

Diamondosomes: Submicron Colloidosomes with Nanodiamond Shells

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Nanodiamonds are a fascinating material that only recently came into the spotlight despite being known for over 60 years. Recent breakthroughs in the last decade regarding processing, purifying, and dispersing nanodiamonds enabled widespread research on diamond nanoparticles and quickly showed the inherent potential of this newly rediscovered nanomaterial.^[1] Among the outstanding properties of the sp^3 -hybridized carbon nanoparticles are inherent fluorescence, excellent biocompatibility, and ease of surface functionalization.^[2] Furthermore, pure detonation nanodiamonds are easy to procure without being prohibitively expensive. Based on these features, nanodiamonds are attractive candidates for biomedical applications such as drug delivery and nanoparticle-assisted diagnostics.^[3–5] In this regard, modern theranostic nanomedicine calls for novel approaches that combine therapeutics and diagnostics in a single vehicle.^[6] A highly promising candidate for this application field are colloidosomes, which are defined as capsules with a shell consisting of individual nanoparticles.^[7] Since basically any kind or combination of nanoparticles can be used to form the shells, colloidosomes are drug carriers with the potential to be designed with multifunctional properties. With this in mind, colloidosomes can be envisioned as a highly versatile platform for the tailored assembly of delivery vehicles for theranostics. However, most of the colloidosomes that are reported in the literature are far too large or too unstable to show the full potential for the biomedical field.^[8,9] In our recent work, we were able to demonstrate for the first time the synthesis of colloidosomes with diameters below 1 μm , thereby positioning colloidosomes within closer reach of biomedical applications. However, although these submicron colloidosomes on the basis of metal oxide nanoparticles and lipids were inherently stable in decane as well as in water, it was only possible to maintain the integrity of dried colloidosomes if very gentle drying methods like freeze-drying or lyophilization were used. Thus, a major goal in colloidosome research is to find a facile way to fortify colloidosome shells without reverting to polymeric additives or aggressive chemical methods that would prevent in situ loading of colloidosomes with sensitive drug molecules or compromise biocompatibility of the capsules.

One feature of nanodiamonds, which for a long time was the main obstacle for the preparation of high quality dispersions of single nanodiamonds ($d = 4\text{--}5\text{ nm}$), is the pronounced aggregation behavior of detonation nanodiamonds. Upon fabrication by detonation of oxygen-deficient $\text{tnt}/\text{hexagon}$ in inert media, nanodiamonds exist in the form of agglutinates, small aggregates of roughly 100 nm that are covered in sooty layers of sp^2 carbon. While most of the sp^2 carbon can be removed from agglutinates by treatment in acid media and annealing, it still takes considerable energy to disintegrate the remaining nanodiamond aggregates. This is usually done by milling in wet zirconia medium and/or by high-energy ultrasonication.^[10] The strong aggregation between detonation nanodiamonds is most likely caused by remarkably strong van der Waals interactions^[11] as well as additional electrostatic attraction due to anisotropy of the faceted diamond surface.^[12] Simulations suggest that this anisotropy adds attractive coulomb interactions owing to differences of the facet's dipole moments.^[13] On the other hand, once the aggregates are separated, nanodiamonds form very stable dispersions with zeta potentials around $\pm 60\text{ mV}$,^[14] depending on the surface configuration. Accordingly, the reaggregation or assembly of dispersed nanodiamonds can potentially form particularly stable nanostructures. In exploiting this effect for the preparation of colloidosomes, we aim to produce outstandingly stable colloidosomes with nanodiamond shells. Because these fortified colloidosomes present a significant step forward and might be used as a basis for introducing further functionality to nanodiamond colloidosomes, we introduce the moniker "diamondosomes."

Generally, the assembly of submicron colloidosomes proceeds through the controlled aggregation of nanoparticles at the surfaces of emulsion droplets. The controlled formation of interfacial films is guided by the presence of oil-soluble surfactants that bear the same sign of charge as the particles.^[15,16] Oppositely charged lipids and nanoparticles cause uncontrolled and excessive agglomeration. This interaction should not be confused with the formation of a lipid bilayer around nanoparticles, which is also reported for nanodiamond–lipid conjugates.^[17–19] In this work, this principle was tested with nanodiamonds (**Figure 1**). After purification (the nanodiamonds were used as received and purification was performed by the manufacturer), the surfaces of detonation diamonds consist of unsaturated sp^2 carbon that is partially oxidized by different oxygen species, mainly carboxylic groups and carboxylic anhydrides.^[20] By annealing the purified nanodiamonds in air, the particle surface becomes fully oxidized and the diamonds are able to form stable dispersions in water, owing to the low zeta potential of up to -60 mV over a broad range pH.^[14] Conversely, if the purified detonation nanodiamonds are annealed using a hydrogen atmosphere, most of the oxygen species will be reduced and

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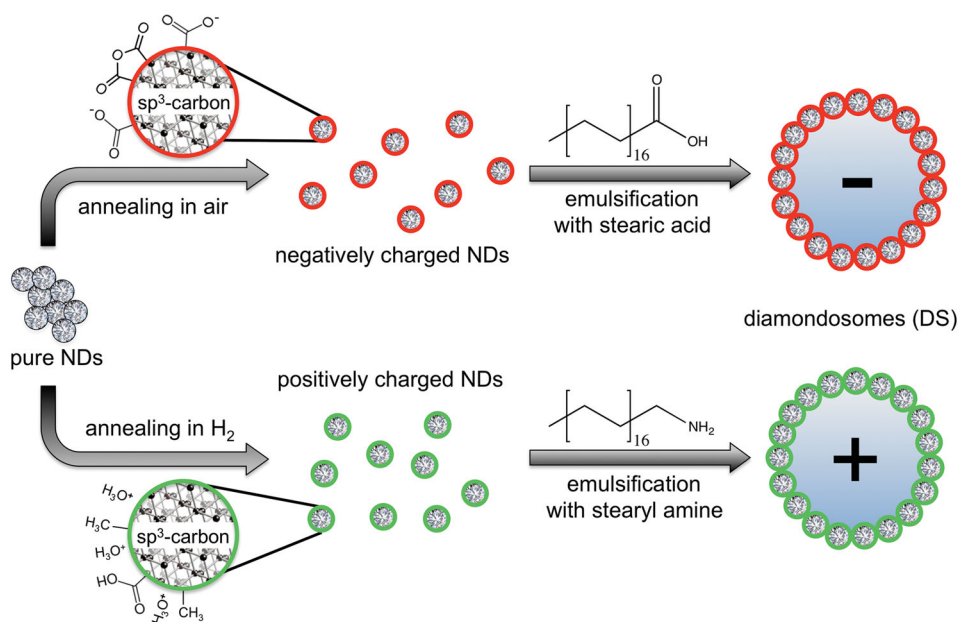


Figure 1. Diamondosomes with negatively charged shells are prepared from nanodiamonds that are annealed in air and subsequently synthesized with stearic acid; positively charged shells can be prepared from nanodiamonds annealed in H_2 atmosphere and produced with stearyl amine.

Table 1. Zeta potential and sizes of nanodiamonds (ND) and diamondosomes (DS) at pH 7.

	ND-	ND+	DS-	DS+
Size	$\approx 4 \text{ nm}^a$	$\approx 4 \text{ nm}^a$	$233 \pm 66 \text{ nm}^b$	$508 \pm 47 \text{ nm}^b$
Zeta potential	$-42 \pm 3 \text{ mV}$	$55 \pm 9 \text{ mV}$	$-22 \pm 3 \text{ mV}$	$45 \pm 8 \text{ mV}$

^a)NLS fit weighted by numbers; ^b)cumulant fit

C–H bonds are formed from the remaining sp^2 carbon. This results in stable dispersions of nanodiamonds with zeta potentials of up to 60 mV. These two nanodiamond dispersions (ND–: negatively charged nanodiamonds, ND+: positively charged nanodiamonds) were used to form colloidosomes. The lipid stearic acid was used to stabilize ND– and stearyl amine for ND+. Colloidosome formation was possible with both combinations of nanoparticles and lipids. The resulting diamondosomes will be called DS– for colloidosomes from ND– and DS+ for colloidosomes from ND+. Size and zeta-potential results of both ND dispersions and diamondosomes are shown in **Table 1**. Note that due to the polydispersity of the DS samples, the cumulant values for the diameters are lower than those found with scanning electron microscopy (SEM).

Figure 2 shows diamondosomes formed from stearic acid and ND– (DS–). DS– taken from the decane phase (**Figure 2a**) have the appearance of a deflated ball or a crumpled paper bag. This affirms that these diamondosomes consist of a thin film of nanodiamonds that is quite flexible and withstands evaporation of the inner water phase. The diameters of DS– that were taken from the aqueous phase (**Figure 2b**) decreased relatively to those dried from decane and better maintained their spherical shape. Apparently, the transfer to the aqueous phase consolidates the capsules. It is of note that it is possible to dry diamondosomes in air at ambient temperature and humidity. This is in

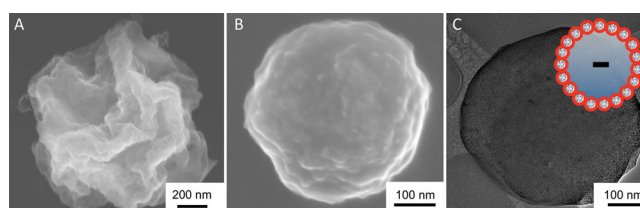


Figure 2. A,B) SEM and C) TEM images of diamondosomes assembled from negatively charged nanodiamonds and stearic acid. Samples A and C were taken from the decane phase, sample B was taken from the aqueous phase.

contrast to the previously described submicron colloidosomes on the basis of metal oxides. These colloidosomes had to be lyophilized to prevent bursting caused by large Laplace pressure differences in the small capsules.^[15]

The same holds true in all aspects for DS+, which are prepared from ND+ and stearyl amine (**Figure 3**). The only obvious difference is an increased roughness of the capsule surfaces.

The components responsible for diamondosome shell formation were studied using interfacial shear rheology. For this,

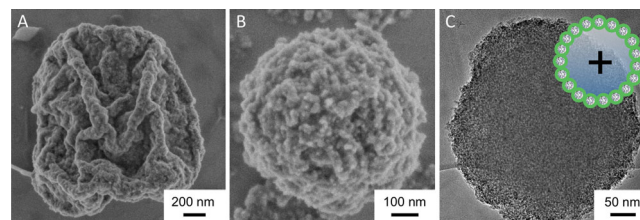


Figure 3. A,B) SEM images of diamondosomes assembled from charged nanodiamonds and stearyl amine. Samples were taken from the decane phase. Samples A and C were taken from the decane phase, sample B was taken from the aqueous phase.

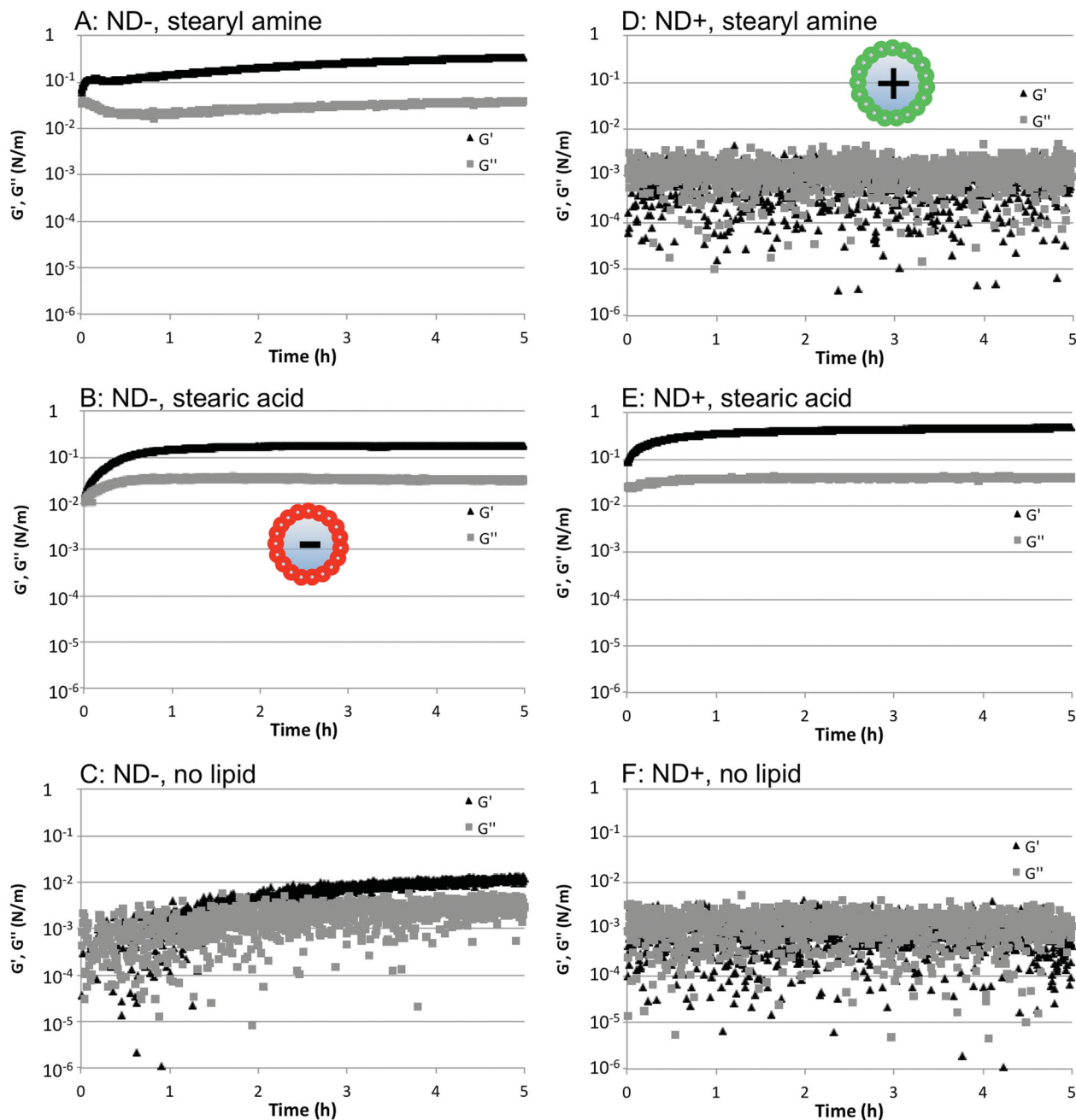


Figure 4. Interfacial shear rheology measurements of negatively charged nanodiamonds and A) stearyl amine, B) stearic acid, and C) no lipid as well as positively charged nanodiamonds and D) stearyl amine, E) stearic acid, and F) no lipid.

the curved interfaces of emulsion droplets were transferred to the planar interface between water and oil and analyzed with a Pt/Ir DuNöuy ring that was positioned exactly at the interface. **Figure 4** shows the increase of the interfacial rheological moduli G' and G'' as a function of time. The results for ND⁻ reveal a slight surface activity of the nanoparticles without lipids, leading to the formation of a weak thin film (Figure 4C). With stearic acid and ND⁻, an elastic thin film grows within about 1 h (Figure 4B). Afterwards, the moduli

remain constant. This behavior most likely corresponds to the controlled film growth that makes submicron colloidosome formation possible. Film growth can also be observed with ND⁻ and stearyl amine (Figure 4A), which is to be expected due to the oppositely charged building blocks. However, the moduli do not exhibit a plateau and increase steadily over time. This continuous aggregation of nanoparticles prevents the formation of discrete colloidosomes and instead causes bulk phase agglomeration.

The interpretation of interfacial film growth with ND+ is less straightforward. While oppositely charged ND+ and stearic acid again show continuous film growth that leads to uncontrolled aggregation during colloidosome synthesis, film growth cannot be measured without lipid and does not occur in the presence of stearyl amine. Instead, only the noise base line of the rheometer is visible. Because diamondosome formation is not possible with both ND- and ND+ in the absence of lipids, it is quite likely that with ND+ and stearyl amine a thin film does form at the interface between decane and water, which cannot be detected by the rheometer. The difference in the moduli of the interfacial films can be explained by the different amounts of charges that are carried by stearic acid and stearyl amine at pH 7. For this pH value, a considerably larger part of the carboxylic head groups of stearic acid are charged compared to the amine groups of stearyl amine. Consequently, film formation with stearic acid is more pronounced with nanodiamonds of the same charge.^[16]

In conclusion, it was possible to validate the principle of colloidosome formation with nanoparticles and lipids of the same charge. Using nanodiamonds, colloidosomes with high shell stability could be formed that represent a new platform for the preparation of more complex, multifunctional diamondosomes.

Experimental Section

Purified detonation diamond nanopowder was purchased from Plasmachem GmbH, Berlin (G01 grade). Stearic acid (octadecyl acid), stearyl amine (octadecyl amine), and decane were purchased at high purity from Sigma-Aldrich GmbH, Taufkirchen and were used without further purification. Dispersion of the nanodiamond powders was carried out according to Hees et al.^[14] To produce negatively charged nanodiamonds, 1 g of powder was annealed for 5 h at 450 °C in air. For positively charged nanodiamonds, 1 g of powder was annealed for 5 h at 500 °C in a 1 bar hydrogen atmosphere. The treated powders were suspended in 100 mL Millipore water and ultrasonicated using a high power disintegrator at 250 W for 3 h. Subsequently, the dispersions were centrifuged twice for 1 h at 16 g. The obtained bluish brown dispersions exhibited nanodiamond concentrations of around 0.5%wt. The particle sizes ((determined by dynamic light scattering (DLS)) were around 60 nm weighted by intensity and around 4 nm weighted by numbers. The zeta potentials were -60 mV, mainly caused by carboxyl groups and +60 mV,^[2] most likely caused by H₃O⁺ adsorption,^[21] at pH 7.

Colloidosome preparation was carried out according to Bollhorst et al.^[15] For the preparation of negatively charged diamondosomes, 10 mL of 1 × 10⁻³ M stearic acid solution in decane 10 mL was given on 10 mL of the aqueous dispersion with negatively charged nanodiamonds (ND-). This two-phase system was sonicated for 1 h in an ultrasound bath resulting in an emulsion of water in oil, which is the template for colloidosome formation. After 1 h resting time, samples were taken from the decane phase. To obtain diamondosomes in water, the remaining decane phase was centrifuged over fresh water for 3 min at 6 g. In the same manner, stearyl amine and positively charged nanodiamonds (ND+) were used for the preparation of positively charged diamondosomes.

Dynamic light scattering was performed using a Beckman-Coulter DelsaNanoC. The experiments were carried out at the backscattering angle of 165°. The data were evaluated via intensity and number distributions.

Zeta-potential measurements of the diamondosomes were also done using the Beckman Coulter DelsaNanoC. Here, the measurements were performed at the scattering angle of 15°. The zeta potential of the nanodiamond dispersions was measured using a colloidal vibration

current probe (DT1200, Quantachrom, Ödelzhausen, Germany) with the exact dispersion concentrations as input parameters. SEM images were acquired using a Zeiss Auriga40 and a Zeiss Supra40. Samples were deposited directly on silicon substrates from either the organic or the aqueous phase. The samples were not sputter coated.

Transmission electron microscopy (TEM) was carried out using an FEI Titan 80/300kV equipped with a cs-corrector for spherical aberration of the objective lens at 300 kV. Samples were deposited on CVD graphene film coated copper grids from Graphene Supermarket (New York, USA).

The surface shear rheological properties of the films were determined using a TA Instruments DHR3 rheometer, which was equipped with a 2d-double Couette shearing system. The measuring cell consisted of a teflon dish with an inner and outer edge and a Pt/Ir-DuNöye ring ($d = 2$ cm), which could be placed exactly at the interface between oil and water. The dish was first filled with the aqueous phase up to a ledge at the half-height of the dish (ca. 6 mL). For measurements at the decane-water interface, the ring was first placed on the aqueous phase and the decane solution was added (4 mL) afterwards. The torque required to rotate the ring with a sinusoidal angular frequency ω and a deformation (or strain) γ was measured. In such experiments, the 2D elastic modulus G' and the 2D viscous modulus G'' can be computed from the amplitudes and phase angles of the stress and deformation signals. To analyze thin film growth, time tests (constant $\omega = 0.1$ rad s⁻¹ and constant $\gamma = 0.1\%$) were performed for 5 h with strains well within the linear-viscoelastic regime. Due to the relatively low vapor pressure of decane (0.13 kPa) and the presence of surfactants at the air/decane interface, no significant evaporation of decane occurred during the tests.

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