Nanodiamonds for Detoxification

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ABSTRACT

Identifying new biocompatible enterosorbents that bind aflotoxins is an active area of research. One particular enterosorbent of interest is nanodiamond particles. We report the first experiments on using nanodiamonds (NDs) as enterosorbents for aflotoxin (Af) adsorption. Because nanodiamonds exhibit low colloidal stability, modification techniques and ways of stabilizing these structures over various pH environments are essential. Analysis of colloidal stability of ND suspensions that have undergone different surface modifications is presented based on measurements of their zeta potentials and on titration experiments.

***Keywords*:** nanodiamonds, bioapplications, aflotoxins, enterosorbents, nanoparticles.

1 INTRODUCTION

Mycotoxins, low molecular weight by-products of mold growth, are known carcinogens. Aflotoxins, a group of mycotoxin, are invisible to the naked eye and may infect humans directly or indirectly by ingestion of moldy foods or infected animal products. Enterosorbents, structures that bind toxins in the gastrointestinal tract, are an effective way to remove mycotoxins. Requirements for a successful enterosorbent include biocompatibility, good dispersivity during ingestion and excellent selectivity as to not interfere with the adsorption of critical nutrients needed in the diet[1]. Furthermore, they should be relatively cheap and abundant, as well as easily transported and administered. Previous studies have shown that hydrated sodium calcium aluminosilicate (HSCAS) clay as a feed additive [1] serves as an effective enterosorbent for the binding of aflotoxins. While HSCAS is effective towards aflotoxin adsorption, it has a low selectivity, which motivates the discovery of more suitable enterosorbents.

Nanodiamonds (NDs) are among one of the materials being researched for enterosorbent applications. ND particles have attractive physicochemical properties that include very high surface densities (300-400m^2/g), rich surface chemistries, and permanent surface charges. Previous studies indicate that these diamond nanoparticles are biocompatible and non-toxic [2,3]. Such properties are critical for their biomedical use. Biocompatibility and non toxicity issues were demonstrated in white mice by substituting ND hydrosols (0.002-0.5 wt. %) for water over a period of 3-6 months [3]. No significant weight changes, problems in reproduction (over 5 generations) or deaths were observed as a result of this study. However, with extended substitution, leukocyte counts were increased, with the level dependent on the concentration of NDs in the hydrosol [2,3]. A second study using intramuscular injection showed no tissue inflammation at the area of injection [2]. Outcomes from these studies encourage further research and indicate that the use of diamond nanoparticles may be promising for binding particular classes of mycotoxins in both animal and potentially human use.

While their physicochemical properties make them attractive candidates as enterosorbents, selection of the appropriate NDs for mycotoxin binding is not straight forward, primarily due to a wide variety of ND types. In gerenal, NDs produced by detonation of carbon-containing explosives were purified from the soot and metallic impurities were removed by a wide variety of methods at an industrial scale. This results in different aggregates size and surface chemistry, which leads to various levels of dispersivity and stability among ND particles. Colloidal stability among vendor received, unmodified nanodimonds in most cases is quite low. Appropriate surface modification significantly increases ND colloidal stability [4]. Additional processing may include fractionation [5] and treatment in atmospheric plasma[6] to produce target surface structures that alter chemical and physical properties that in turn change both the hydrophilic/ hydrophobic and dispersivity characteristics.

With an end goal to develop the optimal enterosorbent, such characteristics as particle size, surface chemistry, and zeta potential across different pH levels have been studied for several types of NDs and are reported below. Preliminary results on binding aflatoxin by ND particles are also discussed.

2 EXPERIMENTAL DETAILS

The ND used in this work was synthesized by the detonation of a mixture of trinitrotoluene (TNT) and 1,3,5-trinitro-1,3,5-*s*-triazine (RDX). The sample denoted Ch-St was purchased from "New Technologies", Chelyabinsk, Russia. This sample was generated by a detonation soot purification process using a mixture of sulfuric acid with chromic anhydride treatment, washed with water, and dried. Sample Ch-St was then additionally purified using ion-exchange resins, heat treated in an air atmosphere and fractionated by centrifugation. This modified sample is called Ch-I6 in the experiments below. Samples denoted Kr-B and Kr-Gr were produced at the Krasnoyarsk Research Center, Russia by explosion of TNT/RDX in a CO_2 atmosphere and oxidized in air in the presence of boric anhydride. The

samples were then modified, as given in reference 4, resulting in a significant increase of the ND dispersivity and hydrosol stability. Sample RUDDM was purchased from Real-Dzerzinsk, LTD. This product was also modified according to the method in reference 4, fractionated and dried. The polydispersed powder (RUDDM) was fractionated to 2 fractions with ND agglomerate size within the 0-250nm range (RUDDM1) and the 150-400nm range (RUDDM2). Prior to Zeta-potential and particle size measurements, the ND suspensions were sonicated. The sonicator was equipped with a tapered titanium horn with a tip diameter of 3 mm (Cole-Parmer® 750-Watt Ultrasonic Homogenizer EW-04711-60, 20 kHz), which was directly immersed into the sample. The output power was 10W and the output intensity was ~100W/cm [2]. Size distributions of the NDs in their hydrosols were measured by dynamic light scattering using a Beckman-Coulter N5 submicron particle size analyzer and Malvern ZetaSizer Nano ZS. Zeta potential values were measured using laser doppler velocimetry (Zetasizer Nano ZS, Malvern Instruments). The pH titrations of 0.1wt% ND suspensions were conducted in several manners: from pH of 12 to 1; from pH of 1 to 12 and, additionally, from the 'natural' pH of suspensions to 1 or to 12. To conduct the pH titration, 0.1M HCl and 0.1M NaOH were used. De-ionized (DI) water with a resistivity of 18MΩ-cm was used to prepare the samples.

Aflatoxin B_1 (Af) was purchased from VNIIVSGE (Russia). A suspension of Af (5μg/ml) in DI water was mixed with a ND hydrosol (10mg/ml) in the ratio 1:1. After a 5min incubation time, Ca ions (5mM) were added to the mixture to promote ND coagulation. After separating the NDs with adsorbed Af using centrifugation (Eppendorf Mini Spin Plus centrifuge), the concentration of the Af in the supernatant was measured and compared with the concentration of the stock Af solution. Adsorption capacity was calculated as the difference between the initial and final concentrations of Af. The concentration of Af in suspensions was measured by two methods: from fluorescence spectra collected with SpectronicUnicam (USA) and by High performance liquid chromatography (HPLC) with a Milichrom A-02 (EcoNova, Novosibirsk). It was noticed that fluorescence of Af nonlinearly depended on a variety of factors such as irradiation time, Af concentration as well as presence of the traces of NDs in the suspension. These factors made interpretation of the results challenging.

3 RESULTS AND DISCUSSION

3.1 ZETA POTENTIAL

The zeta potential is the electrostatic potential (or the net charge) at the particle-slipping plane. The slipping plane is the boundary of a hypothetical sphere enclosing a particle (where loosely bound ions form a stable state and move together with a particle). It is generally stated that colloids with zeta potential values above 30mV or below -30mV are considered stable suspensions because particles electrostatically repel each other and do not agglomerate.

It was important that the sample preparation procedures provide reproducible results. Thus, we studied several factors that can potentially influence readings of the zeta potential, namely the role of the sample sonication and ND concentration. To study the role of sonication time, suspensions of 0.1wt % I6 in DI water were prepared and sonicated for 0, 1, 2, 3, 5, or 10 minutes. The zeta potential of the suspension without agitation was also measured. The ND zeta potential result was the average of six consecutive measurements with a standard deviation (STD) about 1-2mV. The results of this study are illustrated in Figure 1. It can be concluded that sonication time has little affect on the zeta potential readings (Fig.1).

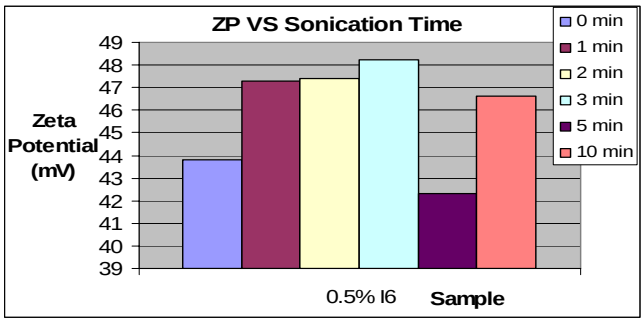

Figure 1: Zeta Potential as a function of sonication time.

The dependence of zeta potential on sample concentration was also studied for the I6 ND suspension (Fig.2). An increase in the absolute value of the zeta potential by several mV was observed as the sample was diluted from ~0.1wt% to ~0.01wt%. A similar tendency was observed for several other ND types. To avoid a number of variables, a standard procedure for performing zeta potential measurements on samples with 0.1wt% was used in further experiments. The results most likely indicate that the number of dissociating surface groups responsible for the surface charge of the ND is concentration-dependent.

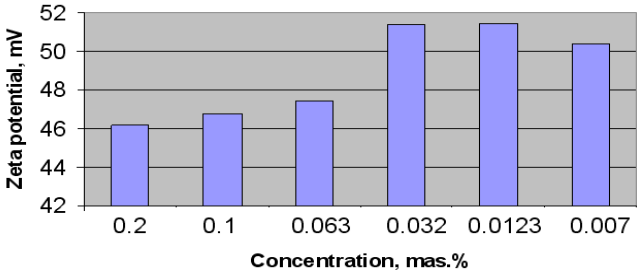

Figure2: Dependence of zeta potential on I6 concentration.

Table 1 below summarizes agglomerate sizes and zeta potentials of several types of NDs. The size result is the average of six consecutive measurements with a standard deviation (STD) of ~3-4nm. All samples, except Ch-St, demonstrate a high zeta potential, in correspondence with the high colloidal stability of these samples. Both positive and negative zeta potentials for the studied samples were

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observed. In principle, this can be important for adsorption of charged toxins by means of electrostatic interactions. The different zeta potential signs for the 2 groups of samples (Ch-St and I6) and (Kr and RUDDM) are caused by different types of the chemical groups at the surface of the particles. These chemical groups originate from different stages of sample purification/modification and, possibly, due to residual metal ions that are incorporated to the particles during synthesis. In the present paper we do not proceed with a detailed discussion of the origin of the sign of zeta potentials of the samples. Nonetheless, it is worth mentioning that the modification[4] itself does not change the zeta potential sign; Ch-St modified by the method in reference 4 demonstrates a zeta-potential of ~ +40mV.

ND, 0.1 wt%	ZP (mV)	ZP STD	Size (nm)
I6	+46.8	0.9	185
Ch-St	+17.4	0.4	300
Kr Black	-47	2.1	80
Kr Grey	-44.7	0.6	397
RUDDM1	-49.3	1.9	75

Table 1: Agglomerate size (nm) and zeta potential (ZP, mV) for 0.1wt% ND suspensions in DI water.

It should be noted also that there is no correlation between aggregate size and zeta potential in the studied samples.

3.2 TITRATION OF ND SUSPENSIONS

Titration studies were carried out to determine the stability of ND suspensions at various pH levels. Due to the proposed end use of ND as gastrointestinal (GI) nanoenterosorbents, the selected pH range was broad enough to simulate conditions in cow GI tract. It has been observed that zeta potential values vary depending on the starting point of the titration. For the five samples under study the titration curves were first taken from initial pH to 1 and initial pH to 12 for 0.1wt% ND suspensions (two independent titrations). The initial pH values of these samples ranged within pH 5-6 (pH of the DI water was ~5.8). The titration curves of these samples are plotted in Figure 3.

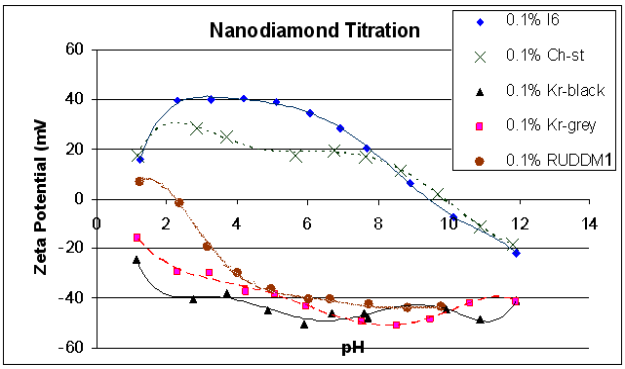
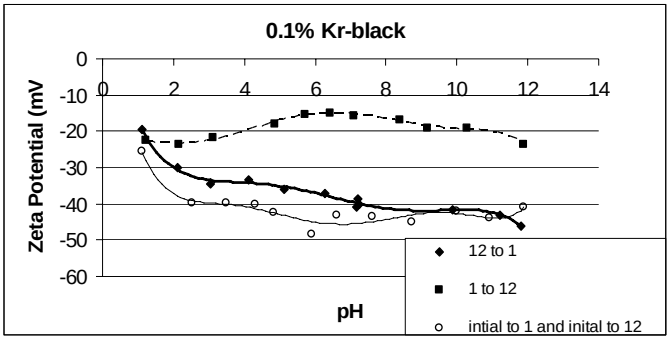


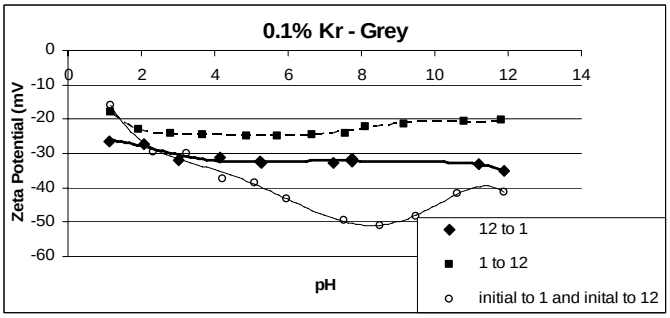
Figure 3: Zeta potential vs. pH for five NDs. Titration was carried out starting at initial pH toward either pH 1 or pH 12 in each case.

During this experiment it was observed that the stability over pH environments depends on the charge of the nanodiamond particle. Those nanodiamonds that were negatively charged showed a decrease in colloidal stability as pH approaches 1 (shown quite prominently for RUDDM1). RUDDM1, which under normal conditions was shown to be the most stable of the nanodiamond types, possesses an isoelectric point (point of least stability) at a pH of ~2.4, and noted to be the least stable of all negatively charged nanodiamonds. For those samples that hold a positive charge, as pH was increased the stability was quickly diminished reaching an isoelectric point around a pH of 9.5 and 10. This experiment suggested that Kr-black was the most stable among the all nanodiamonds titrated.

As previously mentioned, the shape of the titration curve and the stability of the colloid were influenced by the starting pH. Consequently, titration was performed to show the influence of the starting pH. Samples of Kr Black and Kr Grey were prepared at a concentration of 0.1wt%. These samples were then titrated over various 'trajectories' that included: initial pH to 1, initial pH to 12, pH of 1 to 12, and a pH of 12 to 1. The results of this experiment are given in Fig.4.



(a)



(b)

Figure 4: Effect of titration direction on zeta potential of Kr Black (a) and Kr Grey (b).

The outcome of the concentration verses zeta potential reported in Section 3.1 allowed for further experimentation on concentration versus zeta potential under various pH. Full titration (taken from pH 1 to 12 and 12 to 1) was performed for the Kr-black sample at 0.1wt% and 0.01wt% (Fig.5).

Similar to the data found in Section 3.1, the concentration does have a notable effect on the zeta potential. Wherein Figure 2 the zeta potential for positively charged I6 increased with deceasing concentration, the opposite held true for negatively charged Kr-black; when concentration was decreased the absolute value of the zeta potential decreased.

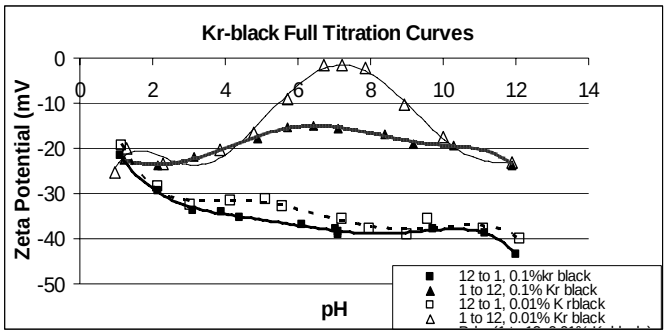


Figure 5: Titration curves for 0.1 and 0.01 wt% of Kr Black.

These experiments suggested that both the direction of the titration and the concentration of the sample have a great affect on zeta potential and, therefore, colloidal stability.

3.3 AFLATOXIN ADSORPTION by ND

Polydispersed ND RUDDM and its two fractions with different particle sizes as well as the Ch-St and I6 samples were studied for Af adsorption. An aim of the experiments was to elucidate the role of the sign of the zeta potential (surface chemistry) and influence of particle size (surface area available for binding) on adsorption. Results of the experiments on sorption capacity of the ND samples defined by two independent methods (fluorescent spectroscopy and HPLC) are given in Table 2. Results of the two independent methods of measurements are consistent.

ND	Fluorescence Spectroscopy	HPLC method
RUDDM	45	42.5
RUDDM1	55	75
RUDDM2	63	45
Ch-St	132	112
Ch-I6	123	106.25

Table 2: Sorption capacity of AfB1 by different types of ND (mg of AfB1 per kg of ND).

The NDs preserved colloidal stability in the presence of Af. A fast adsorption rate is essential for enterosorbents. In our experiments we studied both addition of ND hydrosols and dry powders to Af suspensions (details are not reported). The ability to use enterosorbents either as dried powders or hydrosols is important from a practical viewpoint. Using dried sorbents is most common because for some enterosorbents the presence of water in pores prevents Af penetration to the pores [7]. At the same, time dispersivity in water of the sorbents is higher.

As follows from Table 2, NDs with a positive zeta potential demonstrate higher adsorption toward Afs as compared to a group of NDs with a negative surface charge in an aqueous media. In principle, Af has both positively and negatively charged groups within the molecule, thus, both types of NDs can electrostatically interact with Afs. We can also preliminarily conclude that the size of ND aggregates within both groups of NDs does not play an essential role in the Af sorption capacity.

4 CONCLUSION

Two groups of NDs with positive and negative zeta potentials in neutral media were selected and thoroughly characterized. Titration of the samples demonstrated that for some samples relatively high zeta potentials can be preserved over a wide pH range, indicating high colloidal stability in these conditions. Initial experiments on Af adsorption by NDs demonstrated their ability to adsorb Af. At the same time, NDs from different vendors processed and modified under different conditions demonstrated different sorption capacity toward Af. This suggests that ND surface chemistry can be modified in a controlled manner such that the Af sorption is increased.

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