Synthesis of Microcapsules with Encapsulated Magnetic Nanoparticles for α-amylase Immobilization

Roman Melnyk, Vitalii Serdiuk, Oleh Shevchuk, Viktor Tokarev

Organic Chemistry Department, Lviv Polytechnic National University, UKRAINE, Lviv, S. Bandery street 12, E-mail: roman.i.melnyk@lpnu.ua

Abstract – Polymeric microcapsules with paraffin core containing magnetite nanoparticles modified by oleic acid and functional polymeric shell were synthesized using the technique of "extraction-coacervation" microencapsulation. The influence of process parameters onto colloidal chemical properties (size, dispersity index, surface porosity) of synthesized microcapsules was studied. It was shown that the use of heterofunctional copolymer as microcapsule shell allows the possibility of irreversible immobilization of α-amylase and provide its participation in the reaction of starch catalytic decomposition.

Keywords – magnetite, acrylate copolymer, heterofunctional copolymer, magnetic nanoparticles, encapsulation, microcapsules, enzyme immobilization.

I. Introduction

In past decade, magnetic nanoparticles (MN) with definite size distribution and structure are of a wide attention due their unique physical and chemical properties [1]. Their use as the carriers of diverse bioactive moieties is of special interest due to their unique properties such as superparamagnetism, high surface area, large surface-to-volume ratio, easy separation under external magnetic fields [2]. Compared to porous carriers, such non-porous nanoparticles have no external diffusion problems, making them more competitive especially for large scale industrial usage in solid–liquid systems (e.g., precipitated protein) [3]. However, unmodified magnetite nanoparticles often have high reactivity and easily undergo degradation upon direct exposing to certain environment, leading to poor stability. Hence, the elaboration of the method of obtaining polymeric microcapsules (MC) with functional shell and encapsulated MN is the important scientific and practical problem.

Therefore the aim of this work is to study the processes of obtaining and properties of functionalized polymer microcapsules with paraffin core filled with MN for immobilization of α-amylase enzymes.

II. Experimental part

Heterofunctional tetrapolymer (HFP) – copolymer of acrylonitrile (AN), butyl methacrylate (BMA), styrene (ST) and maleic anhydride (MA), the composition and properties of HFP are presented in Table 1. IR analysis of synthesized HFP proved the presence of corresponding functional groups in HFP structure: 2240 cm^{-1} – valent vibration νC≡N of AN nitrile group; 1856 and 1780 cm^{-1} – νC=O vibrations of MA anhydride groups; 1728 cm^{-1} – νC=O vibrations and 1220 cm^{-1} – νC–O vibrations of ester group in BMA; 3030 cm^{-1} – νC–H vibrations, 1600, 1580, 1460 cm^{-1} – skeleton vibrations of aromatic C–C bonds; 760 and 700 cm^{-1} – δC–H deformation vibrations of ST phenyl groups.

TABLE 1

COMPOSITION AND PROPERTIES OF HFP

COPOLYMER COMPOSITION, % MOLE				M_N	M_W	DISPERSITY INDEX (M_W/ M_N)	T_G, K
AN	BMA	ST	MA				
50.2	27.9	14.3	7.6	28602	53013	1.85	356±1

The method of synthesis of magnetite nanoparticles. The synthesis of Fe_3O_4 nanoparticles modified with oleic acid (OA) was carried out using modified co-precipitation technique [4]. The difference of our modified method from the original technique was that in this case was used crystalline $FeSO_4 \cdot 7H2O$, and the final product synthesis was prepared as a suspension in ethyl acetate.

The technique of MN microencapsulation. In this work we applied "extraction-coacervation" method of MN encapsulation described in [4].

The technique of immobilization of α-amylase onto microcapsules surface. In order to carry out the immobilization of α-amylase onto MC surface we prepared phosphate saline buffer solution (pH=6). This solution (100 ml) with 500 mg of α-amylase enzyme previously solved in it was added to the synthesized MC. The glass with obtained suspension was placed onto water bath with stirring during 3 hours. After that the enzyme solution was separated from MC containing Fe_3O_4 nanoparticles via magnetic separation. Modified MC were washed three times by 0.01% solution of Tween 20 in water.

Methods of analysis of modified MN and MC with encapsulated MN.

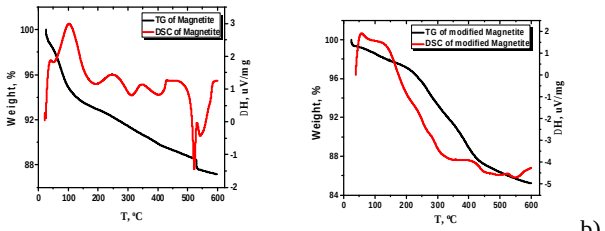
Thermogravimetric analysis (TGA) was carried out using TG 209 F1/Iris device at dynamic mode in air atmosphere and differential-scanning calorimetry (DSC) was performed using DSC 204 F1 Phoenix. Electron microscopic studies were performed using Selmi REM-106I scanning electron microscope (SEM).

Using the results of statistical treatment of the size measurements of 400-500 particles differential curves of MC size distribution were built and number-average (d_n), weight-average (d_w) sizes as well as dispersity indexes (k_p) of synthesized MC were calculated.

III. Results and discussion

Synthesis and modification of MN. At the first stage we have synthesized magnetic nanoparticles with simultaneous their modification by oleic acid. The curves of TGA and DSC of synthesized Fe_3O_4 nanoparticles are presented in Fig. 1. For unmodified magnetite nanoparticles we observed sharp weight decrease in the range from 25 °C to 100 °C that was not observed for modified MN. This is evidently caused due to the elimination of adsorbed water because MN modified by oleic acid have hydrophobic surface and do not adsorb water molecules. Besides, for the sample of modified MN

the sharp weight decrease is observed in the range 250-400 °C (close to the boiling point for oleic acid) that can be explained by elimination of unbonded oleic acid.



a) b)

Fig. 1 The curves of TGA and DSC of magnetite samples: unmodified (a) and modified by oleic acid (b)

MN microencapsulation. The next step of the work was the MN encapsulation in microcapsules with paraffin core and functional polymer shell and the study of dependences of colloidal-chemical properties of synthesized MC, namely of number-average, weight-average sizes as well as polydispersity indexes on the process parameters (Table 2).

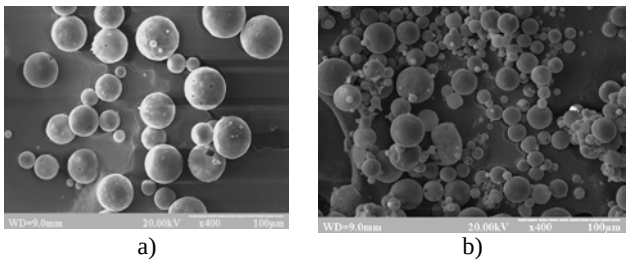
TABLE 2

MC FORMATION CONDITIONS AND CHARACTERISTICS (MN TO PARAFFIN RATIO IS 1:22 WEIGHT PARTS, DISPERGATION RATE – 400 RPM)

SAMPLE	PVA CONTENT, %	T, K	D_N, μM	D_W, μM	K_P
MC1	1.0	328	47.9	78.1	1.63
MC2	2.0	328	37.5	69.6	1.86
MC3	3.0	328	28.3	63.5	2.24

According to obtained data (Table 2) one can conclude that the increase of PVA concentration in stabilizing solution causes the decrease of number-average and weight-average size of MC. At the same time the value of polydispersity index increases.

One can see (Fig. 2), that as a result of encapsulation of magnetic nanoparticles of magnetite microcapsules with regular spherical shape were obtained and agglomeration of particles was not observed.



a) b)

Fig. 2 Microcapsules of sample MC 1 (a), MC 3 (b).

As one can see the porosity of the surface of microcapsules obtained with 3% of PVA in stabilizing solution is less as compare with microcapsules synthesized at the PVA concentration of 1%. It is caused by the fact that greater concentration of PVA leads to the increase of solution viscosity. As a result, during the stage of water addition extraction of ethyl acetate into water phase proceeds slower and the microcapsule surface is formed smoother with less amount of pores. It is necessary to note that obtained MC are rather stable even in acidic medium. After their deposition into 1.5% solution of HCl during one month they keep magnetic properties, that witnesses in favor of dense encapsulation of modified magnetic nanoparticles in the paraffin core.

The next step of the work was the immobilization of α-amylase enzyme onto MC surface using the technique described above. To prove α-amylase immobilization the obtained MC were placed into vial with 5% starch solution (1 ml) and phosphate buffer solution (5 ml). To another vial the same solutions were added but without MC. These vials were deposited into thermostat at 40 °C during 20 hours. After that 1 drop of iodine were added to each vial. In the vial that did not contain MC the appearance of intensive color was observed while the solution in the vial containing MC remained colorless (Fig.3). The test was repeated a few times. Obtained result proves the catalytic action of immobilized α-amylase in the reaction of starch decomposition.

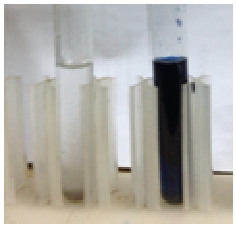


Fig. 3 The vials with MC containing immobilized α-amylase (left) and without MC (right).

Conclusion

The results of performed studies witness that the method proposed allows to obtain microcapsules with paraffin core containing magnetic nanoparticles and functionalized polymeric shell. The influence of process parameters onto colloidal-chemical properties of synthesized microcapsules has been studied. It was shown that MC size and polymeric shell porosity decreases with the increase of polyvinyl alcohol concentration in the stabilizing solution. The presence of functional groups in MC shell structure allows irreversibly immobilization of enzymes that can be used as biocatalysts for industrial application.

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