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Synthesis and Characterization of Polymeric Nanoparticles Formed from Cowry Shells and Acacia Gum Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author KJA designed the study, performed the laboratory syntheses, wrote the protocol and wrote the first draft of the manuscript. Authors GMO and GA supervised the work and interpreted the results. Author IOA managed the analyses of the study and provided technical support. Author IEA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study investigated the morphology, dimension, and composition of polymeric nanomaterials obtained from cowry shells and Acacia tree gum arabic extracts. Chitosan and gum arabic were extracted from cowry shells and Acacia trees respectively using standard chemical methods. These were used to produce the chitosan nanoparticles using ionic gelation technique. Observation with a Jeol JSM 7600F Field Emission Gun Ultra-High Resolution Scanning Electron Microscope confirmed the formation of distinct particles composed of smooth ovals, spheres and short cylinders. The nanoparticles were found to have a mean size of 150 nm as measured from a Philips 120 kV EM420 transmission electron microscope. The chemical analysis result obtained from the

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X-Ray fluorescence studies along with the morphology and dimension of the nanoparticles suggested that the developed nanomaterials are suitable as nanocarriers for targeted drug delivery applications.

Keywords: Cowry shells; chitosan; acacia gum; nanoparticles; ionic gelation; drug delivery.

1. INTRODUCTION

Drug delivery, using nano-scaled drug-carrier materials, has been widely researched [1-7]. A wide range of materials, such as natural and synthetic polymers, lipids, and surfactants have been employed to prepare drug nano carriers [8,9]. Biodegradable nanoparticles have been prepared from a variety of materials such as proteins, polysaccharides and synthetic biodegradable polymers. Some of these have been tested and accepted as drug delivery systems [10,11,12]. Polymeric nanoparticles have received more attention than liposomes because of their therapeutic potential and greater stability in biological fluids as well as during storage [13]. In addition, they show high encapsulation efficiency and protection of unstable drugs against degradation by the external environment in comparison to liposomes [14,15].

Properties of polymeric nanoparticles can be tailored by using different polymers and co-polymers or proteins. The new strategies use new biodegradable synthetic polymers and modified polymers from natural products such as chitosan and albumin.

Chitosan is a nontoxic, biodegradable and biocompatible linear polysaccharide of randomly distributed N-acetyl glucosamine and glucosamine units. Chitosan is a copolymer usually derived from chitin, the second most abundant polysaccharide in nature, after cellulose. Chitin, is present in the exoskeleton of arthropods, crustaceans, yeast and fungi [16,17]. Due to the outstanding biological properties of chitosan, it has found wide applications in pharmaceutics [18,19]. Similarly, its derivatives have shown improved characteristics.

Chitosan forms nanoparticles in solution when reacted with substances such as sodium tripolyphosphate, xanthan gum, and acacia gum Arabic by ionic gelation method. The nature of particle formation mechanism in ionic gelation technique is such that positively charged amine groups of chitosan are neutralized by their interaction with negatively charged Arabic gum polymer [20].

According to the Pharmaceutical Codex [21] Acacia is composed chiefly of the calcium, potassium, and magnesium salts of arabic acid which is a highly branched polysaccharide with uronic groups. Acid hydrolysis with 2% v/v sulphuric acid yields mainly D-galactopyranose, L-arabofuranose, L-thamnopyranose, and D-glucoronic acid, in the approximate molar ratio of 3:3:1:1. According to Avadi et al. [20] the solution of Arabic gum in water dissociates the salts and reveals the negative charge of Arabic gum, which allows the interaction with the positive charge of chitosan. Acacia is used in combination with gelatin to form complex coarcervates, by control of the dispersed state, temperature, and pH and addition of disolvating agents, these colloidal mixtures can be used to micro-encapsulate drugs to ease handling or to control dissolution rate [21].

Past research has also shown cowry shell based biopolymer to be suitable for biomedical applications [22-24]. The present study therefore attempted to take the work on cowry shell based chitosan further by the synthesis of cowry shell based chitosan nanoparticles. Furthermore, the suitability of acacia gum Arabic, extracted from Nigeria based Acacia tree, as a possible crosslinking agent for the production of chitosan nanoparticle was also investigated.

2. EXPERIMENT

Methodology employed for synthesizing and characterizing the nanoparticles are reported in the following paragraphs.

2.1 Isolation of Chitosan from Cowry Shells

Cowry shells sourced from a local market in Ilesa, Nigeria, were thoroughly washed to remove impurities with subsequent drying at 60 ^{o}C to remove moisture. The shells were pulverized with a rock mill and sieved to collect 250 µm sized particles. Deproteinization of the pulverized mass was carried out by the addition of 0.1 M NaOH to the pulverized shell in a conical flask. The mixture was boiled and stirred at 100ºC for 2 hours in a water bath. The

resulting product was washed with water to neutrality and subsequently soaked in in pure acetone for 24 hours to decolorize it. Demineralization was accomplished using 0.5 M Hydrochloric acid to leach out $CaCO_3$ while boiling and stirring the mixture at 100ºC in a water bath for 60 minutes. Upon washing and drying, the residue, which is chitin, was oven-dried at 100ºC for 2 hours. The chitosan was finally obtained by the standard alkaline deacetylation of the isolated chitin.

2.2 Extraction of Gum from Acacia Tree

Collection of gummy exudate from Acacia tree stem was done after incision of some Acacia trees (specie: acacia Senegal). Impurities such as leaves, insects, stone, and bark were physically separated from the exudate. The exudate collected was oven-dried at 30-40ºC for 6 hours followed by washing in acetone to remove impurities such as tannin, natural pigments, trace elements and proteins present in the crude gum [25].

The Acacia gum crumbs obtained was refrigerated for 12 hours to embrittle it in preparatory to grinding. Finally, the gum crumbs were carefully ground in pharmaceutical mortal to pass through a 250 µm sieve.

2.3 Synthesis of Chitosan-acacia Gum Nanoparticles

Chitosan solution was prepared by adding 2 g of chitosan and 50 ml 1% acetic acid solution under magnetic stirring for 12 hours. The resulting solution was filtered and preserved. Similarly, acacia gum solution was prepared by the addition of 4 g of gum arabic to 50 ml of distilled water under magnetic stirring for 6 hours. Chitosan-acacia gum arabic nanoparticles were synthesized by drop-wise addition of acacia gum solution to chitosan solution under vigorous magnetic stirring [26]. The resulting suspension was left to gelate at room temperature for 30 minutes. The recovery of nanoparticles from the jelly solution obtained above was carried out by centrifuging the solution at 7000 rpm for 15 minutes. Oven-drying of the residue was carried out at 30-40ºC to obtain powdered nanoparticles. Chemical characterization of the synthesized chitosan-acacia gum arabic nanoparticles was carried out using Fourier transform infrared spectroscopy (FTIR). Finally, morphological and structural analysis of the synthesized nanoparticles was carried out using a Jeol JSM 7600F Field Emission Gun, Ultra-High Resolution, Scanning Electron Microscope (SEM) and a Philips 120 kV EM420 Transmission Electron Microscope (TEM).

3. RESULTS AND DISCUSSION

Results obtained from various structural and chemical characterization conducted for the nanoparticles are reported and discussed as follow.

3.1 Morphological Characterization

The morphology of the nanoparticles observed with the SEM are presented in Figs. 1 and 2. Fig. 1 shows a cluster of discrete particles. The collection is apparently dense with identifiable shapes for particles near the surface. Prominent among observable shapes are spheres, ovals and short cylinders. All the shapes are generally smooth in appearance as shown in Fig. 2, obtained at higher magnification. The morphology obtained here is similar to that of Avadi et al. [27].

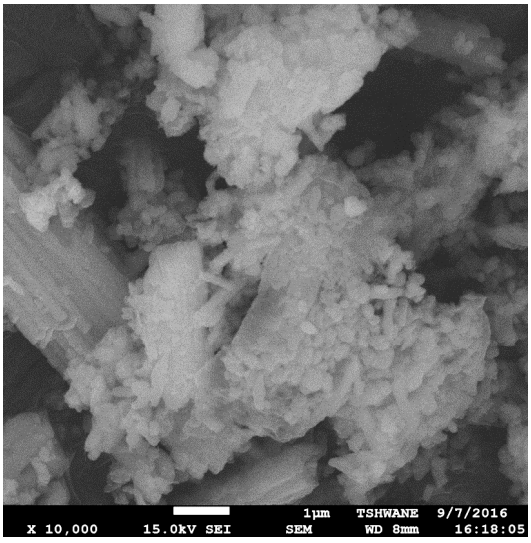


Fig. 1. SEM photomicrograph of chitosan-acacia gum arabic nanoparticles showing a cluster of discrete particles

The general absence of sharp edges indicate a convenient property required for smooth transport within blood vessels. Smooth surface implies low attrition and friction in transport. This again goes to imply a reduced incidence of wear and degradation, which usually occurs with drug loaded nanoparticles when delivering active pharmaceutical ingredient to ailing sites.

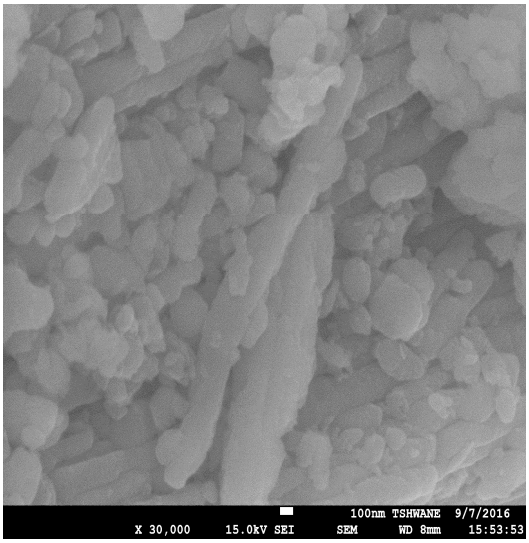


Fig. 2. SEM photomicrograph of chitosan-acacia gum arabic nanoparticles showing absence of sharp edges in particles

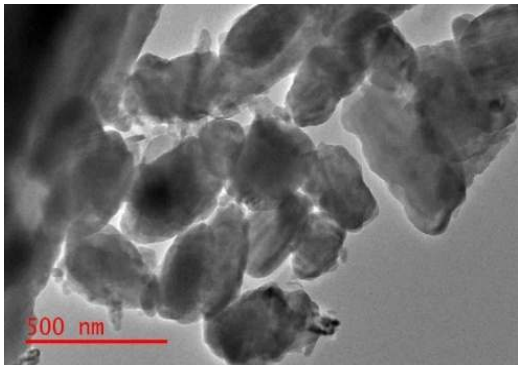
Another advantage derivable from the smoothness of the nanoparticles surface is reduced systemic circulation time. Nanoparticles tend to arrive their tumor-destination with little stress and at a shorter time under active tumor-targeting scheme. An added benefit of the smooth morphology is that cases of premature drug release will be largely forestalled. Sunderland et al. [12] noted that passive targeting of tumors is typically slow, so extended systemic circulation is required to achieve sufficient nanoparticle concentration at tumor site.

3.2 Dimensional Characterization

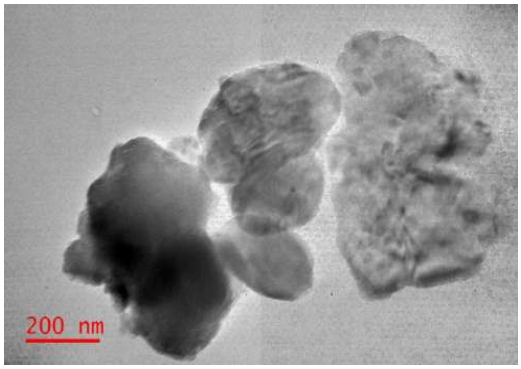
Further investigation made to characterize the chitosan-acacia gum nanoparticles with respect to dimension using the TEM technique established that particle sizes are in the range of 50 – 300 nm, the mean particle size of the nanoparticles was found to be 150 nm (Fig. 3).

Most targeted drug delivery systems based on nanoparticles are in the range of 10-300 nm [10]. Sunderland et al. [12] cautioned that it is important that nanoparticles avoid opsonization and liver uptake during extended systemic circulation. Phagocytosis, which occurs after opsonization, represents the primary mode of particle clearance in vivo [28].

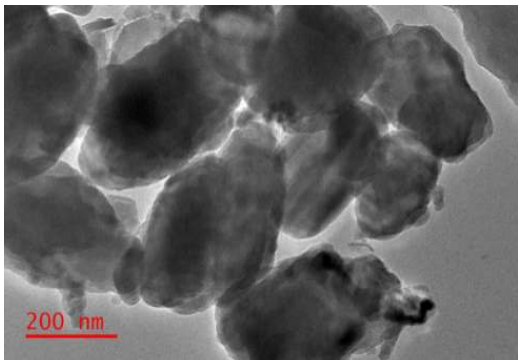
Owens III and Peppas [29] pointed out that size plays a key role in the final biodistribution and blood clearance of stealth particles. Very fine nanoparticles are at greater risk of opsonization clearance by being electrostatically bonded to opsonins exhibiting Brownian motion throughout the blood [29].



(a)



(b)



(c)

Fig. 3. TEM photomicrograph of chitosan-acacia gum nanoparticles showing (a) discrete nanoparticles, (b) associating nanoparticles; and (c) overlapping nanoparticles

Particles with hydrodynamic radii of over 200 nm typically exhibit a lesser rate of clearance than particles with radii under 200 nm [30]. The 50-300 nm particle size obtained is expected to present minimal clearance susceptibility. In Physiology, particles of such size in transport cannot occlude visceral vessels.

It is also envisaged that obtained size range will be capable of encapsulating substantial active pharmaceutical ingredient for therapeutic action. This will go a long way in improving the efficiency of the targeted delivery system.

3.3 Chemical Analysis

The FTIR spectra shows that the chitosan isolated from the cowry shell has three main functional groups, which are the hydroxyl group (O-H), the amino group (N-H) and C=O of an amide group. structure shown in Fig. 4. The strong absorption peak at 1787.54 cm^{-1} is characteristic of chitosan and corresponds to amide (C=O) stretching vibrations. Other main bands appearing in that spectrum are due to stretching vibrations of O-H groups in the range from 3750 cm^{-1} to 3000 cm^{-1}, which are overlapped to the stretching vibration of N-H; and C–H bond in $–CH_2$ (2920 cm^{-1}) and $–CH_3$ (2875 cm^{-1}) groups [31,32].

The striking resemblance between the FT-IR spectra of chitosan in Fig. 4 and chitosan-Acacia gum arabic nanoparticles, Fig. 5, depicts the nanoparticle as a true derivative of chitosan.

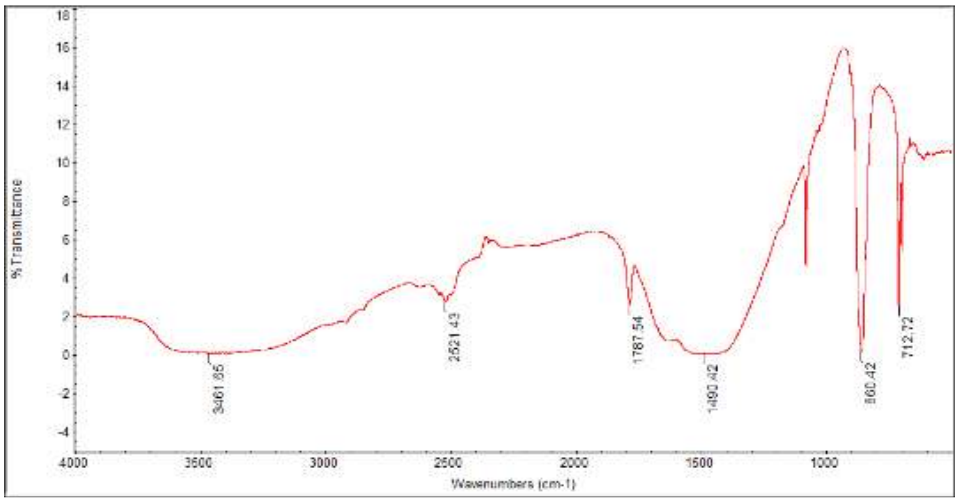


Fig. 4. FT-IR spectra of isolated chitosan

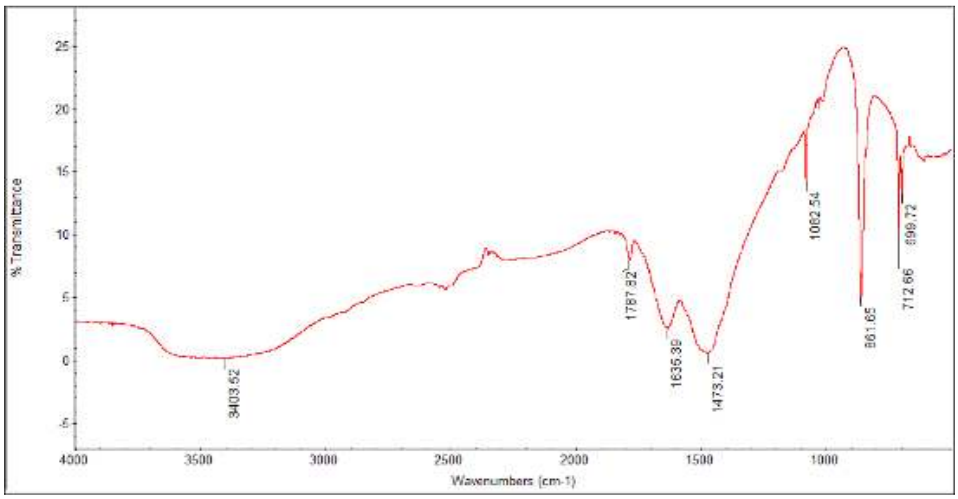


Fig. 5. FT-IR spectra of chitosan-acacia gum arabic nanoparticles

It can be further deduced that variation in the spectra affirms formation of a new substance and not just a physical change in the chitosan structure. This could be related to the type of particle formation mechanism in ionic gelation, where positively charged amine groups of chitosan are neutralized by their interaction with negatively charged Acacia gum polymer [20].

In agreement with the observation of Abdelhalim [32], it can be noted that both the FT-IR of chitosan and its nanoparticle derivative show the existence of extensive hydrogen bonding in the range of 3300 – 3500 cm^{-1}. However, it seems that the chitosan-acacia gum nanoparticles possess relatively higher extents of hydrogen bonding than chitosan.

In addition, X-Ray Fluorescence (XRF) results for the synthesized materials is presented in Table 1. This shows that the synthesized materials have little, or no trace of other elements/ impurities capable of affecting its biocompatibility.

Table 1. X-Ray fluorescence (XRF) results for the synthesized materials

Element	Chitosan (Wt. %)	Gum Arabic (Wt. %)	Chitosan /gum Arabic nanoparticles
P	1.7673	5.5235	1.0798
Cl	1.2200	3.2972	0.9725
K	1.7494	8.6066	1.7445
Ca	35.7397	18.2917	36.6864
V	0.0213	0.0808	0.0185
Mn	0.0210	0.3314	0.0113
Fe	0.1142	1.9781	0.1343
Cu	0.0156	0.5176	0.0142
Zn	0.0156	0.2196	0.0204
Ga	0.0091	0.0781	0.089
Se	0.0043	0.0909	0.0048
Sr	0.0349	0.0686	0.00412
Ti	0.0120	0.2355	0.0191
Mo	0.0033	0.0916	0.0025
Cr	0.0033	0.1568	0.0024
Ni	0.0261	0.4866	0.0295
As	0.0024	0.0308	0.0036
Bi	0.0087	0.2028	0.0087
W	0.0108	0.4078	0.0106
Pb	0.0006	0.0444	0.0010
Nb	0.0034	0.0886	0.0039

4. CONCLUSION

The following conclusions can therefore be drawn from the results of this work.

(1) Chitosan and acacia gum were successfully extracted from cowry shells and Acacia trees of Nigeria origin.
(2) Nanoparticles were formed after acacia gum solution was added to chitosan solution under magnetic stirring at room temperature.
(3) The morphology obtained from scanning electron microscope showed prominent shapes such as spheres, ovals and short cylinders.
(4) All the shapes are visually smooth in appearance with a general absence of sharp edges. This portends a low risk of friction and attrition, a reduced incidence of wear and degradation, short systemic circulation time, and a forestalled premature drug release; if nanoparticle is applied in targeted drug delivery.
(5) The nanoparticles have a mean particle size of 150 nm which is within the suitable range for drug nanocarriers.
(6) In Physiology, particles of such size in transport cannot occlude visceral vessels.
(7) The nanoparticles are considered suitable for application as drug nanocarriers in targeted drug delivery systems.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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