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The Immobilization of Polyethylene Imine Nano and Microspheres on Glass Using High Intensity Ultrasound

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The present article describes the creation and immobilization of Polyethylene imine (PEI) capsules on a glass surface. The synthesis and deposition were accomplished by short-time (3 min) one-step reaction. The preparation and immobilization of PEI spheres, was carried out using an environmental friendly method, the ultrasonic emulsification. The ultrasonic technique enables to control size and fulfillment of the internal part of the immobilized PEI spheres. Moreover, the ultrasonic emulsification method showed 100% efficiency in PEI spheres creation, which means no residues of aqueous PEI and oil solvents remained in the reaction flask after the nanosphere's creation. The immobilized PEI spheres have sizes varied from 50 to 500 nm. The PEI spheres were successfully filled either with organic solvent (hydrophobic) or with water (hydrophilic). This method provides us the perspective for future encapsulation of various molecules which have hydrophobic or hydrophilic nature.

Introduction

The use of cationic polymers confers several advantages, due to the fact that they are durable, inexpensive,

easy to prepare, purify and chemically modify, as well as due to their enormous stability. Polyethylene imine (PEI) polymer is known as a very effective polymer for neutralization of excess anionic colloidal charge, especially under neutral pH conditions.¹ Modified PEI copolymers having relatively high molecular mass can be effective for drainage and pitch control.^{2,3}

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In the present study we have produced positively charged vehicles that will be easily adhered to glass surface. Partially charged PEI (protonated amine groups in aqueous medium) polymer is found to be an excellent choice for positively charged nanoparticles' (NPs) synthesis. The positively charged PEI NPs are easily immobilized on glass surface and thus avoiding aggregation of NPs by promoting electrostatic stabilization.

The PEI NPs creation and glass coating with PEI NPs were performed using high intensity ultrasound. In our previous studies we described the creation of proteinaceous, DNA and RNA microspheres by the ultrasonic emulsification method.⁴⁻⁶ Moreover, the use of ultrasonic waves for micro/nanocapsules deposition on textiles' surfaces and encapsulation of different molecules inside the microspheres was also described.⁷⁻⁹ Ultrasonication was used as an alternative mechanism to heat, pressure, light or electricity to initiate chemical reactions. Under sonication, the chemical reactions-generated products are different from the ones predicted by orbital symmetry rules.¹⁰

In the present work, for the first time, PEI nanospheres (NSs) were created using high intensity ultrasound and deposited on glass surface in a one-step process. The sonochemically formed PEI NSs and glass surfaces coated with PEI NSs were analyzed and characterized using different microscopic and spectroscopic techniques. The mechanism of PEI NSs formation and immobilization was studied and proposed. In addition, we succeeded to control the size and composition of immobilized PEI nanospheres. There are many types of PEI containing nanoparticles for drug delivery applications that were synthesized by scientists.¹¹⁻¹³ However, until now, there are no studies made on controlling the size and composition of pristine PEI spheres.

The coated glass with PEI NSs have a potential application as an antibacterial carrier surface where the rate of the quantities of released antibacterial agent could be controlled by modification of immobilization of PEI NSs.

Experimental Section

Sonochemical Preparation of Polyethylene Imine Nanospheres and Immobilization of Polyethylene Imine NSs on Glass Surface

All the ingredients were purchased in Sigma-Aldrich company (Sigma-Aldrich Israel, Rehovot, Israel). To

immobilize PEI nanospheres onto glass surface, soya bean oil (6.7 mL) was layered over 10 mL of a 5% w/v aqueous PEI (polyethylenimine polymer, Mw 13000) solution in the presence of a (5 × 5 cm.) glass slide.

The solution was sonicated for 3 min with a high-intensity ultrasonic probe (Sonic and Materials, VC-600, 20 kHz, 0.5 in a Ti horn, at 30% amplitude). The bottom of the high-intensity ultrasonic horn was positioned at the aqueous-organic interface (where the piece of glass slide was fixed), employing an acoustic power of ≈ 58 W/cm² with an initial temperature of 22°C in the reaction cell. An ice-cooling bath was used to keep the temperature constant. At the end of the reaction, the glass surface with immobilized PEI nanospheres was washed 3 times with 5 mL of doubly distilled water to remove the residue of the unattached nanospheres and the pristine polymeric molecules.

Coloring Test

For a "coloring test," Nile-red dye (Sigma-Aldrich) was used. The dye was added to the precursor mixture (1 μ g/mL) of the reaction, the immobilization of PEI nanospheres on glass surface using high intensity ultrasound. The resulting surfaces were then compared by using fluorescent and confocal microscopy.

Characterization Methods

The PEI spheres formed in the reaction cell (during sonochemical coating of glass surface with PEI NSs) were analyzed and characterized using SEM (scanning electron microscopy, JSM-840; JEOL, Tokyo, Japan), light microscopy (Apo-Tome microscope; Zeiss, Jena, Germany), and dynamic light scattering (DLS) measurements.

Dynamic light scattering measurements and z-potential measurements were carried out on an ALV/CGS-3 compact goniometer system equipped with an ALV/LSE-5003 light scattering electronic and a multiple s digital correlator, and a 632.8 nm JDSU laser 1145P. DLS and z-potential experiments were carried out on a doubly diluted as-separated NSs solution, that is the NSs removed after the sonication without the glass slide, were diluted with an equal amount of doubly distilled water (DDW). Each measurement took 10 s; particle distribution and electrical charge distribution were obtained by averaging ten DLS measurements.

For light microscopy (Apo-Tome Microscope; Zeiss) and confocal microscopy (Confocal Zeiss), samples were prepared by gluing the coated glass to another glass-slide.

Wide-field fluorescence images were obtained using the cell-R system based on an NIKON 90i fully motorized inverted microscope ($60\times$ Plan Apo objective, 1.42 numerical aperture [NA]) fitted with an Orca-AG charge-coupled device camera (Hamamatsu, Bridgewater, NJ), rapid wavelength switching, and driven by the cell-R software (Olympus Biosystems, Planegg, Germany). Samples were washed 3 times with double-distilled water (DDW) before each measurement.

Results and Discussion

In the first stage in the characterization process of the products we have checked the efficiency of sonochemical method in converting PEI polymer to PEI nanospheres and immobilization of PEI NSs onto the glass. After the sonochemical reaction was accomplished the dense crème-like solution was observed. To determine the location of the nanospheres on the glass surface, the PEI polymer was coupled with 1,4-dichloroisothiocyanate dye (FITC) which has fluorescent signal in the green portion of the fluorescent spectrum. The excitation maximum of FITC dye is at 488 nm and its emission peak is at 520 nm. The glass surface was coated with FITC-PEI NSs and emitted green signal from the surface. The green signal emitted from the surface of the glass confirms the presence of the FITC-PEI polymer on the surface of the glass.

The morphology of coated glass surfaces was analyzed using scanning electron microscopy. The glass was found to be coated with spherical particles which are PEI nanospheres. Figures 1a and b shows uncoated and coated glass surfaces. The immobilized NSs, which are presented in Fig. 1b, have an average size of 250 nm which is respective to 5:6 (mL/mL) oil:aqueous PEI starting solutions ratio.

The next question was: How does the change of some fundamental parameters affect the efficiency of sonochemical PEI NSs immobilization process? The following parameters were studied to find out the efficiency of using sonochemical method in coating glass surfaces with PEI NSs: (i) intensity of applied ultrasonic waves, (ii) time of sonication process, and (iii) the ratio of organic solvent/water solution of PEI. Changes in two of the three parameters do not affect much the efficiency of PEI NSs coating. The efficiency was studied by following PEI NSs formation and immobilization using electron and light microscopy analysis. The creation and immobilization of PEI NSs on glass surface were successfully achieved when the intensity of applied ultrasonic waves varied from 20% to 50% amplitude which equal to 38.6 and 96.6 W/cm² acoustic power, respectively. Under lower intensities than 38.6 W/cm² or at higher intensities than 96.6 W/cm² acoustic powers, PEI nanospheres were not formed. The time frame of the sonochemical reaction for the formation of PEI NSs was found to be from 2 to 5 min. The change in time or acoustic power of ultrasound does not have effect on shape or size of the immobilized spheres. When the ratio of oil/water solution of PEI was changed a very dramatic

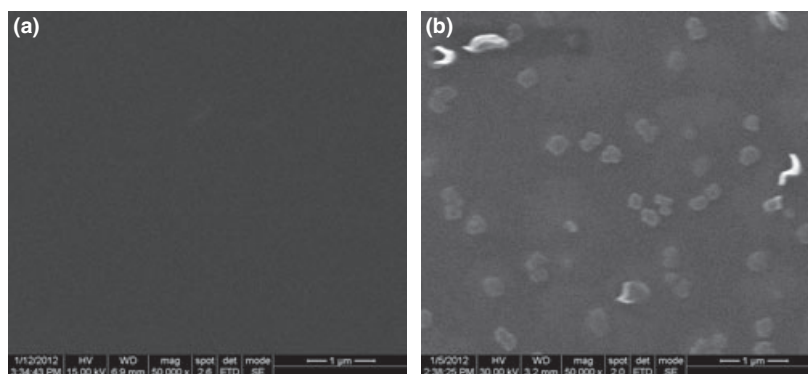


Fig. 1. SEM images of: (a) uncoated glass surface and (b) PEI NSs' coated glass surface. The average size of immobilized PEI NSs is around 250 nm. The ratio of the starting aqueous/oil solution was 5:6 mL. Scale Bar = 1 μm.

size dependence of the immobilized PEI NSs was observed. The size for immobilized PEI NSs was measured using electron microscopy and confirmed by DLS measurements. DLS measurements were performed for the PEI NSs which were not adhered to the glass surface and were found in the residue solution. The average size and zeta-potential measurements were performed on the DLS apparatus and are in good agreement with SEM measurements. The results are summarized in Table I. The SEM images of glass surfaces coated with PEI NSs are presented in Fig. 2. It is worth mentioning that the solution samples for the DLS measurements and the glass surfaces were fabricated under identical conditions. The difference among the three images presented in Figs. 2a–c is in the size of immobilized PEI NSs. Figure 2a shows the PEI NSs coating formed by applying ultrasonic waves to the starting solution with ratio (ratio of aqueous PEI/oil) 10:1 (mL). The average size of the immobilized NSs is found to be 500 nm. The same size PEI NSs coating is obtained for the ratio 9:2 of starting solutions.

Figure 2b represents the PEI NSs immobilized on glass surface when the starting aqueous PEI/oil ratio was 8:3. The average size of immobilized PEI nanospheres was measured to be 250 nm. The same morphology and average size was obtained for PEI NSs coated glass surface when the starting solutions ratios were 7:4, 6:5, 5.5:5.5, and 5:6. When the starting solvents ratio reached 4:7 and 3:8 the size of immobilized spheres decreased to about 60 nm (See Fig. 2c). At initial ratios of 2:9 and 1:10 the nanospheres sizes are about 500 nm. The change in average sizes of immobilized NSs could be explained by change in nanospheres' composition as a function of aqueous PEI/oil ratio. All the PEI NSs formed from different starting solution ratios have a positive charge of around +35 mV. The advantage of the obtained (changes in size) results is in its ability to control over the size of immobilized PEI NSs. Size control opens the possibility to perform surface modifications and to control over the functionality of the coated surfaces.

The PEI NSs presented in Figs. 2a and b do not have perfect spherical shape. To study the morphology

Table I. Summary of Size and Zeta-Potential (Positive Values are Presented in the Table) Changes as a Function of Aqueous/Oil Starting Solutions Ratio Changes

Water solution of PEI: Oil ratio (mL)	10:1	9:2	8:3	7:4	6:5	5.5:5.5	5:6	4:7	3:8	2:9	1:10
Size (nm)	523	518	250	246	243	254	251	58	70	503	506
Zeta-potential (mV)	35	32	39	34	31	41	35	36	35	34	35

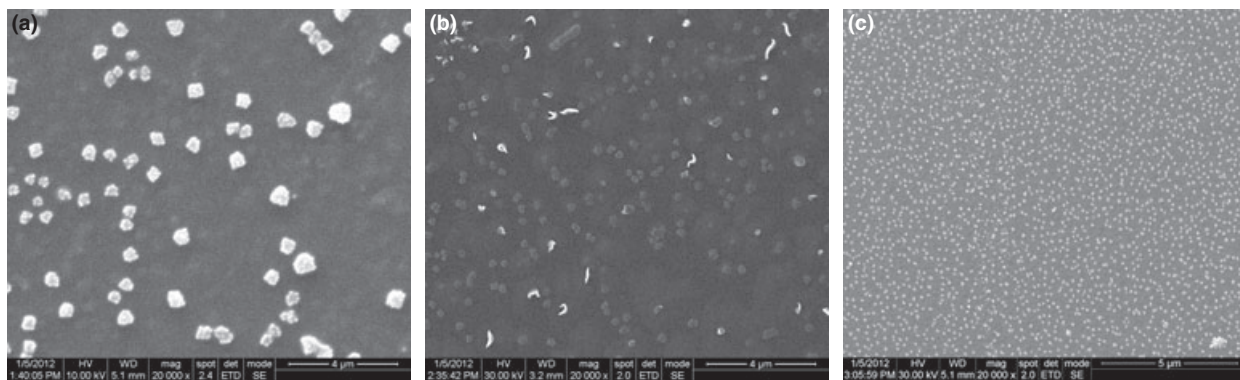


Fig. 2. SEM images of immobilized PEI NSs on glass surfaces. The NSs were obtained starting with different aqueous/oil solution ratios: (a) 10:1 (mL). The created and immobilized NSs have an average size around 500 nm; (b) 8:3 (mL). glass-coated surface with average size of immobilized PEI NSs of 250 nm; (c) 4:7 (mL) with a PEI NS average size around 60 nm. Scale Bar = 4 μ m.

of the nanospheres presented in Figs. 2a and b HRSEM analysis (high resolution scanning electron microscopy measurements) was done. The HRSEM measurement results are presented in Fig. 3. For both cases (Figs. 2a and b) PEI NSs obtained are aggregates, the difference between two samples (Figs. 2a and b) is in the size of aggregates. Each of the aggregates consists of PEI NSs with an average size of 60 nm (the same size of the nanospheres presented in Fig. 2c).

To study the PEI nanospheres composition the samples were colored with dye and analyzed using confocal microscopy. For this purpose the coloring test with Nile Red dye (See Experimental Section) was performed. Nile Red is a dye with a solvent-dependent absorption spectrum. It is an uncharged hydrophobic molecule whose fluorescence is strongly influenced by the polarity of its environment. With decrease in polarity of the environment, the emission maximum shifts to a shorter wavelength (from red in water to green and yellow in oil).¹⁴ The dye is photostable, the working wavelength range is broad and removed from wavelengths at which many biomolecules absorb. The fluorescence of Nile Red is unaffected in the pH range between 4.5 and 8.5.¹⁵ Nile Red is widely used to detect the hydrophobicity/hydrophilicity of the environ-

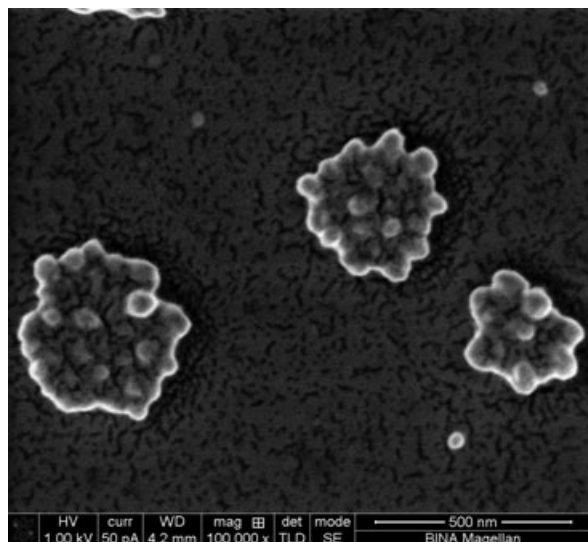


Fig. 3. High resolution scanning electron microscopy measurements image of aggregates of PEI NSs. Each of the aggregates consists of small PEI NSs with an average size of 60 nm. The aggregates presented on the image have an average size of 500 nm. Scale Bar = 500 nm.

ment. The changes in inner environment of PEI spheres was detected by confocal microscopy and presented in Fig. 4. With a change of solvent ratio, the environment of the inner part of the spheres was also changed. The following microscope fluorescent filters were used for the current analysis: Rhod-red color (excitation 550 nm, emission 573 nm), and GFP-green color (excitation 489 nm, emission 509 nm). Figures 4a–e show fluorescent images of PEI spheres colored with Nile Red dye. Fluorescence appearing at longer wavelengths (red fluorescence) indicates a more hydrophilic environment. The green fluorescent signal with emission at shorter (compared to red) wavelength, indicates a more hydrophobic environment.

Figure 4a represents the confocal microscopy image that was observed for Nile Red dyed PEI NSs of samples with aqueous PEI/oil solutions ratios of 10:1 and 9:2. The analyzed spheres are formed on the glass slide in the reaction cell but were not bonded to the glass surface (remained in residues solution). It is clearly observed in Fig. 4a that the nature of the inner part of PEI sphere is hydrophilic and hydrophobic (has a red [Rhod] and green [GFP] fluorescent signal emits from inner part). This means that the spheres are filled with both solvents: oil and water. When the aqueous/oil ratio reached 8:3 the hydrophobicity of the environment of the inner part of the sphere changed significantly to be less hydrophobic and the hydrophobic signal (green) became denser at the shell of the PEI sphere (See Fig. 4b). Figure 4c represents PEI spheres prepared with water/oil ratio 6:5. In this case the green color emits only from the shell of the sphere (hydrophobic nature) and the red color is from the inner part of the sphere (hydrophilic part). The conclusion is that the PEI sphere that we observed for the sample with ratio 6:5 filled only with aqueous solution because green color emits from the shell and red from the inner part. With increasing portion of organic solvent to 3:8 (Fig. 4d) the construction of the sphere became identical to the sphere with solvent ratio 8:3, where the green (hydrophobic) signal emission is denser near the shell and red (hydrophilic) signal emits from the inner part of the sphere. The PEI sphere construction completely changed at water/oil ratio of 2:9 (Fig. 4e). The red color emits only from the shell of the sphere and the green color emits from the inner part as well as from the shell of PEI sphere, meaning that PEI sphere filled with oil only. The results of fulfillment studies open possibility to encapsulate different drugs which have

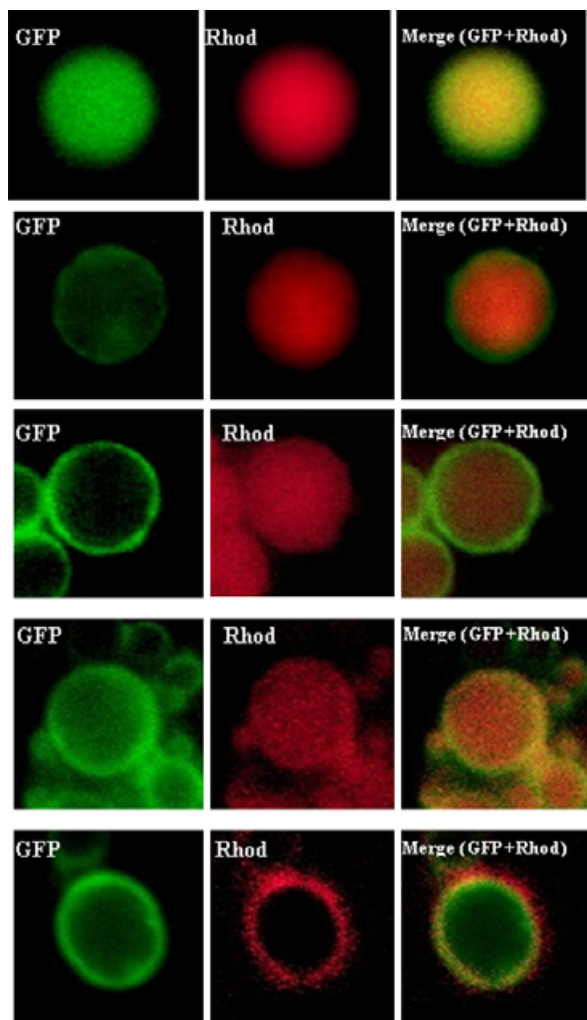


Fig. 4. Confocal images of Nile-Red colored PEI nanospheres. Two filters were used for confocal microscopy analysis: GFP-green signal with an emission peak at 520 nm and a Rhod.-red signal with an emission peak at 573 nm. (a) Confocal image of a Nile-Red-colored PEI nanosphere which was formed from a starting solution ratio of 10:1 (aqueous solution PEI/oil); (b) a PEI nanosphere which was formed from a starting solution ratio of 8:3; (c) a PEI nanosphere formed from starting solutions ratio of 6:5; (d) PEI nanosphere formed from starting solution ratio of 3:8; (e) a PEI nanosphere formed from a starting solution ratio of 2:9. Relatively big PEI spheres are presented on the images to show the PEI sphere composition in a better way.

hydrophilic or hydrophobic nature. The surface coating of nanoparticles is crucial to determining their properties. In particular, the surface coating can regulate stability, solubility, and targeting. The control over the

size of immobilized PEI NSs enables to establish the thickness of the coating. The control over fulfillment of NSs enables to encapsulate species which have different sizes as well as different natures. For example for fragrance encapsulation like “Limonene” (has hydrophobic nature) or “Vanillin” (has hydrophilic nature) the smaller size PEI NSs is supposed to be used due to the small size of the fragrance molecule. For encapsulation of drugs (antibiotics) the bigger size of the PEI NSs would be synthesized. In summary, the control over the size and fulfillment of the PEI NSs allow to create “smart” surface coatings which will have different functionalities.

The mechanism of PEI nanospheres formation and immobilization was studied by FTIR measurements. Three samples were analyzed and compared: (i) aqueous solution of 5% PEI polymer, (ii) oil, and (iii) PEI NSs. There were no new peaks found in FTIR spectra. From this information we concluded that the PEI polymer does not form any new bonds and polymerization process to form PEI sphere occurs between PEI polymer chains. For the immobilization mechanism, electrostatic interactions between the positively charged PEI nanospheres and oxidized glass surface are responsible. To confirm our hypothesis the PEI aqueous solution (5%) was mixed with aqueous solution (5%) of BSA protein and sonicated with organic solvent (soya bean oil) during 3 min with the presence of glass slide in the reaction cell. After reaction was accomplished, the PEI-BSA spheres were detected (in the residues solution) with an average size of 1.2 μm and z-potential charge of -18 mV. The average size and zeta-potential charge were measured using DLS technique (the sample for measurement was taken from the residue’s solution phase). The glass slide which was placed in the reaction cell for PEI-BSA spheres coating was washed after reaction accomplished 3 times with DDW. After washing no spheres immobilized on the surface of the glass were detected (using electron and light microscopy). The results confirm that only positively charged PEI NSs are possible to immobilize on the surface of the glass. When the charge of the spheres became negative, the spheres were not adhered to the surface. Anyway the encapsulation of the different drugs inside the PEI nanoparticles and BSA spheres (separately) is possible.^{16,17} The surface charge does not affect the encapsulation abilities of the spheres. The surface charge set the ability of the sphere to adhere to the glass surface. Moreover, ultrasonic waves

are necessary for imbedding PEI NSs onto the glass surface.

Conclusion

In the current research we demonstrate a one step synthesis and glass surface coating with PEI nanospheres by using an environmentally friendly method named ultrasonic emulsification. The immobilized PEI NSs were obtained with different nanometric sizes that varied from 50 to 500 nm. The control over the size of NSs is possible due to change in starting solutions ratios (aqueous/oil ratio). Moreover, we demonstrated that change in solution ratios has an effect on PEI NSs composition. By changing the solvents ratio we enable control of fulfillment of the spheres. In future work, we plan to explore the above-described method for encapsulation of different “drugs” (which have either hydrophobic or hydrophilic nature) inside the immobilized PEI nanospheres.

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