TURLI MANBALARDAN XITOZAN SINTEZI VA XOSSALARI

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Annotatsiya. Qisqichbaqa qobig‘idan uch bosqichli deproteinlash, demineralash va deasetillash jarayonlari orqali olingan xitozan namunalarining deasetillanish darajasi 84% ga tengligi konduktometrik titrlash usuli yordamida, molekulyar massasi 788 kDa ga tengligi esa viskozimetrik o‘lchashlar orqali aniqlandi hamda fizikaviy va biologik xossalari o‘rganildi. Dinamik yorug‘lik tarqalishi (DLS) usuli asosida zarracha o‘lchami 28–384 nm oralig‘ida, asosiy taqsimot 384 nm (89,9%) atrofida ekanligi aniqlandi.

Kalit so‘zlar: Qisqichbaqa qobig‘idan uch bosqichli deproteinlash, demineralash va deasetillash, xitozan *Bombyx mori*

Abstract. Chitosan samples obtained from crayfish shells through the three-step processes of deproteinization, demineralization, and deacetylation were analyzed for their degree of deacetylation and molecular weight. The degree of deacetylation determined by conductometric titration was 84%, while the molecular weight measured by the viscometric method was 788 kDa. Their physical and biological properties were also studied.According to the data obtained by the dynamic light scattering (DLS) method, the particle size ranged from 28 to 384 nm, with the main distribution corresponding to 384 nm (89.9%).

Keywords: Three-step deproteinization, demineralization, and deacetylation of crab shell, chitosan from *Bombyx mori*

Аннотация. Образцы хитозана, полученные из панцирей раков посредством трёхступенчатых процессов - депротеинизации, деминерализации и деацетилирования, были исследованы на степень деацетилирования и молекулярную массу. Степень деацетилирования, определённая методом кондуктометрического титрования, составила 84%, а молекулярная масса, измеренная вискозиметрическим методом, - 788 kDa. Также были изучены их физические и биологические свойства.

Согласно данным метода динамического рассеяния света (DLS), размер частиц находился в диапазоне 28–384 нм, при этом основное распределение приходилось на размер 384 нм (89,9%).

Ключевые слова: Трёхэтапное депротеинирование, деминерализация и деацетилирование раковины краба, хитозан из *Bombyx mori*

Kirish

Bugungi kunda tut ipak qurti g`umbagi, nobud bo`lgan asalari qoldiqlari, zamburuglar, qisqichbaqalar, ba’zi suvo’tlari, kabi manbalardan xitozan (XZ)

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ajratib olish va ular asosidagi preparatlarning xalq xo‘jaligining turli tarmoqlarida qo‘llanilish ko‘lami kengayib bormoqda. Xitozan namunalarining qaysi manbadan olinishidan qattiy nazar ular biomoyilligi, bioparchalanuvchanligi, biologik faolligi, ekologik xavfsizlik singari xossalarni namoyon qiladi.

Jumladan, daryo qisqichbaqasi qobig‘idan olingan *Crab Shells* xitozani ikki bosqichda olingan. Birinchi bosqichda xitin olish jarayoni 7% 60°C NaOH eritmasi bilan 100°C da 24 soat davomida amalga oshirildi. Ikkinchi bosqichda esa xitozan olish jarayoni 50% NaOH eritmasi bilan 100°C da 10 soat davomida amalga oshirildi. Olingan xitozan deasetillanish darajasi 84% ga tengligi konduktometrik titrlash usuli yordamida, molekulyar massasi 788 kDa ga tengligi esa viskozimetrik o‘lchashlar orqali aniqlandi. IQ-spektroskopik usulda o‘rganilgan. Bunga ko‘ra, 1220 sm^{-1} va 1020 sm^{-1} diapazonlarda kuzatilgan yutilish sohalari XZ tarkibidagi erkin aminoguruhlarni (-NH_2) ifodalaydi. Daryo qisqichbaqasi qobig‘ining 40 g kukunida mavjud bo‘lgan minerallarni deminerallashdan so‘ng 23 g xitin olingan va umumiy quruq vaznning (39,53%) ni tashkil etgan [1].

ADABIYOTLAR TAHLILI

Adabiyotlarga ko‘ra, qisqichbaqa chig‘anoqlaridan *Crab Shells* xitozanini olish deproteinlash, deminerallash va deasetillash bosqichlaridan iborat [2]. Mualliflar qisqichbaqa qobig‘i uchun uch xil ya’ni mangrov qisqichbaqasi qobig‘i (K1), mayda qisqichbaqa qobig‘i (K2) va kappa qisqichbaqasi qobig‘ini (K3) xom-ashyo sifatida olib, xitozanni bosqichma-bosqich ajratish bo‘yicha tadqiqotlar o‘tkazishgan (1-jadval).

1-jadval.

***Crab Shells* xitozan namunasi natijalari [2]**

namuna	xom-ashyo, (g)	deminerallash, g	deproteinlash, g	deatsetillash, g
K1	100	57,4	50,5	38,5
K2	100	60,6	50,4	44,0
K3	100	59,0	50,4	41,0

K2 namunadan nisbatan ko‘proq *Crab Shells* xitozani olingani jadvalda keltirilgan daryo qisqichbaqasi chig‘anog‘i tarkibida 20-30% xitin mavjud bo‘lishi aniqlangan [3]. Qisqichbaqa chig‘anoqlari 3,5% NaOH eritmasi bilan 65°C da 2 soat davomida aralashtirib deproteinlangan. So‘ngra xona haroratida 30 daqiqa davomida 1N HCl bilan deminerallab, xona haroratida 2 soat davomida quritilgan. Shundan so‘ng, 5 daqiqa 0,315% natriy gipoxlorit eritmasi bilan oqartirilgan. Deasetillash 50% NaOH eritmasi bilan 100°C da 5-6 soat davomida amalga oshirilib, dist. suv bilan yuvilgan va pechda 24 soat davomida 60°C da quritilgan [4] sintez qilingan *Crab Shells* xitozanining deatsetillanish darajasi 79,68% [5] molekulyar massasi 450 kDa [6] ga tengligi aniqlangan.

ISSN 3030-3907

Crab Shells daryo qisqichbaqasi va *Aspergillus niger* qo‘ziqorinlarning qobig‘idan 37,5% va 39,3% unum bilan xitozan olingan va IQ-spektroskopiyasi yordamida xarakterlangan. *Crab Shells* xitozanining IQ-spektri 500,05 sm^{-1} va 3880,60 sm^{-1} diapazonda, *Aspergillus niger* xitozanining FT-IR spektri 564,67 sm^{-1} va 3451,92 sm^{-1} diapazonda joylashgan asosiy cho‘qqilarni qayd etgan. Xitozanning spektr cho‘qqilari yordamida *Crab Shells* va *Aspergillus niger* xitozanlarning deatsetillanish darajasi 90% va 60% ga tengligi aniqlangan [7].

Suvda eruvchan xitozan (XZ) bir necha jarayonlar yordamida qisqichbaqa qobig‘i chiqindilaridan sintez qilingan. Xitinni deatsetillash har xil haroratlarda, ya’ni 50÷100°C da amalga oshirilib, eruvchan XZ ning optimal rentabelligi 90°C haroratda olingan. XZ ning eng yuqori DD-84,34% ni tashkil etib, bu 90°C da deatsetillanish natijasida olingan. Xona haroratida XZ ning optimal eruvchanligi 1,842 g/L, 40°C da 1,846 g/L ni tashkil etgan bo‘lib, bu 90°C da XZ ni deatsetillash natijasida olinganligi aniqlangan [8]. Qisqichbaqa chig‘anoqlari harorat va vaqtning turli xil ishlov berish sharoitlarda NaOH va HCI bilan uch bosqichda: deproteinlash, deminerallash va deatsetillab XZ olingan. Olingan XZ dinamik yorug'lik tarqalishi (DLS), Brunauer Emmett Teller (BET) va termogravimetrik analiz (TGA) yordamida tavsiflangan. DLS, BET va TGA mos ravishda olingan XZ ning o‘rtacha zarracha hajmi 729 nm bo‘lib o‘rtacha polidispers, sirt maydoni 12,67 m^2/g ga teng tabiatda mezoporoz va 143°C gacha termik barqarorligiga ega ekanligi aniqlangan [9].

Xitozan turli xil boshlang‘ich xomashyolardan: baliq tangachalari, qisqichbaqalar qobig‘idan xitinni deatsetillanish jarayoni yordamida 6 soat davomida 90°C da 40% KOH eritmasi bilan olingan. Olingan xitozan IQ-spektroskopik tahlili bilan tavsiflangan. Aminoguruh mavjudligi sintez qilingan XZ ning IQ-spektrlari tasdiqlangan va baliq tangachalari bilan qisqichbaqa qobig'idan olingan XZ ning IQ-spektrlari usuli bilan hisoblangan DD mos ravishda 75% va 78% ni tashkil etgan [10].

Ushbu ishda ko‘k qisqichbaqa qobig‘i chiqindilaridan sintez qilingan XZ ning antioksidant va antibakterial potensiallari o‘rganilgan. Sintezlangan XZ ning antioksidant faolligi askorbin kislota standarti (87,61±1,34) bilan solishtirganda o‘rtacha (55,30±5,05%) ekanligi aniqlangan, shuningdek, *Staphylococcus aureus* va *Escherichia coli* ga qarshi o‘rtacha antibakterial ta‘sir ko‘rsatgan [11].

TADQIQOT METODOLOGIYASI

Mazkur tadqiqotni bajarishda *Sigma Aldrich* firmasiga tegishli “kimyoviy toza” markali NaOH, H_2O_2, HCl, CH_3COOH singari kimyoviy reagentlardan foydalanildi.

Viskozimetrik tadqiqotlar

ISSN 3030-3907

Xitozanning molekulyar massasi (MM) viskozometrik usul yordamida aniqlanadi. Qovushqoqlikni aniqlash uchun 0,5% li xitozan eritmasi 0,33 M atsetat bufer eritmasida eritildi. Mark-Kun-Houwink formulasi yordamida MM hisoblangan:

$$[\eta] = K \cdot M^{\alpha}$$

Namunalarning xarakteristik qovushqoqliklari Ubbelode viskozimetrida 25°C da termostatda erituvchining oqib o‘tish vaqti bilan o‘lchandi. Bunday sharoitda α va K indekslari α=0,83; $K=1,4\cdot10^{-4}$ ga teng bo‘ladi.

Konduktometrik titrlash

Xitozanning deatsetillanish darajasini aniqlash uchun $9,2\cdot10^{-2}$ g xitozan o‘lchab olindi va 20 ml 0,02 N HCl eritmasida eritildi, eritmaning boshlang‘ich pH qiymati 1,6 ga teng. Keyin xitozan eritmasi har 30 sekundda 0,1 M NaOH fiksanal eritmasi bilan titrlanib, sarflangan ishqor miqdori va eritmaning elektro‘tkazuvchanligi aniqlandi.

IQ-spektroskopik tahlillar

IR Tracer-100 spektrometri BRUKER (Germaniya) yordamida xitozanning IQ-spektroskopik tadqiqotlari amalga oshirildi. *IRTracer-100* 60000:1 yuqori nisbatdagi tovush/shovqin, 0,25 sm^{-1} o‘tkazuvchanlikdagi va soniyada 20 spektrgacha qayd qilish imkonini beruvchi tezkor skanerlash rejimida ishlash qobiliyati bilan ajralib turadi.

AKM-tadqiqotlar

Ph. Eur±0,01 ga muvofiq kaliy dixromat bilan UV fotometriyasining aniqligi 22°C haroratda *AFM Agilent* 5500 atom kuchlanishli mikroskop (AQSh) yordamida *Crab Shells* xitozani plyonkalarning morfologiyasi o‘rganildi. Ishni bajarishda qattiqligi 9,5N/m va 145 кГц ga teng bo‘lgan kremniy idishlar ishlatildi. X,Y da ASM ni maksimal ko‘rish maydoni 15x15 mkm^2, Z da - 1 mkm.

DLS-o`lchashlar

Xitozan namunalarining biodegradatsiyalanishi suvli muhitda zarrachalar tarqalishi, taqsimlanishi va zarra o‘lchamlari *Photocor* analizatori yordamida dinamik yorug‘lik tarqalishini tadqiq qilish orqali aniqlandi. Ushbu usul tarqalgan yorug‘lik intensivligidagi tebranishlarni qayd etish orqali dispers zarrachalarning o‘rtacha diffuziya tezligini o‘lchashga asoslangan. Bu usul yordamida eritmadagi dispers zarrachalarning diffuziya koeffitsientini bevosita o‘lchash orqali zarracha hajmini hisoblab topish mumkin. Amaldagi *Photocor* analizatori yordamida 278-363 K harorat intervalida zarralar, agregatlar, komplekslarning hosil bo‘lish va parchalanish jarayonlarini o‘rganish imkonini beradi.

Rentgeno-strukturaviy tadqiqotlar

ISSN 3030-3907

Rentgenografik tadqiqotlar XRD-6100 (*Shimadzu, Yaponiya*) qurilmasida qayd etilgan diffraktogrammalar asosida amalga oshirildi. CuKα-nurlanish (β-filtr, Ni, 1,54178 Å) tok rejimida va trubka kuchlanishi 30 mA, 30 kV da amalga oshirildi. 0,02 darajali, 4 daraja/min qadam bilan doimiy detektor aylanish tezligi qo‘llanilgan (ω/2θ-bog‘lanish) va skanerlash burchagi 4 dan 80° gacha o‘zgartirildi.

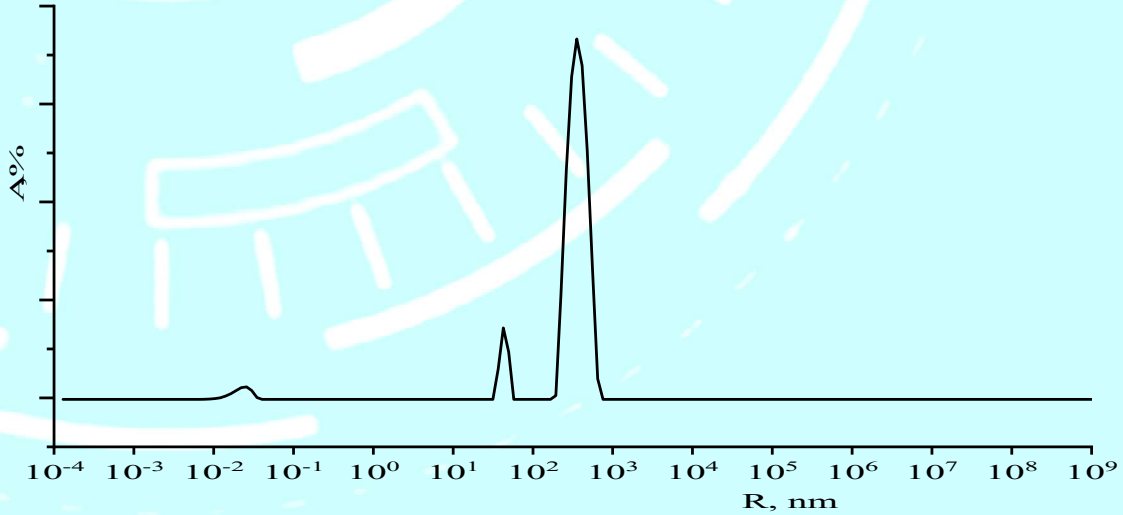
Termogravimetrik va differentsial termik tahlillar

XZ namunalarining termogravimetrik va differentsial termik tahlili TG 209 F1 termogravimetrik analizatorida o‘rganildi. Kimyoviy barqarorlik turli kontsentratsiyali kuchli oksidlovchi kislotalarga hamda yuqori haroratda kuchli asosga nisbatan o‘rganildi.

MUHOKAMA VA NATIJALAR

DLS analiz natijalari

Crab Shells xitozanining eritmalaridagi zarrachalar o‘lchami *DLS* analizatori yordamida tekshirildi [13].



3-rasm. *Crab Shells* xitozani eritmasining *DLS* analiz natijalari

3-jadval.

***Crab Shells* xitozanining eritmadagi zarracha o‘lchamlari**

Peak Num	Area	Mean	Position	STD
1	0.024	0.025	0.028	0.005
2	0.077	47.03	46.16	6.231
3	0.899	401.3	383.6	108.5

Crab Shells xitozani eritmasining zarracha hajmi va rangi barqaror bo‘lgach, zarrachalarning o‘lchami aniqlandi. Zarrachalarning eritmadagi tarqalish gistogrammalari bo‘yicha tajriba natijalari shuni ko‘rsatadiki, xitozan eritmasidagi zarrachalar gidrodinamik radiusi o‘rtacha 28-384 nm ga tengligi ko`rsatildi. Sintez qilingan namunaning 2,4% zarrachalari 28 nm gidrodinamik radiusga ega

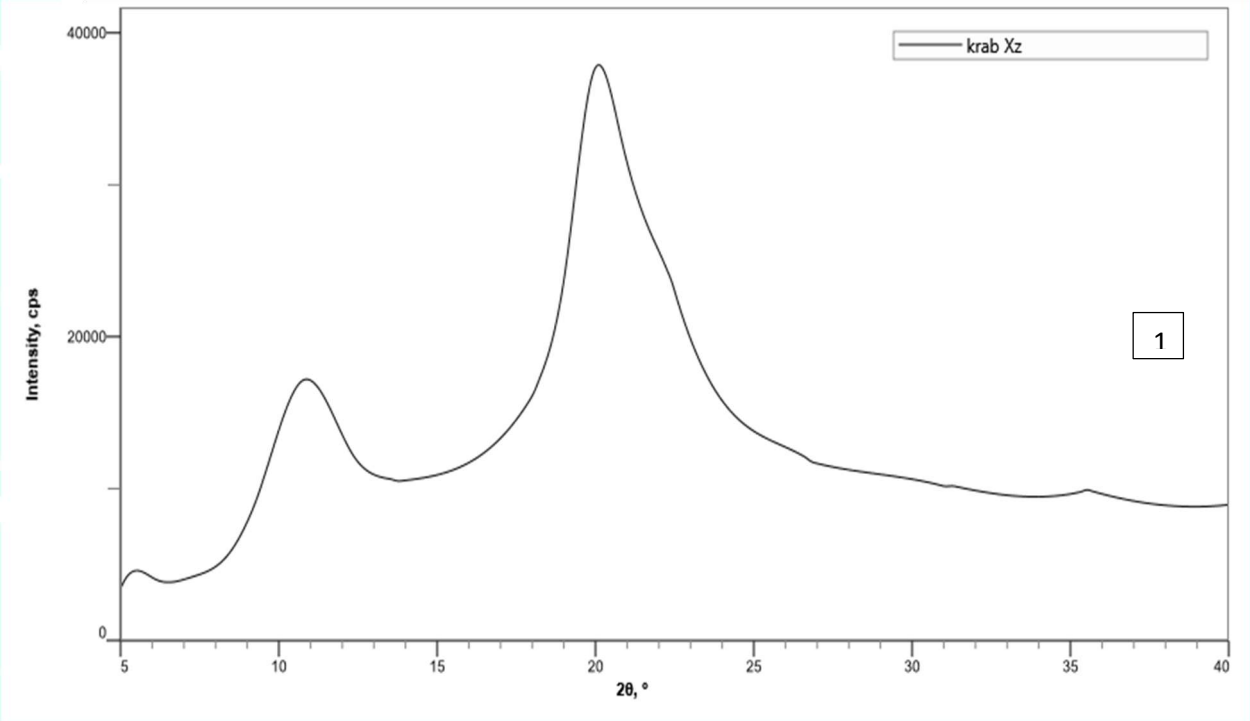
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bo‘ldi, 7,7% zarrachalar esa 46,16 nm hamda 89,9% zarrachalar 384 nm o‘lchamda ekanligi aniqlandi.

4-jadval.

***Crab Shells* xitozanining rentgen fazaviy analizi**

№	2θ, °	d, Å	FWHM, °	Size, Å
1	9.505(11)	9.297(11)	1.24(3)	67.3 (16)
2	12.39 (12)	7.14(7)	3.0(5)	28(4)
3	19.348(3)	4.5840(6)	2.629(13)	32.02(16)
4	28.40(17)	3.140(18)	5.4(5)	15.9(16)
5	38.97(9)	2.309(5)	3.76(16)	232.4(10)



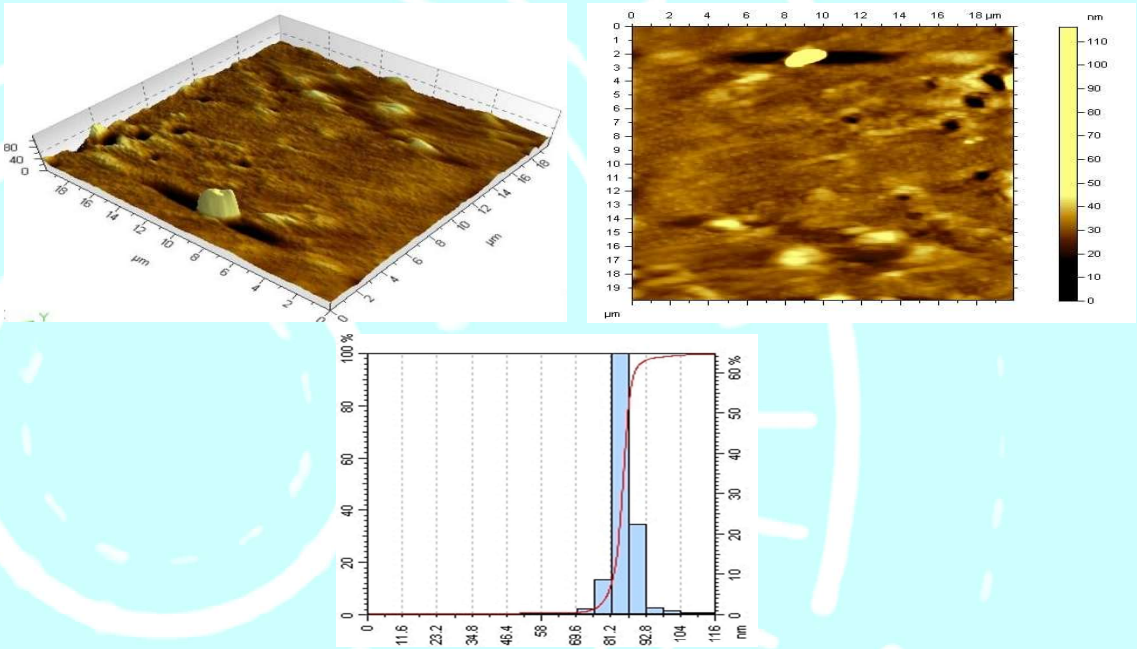
4-rasm. *Crab Shells* xitozani difraktogrammasi

Crab Shells xitozanining namunasi bo‘yicha eksperimental ma’lumotlardan ko‘rinib turibdiki, xitozan ikkita xarakterli cho‘qqilarni ko‘rsatadi, biri 2θ =20° da kuchli cho‘qqi, ikkinchisi 2θ=10° da zaif cho‘qqi borligi aniqlandi. 20°da cho‘qqining mavjudligi aminoguruhlar (-NH_2) borligi aniqlandi va adabiyotda [14] keltirilgan ma’lumot bilan taqqoslaganda mosligi o‘rganildi.

***Crab Shells* xitozanining atom kuchlanishli mikroskopik (AKM) tahlili**

Crab Shells xitozani plyonkasi olinib, uning morfologiyasi AKM vositasida o‘rganildi (6-rasm).

ISSN 3030-3907



5-rasm. *Crab Shells* xitozani matritsasi bo‘ylab zarralar tarqalishining gistogrammalari

Olingan natijalar plyonkalar yuzasida turli o‘lchamdagi sferik va nosferik zarrachalar mavjudligini ko‘rsatadi (6-rasm). Analiz natijalarida o‘lchami 69,6÷116 nm gacha, jumladan, o‘rtacha 82,5 nm bo‘lgan nanozarrachalar hosil bo‘lganligi aniqlandi. Nanozarrachalar hosil bo‘lishi yuza sirtini oshiradi, bu esa reagent sirtiga boshqa reagent qo‘llaganda diffuziyani kuchaytirib beradi. 1 mikrongacha bo‘lgan diapazonda zarrachalar agregativ barqaror bo‘ladi.

XULOSA VA TAKLIFLAR

Shunday qilib, daryo qisqichbaqasi qobig‘idan uch bosqichda: deproteinlash, deminerallash va deasetillash orqali *Crab Shells* xitozani olindi. XZ makromolekulasida 3357 sm^{-1} teskari ishorada kuchli cho‘qqi $–NH_2$ guruhning mavjudligi o‘rganildi. Tanlangan sintez sharoitida nanozarrachalar o‘lchami 69,6÷116 nm gacha intervalda hosil bo‘lishi aniqlandi. *Crab Shells* xitozani plyonkasi olinib, uning morfologiyasi AKM vositasida o‘rganildi Analiz natijalariga ko`ra, o‘rtacha o`lchami 82,5 nm bo‘lgan nanozarrachalar hosil bo‘lganligi aniqlandi.

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