Electronic supplementary materials *Mendeleev Commun.*, 2021, **31**, 869-871

Controlled release of α-amylase from microchamber arrays containing carbon nanoparticle aggregates

Ekaterina A. Mordovina, Olga A. Sindeeva, Anna M. Abramova, Daria V. Tsyupka, Vsevolod S. Atkin, Daniil N. Bratashov, Irina Yu. Goryacheva and Gleb B. Sukhorukov

1. Synthesis of carbon nanoparticles

To obtain CNPs, a weighed portion of 0.250 g DSS was dissolved in 50 ml of bidistilled water. The resulting solution with a concentration of 5 mg/ml was transferred into a Teflon pot with a tight-fitting lid and placed in a stainless steel autoclave, then autoclaved at 200 ℃ for 3 hours [Figure S1*(a)*]. One Teflon pot allows autoclaving 10-15 ml of the resulting solution, therefore it is necessary to use several autoclaves. After autoclaving, the resulting suspension CNPs was taken and transferred into a 50 ml tube.

The obtained CNPs were characterized by optical spectroscopy and transmission electron microscopy (TEM) in the work[1] of Kokorina et al. CNPs have an emission maximum in the region of 500 nm (absorption and fluorescence spectra are shown in the Figure S1*(a)* on the right. Also, according to TEM and gel exclusion chromatography data, CNPs are heterogeneous.

2. Preparation of a composite of polylactic acid and carbon nanoparticles

To obtain a PLA solution containing CNPs, CNPs suspension was dried first. For this, a solution of CNPs and ethanol was dropped into a Petri dish and dried at 50-60 ℃ until the liquid completely evaporated. After complete evaporation of the liquid, the resulting powder was dissolved in alcohol and dried under the same conditions; this operation was repeated several times. After the CNPs powder ceased to dissolve in alcohol, the drying process was completed. The resulting powder was placed in a glass vessel, 15 ml of chloroform and a weighed portion of PLA corresponding to a 1% solution were added [Figure S1*(b)*]. The yield of CNPs after each synthesis is different, and on average amounts to 100-150 mg.

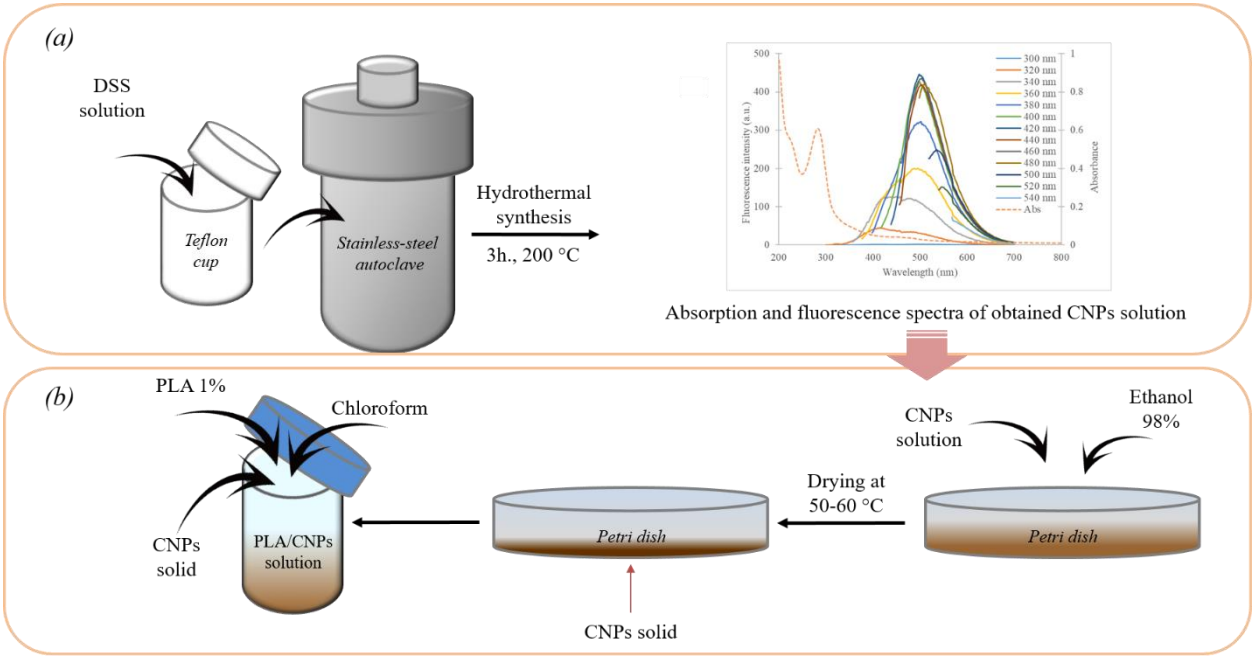

Figure S1 *(a)* Synthesis of CNPs and *(b)* preparation of a composite of polylactic acid and carbon nanoparticles

3. Colorimetric determination of α-amylase

Evaluation of the enzymatic action of α-amylase on starch and its complex with iodine was carried out according by visual observation of color changes[2-4].

In the wells of the microplate, 100 µl of a 0.5% starch solution was dispensed. Then, 10 µl of iodine solution and 10 µl of bidistilled water were added to the control wells (red area in Figure S2). To assess the effect of the enzyme on the starch iodine complex, 10 µl of iodine and 10 µl of α-amylase of various concentrations were added to the starch (yellow area in Figure S2). To assess the effect of the enzyme on starch, 10 µl of α-amylase of various concentrations was added to the wells to the starch (green area in Figure S2). After 30 minutes, 10 µl of iodine was added to the wells of the green area and the color of the solutions was observed in all wells.

As a result of the interaction of amylase and starch, with the subsequent addition of iodine, as well as amylase and starch iodine complex, the color intensity changes. These changes indicate the enzymatic destruction of starch and its complex with iodine. However, when the complex of starch with iodine is destroyed, the color change occurs more slowly, which may indicate different kinetics of the enzymatic reaction.

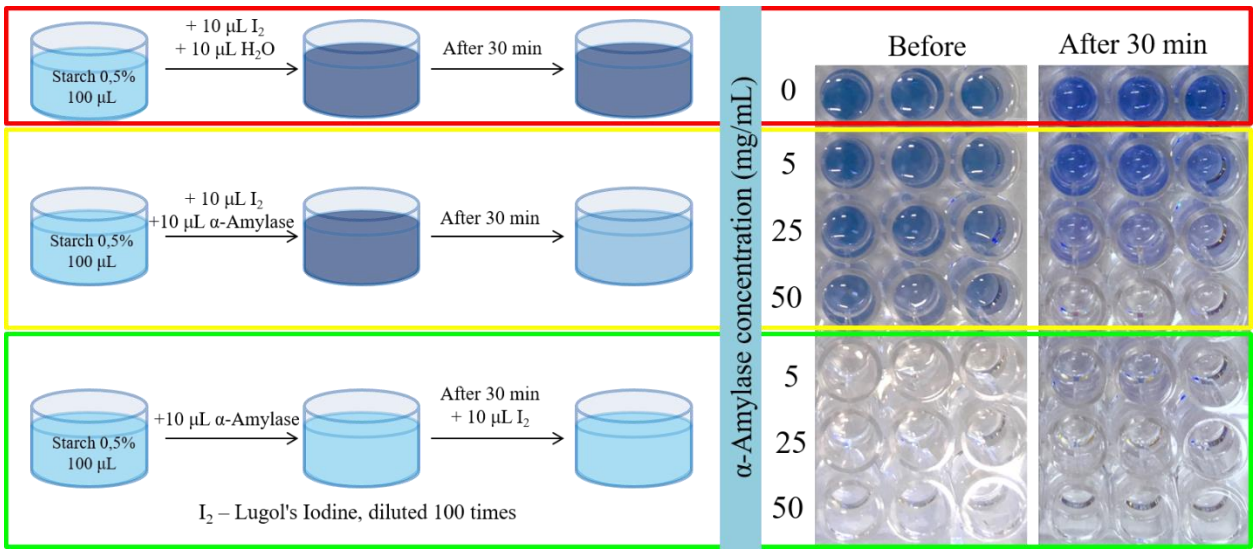

Figure S2 Scheme (left) and result (right) of colorimetric assessment of the enzymatic action of α-amylase on starch and its complex with iodine

4. Controlled release of α-amylase

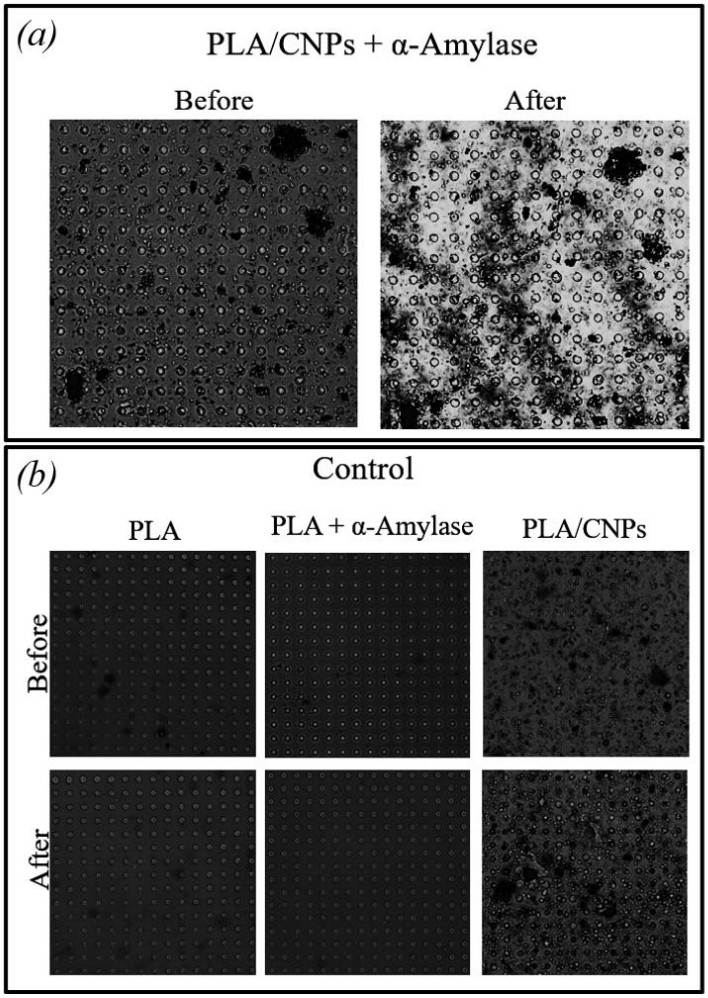

Figure S3 *(a)* Black and white images MCA from an optical microscope of before and after laser exposure for PLA/CNPs with α-amylase as cargo and (b) control samples

References

1. A. A. Kokorina, E. S. Prikhozhdenko, N. V. Tarakina, A. V. Sapelkin, G. B. Sukhorukov and I. Y. Goryacheva, *Carbon*, 2018, **127**, 541.
2. C. Huggins and P. S. Russell, *Ann. Surg.*, 1948, **128**, 668.
3. N. R. Pimstone, *Clinical Chemistry*, 1964, **10**, 891.
4. B. Cochran, D. Lunday and F. Miskevich, *J. Chem. Educ*., 2008, **85**, 401.