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Nano and Microencapsulation of Cephalosporin Antibiotics

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Abstract: Problems of microencapsulation of drugs are mainly deal with not only the methods of their production, but mostly they are linked with the evidentiary tools of the particles. In this paper there are considered the assessments of the micro-and nanocapsules containing a cephalosporin antibiotic, namely, cefotaxime by atomic force, confocal and electron microscopy, IR, Raman and UV spectroscopy. The findings indicate that these methods are applicable for identification of micro-and nanocapsulative medicines.

Key words: Antibiotic · Cefotaxime · Ceftriaxone · Cefazolin · Polyvinyl alcohol (PVA) · Nanoencapsulation · Microencapsulation

INTRODUCTION

Micro-and nanoencapsulation is the most widely-used direction in the pharmaceutical industry and this is due to the fact that the polymer nanoparticles are universal in orientation of tissues, they are characterized by controlled release, that helps to solve the problem of drug delivery to necessary place. Today in pharmaceutics and medicine one of the most poorly solved problems (one of the problems which is solved with difficulties) is the stability of drugs. Polymeric micelles from this side have uncontested advantages: high stability both *in vitro* and *in vivo*, rather narrow size distribution, unique architecture - the «core-shell», all these facts protect unstable drugs from chemical degradation [1]. Microencapsulation is the direction, which is applicable in various industries and fields of study. Microcapsules are possible to study with the various methods of analytical chemistry, they may be the subject of analytical chemistry studies, if the research deals with the phenomena occurring at (taking place in) the phase boundary during encapsulation products may be the objects of colloid chemistry researches. List of Sciences, where micro-and nanocapsules may be the objects of study is not limited to the above ones. As the encapsulated particles are different from the parent compounds in properties, they can be attributed to polymer modification. Based on their architecture «core-shell», the microcapsules can be called supramolecular assemblies and to be the object of supramolecular chemistry.

In this article there are considered researches of polymeric micro- and nanocapsules, where shell is PVA and core is an antibiotic cefotaxime of the group of cephalosporins. The nature of the polymer shell makes it necessary of application of methods with minimal damaging effects on the chemical structure for the study of micro-and nanocapsules. In this instance, Raman spectroscopy, atomic force and confocal microscopy have uncontested advantages from all the arsenal of the modern methods of learning.

Vibrational spectroscopy methods are widely used by organic chemists to identify compounds and by pharmacists to determine the quality of drug products [2-5]. Methods of IR spectroscopy, much less KRS are traditionally used to study the process of formation of capsules based on organic medicines. The main advantages of this method are its non-invasiveness, selectivity, sensitivity and speed measurements [6].

UV spectroscopy is widely used in the pharmaceutical industry for qualitative and quantitative determination. As UV spectrum can be done only for a solute or gaseous compounds (fluid), solid substances must be dissolved, so the analysis of the microcapsules is quite possible for the qualitative and quantitative determination of the structure of the nucleus

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[7]. In this case it is possible to prove the structure of the microcapsules by spectroscopic methods, which are widely used in the pharmaceutical industry (IR, Raman, UV-spectroscopy). Microscopic analysis methods are used for investigation of the surface structure, shape and size of products encapsulation. As the use of every single method gives only an indirect confirmation of the structure, in practice it is necessary to combine the results of the analysis of several methods.

MATERIALS AND METHODS

Cephalosporin antibiotics, particularly cefotaxime, are produced in the form of powder due to the fact that their solutes are not stable. That is why the task of encapsulation of this substance was set, because according to the literature, polymeric micelles are more stable and have a number of other features that are useful for the pharmaceutical and medical industries. Choice of PVA as capsule shell is due to the availability of the polymer and its wide use in the pharmaceutical industry. It should be noted that the preparation of microcapsules of water-soluble compounds in water-soluble high-molecular compounds is a particularly difficult task, as its solution should be done with certain conditions. Our methods allow to solve this kind of problem. The nature of polymer shell makes it necessary to use methods with minimal damaging effects on the chemical structure to study micro-and nanocapsules. In this regard, Raman (Raman) spectroscopy, atomic force and confocal microscopy have uncontested advantages from all the arsenal of the modern methods of learning.

Raman and fluorescence confocal laser scanning confocal microscopy were carried out on microspectrometer Omega Scope, production AIST-NT (*Russia*), combined with a confocal microscope. IR-spectrology-on IR Fourier spectrometer "Bruker Tensor 27" (*Germany*) with validated software OPUS-NT", device for elimination of IR radiation by method of NPVO PIKEMIRacle (Germanium cristal) (*USA*) and IR Fourier spectrometer "AVATAR 360 FT-IRE. S.P. Nicolet", device for elimination of IR radiation by method of NPVO Thermo Scientific"(Selenide of Zink cristal). UV specters were obtained by "Evolution Array UV-Visible Spectrophotometer", device for elimination of UV specters "Thermo Scientific". Atom-power scanned images were obtained with the use of "ACM Smart SPMAIST-NT" (*Russia*). Scanning electronic microscopy was performed on electronic microscope SH-1500(*Japan*).

DISCUSSION

In determining the most effective method for each case it should be based on the certain properties of the final product, the cost of the process and many other factors [8]. But mostly, the choice of method is determined by the properties of original encapsulated substance. According to our original method there were made (obtained) microcapsules of antibiotic cephalosporin group of 3-d generation - cefotaxime. Given the fact that one of the important characteristics of encapsulation is output (yield) by weight the results of microencapsulation in different ratios core/polymer in cefotaxime, ceftriaxone and cefazolin in the PVA are showed in Table 1.

The Table 1 shows that we obtained microcapsules with enough high yields, which range from 84 to 90%. This is a good indication of the method of encapsulation, because the losses are minimal.

The data of confocal microscopy show that the particles of encapsulated cefotaxime have a spherical shape and their size is less than 1 nanometer, FKLSM-size of nanocapsules' agglomerate is 6 nanometer.

The image of atomic force microscope was processed using Gwiddion 2.26, 3D - model of the surface was built (Fig. 4). Curve of particles distribution of cefotaxime encapsulated by the polyvinyl alcohol is shown in Fig. 5 where their average diameter is determined.

Physical size - r is defined by radius of the probe cantilever - R = 10 - 25 nm, that is measured by AFM probe size - r and c are calculated taking into account the simple geometrical constructions (Fig. 5), from (1):

$$r = r_c^2/((2^{1/2})\times R)$$

Physical size, calculated from equation (1) was 232 nm.

Table 1: Results of the encapsulation of cefotaxime in PVA

	Ratio core / polymer								
	1:5	1:4	1:3	1:2	1:1	2:1	3:1	4:1	5:1
output (yield) byweight, %	90		89		85				84

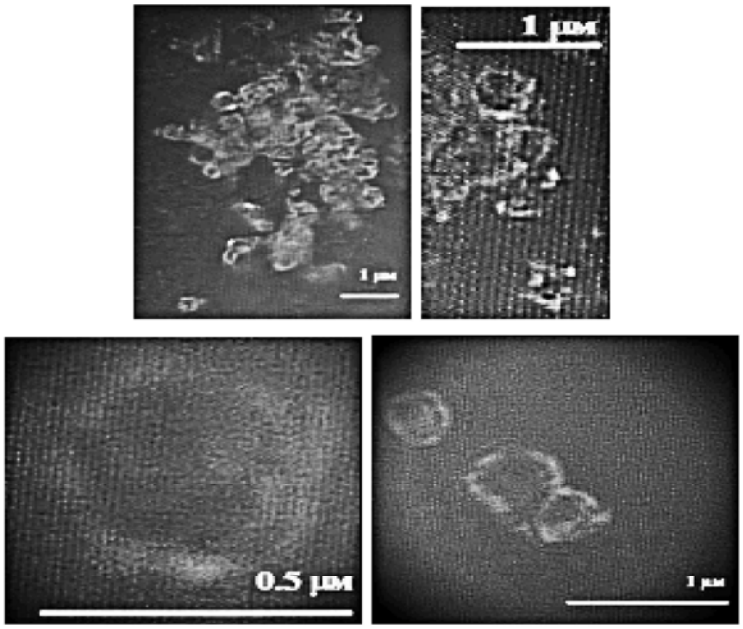

Fig. 1: Confocal microscopy of cefotaxime encapsulated in PVA, ratio 3:1

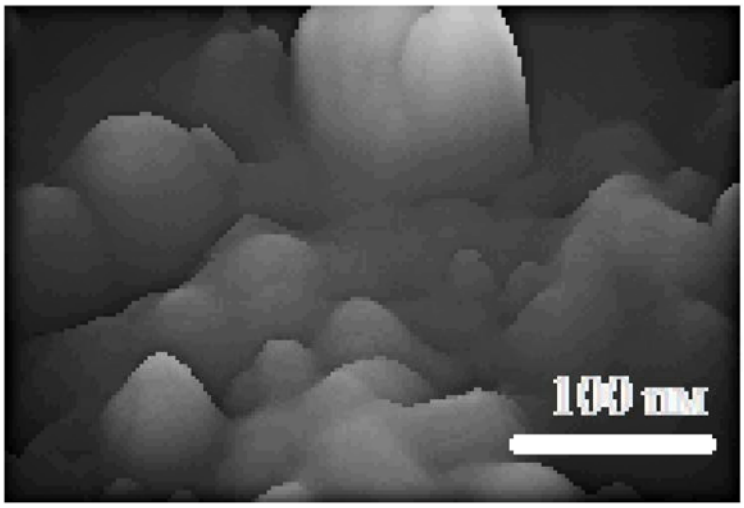

Fig. 2: 3D-model of the particles surface of cefotaxime encapsulated by polyvinyl alcohol

The results of atomic force and confocal microscopy show that the size of cefotaxime nanocapsules is less than 1 nanometer.

Raman spectra were made at two wavelengths $\lambda = 532$ nm, $\lambda = 473$ nm and are shown in Fig. 6.7 and the data - in table 2. In the Raman spectrum of the microcapsules of cefotaxime, ceftriaxone, cefazolin encapsulated by polyvinyl alcohol in all the ratios core / polymer there are spectral lines which is typical for PVA: 2921 CM^{-1} - CH_2 (V_{ac}); 1452 CM^{-1}CH (δ_{CH}); 1371 CM^{-1} - OH (δ_{OH}). The data presented in table 2 show that during decreasing the shell thickness of the microcapsules the giant Raman jump takes place-there is increasing of intensity in the Raman spectrum to 10^6 times [9].

The IR spectra of the microcapsules was investigated and the data of UV-visible spectroscopy of the microcapsules are shown in Table 3.

In the IR spectra of the microcapsules of cefotaxime in the polyvinyl alcohol in the ratio core / polymer there were found absorption bands which are characterised for the PVA : 3327 CM^{-1} - OH (V_{OH}), 2921 CM^{-1} - CH_2 (V_{ac}); 1452 CM^{-1} - CH (δ_{CH}); 1371 CM^{-1} - OH (δ_{OH}).

Table 2: Raman spectra cefotaxime encapsulated in PVA in different ratios core/polymer

Laser wave length	Ratio of core / polymer	Intensity at a frequency of 2921 cm -1 in the spectrum	Intensity at a frequency of 1452 cm -1 in the spectrum	Intensity at a frequency of 1371 cm -1 in the spectrum
473 nm	1:5	2622	387	330
	1:4	848	289	272
	1:3	1246	782	719
	1:2	2121	1229	1169
	1:1	2755	1233	1233
	2:1	4023	2184	2058
	3:1	6813	3262	3135
	4:1	7637	4847	4657
	5:1	18160	10870	10360
532 nm	1:5	617	258	230
	1:4	1004	469	429
	1:3	377	324	306
	1:2	549	472	450
	1:1	1207	819	710
	2:1	443	450	401
	3:1	5183	1886	1664
	4:1	2308	1791	1746
	5:1	25800	24500	22786

Table 3: The peaks of cefotaxime encapsulated by polyvinyl alcohol in different ratios core/polymer in the UV spectra

Name		The absorption maximum (nm)	The absorption maximum(AU)	Minimum absorption (nm)	Minimum absorption (AU)
The ratio of core / polymer	1:5	234,20	1,0937	217,6000	1,0226
of microcapsules	1:4	234,21	0,8738	220,2000	0,8315
	1:3	233,90	1,7371	217,4000	1,5887
	1:2	233,70	1,2367	215,5000	1,1089
	1:1	235,40	1,1910	217,7000	1,0811
	2:1	235,50	0,9784	216,7000	0,8654
	3:1	234,80	1,0315	216,9000	0,9080
	4:1	234,60	1,2029	217,3000	1,0635
	5:1	235,00	1,2393	219,8000	1,1625
Cefotaxime	234,70	1,9774	216,3000	1,7164	

As can be seen from Table 3 in UV spectra of samples of cefotaxime encapsulated in PVA in different ratios core/polymer there is the maximum absorption at a wavelength of 234 ± 1 nm, which is typical for cefotaxime. This indicates that there is encapsulated antibiotic in the microcapsules of the samples.

Scanning electron microscopy (SEM) of the objects was shown butdata about the surface structure of cefotaximenanocapsules were not obtained due to the low image clarity with an increase in 10,000 times in a scanning electron microscope. So from the results of SEM for cefotaxime encapsulated in PVA it can be concluded that the particles have a spherical shape and their size is dozens times smaller than the size of the microcapsules of ceftriaxone in PVA.

Supramolecular chemistry uses the laws of organic synthetic chemistry for getting supramolecular assemblies, the laws of coordination chemistry of complexes and physical chemistry for study of the components interactions, the laws of biochemistry for review of the supramolecular assemblies' functioning. Self-assembly and self-organization are supramolecular properties [10, 11]. In supramolecular chemistry to achieve the controlled assembly of molecular segments and spontaneous organization of molecules in a stable structure non-covalent interactions are used [12, 13].

Table 4: Analysis of fractals, shown in Fig. 3

Fractal number	Branch 1	branch 2	branch 3	branch 4	branch 5	branch 6	branch 7	branch 8	branch 9
1	62,49	17,76	17,12	42,27	19,81	14,69	11,26	9,49	10,54
2	34,05	11,68	13,49	14,68	13,18	10,74	12,17	6,12	6,53

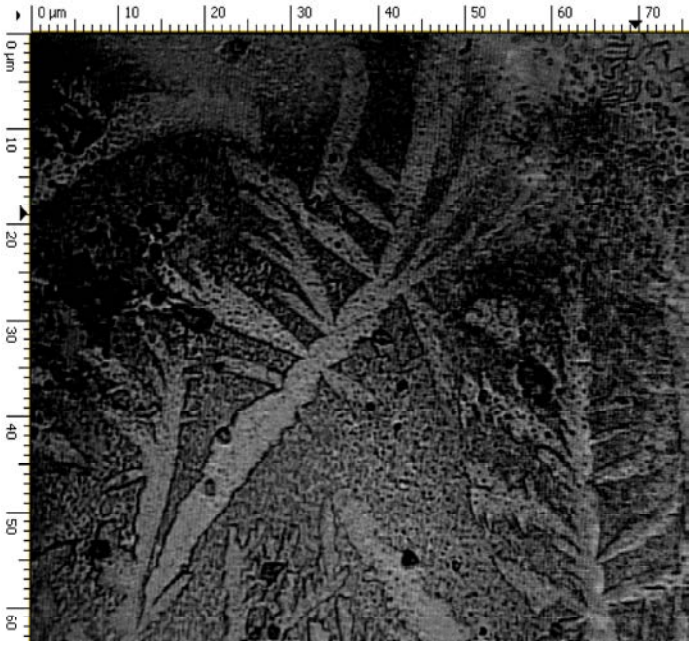

Fig. 3: Confocal image of fractal contexture from microcapsules cefotaxime solute with an increase of 2830 times

Self-assembled structures can be interpreted as aspects of biological systems: artificial cells of membranes and enzymes, or channels [14].

The word Fractal comes from the Latin word "fractal" - separate, not integer (entire). A plurality (accumulation) is called fractal if it has a nonintegral dimension, which is defined by the formula (2):

$$D=\lim_{e\to 0}\frac{\log_c N(e)}{\log_c\left(\frac{1}{e}\right)} \quad (2)$$

where N (e) - the number of cells that can be covered by a fractal image, e - cell size [15]. Formation of fractal compositions was found at a concentration of cefotaxime microcapsules in aqueous solution of 0.05% and was photographed with an increase in 2830 times (Figure 3).

Fractal dimension calculated for structures which are shown in Fig. 13 by (2) was 3.897. Thus, the examined structures are really fractals, because the fractal dimension is not an integer (whole number). Seeing the fact that in the aqueous solution of the microcapsules with enough low concentration fractal composition are detected, they have self-organization. Formation of microcapsules occurs spontaneously on account of noncovalent interactions and it says that they are characterized by self-assembly. Therefore, cefotaxime encapsulated in PVA has supra molecular properties.

CONCLUSIONS

Nanocapsules are the capsules when their size is less than 1 micrometer [16]. Data which we obtained from the image of atomic force microscope says that the average diameter of the capsule is 232 nm, that is less than 1000 nm. According to electron microscopy, particle size is 50 micrometers, therefore, samples can be considered microcapsules where the ratio core /shell 4:1. Information of IR spectra shows that the shell of the particles is the PVA, as there are absorption bands characterized for this polymer in the IR spectra of the surface of all the ratios core/polymer. The results of the UV spectra indicate the presence of cefotaxime in nanocapsules in all these ratios. There are peaks characterized for the PVA in the Raman spectra of all samples. Based on the researches it was revealed that the method of Raman spectroscopy is applied for analyzing of cefotaxime encapsulated in PVA, because of its rapidity, accuracy and applicability when

working with biological objects. Thus, the method of KRS is the most convenient of all we used in this work to research nanocapsules containing biologically active substances. Supramolecular properties of the nanocapsules were studied. The results show that the microcapsules of cefotaxime have supramolecular properties - self-assembly and self-organization. Based on this study it can be concluded that the encapsulation is closely connected with the chemistry of high-molecular compounds, analytical chemistry [16, 17], as to confirm the structure of synthesized particles we need to develop methods for their analyses and the encapsulated material (microcapsules) can be attributed to the objects of the supramolecular chemistry study according to the literature [18, 19].

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