Carbon Nanosorbent for Purification Different Biomolecules

Z.A. Mansurov[1*], A.R. Kerimkulova[1], S.A. Ibragimova[2], E.Y. Gukenheimer[2]

[1]The Institute of Combustion Problems, Almaty, Kazakhstan
[2]M.A. Aytkhozhin's Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan

Abstract

The article presents the results of physico-chemical studies on the development of nanostructured carbon materials from domestic raw materials. Were obtained and tested micro-mesoporous carbon sorbents for molecular-sieve chromatography of markers and investigated the applicability of carbon sorbents for the separation of protein-lipid complex, and plant bio-stimulator.

Carbon sorbents have well-developed porous structure but their disadvantage is the weak mechanical strength. Recently it was shown that some carbon nanostructures have enormous strength. Thus arose the need to give the nano structured elements to carbon sorbent. Creating carbon sorbents containing nanocarbon structure was the aim of our study, as these by sorbents will be very useful for large-scale purification of biomolecules.

The new carbon nanosorbent was prepared by carbonization of the stones of abricot seeds. The physico - chemical characteristics of nanostructured carbon sorbent was investigated by modern methods like scanning electrone microscope and infra red spectrophotometry. Based on the goal, nano-carbon materials in the laboratory of the Institute of Combustion Problems, Al-Farabi Kazakh National University synthesized nanostructured carbon sorbents – 'Nanokarbosorb' type for chromatography of biomolecules.

It should be noted that the nanostructured elements of the frame attached to an unusually high mechanical strength to nanokarbosorb. Because of this, this sorbent can withstand high fluid pressure at work and has high durability, therefore, it can be used over the years. The sorbent has a very large porosity and large internal surface and, accordingly, a large capacity and has no parasitic sorption. Studies have shown that "Nanokarbosorb" suitable for purification of a powerful biostimulator plants.

In this regard, of great interest represents nanostructured carbon sorbents with improved chromatography characteristics. Carbon sorbents known to mankind over thousands of years. They are widely used for purification of alcohol and other solutions.

They are mechanically very weak and quickly attacked by fungi and bacteria. In this reason they are unconvenient large scale purification biomolecules. However, further improvement of chromatographic sorbents is impossible without the use of ideas and techniques of nanotechnology.

Introduction

Impressive achievements in the field of proteomics, enzyme engineering and biotechnology have allowed to create a range of innovative and highly effective biological products, the use of which has had a revolutionary influence on medicine and agriculture. Therefore it is time for the industrial preparation of these biological preparation: bio-organic substances, proteins and even biostructures [1, 2]. Existing Sorbents: sephadex, sepharose and sorbents for hydrophobic chromatography – octyl and phenyl agarose gels have a number of serious disadvantages. They are mechanically very weak and quickly attacked by fungi and bacteria. In this reason they are unconvenient large scale purification biomolecules. However, further improvement of chromatographic sorbents is impossible without the use of ideas and techniques of nanotechnology.

*corresponding author. Email: zmansurov@kaznu.kz

In this regard, of great interest represents nanostructured carbon sorbents with improved chromatography characteristics. Carbon sorbents known to mankind over thousands of years. They are widely used for purification of alcohol and other solutions. Carbon sorbents have well-developed porous structure but their disadvantage is the weak mechanical strength. Recently it was shown that some carbon nanostructures have enormous strength. Thus arose the need to give the nano structured elements to carbon sorbent.

Creating carbon sorbents containing nanocarbon structure was the aim of our study, as these by sorbents will be very useful for large-scale purification of biomolecules.

Experimental

The object of the study were dry and a three-day germinated seeds of wheat (Triticum aestivum) ‘Steklovidnaya-24’ cultivar. Seeds were germinated in sterile conditions on filter paper in Petri dishes at 20°C in an incubator. Cell-free extracts of the studied plants were obtained by homogenizing them in a chilled porcelain mortar in 0.05 M Tris-HCl buffer, pH 7.4. The homogenate was centrifuged at 10000 xg during 10 minutes in refrigerated centrifuge type "K-24 (Germany).

Chromatographic separation technique is described in detail in the book of Gilmanov et al [3]. To control the chromatographic separation was carried out on UV monitor type Uvicord SII produced by LKB (Sweden). Fraction was purified spherasomes was obtained by method Dilbarkanova R. and Gilmanov M.K. [4].

Microscope, type JEOL SUPER PROBE 733 (Japan) was used for scanning electron microscopy.

Results and Discussion

The new carbon nanosorbent was prepared by carbonization of the stones of abricot seeds. The physico-chemical characteristics of nanostructured carbon sorbent was investigated by modern methods like scanning electrone microscope and infra red spectrophotometry. Based on the goal, nano-carbon materials in the laboratory of the Institute of Combustion Problems, Al-Farabi Kazakh National University synthesized nanostructured carbon sorbents – ‘Nanokarbosorb’ type for chromatography of biomolecules [5-7]. It should be noted that the nanostructured elements of the frame attached to an unusually high mechanical strength to nanokarbosorb. Because of this, this sorbent can withstand high fluid pressure at work and has high durability, therefore, it can be used over the years. The sorbent has a very large porosity and large internal surface and, accordingly, a large capacity and has no parasitic sorption. Studies have shown that "Nanokarbosorb" suitable for purification of a powerful biostimulator plants. For purification of the biostimulator took three days germinated wheat seeds were gomogenised in 70% ethanol. The ethanol extract was purified on a column of ‘Nanocarbosorb’. Biostimulator desorption from the column was carried out by 50% ethanol. (Figure 1). The effectiveness of this biostimulator says that for pre-processing of seeds to plant 100 hectares spent only 2 g of the biostimulator. Biostimulator increases productivity of winter rye on 40%, winter wheat on 30% and sugar beet on 20% (Table 1) and also accelerates the ripening of winter wheat on 10 days. Figure 2 shows the effect of biostimulator on the development of roots of the sugar beet. Effective sorbents are also needed for blood purification in connection with the problem of sepsis. It seems that every year around the world recorded over 18 million cases of sepsis, many of which are letal [8, 9]. Thus, the urgent task is to remove from the blood of an excess of inflammatory cytokines which is the cause of death. Unfortunately, nowadays there aren’t the effective sorbents for removing cytokines from the blood of patients.Due to this, we suggest the ‘Nanocarbosorb’ for removing cytokines from the blood of patients. For the experiment we used a homogenous commercial preparation of cytokine – ‘Roncoleukin’. Our results say about fast sorption Roncoleukin by "Nanokarbosorb’. This opens the real way to fight sepsis, because "Nanokarbosorb" can be used for removing excess blood cytokines. It is very important that the nanokarbosorb can be used for large-scale purification of cytokines which very necessary for the treatment of many immune diseases. The big interest represents the study of the use of "Nanokarbosorb" for purification of lipid-protein complexes – spherasomes. During chromatographic purification the spherosomes are placed at the first chromatographic peak. It was carried out the electron microscopy of fractions of this peak. As shown at Figure 3, this fraction actually containes only spherosomes.

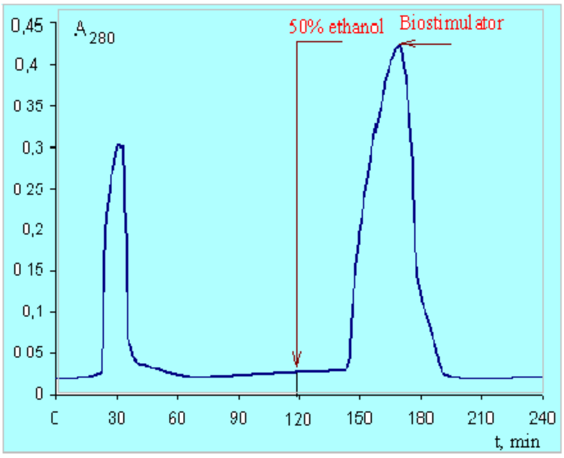

Fig. 1. The separation of biostimulator on nanocarbosorb column

Fig. 2. The effect of biostimulator on the development of roots of the sugar beet 1-control, 2-experiment.

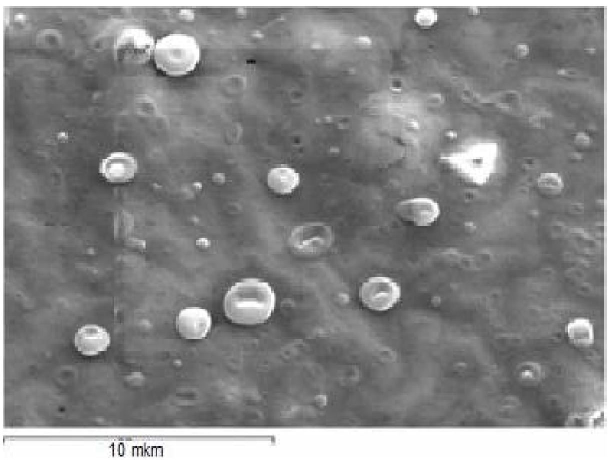

Fig.3 Electron microscopy of the purified spherosomes.

Table 1
Influence of biostimulator's on increasing yields of winter rye and sugar beet

Variants	Increase the yield of winter rye, kg/ha			
	swath			average
Control	19,9	20,0	20,1	20,0±0,1
Experimental	27,9	28,0	28,1	28,0±0,1
Productivity %	40,2	40	39,8	40±0,2
Variants	Increasing yields of sugar beet, kg/ha			
	swath			average
Control	227	265	273	255±18
Experimental	288	302	321	303±17
Productivity %	26,8	14	17,6	20±6,8

Spherasomes very promising for creating high-sensitive biosensors for ecological monitoring of pollution sources of drinking water by sewage water.

References

1. Twyman R.M. Principles of Proteomics, BIOS Scintific Publishers. 2004: 217-234.
2. Patterson S.D. and Aebersold R.H. (2003) Proteomics: the first decade and beyond. Nature Genet 33 (Suppl): 311-323.
3. M. Gilmanov, O. Fursov, A. Frantcev. Methods of purification and the study of plant enzymes. Science, 1981. 91p.
4. Dilbarkanova R., Gilmanov M.K. Spherosomes of the plant cells. Monograph, Almaty "Gylym" - 1997 (in Russian)
5. Z.A. Mansurov, M.K. Gilmanov. Nanostructural Carbon Sorbents for Different Functional Application/ in the book Sorbents: Properties, Materials and Applications, 2009 “Nova Science Publishers, Inc (New York). Editor: Thomas P. Willis. Chapter 7. pp 217-284.

6. Mansurova R.M. Physical-chemical basis of the synthesis of carbon-containing composition. // Monograph, Almaty, 2001.180p.
7. Z.A. Mansurov. Some Applications of Nanocarbon Materials for Novel Devices // R. Gross et al (eds.), Nonoscale-Devices - Fundamentals, 355-368., 2006, Springer, p. 355-368.
8. Slade E, Tamber P.S., Vincent J.L. The surviving sepsis campaign: raising awareness to reduce mortality. Crit Care 2003;7(1):1-2.
9. Gleb Yashin, Yury Gogotsi. Mesoporous carbide-derived carbon with porosity tuned for efficient adsorption of cytokines// Biomaterials 27 (2006) 5755-5762

Received 2 September 2011