ejpmr, 2018,5(6), 162-166

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

MORPHOLOGICAL ANALYSIS OFCHITOSAN-ACACIA GUM NANOPARTICLES LOADED AND UNLOADED WITH CISPLATIN

Kunle Joseph Akinluwade*[1,2], Grace Modupe Oyatogun[1], Gbenga Alebiowu[3] and Abimbola Patricia Popoola[4]

[1]Department of Materials Science and Engineering, Obafemi Awolowo University (OAU), Ile-Ife, 220282, Nigeria.
[2]Department of Research and Development, Prototype Engineering Development Institute, (National Agency for Science and Engineering Infrastructure, NASENI), Ilesa, 233036, Nigeria.
[3]Department of Pharmaceutics, Obafemi Awolowo University (OAU), Ile-Ife, 220282, Nigeria.
[4]Department of Chemical Metallurgical and Materials Engineering, Faculty of Engineering and the Built Environment, Tshwane University of Technology, Pretoria, South Africa.

***Corresponding Author: Dr. Kunle Joseph Akinluwade**

Department of Materials Science and Engineering, Obafemi Awolowo University (OAU), Ile-Ife, 220282, Nigeria.

Article Received on 04/04/2018 **Article Revised on 25/04/2018** **Article Accepted on 15/05/2018**

ABSTRACT

In this study, chitosan-based and cisplatin-laden nanoparticles formed from cowry shells and acacia gum were synthesized with an investigation of the morphology and dimension of the nanoparticles. Chitosan and gum arabic were extracted from cowry shells and Acacia trees respectively to prepare nanoparticles using standard chemical methods. Observation with a Jeol JSM 7600F Field Emission Gun Ultra-High Resolution Scanning Electron Microscope confirmed the formation of particles composed of distinct spheres, ovals, and short cylinders. The study further investigated the morphology of the nanoparticles before and after loading with cisplatin, a multipurpose anti-cancer drug. Quantitative morphological description of nanoparticles was done using shape factors such as aspect ratio, roundness, irregularity and equivalent circle diameter (ECD), which were obtained from Media cybernetics software analysis while qualitative description was done on the basis of visual observation of acquired SEM photomicrographs. Results showed that the respective values of aspect ratio (0.67 and 0.68), roundness (0.91 and 0.95), irregularity (2.69 and 2.80) and ECD (0.67 and 0.80) obtained for cisplatin-unloaded and cisplatin-loaded nanoparticles are strikingly comparable providing evidence that drug encapsulation did not impair the morphological attributes of the nanoparticles significantly. The developed nanomaterials are thus considered suitable as nanocarriers for targeted drug delivery applications.

KEYWORDS: Cowry shells, cisplatin, Acacia gum, nanoparticles, shape factors, Media cybernetics

1. INTRODUCTION

Drug delivery systems are engineered technologies for the targeted delivery and/ or controlled release of therapeutic agents.[1] Drug delivery systems control the rate at which a drug is released and the location in the body where it is released. However, some systems can control both.[1] Particular attention has been on the use of microelectro-mechanical-systems, cyclodextrins (the most representative of molecular drug delivery systems), microemulsion and organogel (supramolecular aggregates), and colloidal carriers (liposomes, niosomes, ethosomes, ultra-deformable vesicles, nanoparticles) for delivery of drugs.[2]

Recent developments in nanotechnology have shown that nanoparticles have a great potential as drug carriers [3-5]. Due to their sizes, they exhibit unique physicochemical and biological properties such as enhanced reactive area and the ability to cross cell and tissue barriers.[1-5] These attributes make them a favorable material for pharmaceutical applications.[2-8]

A wide range of materials, such as natural and synthetic polymers, lipids, and surfactants, have been employed to prepare drug nano carriers.[3] Biodegradable nanoparticles have been prepared from a variety of materials such as proteins, polysaccharides and synthetic biodegradable polymers.[5]

Polymeric nanoparticles have received more attention than others because of their therapeutic potential and greater stability in biological fluids as well as during storage.[5-8] In addition, they show high encapsulation efficiency and protection of unstable drugs against degradation by the external environment in comparison to others.[7, 8] The utilization of Chitosan as drug delivery system has been reported.[9-13] Over the last few decades, investigations had been focused on applications of chitosan and its derivatives in drug delivery.[14-18]

Chitosan is a nontoxic, biodegradable and biocompatible linear polysaccharide of randomly distributed N-acetyl glucosamine and glucosamine units.[12] Chitosan is usually derived from chitin, which is present in the exo-skeleton of arthropods, crustaceans, yeast and fungi.[12-16] Chitosan nanoparticles derivative is usually obtained by the ionic gelation with polyphosphates, nucleic acids, sodium tripolyphosphate, xanthan gum, and acacia gum Arabic.[6,9]

Past research has shown cowry shell based materials to be suitable for biomedical applications.[18,20] In addition, the biopolymer extract of cowry shell, chitosan, has been found to be suitable for pharmaceutical applications.[9,10,20]

The present study therefore attempted to take the work further by synthesizing cowry shell based chitosan nanoparticles for pharmaceutical application. The suitability of the synthesized nanoparticles as a nano carrier was also studied by analyzing the morphological parameters such as aspect ratio, roundness, irregularity and equivalent circle diameter, of chitosan-acacia gum nanoparticles using Media cybernetics software.

2. MATERIALS AND METHOD

2.1 Materials

Materials used for this study include cowry shells (Ilesa, Nigeria), gum Arabic exudate (Kano, Nigeria), cisplatin and phosphate buffered saline (PBS) (Sigma-Aldrich Laborchemickalien GMBH,Seelze, Germany), hydrochloric acid, sodium hydroxide, acetic acid, acetone (BDHChemicals Ltd Poole, England) and distilled water.

2.2 Synthesis of cisplatin-loaded nanoparticles

Chitosan-acacia gum nanoparticles were prepared from cowry shells and acacia gum using the standard ionic gelation technique [9]. Cisplatin, a multipurpose anti-cancer drug, was loaded onto chitosan-acacia gum nanoparticles by encapsulation mechanism using the standard ionic gelation method.[21-25] This entails the preparation of chitosan, acacia gum and drug solution. The chitosan solution was prepared by adding 2 g of chitosan to 50 ml of 1% acetic acid acid solution under magnetic stirring for 12 hours. Similarly, the gum arabic solution was prepared by the addition of 4 g of gum arabic to 50 ml of distilled water under magnetic stirring for 6 hours. Powdered cisplatin from a 25 mg drug stock of the active pharmaceutical ingredient (API), was dissolved in distilled water to form a solution having a mass concentration of 0.5 mg/ml. The drug solution so formed was added directly to the chitosan solution under a gentle magnetic stirring to form chitosan-drug solution. Drug-laden nanoparticles were synthesized by drop-wise addition of acacia gum solution to chitosan-drug solution under vigorous magnetic stirring. The resulting drug-loaded nanoparticles were recovered by centrifuging the solution at 7000 rpm for15 minutes.

2.3 Scanning Electron Microscopy

The morphology of the synthesized nanoparticle samples were studied using the Jeol JSM 7600F Field Emission Gun Ultra-High Resolution Scanning Electron Microscope. The nanoparticle samples were imaged with a beam of electrons, scanned across, creating an image of the surface of the sample. The image was achieved via the detection of secondary electrons that were released from the specimen as a result of it being scanned by very high energy primary electrons emitted from the electron gun in the SEM. The mean dimension of the synthesized nanoparticles has been determined to be 150 nm.[9]

2.4 Photomicrograph Analysis

The SEM images of chitosan-acacia gum nanoparticles and drug-loaded nanoparticles were further analyzed on a personal computer using Image Pro Premier software (Media Cybernetics, Bethesda, MD, USA) to determine the particle descriptors of major and minor axis length, perimeter and projected area from which shape factors of aspect ratio, roundness, irregularity and equivalent diameter were determined.[25-26]

3. RESULTS AND DISCUSSION

Results obtained from SEM characterization and morphological analysis conducted for the nanoparticles are discussed in this section. Figures 1 and 2 show the SEM micrographs of synthesized chitosan-acacia gum nanoparticles and cisplatin-loaded chitosan-acacia gum nanoparticles respectively. Morphological parameters of chitosan-acacia gum nanoparticles and drug-loaded nanoparticles as analysed by Media cybermetrics software are presented in Table 1.

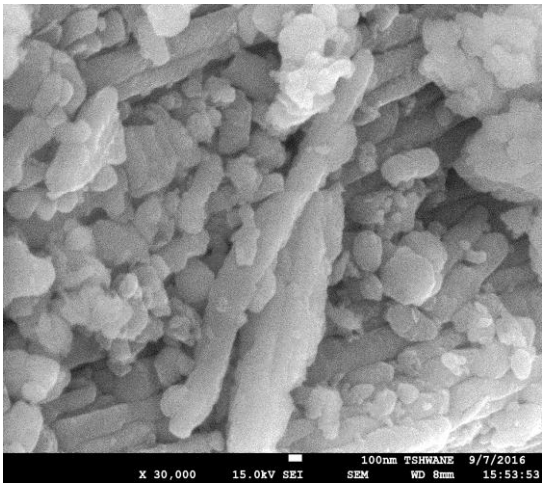

Figure 1: SEM micrograph of chitosan-acacia gum arabic nanoparticles

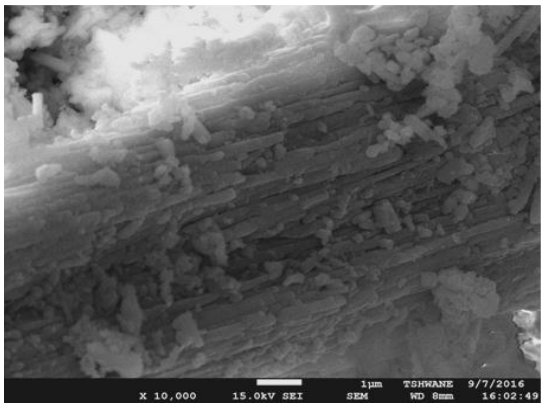

Figure 2: SEM photomicrograph of cisplatin-loaded nanoparticles.

Table 1: Morphological parameters of chitosan-acacia gum nanoparticles and drug-loaded nanoparticles

Shape factors	Cisplatin-free nanoparticle	Cisplatin-loaded nanoparticle
Aspect ratio	0.67	0.68
Roundness	0.91	0.95
Irregularity	2.69	2.8
Equivalent Circle Diameter	0.67	0.8

3.1 Morphological Characterization

Morphological shapes of synthesized nanoparticles obtained by SEM photomicrographs in Figures 1 and 2 are described both quantitatively and qualitatively. Quantitative description of synthesized nanoparticles is done using morphological descriptors such as aspect ratio, roundness, irregularity and equivalent circle diameter (ECD), which were obtained from Media cybernetics software analysis as presented in Table 1. The morphological descriptors have been proposed and used by different image analysis software.[27-32] On the other hand, qualitative description is on the basis of visual observation of the SEM photomicrographs.[33]

From Table 1, the synthesized chitosan-acacia gum nanoparticles and drug-loaded nanoparticles have aspect ratios of 0.67 and 0.68 respectively. The aspect ratio, with a range of 0-1, is a measure of how elongated a particle is with a perfect circle having a roundness value of one.[29,31] Thus, both nanoparticles are more spherical than elongated. In addition, both are comparatively spherical. The aspect ratio values obtained are high enough to impact better powder packing and good flow rate to the synthesized nanoparticles because the closer the aspect ratio is to 1 the spherical is the particle.

Roundness, is a measure of how closely the projected area of a particle resembles a perfect circle; with a perfect circle having a roundness value of 1.[32, 30] The drug-loaded nanoparticles have the higher roundness value of 0.95, while chitosan-acacia gum nanoparticles have the lower roundness value of 0.91.

Roundness, or smoothness, is different from aspect ratio in that it takes into consideration the projected area of the particle while aspect ratio uses the maximum and minimum Feret's diameter in its calculation of length and breadth of the particle which is then used to calculate the aspect ratio.[27-29] Roundness particularly describes the surface smoothness in terms of surface asperity.

Irregularity indicates whether the particle is elongated or irregular.[30] It therefore measures the surface area compared to the size of the particle with a perfect circle having irregularity of π.[26,27,31] Irregularity values higher than π suggests that the particles are irregular—a situation in which so many different shapes are present that a particular shape cannot be said to be predominant. The drug-loaded nanoparticle has the higher irregularity value of 2.8 while chitosan-acacia gum nanoparticles has the lower, 2.69, both lower than π. These results further support the claim that the nanoparticles have distinct and identifiable shapes viz ovals, spheres and short cylinders.

It is worthy of notice that the values of aspect ratio (0.67 and 0.68), roundness (0.91 and 0.95), irregularity (2.69 and 2.80) and ECD (0.67 and 0.80) for chitosan-acacia gum nanoparticles and drug-loaded nanoparticles respectively are strikingly comparable. This provides evidence that drug encapsulation did not impair the morphological parameters of the nanoparticles significantly. In addition, it may be observed that, the ability of chitosan and acacia gum to form smooth nanoparticles by the ionic gelation technique was not retarded by the encapsulation of cisplatin.

On a qualitative sense, a general absence of sharp edges can be seen to characterize the nanoparticles whose SEM photomicrograph was presented in Figure 1. This indicates a convenient property required for smooth transport within blood vessels.[26,27,29-32] Smooth surface implies low attrition and friction in transport. This again goes to imply a reduced incidence of wear and degradation when the nanoparticles are loaded with drug. Since the nanoparticles will essentially serve as a carrier—more like a vehicle—delivering active pharmaceutical ingredient to ailing sites, wear and degradation may occur over time.[31-32]

The drug-loaded nanoparticles in Figure 2 are similar to the drug-free nanoparticles in Figure 1 in terms of shape and surface smoothness.

The general absence of sharp edges indicate a convenient property required for smooth transport within blood vessels.[26-27, 30-32] Another advantage derivable from the smoothness of the nanoparticles surface is reduced systemic circulation time.[29, 30-32] 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.

The particles in Figure 1 are mainly spheres, ovals and short cylinders but the short cylinders seem to predominate. The formation of smooth short cylinders appeared to be more favourable at the synthesis condition - gentle vibration of the magnetic stirrer and centrifuging speed of 7000 rpm.

4. CONCLUSION

The following conclusions are drawn from the results of this study.

(1) Nanoparticles were formed after acacia gum solution was added to chitosan-cisplatin solution under gentle magnetic stirring by the ionic gelation method. The particles showed distinct spheres, ovals and short cylinders.

(2) The morphology obtained from scanning electron microscope showed distinct shapes which could be readily analyzed for shape factors like aspect ratio, roundness, irregularity and equivalent circle diameter using Premier version of Media cybernetics.

(3) The respective values of aspect ratio (0.67 and 0.68), roundness (0.91 and 0.95), irregularity (2.69 and 2.80) and ECD (0.67 and 0.80) obtained for cisplatin-free and cisplatin-loaded nanoparticles are strikingly comparable providing evidence that drug encapsulation did not impair the morphological properties of the nanoparticles significantly.

(4) The values obtained for the shape factors present the nanoparticles as suitable particles for application as drug nanocarriers in targeted drug delivery systems.

ACKNOWLEDGEMENTS

The authors wish to appreciate the following at the indicated capacities.

Sponsor

Pan African Materials Institute (PAMI), African Centre of Excellence, African University of Science and Technology (AUST), Abuja

Technical Partners

1) Media Cybernetics Inc., Cambridge, provided Image-Pro Premier software.
2) Messrs Folahan Ibitoye and Ifeoluwa Adewole provided technical assistance on the use of Image-Pro Premier software.
3) Dr. Oladeji Ige provided technical assistance on SEM and TEM characterization of nanoparticles.

REFERENCES

1. NIBIB (2016) Drug Delivery Systems: Getting Drugs to Their Targets in a Controlled Manner. https://www.nibib.nih.gov/science-education/science-topics/drug-delivery-systems-getting-drugs-their-targets-controlled-manner. Accessed April 2017.
2. Paolino, D., Sinha, P., Fresta, M. and Ferrari, M. (2006). "Drug Delivery Systems" In: Encyclopedia of Medical Devices and Instrumentation, 2nd edition. Webster, J. G. (Ed). John Wiley & Sons, Inc.
3. Blasi, P., Schoubben, A., Romano, G. V., Giovagnoli, S., DiMichele, A., Ricci, M. (2013) Lipid Nanoparticles for Brain Targeting II. Technological characterization. *Colloid surf. Biointerfaces*, 110: 130-137.
4. Duncan, R. (2003) The Dawning Era of Polymer Therapeutics. *Nat. Rev. Drug Discov*. 2, 347-360, doi: 10.1038/nrd1088.
5. Mahapatro, A. and Singh, D. K. (2011) Biodegradable Nanoparticles Are Excellent Vehicle for Site Directed in-vivo Delivery of Drugs and Vaccines. *Journal of Nanobiotechnology*, 9(55): 1-11, doi: 10.1186/1477-3155-9-55.
6. Pinto, R. C., Neufeld, R. J., Ribeiro, A. J., Veiga, F (2006) Nanoencapsulation I. Methods for Preparation of Drug-loaded Polymeric Nanoparticles. Nanomedicine: *Nanotechnology, Biology and Medicine*, 2(1): 8-21.
7. Korting, H. C. and Schafer-Korting, M. (2010) "Carriers in the Topical Treatment of Skin Disease," Handbook of Experimental Pharmacology, 197: 435-468.
8. Alvarez-Roman, R., Naik, A., Kalia, Y. N., Guy, R. H. and Fessi, H. (2004) Enhancement of Topical Delivery from *Biodegradable Nanoparticles. Pharmaceutical Research*, 21(10): 1818-1825. doi:10.1023/B:PHAM.0000045235.86197.ef
9. Akinluwade, K. J., Oyatogun, G. M., Alebiowu, G., Adeyemi, I. O., Akinwole, I. E. (2017) Synthesis and characterization of Polymeric Nanoparticles formed from Cowry Shells and Acacia Gum Extracts. *Journal of Advances in Biology and Biotechnology*, 14(1): 1-8.
10. Akinwole, I. E. (2015) Investigation of the Suitability of Biopolymer Extracts of Cowry and Crab Shell for Controlled Released Drugs. Unpublished M. Sc. Thesis of the Obafemi Awolowo University, Ile-Ife. Nigeria.
11. Phromsopha, T. and Baimark, Y. (2010) Chitosan Microparticles Prepared by The Water-In-Oil Emulsion Solvent Diffusion Method for Drug Delivery. *Biotechnology*, 9: 61-66
12. Kumar, MNVR. (2000): A Review of Chitin and Chitosan Applications. *Reactive & Functional Polymers*, 46(1): 1–27.
13. Calvo, P., Remunan-lopez, C., Vila-jato, J. L. and Aloso, M. J. (1997) Novel Hydrophilic Chitosan–Polyethylene Oxide Nanoparticles as Protein Carriers. *Journal of Applied Polymer Science*, 63(1): 125–132.

14. Bernkop-Schnürch, A. and Dünnhaupt, S. (2012) Chitosan-Based Drug Delivery Systems. *Eur J Pharm Biopharm*, 81(3): 463-469.
15. Garcia-Fuentes, M., Alonso, M. J. (2012) Chitosan-Based Drug Nanocarriers: Where Do We Stand? *J Control Release*, 161(2): 496-504.
16. Zeng, J. B., He, Y. S., Li, S. L., Wang, Y. Z. (2012). Chitin Whiskers: An Overview. *Biomacromolecules*, 13(1): 1-11.
17. Wilczewska, A. Z., Niemirowicz, K., Markiewicz, K. H., Car, H. (2012) Nanoparticles as Drug Delivery Systems. *Pharmacological Reports*, 64(5): 1020-1037.
18. Oyatogun, G. M., Esan, T. A., Oziegbe, E. O., Adebiyi, K. E., Togun, R. O., Dare E. O., and Adeoye, M. O., 2011, "The Development, Characterization and In-vivo Testing of Cowry Based Materials for Dental Application," Proceedings, Faculty of Technology Conference, 142-147.
19. Oyatogun, G. M., Esan, T. A., Oziegbe, E. O., Adebiyi, A. O., Togun, R. O. and Adeoye, M. O., (2012) Processing, Characterization and Investigation of Suitability of Cowry Shells for Bone Graft Application. *Journal of Osteology and Biomaterials*, 3(1): 21-27.
20. Akinwole, I. E., Alebiowu, G., Oyatogun, G. M., Abere, D. V., Oluwasegun, K. M., Oyatogun, A. O., Abioye, A. A., Abioye, O. P., Adenigba, A. E. and Ayodele, T. J., (2018) Synthesis and Characterization of Cowry and Crab Shells Based Chitosan for Drug Delivery. *Bioceram Dev Appl*, 8: 107. doi: 10.4172/2090-5025.1000107.
21. Fricker, G., Kromp, T., Wendel, A., Blume, A. and Zirkel, J. (2010) Phospholipids and Lipid-Based Formulations in Oral Drug Delivery. *Pharmaceutical Research*, 27(8): 1469-1486, doi:10.1007/s11095-010-0130-x
22. Nafee, N., Schneider, M., Schaefer, U. F. and Lehr, C. M. (2009) Relevance of the Colloidal Stability of Chitosan/PLGA Nanoparticles on Their Cytotoxicity Profile. International *Journal of Pharmaceutics*, 381(2): 130-139, doi:10.1016/j.ijpharm.2009.04.049.
23. Rampino, A., Bogogna, M., Blasi, P., Bellich, B. and Cesaro, A. (2013) Chitosan Nanoparticles: Preparation, Size Evolution and Stability. *International Journal of Pharmaceutics*, 455(2013): 219-228.
24. Avadi, M. R., Sadeghi, A. M. M., Dounighi, N. M., Dinarvand, R., Atyabi, F. and Rafiee-Tehrani, M. (2011) Ex Vivo Evaluation of Insulin Nanoparticles Using Chitosan and Arabic Gum. *International Scholarly Research Network (ISRN) Pharmaceutics*, 2011, doi:10.5402/2011/860109.
25. Avadi, M. R., Sadeghi, A. M. M., Mohammadpour, N., Abedin, S., Atyabi, F., Dinarvand, R., and Rafiee-Tehrani, M. (2010) Preparation and Characterization of Insulin Nanoparticles using Chitosan and Arabic Gum with Ionic Gelation Method. *Nanomedicine*, 6(1): e58–e63.
26. Sadler, N. and Wilson, D. (2010) Prediction of Granule Packing and Flow Behaviour Based on Particle Size and Shape Analysis. *J. Pharm Sci*, 99(2): 958-968
27. Ogunjimi, A. T. and Alebiowu, G. (2013) Flow and Consolidation Properties of Neem Gum Coprocessed with Two Pharmaceutical Excipients. *Powder Technol*, 243: 187-192
28. Pons, M. N., Vivier, H., Delour, V. Authelin, J. R. and Paille'res-Hubert L. (2002). Morphological Analysis of Pharmaceutical Powders. *Powder Technology*, 128: 276-286.
29. Almeida-Prieto, S., Blanco-Mendez, J. Otero-Espinar, F. J. (2007) Microscopic Image Analysis Techniques for Morphological Characteisation of Pharmaceutical Particles: influence of 170 software and the factor algorithms used in the shape factor estimation. *European Journal of Phamaceutics and Biopharmaceutcs*, 67: 766-776.
30. Adeoye, O. and Alebiowu, G. (2013) Flow, Packing and Compaction Properties of Novel Coprocessed Multifunctional Directly Compressible Excipients Prepared from Tapioca Starch and Mannitol. *Pharmaceutical Development and Technology*, 1-10. Doi: 10.3109/10837450.2013.840843,
31. Bodhmage, A. (2006) Correlation between physical properties and flowability indicators for fine powders. M.Sc. Thesis of Saskatchewan University, Canada.
32. Amidon, G. E., Secreast, P. J. and Mudie, D. (2009) Particle and Compact Characterization. In: Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice. Qiu, Y., Chen, Y. and Zhang, G. Z. eds. New York, 163-186.
33. Wojnar, L. (1999): Image analysis, CRL Press, Washington, D.C.