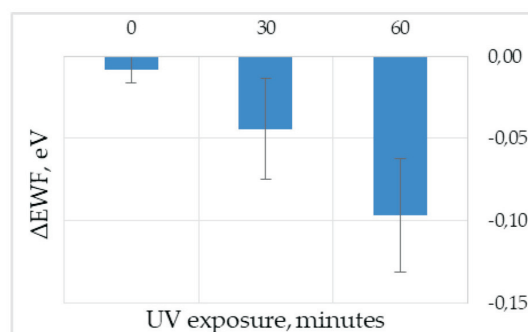


Fig. 1. EWF value change for SiO₂ nanoparticles after UV exposure.

The results of the measurements are shown in Figure 2 for insulin and Figure 3 for immunoglobulin, with mean values of sedimentation rates with error bars representing the standard deviation.

When proteins and nanoparticles are mixed, they form complexes through various interactions, including electrostatic, hydrophobic, and hydrogen bonding interactions. These complexes can be larger and heavier than individual particles, causing them to sediment more rapidly under the influence of gravity. Thus, an increase in sedimentation rate may suggest that more complexes have formed, indicating a higher level of protein-nanoparticle adsorption.

The results from Figure 2 showed that the sedimentation rate of insulin alone was 0.0002 a.u./hour, while that of SiO₂ nanoparticles alone was 0.0532 a.u./hour. When mixed, the rate increased significantly to 0.1134 a.u./hour, indicating that the insulin and nanoparticles were adsorbing onto each other. Exposing nanoparticles to UV light for 30 and 60 minutes gave sedimentation rates of these mixed solutions of 0.0428 and 0.0815 a.u./hour, respectively, which were lower than the rate of insulin mixed with non-exposed nanoparticles, meaning that UV exposure most likely hindered the protein adsorption of nanoparticles. It is worth noting that the UV exposure for 30 minutes performed worse than 60-minute exposure. A t-test showed significance of only exposure of 30 minutes, while sedimentation with 60-minute UV exposure was insignificant comparing to non-exposed SiO₂ particles.

Immunoglobulin had a sedimentation rate of 0.0055 a.u. per hour, while the SiO₂ nanoparticles had a much higher rate of 0.1743 a.u. per hour. When the two substances were mixed, the sedimentation rate was lower — 0.0417 a.u. per hour. This indicates that the immunoglobulin and SiO₂ nanoparticles did not adsorb to each other as well as insulin, perhaps due to repulsive forces. Exposure to UV gave sedimentation rate 0.0627 and 0.0499 a.u. per hour for 30 and 60 minutes respectively, which were within error bars. Also, a t-test shows insignificance of results comparing both 30- and 60-minute UV exposures to non-exposure results. Thus, in the case of immunoglobulin, UV exposure did not affect protein-nanoparticles adsorption.

Temperatures and pH measurements. Data on measured temperatures and pH values during experiment are summarised in Table 1.

DISCUSSION

The measured temperatures (Table 1) varied significantly due to differences in the storage, preparation, mixing, and measurement conditions of the substances. The measurements were conducted for at least an hour at room temperature in small 2 ml cuvettes, allowing the heat to dissipate and reach equilibrium with the environment (~20–22 °C). Therefore, temperature can be excluded as a factor influencing protein-nanoparticle adsorption in this experiment.

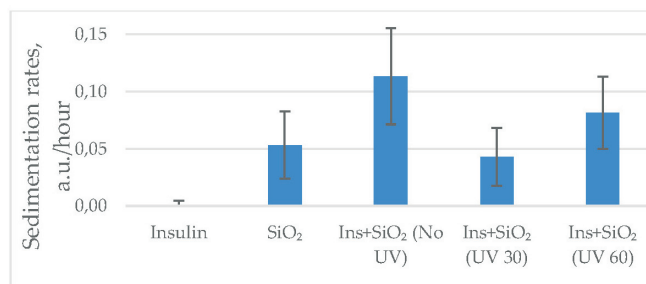


Fig. 2. Sedimentation rates, insulin.

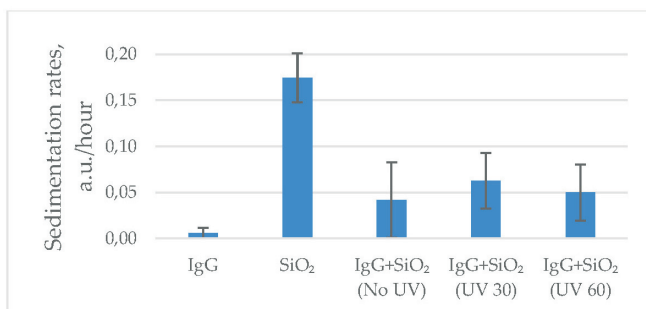


Fig. 3. Sedimentation rates, immunoglobulin.

Table 1. Temperatures and pH

	Insulin		Immunoglobulin	
	Temp., °C	pH	Temp., °C	pH
Buffer	22.0	2.02	22.0	9.29
Protein (test tube)	10.8	1.81	11.5	9.02
Protein (cuvette)	16.2	–	18.4	–
SiO ₂ sonication (test tube)	34.8	1.89	29.9	9.45
SiO ₂ sonication (cuvette)	29.4	–	21.8	–
Protein + SiO ₂ (cuvette)	20.5	–	22.0	–

The pH values of solutions were acidic for insulin and basic for immunoglobulin. Insulin solution and SiO₂ nanoparticles had positive charges due to their pH values being lower than their isoelectric points. This should have caused repulsion between insulin and SiO₂ nanoparticles; however, the measured pH of the buffer was close to the isoelectric point of SiO₂ nanoparticles, potentially resulting in a negative net charge on the nanoparticles. The negative charge on the nanoparticles may explain the increased protein-nanoparticle adsorption. Longer exposure time (60 minutes) resulted in more protein-nanoparticle adsorption, supporting the idea that increased negativity of the surface charge attracts more proteins.

In the case of immunoglobulin, based on the pH of the medium, both proteins and SiO₂ nanoparticles had a net negative charge, and thus they should repel. This may be the reason why the adsorption of mixed proteins with nanoparticles was lower than with nanoparticles alone. Also, this could explain why charging nanoparticles more negatively did not have effect on protein-nanoparticles adsorption.

The ionic strength of 0.01 M HCl, used for insulin, was relatively low — 0.01 M. The low ionic strength may result in

stronger electrostatic interactions between charged particles, such as the adsorption of insulin onto negatively charged SiO₂ nanoparticles. This was confirmed experimentally, as the addition of nanoparticles to the insulin solution significantly improved the sedimentation rate.

The ionic strength of 0.9% NaCl solution used for immunoglobulin was about 0.15 M. SiO₂ nanoparticles in a 0.9% NaCl solution can experience a reduction in their surface charge density due to the high ionic strength. This is because the salt ions in the solution can compete with the surface charges on nanoparticles, shielding them and reducing their effect. As a result, the nanoparticles may be less likely to adsorb proteins, as the reduced surface charge may not provide as strong an attractive force. This also complies with the experimental results, as the sedimentation rate of immunoglobulin with nanoparticles decreased in comparison with the sedimentation rate of SiO₂ nanoparticles alone.

Insulin was adsorbed better on non-exposed nanoparticles, compared to particles exposed to UV for 30 minutes, and 60-minute exposure increased the sedimentation rate, suggesting that an optimal surface charge balance is required for efficient protein adsorption.

CONCLUSIONS

The experiment investigated the interaction between proteins (insulin and immunoglobulin) and SiO₂ nanoparticles under varying UV exposure durations. For insulin, UV exposure hindered protein adsorption, with a decrease in sedimentation rates of approximately –62.23% (UV 30) and –28.10% (UV 60) compared to non-exposed nanoparticles. The pH values and surface charges played pivotal roles; despite the expected repulsion between insulin and SiO₂ due to their positive charges, the negative charge on SiO₂ facilitated increased adsorption. 60-minute exposure intensified this adsorption, implying effect of surface charge on protein binding. In contrast, immunoglobulin's adsorption with

SiO₂ was less affected by UV exposure, exhibiting changes within error margins (50.36% increase for UV 30 and 19.63% for UV 60). The negatively charged nature of both proteins and nanoparticles likely resulted in repulsion, explaining the decreased adsorption compared to nanoparticles alone. The ionic strengths of solutions also influenced adsorption, with low ionic strength favouring insulin-nanoparticle binding and higher ionic strength hindering immunoglobulin-nanoparticle adsorption. Achieving an optimal surface charge balance might be crucial for efficient protein adsorption onto nanoparticles.

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UV APSTAROTAS SiO₂ NANODAĻIŅAS KĀ INSULĪNA UN IMŪNGLOBULĪNA MOLEKULU NESĒJI

Insulīna un imūnglobulīna terapiju parasti veic ar injekcijām, kas ir invazīvas un sāpīgas. Tos nevar lietot perorāli, jo gremošanas enzīmi tos noārdīs. Lai aizsargātu pret enzīmiem, zāļu molekulas var tikt “iesaiņotas”, kas samazina molekulas aktīvo virsmu, kas mijiedarbojas ar vidi. Lai pievienotu olbaltumvielas nanodaļiņām, lai izmantotu zāļu ievadīšanai, var izmantot silīcija dioksīda nanodaļiņas (SiO₂). Tās ir stabilas un bioloģiski saderīgas. SiO₂ nanodaļiņu pakļaušana UV starojumam var kontrolēt molekulu–nanodaļiņu piesaisti to elektrostātiskās mijiedarbības dēļ. Rakstā tiek demonstrēta UV apstarotu SiO₂ nanodaļiņu ietekme uz insulīna un imūnglobulīna molekulu piesaisti. SiO₂ nanodaļiņu virsmas elektriskā potenciāla izmaiņas UV starojuma dēļ tika identificētas, izmantojot elektronu izejas darba mērījumus. UV starojuma ietekme uz molekulu–nanodaļiņu kompleksu veidošanos tika novērtēta, ņemot vērā to sedimentācijas ātrumu šķīdumā, izmantojot spektrofotometriju. Palielinot UV iedarbības laiku, SiO₂ nanodaļiņu virsmas tiek negatīvi uzlādētas. Insulīnam ir labāka adsorbējamība ar SiO₂ nanodaļiņām, salīdzinot ar imūnglobulīnu, iespējams, labvēlīgo apstākļu dēļ, kas veicina pievelkošu elektrostātisko mijiedarbību. Ilgāka UV iedarbība vājina insulīna adsorbējamību, bet būtiski neietekmē imūnglobulīna adsorbējamību.