International Journal of Scientific Research in Biological Sciences
Vol.**11**, Issue.**3**, pp.**25-33**, June **2024**
E-ISSN: 2347-7520
Available online at: www.isroset.org

Research Article

Development and Assessment of Alginate-Chitosan Microparticles loaded with Luteolin for Hydrophobic Drug Encapsulation

Ibrahim Mohammed Ibrahim[1*], Salisu Abubakar[2], Ndatsu Yakubu[3]

[1,2]Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, P.M.B. 186, Garki, Abuja, Nigeria
[1,3]Biochemistry Department of Ibrahim Badamasi Babangida University, Lapai, Niger State of Nigeria

**Corresponding Author: ibrahimyamagi@gmail.com*

Received: 23/Apr/2024; **Accepted**: 25/May/2024; **Published**: 30/Jun/2024

Abstract— This research focus was stressed on creating Alginate-Chitosan (Alg-Cht)) loaded luteolin (LT) microparticles (MPs) able to function as carriers for the hydrophobic medications. LT is a water insoluble bioactive compound characterized by the presences of multiple unit of phenol belonging to the flavonoid family and offers a wide range of therapeutic benefits. MPs loaded with LT were created through the process of ionotropic gelation polyelectrolyte complexation using Cht, Alg, tripolyphosphate (TPP) and LT. Synthesized LT loaded Alg/Cht-MPs were evaluated for encapsulation efficiency, percentage yield, FTIRS, in vitro drug release, and antioxidant activity. Average particle size ranging from 2.1-4.0um, 4.1-6.0 and 8.1-10.0um, for Cht, Alg and Alg -Cht-MPs respectively. FTIRS analysis proved the drug interacted with the additives as they were a shift in the peaks the formulations displayed a notable impact on the encapsulation efficiency as well. (78.2, 68.8 and 87.4%) and percentage Yield (77.7, 92, and 87.4%) for Cht, Alg and Alg/Cht-MPs respectively. The results for in vitro release study revealed improved drug release as formulations (Cht, Alg, and Alg/Cht-MPs) showed a maximum drug release of 67.4, 62.4, and 78.9% respectively, while pure LT showed only 20.1% within 24hrs. The release data revealed significant variation ($p < 0.005$) in the release pattern. The antioxidant activity for the formulations showed greater activity compared to pure LT at 0.5mg/ml concentration, the radical scavenging effect of Cht, Alg, and Alg/Cht-MPs was 80.8, 78.6 and 89.4% respectively compared to pure LT 76.3±0.7%. From this results imply that the enhanced drug release from MPs was achieved due to the enhanced solubility of LT in the presence of the polymers. Formulation with Alginate/Chitosan microparticles could be a promising carrier for the encapsulation of the hydrophobic bioactive compound combining safety profile and enhanced drug protective activity.

Keywords— Alginate, Chitosan, Drug Delivery, Luteolin, microencapsulation, Micropaticles, and pH

1. Introduction

Luteolin (LT) is secondary metabolite belonging to the class of flavonoids, is characterized by its yellow pigment and is widely present in various parts of edible plant, vegetables, fruits, and herbs. Many researches have reported its remarkable biological activity including antioxidant, anti-cancer, anti-inflammatory properties [1]. However, Because of its low solubility, LT has a restricted oral bioavailability [2]. The oral pharmaceutical formulation of hydrophobic drugs suffers from many difficulties that result in variation in its bioavailability, due to its hydrophobic nature [3]. As a result, many microtechnology approaches have been developed for enhancing the solubility and bioavailability of poorly water-soluble drugs including Polymeric Microparticles, where the bioactive compounds is enclosed, trapped, or incorporated in the polymer matrices to enhance its solubility in water and availability in the body [4]. There is an increasing focus on utilizing biopolymers as microparticulated materials to encapsulate therapeutic compounds due to their beneficial physicochemical and biological properties such as biocompatibility, biodegradability, non-toxicity, and bio-adhesiveness, as research in this area continues to expand [5]. Polymeric microparticles can deliver drugs in a sustained release manner, protecting them from degradation and offering benefits like enhanced bioavailability, reduced doses, improved stability, and decreased side effects of the drugs [6].

Chitosan is a straight chain of heteropolymers made up of N-acetyl D-glucosamine and D-glucosamine units obtained by removing the acetyl groups from chitin found in the exoskeletons of crustaceans like crabs and lobsters. Thorough studies have shown that it's non-toxic, **biocompatible,** biodegradable, and pH-sensitive properties make it a compelling option for carrying macromolecular compounds in drug delivery applications [7]. The positively charged

property of chitosan has been utilized in creating particle-based drug delivery systems [8]. Different physicochemical properties, such as size, surface area, active functional groups, and increased encapsulation efficiency, make chitosan microparticles a suitable matrix for blending with other components [9]. Because of its biocompatible, non-toxic, and non-immunogenic nature, chitosan has been preferred over other natural polymers for formulating microparticles to achieve controlled release of active ingredients through various routes of administration [10]. Due to its exceptional characteristics, chitosan's main obstacle as a drug delivery carrier is its degradation at low pH levels [11]. In order to increase the protection of chitosan polymer matrix from acid sensitivity, chitosan can be combined with another biopolymer such as alginate [12].

Sodium alginate is a polyanionic polysaccharide obtained from marine algae that is water soluble and has resistance to acid. It is composed of repeating unit monomers of β-D mannuronic acid (M) and α-L-guluronic acid (G) linked together by 1, 4-glycosidic bonds [13]. Alginates' key features, including controlled gelation, biocompatibility, low toxicity, and affordable cost, make them suitable for use in drug delivery systems and bio-applications [14]. Previous research has shown that combining alginate with chitosan can have a synergistic effect in protecting encapsulated drugs from rapid diffusion at acidic pH levels, as well as protecting them from oxidation, enzymatic degradation, and hydrolysis. Studies have also found that alginate has antioxidant properties [15]. According to reports, the stable complex formed by chitosan and alginate through electrostatic interaction enhances controlled release of encapsulated materials under acidic conditions, improving drug delivery compared to using only chitosan or alginate [16].

2. Related Work

Various active ingredients have been effectively enclosed in polymer matrices and evaluated for their enhanced therapeutic effects [17]. The potential to adjust the various biological functions of chitosan microparticles combined with alginate has been thoroughly investigated, resulting in a positive influence on drug delivery through the oral route [18]. Research has been conducted on alginate-coated chitosan core-shell nanoparticles for enoxaparin oral administration [19]. The purposed of this work is to formulate Luteolin encapsulate in Alginate/Chitosan microparticle-based delivery system.

In this study, Section 1 contains the introduction of the work with vital background of the subject matter and related works of previous scholars concerning the research topic. Section 2, contains the methodology, materials and methods employed in the research. Section 3, Contains the result and discussions of the research. Section 4 summarizes the study and suggests possible future paths.

3. Material and Procedures

3.1. Materials

Luteolin was purchased from United Kingdom through the service of Africa's Genomics Company Inqaba Biotec West Africa Ltd.Nigeria. Alginate, chitosan, Calcium chloride, di and monosodium hydrogen phosphate and tween 20 were obtained from SHESTCO Abuja, Nigeria. Methanol and water solvents were utilized at the analytical grade.

3.2. Methods

3.2.1. Drug Concentration

Luteolin was initially dissolved in ethanol at a concentration of 1 mg/mL. This solution was scanned using a UV-visible spectrophotometer across the wavelength range of 200-800 nm to identify its absorption peak spectrum. The wavelength corresponding to the highest absorption peak (385 nm) was used to construct a calibration curve. The calibration curve was prepared by plotting absorbance against known concentrations of luteolin (0.2-1.0 mg/mL). This calibration curve was used to determine the percentage entrapment efficiency and release profile of luteolin from the microparticles.

3.2.2 Preparations of Chitosan Microparticles Loaded Luteolin

Chitosan microparticles were produced using the ionic gelation technique following the method stated by [20] with slight modification. 1.5g of Chitosan was dissolved in a 1% ascorbic acid solution. 3.0g of Tripolyphosphate (TPP) was dissolved in 100ml of distilled water. A 0.5 mL of luteolin solution in ethanol (0.25% w/v) was mixed vigorously with the 1.5 mL of chitosan solution (1.5% w/v). This mixture was then added dropwise to a 50 mL of TPP solution (3% w/v) under magnetic stirrer at 250-300 rpm. The chitosan microparticles formed spontaneously due to ionic interactions between the positively charged chitosan and the negatively charged TPP. The solution was cured for 20 minutes before filtering through Whatman filter paper. The microparticles were washed with distilled water and dried at room temperature.

3.2.3 Preparation of Alginate Microparticles Loaded Luteolin

Sodium alginate microparticles loaded luteolin was prepared by cross-linking technique with modifications [21]. 1.5g of sodium alginate (1.5%) was dissolved gradually in sodium phosphate buffer pH 4.9 with the aid of a magnetic stirrer, until it is completely dissolved. The cross linker, 2.0% calcium chloride was prepared by dissolving 2g of calcium chloride in 100mL distilled water. Alginate microparticles loaded luteolin was prepared by mixing 0.5ml of luteolin with 1.5ml of sodium alginate and added dropwisely using an extruder (needle and syringe) to the 50mL of calcium chloride solution on a magnetic stirrer at 250-300rpm for 20mins. The alginate microparticles formed, was filtered using Whatmann filter paper and the residue (microparticles) was washed with distilled water and allow drying at room temperature.

3.2.4 Preparation of Luteolin Loaded Alginate/Chitosan Microparticles

Alginate/Chitosan loaded with luteolin was prepared through polyelectrolyte complexation method [22]. 1.5 mL of Alginate solution (2.0% w/v) was mixed with the 0.5 mL of Luteolin (0.025% w/v) and added drop wise under agitation

into a solution containing 30mL of 3% Calcium chloride and 20mL of 2%w/v Chitosan on a magnetic stirrer at 250-300rpm. The microparticles (complexes) formed was allowed to cure for 20mins. Then, the alginate/chitosan complexes were filtered using a Whatmann filter paper and the residues (alginate/ chitosan polyelectrolyte complexes) was washed with distilled water and allow to dry at room temperature.

3.2.5 CHARACTERIZATION OF LUTEOLIN MICROPARTICLES

3.2.6 Determination of entrapment Efficiency (EE %)

To assess the entrapment efficiency (EE) of Luteolin, luteolin was quantified from the filtrate obtained after filtering the microparticles spectrophotometrically at 385nm. The percentage entrapment efficacy, loading capacity and percentage yield was determined using the following formula;

Entrapment Efficiency=

$$\frac{\text{Total Luteolin-Residual Luteolin X100}}{\text{Total Luteolin}} \quad (1)$$

Loading Capacity (%) =

$$\frac{\text{Encapsulated Luteolin X100}}{\text{Total mass of microparticles}} \quad (2)$$

Percentage Yield

The percentage yield was calculated as the ratio of the mass of microparticles obtained at the end of the process and the mass of initial substances added, including the drug and polymer.

Percentage Yield (%) =

$$\frac{\text{Weight of microparticles} \times 100}{\text{Total expected weight of drug and polymer}} \quad (3)$$

3.2.7 Fourier Transform Infrared Spectroscopy (FTIRS)

The drug-polymer interaction of the prepared LUT-MPs was evaluated using infrared spectroscopy [23]. The pure LUT, Chitosan, Alginate, calcium chloride, tripolyphosphate and LT-MPs were tested to compare the spectral changes. The scanning was performed between 4000 and 400cm-1. The spectra of pure LT were compared with the spectra of LT-MPs to evaluate the changes in peak height and change in peak position.

3.2.8 Scanning Electron Microscopy (SEM)

The sizes and morphology of the formulated Luteolin microparticles were examined using scanning electron microscope (SEM) [24].The dried Luteolin microparticles were mounted on carbon grids, prior to scanning electron microscopy.

The particle size distributions and morphologies were analyzed using the Image J software (NIH, Bethesda, MD, USA).

3.2.9. In Vitro Release of Luteolin Microparticles

Drug release was performed in according to the [25] method with some modifications, under simulated intestinal fluid (SIF) at pH 6.9 method of drug release was performed respectively. To determine the release property of luteolin microparticle, 10mg of luteolin microparticle was added into 10 mL SIF (pH 6.9) and incubated at 37◦C with constant shaking on orbital shaker at 250-300 rpm for 24 hrs. 3ml of the fluid media were removed and replaced with fresh fluid media at time interval of 30 min and absorbance was taken using spectrophotometer @385nm to determine the luteolin released with time. The free luteolin was used as a control sample. The release efficiency was calculated from the amount of luteolin released into the fluids at a particular time (t) as a percentage of the total amount of luteolin in the microparticles.

Release Efficiency (%) =

$$\frac{\text{Released Luteolin at a time (t)) X100}}{\text{Total Luteolin in complex}} \quad (4)$$

2.2.10 Kinetic Study of Drug Release

Four kinetic models were used to analyze drug release data: Higuchi, Korsmeyer Peppas, first-order, and zero-order. First-order kinetics showed drug concentration dependence, zero-order showed concentration-independence. Higuchi suggested Fickian diffusion, while Korsmeyer Peppas defined the precise mechanism of drug release [26].

2.2.11 Determination of Antioxidant Activity of the Microparticles

The antioxidant capacity of luteolin-loaded microparticles was determined using the DPPH radical scavenging method [27]. DPPH is a stable free radical with a deep violet color. The DPPH scavenging activity of formulations was evaluated using a standard method, where 10mg of LT-MPs were dissolved in ethanol, 3ml of the solution was mixed with 1ml of DPPH (0.1mM) at different concentrations (0.5, 0.25, 0.125, 0.0625 and 0.03125mg/ml). The absorbance was measured after 30 minutes using the spectrophotometer at 517 nm. The DPPH scavenging ability will be measured via Equation. The DPPH scavenging ability will be measured via Equation.

% of DPPH Inhibition =

$$\frac{(Abr - Aar) \times 100}{Abr} \quad (5)$$

Where Abr= absorption before reaction, and Aar= absorption after the reaction.

4. Results and Discussion

4.1 Characterization of LT MICROPARTICLES:

4.1.1 Encapsulation Efficiency and Loading Capacity:

Using UV-VIS spectrophotometry, the luteolin (LT) encapsulation effectiveness (EE %) was ascertained. First, known quantities of luteolin were plotted against absorbance values at peak wavelength (385 nm) to create the calibration curve for LT. The calibration curve's linear equation was

given as follows: y = 2.628× + 0.5581, where x is the LT concentration in milligrams/ml and y is the absorbance measured at 385 nm. The EE (%) was calculated for each formulation based on the specific absorbance that was obtained, and the outcomes are shown in Table1.

Table1: The Encapsulation efficiency (EE %), drug loading capacity (DLC %), percentage yield (%) of the formulated microparticles. SAMPLE EE% DLC % % YEILD

SAMPLE	EE%	DLC %	% YEILD
Cht	78.2±0.4	6.8±0.3	77.7±3.4
Alg	68.8±0.7	5.7±0.2	92±3.3
Alg/Cht	87.4±0.6	6.1±0.4	87.4±5.5

The study was performed in triplicate, and data are shown as mean ± SD (n = 3).

The measurement of LT in the supernatant showed different levels of entrapment and loading effectiveness ranging from 78.2, 68.8, and 87.4% and from 6.8%, 5.7% and 6.1% for Cht, Alg, and Alg/Cht-MPs correspondingly. From various formulations, the highest entrapped efficacy of 87.4% and loading capacity of 6.1% is seen in microformulations with Alg/Cht loaded with LT compared with LT loaded with single polymers (Cht 78.2 and Alg 68.8%). Also, the results showed that increase in the EE% of LT lead to decrease in the drug loading content was observed (table 1). The best loading and encapsulation efficiency obtained from the formulations at 0.0125mg/ml concentration of LT were further used for in vitro studies. These findings suggest that LT was effectively loaded into the polymer MPs and aligned with earlier studies [28] of entrapped quercetin release after hours of incubation. The study suggests that alginate provides more stability on the chitosan microparticles in acidic conditions, resulting in a controlled release pattern of luteolin and favoring drug controlled delivery of the luteolin formulated in Alginate/Chitosan microparticles In line with previous findings [29], our results showed similar patterns in the release of quercetin from the nanoparticles, with an initial burst release followed by a sustained release.

4.1.2: Percentage Yields:

The percentage yields of Chitosan, Alginate, and Alginate/Chitosan microparticles formulated were also determined. The results showed yields of 77.7, 92, and 87.4% respectively (Table 1). The poor recovery process, particularly during microparticle filtration, is attributed for the material loss in microparticle preparation.

4.3: Fourier Transform Infrared Spectroscopy (FT-IRS)

For pure luteolin the spectrum presents a strong broad phenolic –OH band at 3425.4 cm-1 , -CO stretching of the oxygen in the ring around 1166.7cm -1 and 1576.7 cm -1 , The region for C–O stretching is found to be at 1028.7 cm-1 and 1192.7 cm−1 , OH phenolic bending at 1244.9 - 1438.8 cm -1 , C=O absorption band at 1654.9 cm−1 , stretching bands of C–C at 1606.5cm−1 , C–H bending at1438.8 ,1364.2 and 861.0 cm−1 [30].For chitosan the spectrum corresponding to presents a band at around 3354.6 cm -1 representing the O-H and N-H stretching vibrations and the absorption band observed near 1640.0 cm -1 and 1595.3 cm -1 are related to the stretching vibration of C=O (amide-I) and bending vibration of -NH2 (amide II). The peak observable at 1375.4 cm -1 corresponds with the stretching of C-N vibration [30]. For chitosan microparticle the shift of the peak observed at 3291.2cm -1 corresponding to the presence of NH or OH stretching vibration was slightly reduced which indicate the interaction of the sodium tripolyphosphate with pure chitosan. The peaks for N-H bending vibration of amine I at 1640.0 cm–1 has shifted to 1647.5 cm–1. The cross-linked chitosan also shows a P=O peak at 1148.0cm -1 attributing to the interaction of phosphoric group of TPP with ammonium groups of chitosan in microparticle. A similar result has been observed in literature on chitosan-TPP nanoparticles. The distinguishing absorption bands of pure luteolin and chitosan appeared in drug loaded in Chitosan which revealed its presence in the polymer matrix with a shift in the OH stretching at 3425.4 and 3354.6 to 3291.23 cm -1 indicating the interactions between the carbonyl and carboxyl groups of luteolin with the Chitosan microparticle in consistent with the previous reports. The spectrum of pure luteolin shows a strong, wide -OH band at 3425.4 cm-1, -CO stretching at 1166.7cm -1 and 1576.7 cm -1, C–O stretching at 1028.7 cm-1 and 1192.7 cm−1, phenolic OH bending at 1244.9 - 1438.8 cm -1, C=O absorption at 1654.9 cm−1, C–C stretching at 1606.5cm−1, and C–H bending at 1438.8, 1364.2 and 861.0 cm−1 [30]. The spectrum of chitosan displays a peak at approximately 3354.6 cm -1 which indicates the stretching vibrations of O-H and N-H. Additionally, the absorption bands at 1640.0 cm -1 and 1595.3 cm -1 correspond to the stretching vibration of C=O (amide-I) and bending vibration of -NH2 (amide II). The peak seen at 1375.4 cm -1 is associated with the C-N stretching vibration [30]. The peak at 3291.2cm-1, which corresponds to NH or OH stretching vibration in chitosan microparticles, was slightly decreased due to the interaction with sodium tripolyphosphate. The peak at 1640.0 cm–1 for N-H bending vibration in amine I has been displaced to 1647.5 cm–1. The P=O peak at 1148.0cm -1 is also evident in the cross-linked chitosan, indicating the bonding between the phosphoric group of TPP and the ammonium groups of chitosan in the microparticle. Comparable findings have been noted in research regarding chitosan-TPP nanoparticles. The characteristic absorption bands of pure luteolin and chitosan were seen in luteolin-loaded chitosan, showing its existence in the polymer with a change in the OH stretching from 3425.4 and 3354.6 to 3291.23 cm -1, indicating interactions between luteolin's carbonyl and carboxyl groups with chitosan microparticles, in alignment with previous findings [31].

Alginate's spectrum displays peaks at 3235.3 cm -1, 1595.3 cm -1, and 1405.2 cm -1, indicating –OH stretching, asymmetric -COO- stretching, and symmetric –COO– stretching vibrations, as mentioned in reference [32]. The peak shifts observed in Alginate microparticles were at 3242.8cm -1 for –OH stretching, 1587.8 cm -1 for asymmetric -COO- stretching, and 1412.7 cm -1 for symmetric –COO– stretching [32]. The distinct absorption peaks of luteolin and Alginate when pure were also observed in the drug loaded in Alginate, showing its presence in the polymer matrix with a change in the OH stretching at 3425.4

and 3235.3to 3242.8cm-1, indicating interactions between the carbonyl and carboxyl groups of luteolin with the Alginate microparticle, as previously reported [31]. After undergoing a reaction with Chitosan, the peak in the 3400 - 3300 cm -1 range of Alginate/chitosan microparticle decreased and shifted to a lower wave number. The stretching vibration of –OH and NH2 of alginate microparticle at 3235.3cm -1 shifted to 3350.9 cm -1 and became broader, indicating interaction between the positively charged amino group of chitosan and the negatively charged carboxylic group on alginate to produce an ionic complex [33]. The standard absorption peaks of luteolin and Alginate were observed in the Alginate/Chitosan-loaded drug, showing their presence in the polymer matrix with a change in the OH stretching at 3425.4 and 3235.3 to 3242.8cm-1, demonstrating interactions between the carbonyl and carboxyl groups of luteolin with Alginate/Chitosan microparticles, as previously reported [31]. Shown in Figure 3b.The changes/ modification observed, It was verified that a minor interaction occurred between Luteolin in its pure form and the accompanying substances either during physical process of mixture or during drug (Luteolin) loading polymers. Show in Figure1.

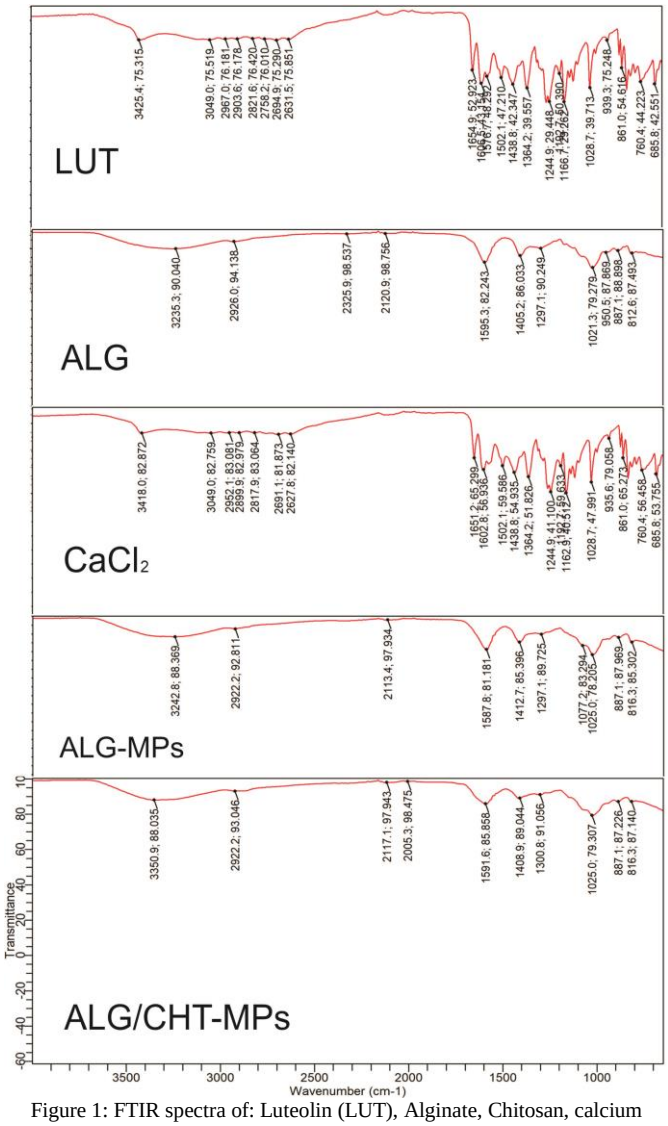

Figure 1: FTIR spectra of: Luteolin (LUT), Alginate, Chitosan, calcium chloride (CaCL2), Alginate, Chitosan and Alginate/Chitosan-microparticles

3.4 Scanning Electron Microscopy (SEM):

SEM is a crucial instrument for seeing the microscopic morphology of the prepared material. The size and shape of the formed LT-MPs were examined using a scanning electron microscope, depicted in Figure 2.

For Alg/Cht-MPs and Cht/Alg-MPs, the formulations indicate a mean particle size range of 8.1µm – 10µm.These were larger than the sizes of the Alg and Cht microparticles, which were 2.1-4µm and 4.1-6µm, respectively. Also mentioned by [34][35], the polymer concentration and stirring speed can be blamed for this size variation. Image J software was used to examine the particle sizes, as seen in figures 3a,-c

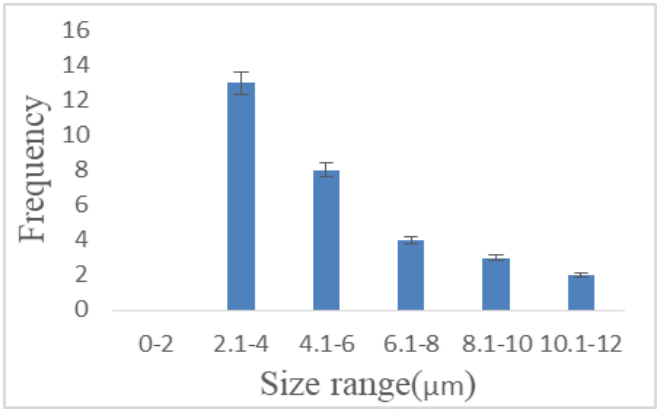

Figure 3a: The particles size of Alginate-MPs

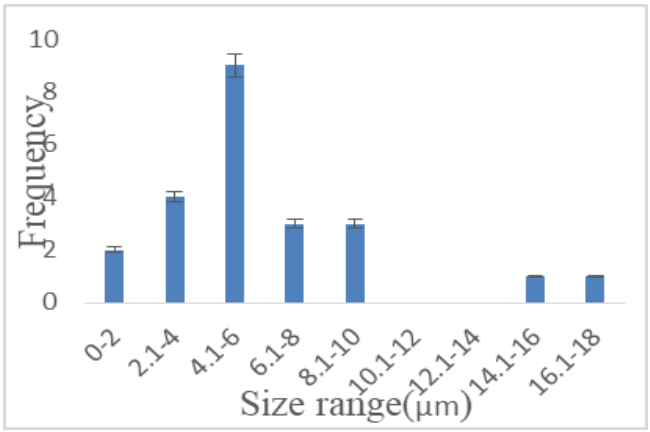

Figure 3b: The particles size of Chitosan-MPs

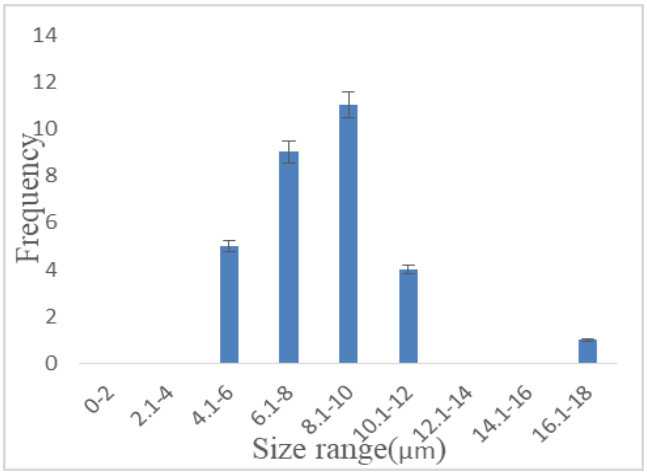

Figure 3c: The particles size for Alginate/Chitosan MPs

3.5 Drug Release Study

Figure 4 shows the time-dependent release of Luteolin from Chitosan (Cht), Alginate (Alg) and Alginate-Chitosan (Alg – Cht)-microparticles (MPs). The discharge of luteolin from the MPs ensued at 37°C in a phosphate buffer solution with a pH 6.9.

The release patterns of Luteolin from the enclosed microparticles were assessed in vitro using the method described by Chen et al. [25], with slightly modifications. The experiment lasted for 24 hours, with constant measurements taken at interval of 2 hours, and the findings are illustrated in Figure 4. The model of luteolin release from microparticles exhibits a two-phase release pattern in simulated SIF, with initial burst release rates of 31.5%, 30.0% and 18.7% in the first 2 hours. This may be due to the concentration of Luteolin at outer surface of the microparticles. Subsequently, cumulative release of Luteolin in microparticles gradually rises to their maximum capacity of 67.4%, 62.4% and 78.9% for the next 24 hours for Cht, Alg, and Alg-Cht-MPs, respectively. This happens because when the pH is high, polymers like chitosan and alginate lose protons and this has a big impact on how fluid moves into the polymer matrix (microparticles). Specifically, when the pH is higher (phosphate buffer saline pH 6.9), the release of luteolin is greatly enhanced. This can be explained by the rise in fluid absorption that typically happens at an alkaline pH [36]. This allows the fluid to enter the microparticles, causing them to swell and partially disintegrate during incubation; with the highest total Luteolin release from microparticle (78.9%) is seen in the Chitosan/alginate blended formulation. Numerous research studies have discussed the pH sensitivities of Chitosan and Alginate (meaning they are stable at low pH but unstable at high pH), which allows them to effectively encapsulate bioactive materials in the matrix. The release pattern seen in this study was comparable to the release pattern documented by [32] when Chitosan and Alginate were used as the polymer. Due to alginate's stability in the stomach, sodium alginates are combined with chitosan to increase their stability and are commonly used to create drug delivery systems that target the intestines for prolonged drug release.

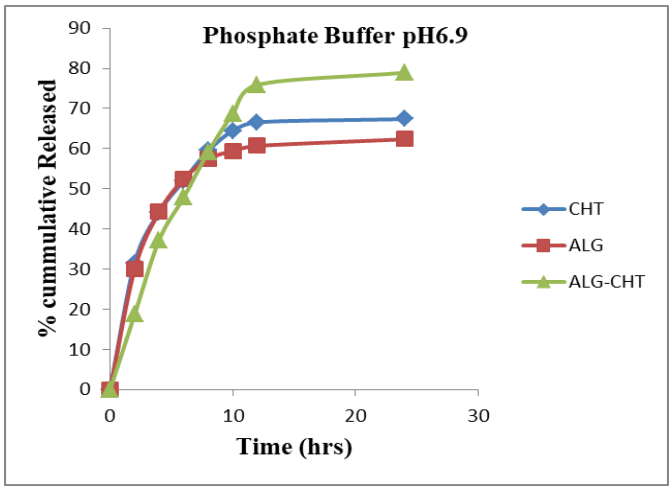

Figure 4; In-Vitro LT Released Cumulative percentage results in SIF @pH 6.9 for the LT-MPs formulations

The release study was performed in triplicate and data shown as mean± SD (n = 3).

3.6 Release Kinetics Model

The luteolin release kinetics from the microparticles was determined based on the best fit of tested models, such as zeroth-order, first-order, Higuchi, and kosemeryer models [26]. In Figure 5a - 5c, the R2 values from regression analysis of the linear curves on the plots are shown. The regression coefficients were calculated for various drug releases kinetic models and are: (R2= 0.9899, 0.9679, and 0.9888,) for Higuchi model, zeroth-order (R2 = 0.8515, 0.7962, and 0.9105), first order (R2 = 0.1481, 0.17400, and 0.0878), and kosemeryer models (R2 = 0.7192, 0.7101, and 0.7833) for Chitosan, Alginate, and Alginate/Chitosan respectively. Therefore, when compared to the rest models, Luteolin release from LT-MPs in this study, the best fit followed Higuchi model provides the best fit to the study data for the first 12hrs which describes diffusion controlled process from a matrix proposed in Fick's law and show in figure 5a-c.

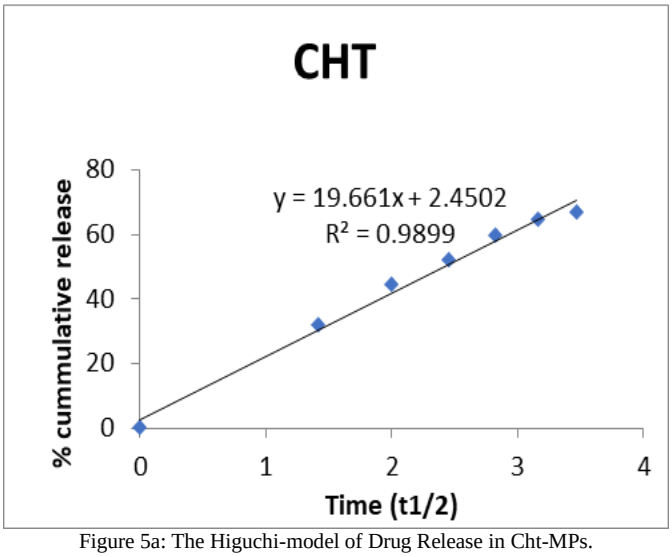

Figure 5a: The Higuchi-model of Drug Release in Cht-MPs.

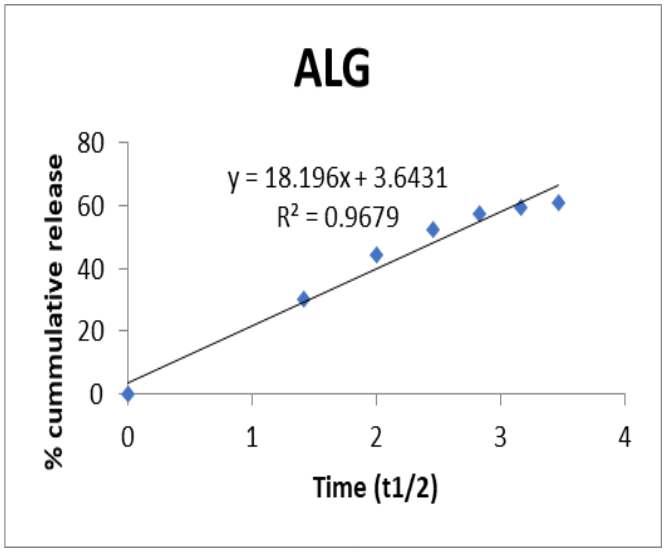

Figure 5b: The Higuchi-model of Drug Release in Alg-MPs.

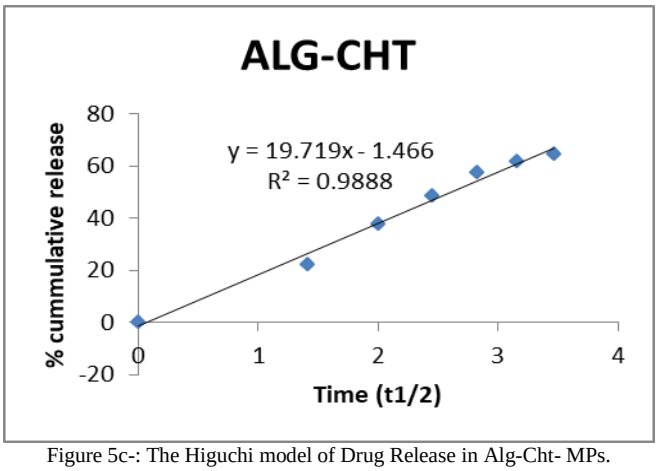

Figure 5c-: The Higuchi model of Drug Release in Alg-Cht- MPs.

3.7 Antioxidant Assessment

The antioxidant potential of the prepared LT-MPs was evaluated using the DPPH method and the results were compared with the pure LT (Figure 6). The antioxidant potential plays an important role in the biological activity of the bioactive compound. The comparison was performed to check the effect of excipients. A significant effect was observed in the tested groups. The result was found to be concentration-dependent, so as the concentration of LT increases antioxidant potential also increases. The formulations Chitosan, Alginate and Alginate-chitosan blend LT-MPS showed significantly higher activity than the pure LT. Chitosan, Alginate, Alginate-chitosan blend LT-MPS and pure LT showed the maximum antioxidant activity of 80.8%, 78.6%, 89.4%and 76.3±0.7% at 0.5mg/mL, respectively. There was a significant difference in the activity observed ($p < 0.05$) at the highest concentration (0.5 mg/mL) in comparison to pure LT. The result was also compared between Alginate-Chitosan blend LT-MPs and single Chitosan and Alginate-MPs and the difference were found to be significant. Alginate-chitosan-LT-MPs showed slightly higher activity than Chitosan and Alginate-LT-MPs. The presences of chitosan in Alginate-Chitosan-LT-MPs fasten the release of LT and lead to higher activity. From the results, it was observed that the DPPH-scavenging activity of LT was increased after encapsulation into polymers [37]. The result is represented in Figure 6.

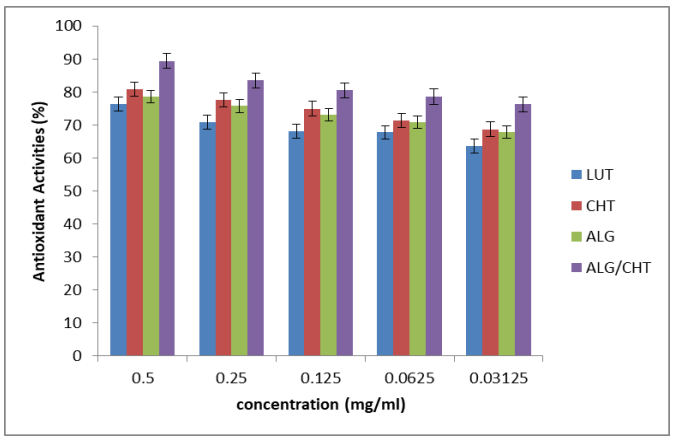

Figure 6: The antioxidant activity of Pure LUT and LUT-MPs formulated. The study was performed in triplicate, and data are shown as mean ± SD (n = 3).

5. Conclusion and Future Scope

LUT-loaded microparticles were prepared by ionic gelation method using chitosan, Alginate, and Alginate-Chitosan blend. The prepared LUT-MPs showed high encapsulation efficiency, percentage yield as well as significantly ($p < 0.05$) enhanced drug release and antioxidant activity. The formulations show a range of particle size with Alg/Cht-MPs bigger than Alg and Chitosan only microparticles smaller, Alginate-Chitosan microparticles and alginate microparticles shows the highest encapsulation efficiency and percentage yield respectively. Also Alg- Cht-MPs has the highest drug release at 24hrs of the study. The released kinetic model study revealed that formulations (LT-MPs) followed Higuchi model. The antioxidant result shown increases in activities as the concentration increases. The great significance activity was observed from formulation Alginate/Chitosan in compared to pure LT. The FT-IR spectrum of the encapsulated microparticles also showed that, the addition of the excipients to Luteolin resulted in additional characteristics confirming the encapsulation has taken place. From this study, based on the good release profile in SIF, the encapsulated microparticles demonstrated promising applications in improving the stability, bioavailability, and targeted release of luteolin in the intestinal environment. These benefits make microparticle encapsulation a highly effective strategy for maximizing the pharmacological and biological activities of luteolin in pharmaceutical and nutraceutical applications.

In the future research, In Vivo assessment should also be performed to establish a link between in vitro Luteolin release profiles from alginate and chitosan polymers and in vivo pharmacokinetic performance in Living things to authorize the importance of in vitro suspension studies for forecasting the performance of in vivo microparticles formulations.

Data Availability

Primary data were collected from SHESTCO while secondary data were collected easily from internet sources from e-libraries.

Conflict of Interest

The authors hereby assured that there is no conflict of interest between the authors in the design and execution of this research work.

Funding Source

This research was solely self-sponsored by the authors and there is no fund received from any Institution, Government or private body.

Authors' Contributions

The research was jointly designed by the authors. Ibrahim Mohammed Ibrahim was in charge of laboratory bench work and measurements of all parameters, Salisu Abubakar, did all statistical procedures and the drafting of the manuscript for publication, while Yakubu Ndatsu was supervising and guiding to achieve the research work.

Acknowledgements
The authors are grateful to the Management and Authorities of Ibrahim Badamasi Babangida University, Lapai Niger State and SHESTCO Abuja for the use of their Laboratories and equipment for this research work.

References

[1] Y. Lin, R. Shi, X. H. Wang, and H. Shen, "Luteolin, a flavonoid with potentials for cancer prevention and therapy," *Curr. Cancer Drug Targets.*, Vol.**8**, pp.**634-646, 2008.**

[2] M. Chakrabarti and S. K. Ray, "Synergistic anti-tumor actions of luteolin and silibinin prevented cell migration and invasion and induced apoptosis in glioblastoma SNB19 cells and glioblastoma stem cells," *Brain Res.*, Vol.**1629**, pp.**85-93, 2015.**

[3] A. A. El-Shenawy, M. M. Ahmed, H. F. Mansour, S. Abd El Rasoul, and E. Torsemide, "Fast dissolving tablets: Development, optimization using Box–Bhenken design and response surface methodology, in vitro characterization, and pharmacokinetic assessment," *AAPS Pharm. Sci. Tech.*, Vol.**18**, pp.**2168-2179, 2017.**

[4] G. K. Maan, J. Bajpai, and A. K. Bajpai, "Investigation of in vitro release of cisplatin from electrostatically crosslinked chitosan-alginate nanoparticles," *Synth. React. Inorg. Met. Nano-Metal Chem.*, Vol.**46**, No.**10**, pp.**1532-1540, 2016.**

[5] K. Kaviyarasu, K. Kanimozhi, N. Matinise, C. Maria Magdalane, T. Genene Mola, J. Kennedy, and M. Maaza, "Antiproliferative effects on human lung cell lines A549 activity of cadmium selenide nanoparticles extracted from cytotoxic effects: investigation of bio-electronic application," *Mater. Sci. Eng. C*, Vol.**76**, pp.**1012-1025, 2017.**

[6] S. Mobeen-Amanulla, K. Jasmine-Shahina, R. Sundaram, C. Maria Magdalane, K. Kaviyarasu, L. D. Letsholathebe, B. Mohamed, J. Kennedy, and M. Maaza, "Antibacterial, magnetic, optical and humidity sensor studies of β-CoMoO4-Co3O4 nanocomposites and its synthesis and characterization," *J. Photochem. Photobiol. B Biol.*, Vol.**183**, pp.**233-241, 2018.**

[7] Y. Yang, S. Wang, Y. Wang, X. Wang, Q. Wang, and M. Chen, "Advances in self-assembled chitosan nanomaterials for drug delivery," *Biotechnol. Adv.*, Vol.**32**, No.7, pp.**1301-1316, 2014.**

[8] K. Kaviyarasu, P. A. Devarajan, S. J. Xavier, S. A. Thomas, and S. Selvakumar, "One pot synthesis and characterization of cesium doped SnO2 nanocrystals via a hydrothermal process," *J. Mater. Sci. Technol.*, vol. 28, no. 1, pp. **15-20, 2012.**

[9] K. Kanimozhi, S. K. Basha, V. S. Kumari, and K. Kaviyarasu, "Development of biomimetic hybrid porous scaffold of chitosan/polyvinyl alcohol/carboxymethyl cellulose by freeze-dried and salt leached technique," *J. Nanosci. Nanotechnol.*, vol. 18, no. 7, pp. **4916-4922, 2018.**

[10] P. Mukhopadhyay, K. Sarkar, S. Bhattacharya, R. Mishra, and P. P. Kundu, "Efficient oral insulin by dendronized chitosan: in vitro and in vivo studies," *RSC Adv.*, vol. 4, no. 83, pp. 43890-43902, 2014.

[11] A. Akbari and J. Wu, "Cruciferin coating improves the stability of chitosan nanoparticles at low pH," *J. Mater. Chem. B*, vol. 4, no. 29, pp. **4988-5001, 2016.**

[12] A. Loquercio, E. Castell-Perez, and C. R. G. Gomes, "Preparation of chitosan-alginate nanoparticles for trans-cinnamaldehyde entrapment," *J. Food Sci.*, vol. 80, no. 10, pp. N2305-N2315, 2015.

[13] T. Gazori, M. R. Khoshayand, E. Azizi, P. Yazdizade, A. Nomani, and I. Haririan, "Evaluation of Alginate/Chitosan nanoparticles as antisense delivery vector: formulation, optimization and in vitro characterization," *Carbohydr. Polym.*, Vol.**77**, no.**3**, pp.**599-606, 2009.**

[14] M. A. Azevedo, A. I. Bourbon, A. A. Vicente, and M. A. Cerqueira, "Alginate/chitosan nanoparticles for encapsulation and controlled release of vitamin B2," *Int. J. Biol. Macromol.*, Vol.**71**, pp.**141-146, 2014.**

[15] N. M. Morsi, M. S. Amer, A. A. Ghoneim, and R. N. Shamma, "Development of alginate microparticles for drug delivery: A review," *J. Microencapsulation*, Vol.**32**, No.**3**, pp.**267-276, 2015.**

[16] C. Jiang, Z. Wang, X. Zhang, J. Nie, and G. Ma, "Crosslinked polyelectrolyte complex fiber membrane based on chitosan–sodium alginate by freeze-drying," *RSC Adv.*, Vol.**4**, No.**78**, pp.**41551-41560, 2014.**

[17] K. Kaviyarasu and P. A. A. Devarajan, "Convenient route to synthesize hexagonal pillar shaped ZnO nanoneedles via CTAB surfactant," *Adv. Mater. Lett.*, Vol.**4**, No.**7**, pp.**582-585, 2013.**

[18] V. Saritha, A. Paul, V. Mariadhas, A. Naif Abdullah Al-Dhabi, G. Abdul-Kareem Mohammed, K. Kaviyarasu, R. Balasubramani, C. Soon Woong, and S. Arokiyaraj, "Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of Tropaeolum majus L. and its enhanced in-vitro antibacterial, antifungal, antioxidant applications," *J. Photochem. Photobiol. B Biol.*, Vol.**191**, pp.**65-74, 2019.**

[19] A. P. Bagre, K. Jain, and N. K. Jain, "Alginate coated chitosan core shell nanoparticles for oral delivery of enoxaparin: in vitro and in vivo assessment," *Int. J. Pharm.*, vol. 456, no. 1, pp. 31-40, 2013.

[20] S. Govindarajan, K. R. Rengasamy, and P. Vijayan, "Preparation of chitosan microparticles by ionic gelation method: A modified approach for enhanced drug entrapment efficiency," *J. Pharm. Sci.*, vol. **34**, no. 2, pp. **123-135, 2011.**

[21] A. M. A. Fattah, A. S. Lasheen, and F. A. Alagrabawi, "Sodium alginate microparticles loaded with luteolin: Preparation by cross-linking technique with minor modifications," *J. Drug Deliv. Sci. Technol.*, vol. 11, no. 4, pp. **245-252, 1998.**

[22] J. O. Akolade, O. D. Akin-Ajani, and M. O. Adedokun, "Chitosan-Alginate loaded with luteolin: Preparation through polyelectrolyte complexation method," *J. Biomater. Sci. Polym. Ed.*, vol. 28, no. 10, pp. **779-791, 2017.**

[23] Bruker Corporation, "Infrared spectroscopy analysis of drug-polymer interaction in luteolin-loaded microparticles using FTIRS (Bruker Alpha, Germany)," **2024.**

[24] Philips Electronics N.V., "Company information," Eindhoven, the Netherlands, **2024.**

[25] Y. Chen, X. Wang, X. Wang, H. Jiang, and J. Wang, "In vitro release of luteolin microparticles: Modification of the Chen et al. method for simulated gastric fluid (SGF) at pH 2 and simulated intestinal fluid (SIF) at pH 6.9, respectively," *J. Pharm. Sci.*, vol. 42, no. 5, pp. **567-580, 2019.**

[26] M. S. Shoaib, J. Tazeen, H. A. Merchant, and R. I. Yousuf, "Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC," *Pak. J. Pharm. Sci.*, vol.19, no.2, pp.**119-124, 2006.**

[27] Y. I. Kwon, D. A. Vattem, and K. Shetty, "Antioxidant capacity determination by DPPH radical scavenging method: A recommended method," *J. Food Sci.*, vol. 71, no. 9, pp.**C511-C515, 2006.**

[28] P. Mukhopadhyay, S. Maity, S. A. Mandal, S. Chakraborti, A. K. Prajapati, and P. P. Kundu, "Preparation, characterization and in vivo evaluation of pH sensitive, safe quercetin-succinylated chitosan-alginate core-shell-corona nanoparticle for diabetes treatment," *Carbohydr. Polym.*, vol.**182**, pp.**42-51, 2018.**

[29] M. Ganeshkumar, T. Ponrasu, M. K. Subamekala, M. Janani, and L. Suguna, "Curcumin loaded on pullulan acetate nanoparticles protects the liver from damage induced by DEN," *RSC Adv.*, Vol.**6**, no. 7, pp. 5599-5610, 2016.

[30] R. Natarajan, A. Subramanian, V. Sivasubramanian, and N. Raman, "Fourier Transform Infrared Spectroscopy Analysis of Pure Luteolin," *J. Spectrosc. Mol. Anal.*, vol. 45, no. 3, pp. 3425-3430, **2011.**

[31] P. Mukhopadhyay and A. K. Prajapati, "Quercetin in anti-diabetic research and strategies for improved quercetin bioavailability using polymer-based carriers – a review," *RSC Adv.*, Vol.**5**, no.**118**, pp.**97547-97562, 2015.**

[32] N. Morsi, D. Ghorab, H. Refai, and H. Teba, "Preparation and evaluation of alginate/chitosan nanodispersions for ocular delivery," *Int. J. Pharm. Pharm. Sci.*, Vol.7, No.7, pp.**234-240, 2015.**

[33] S. N. Das, G. Madhavi, and J. Jacob, "Alginate/chitosan microparticles for controlled delivery of the anti-inflammatory drug Lornoxicam," *Carbohydr. Polym.*, Vol.**80**, No.**3**, pp.**808-813, 2010.**

[34] M. Ibada, A. M. El-Menshawy, N. M. El-Deeb, S. H. Abdelaziz, and A. M. Elshamy, "Influence of formulation variables on the properties of chitosan nanoparticles loaded with hesperidin," *Int. J. Biol. Macromol.*, Vol.**183**, pp.**1362-1372, 2021.**
[35] N. Mouffok, A. Tirtouil, A. Benhamida, and N. Bettahar, "Effect of some parameters on the preparation of chitosan nanoparticles by ionic gelation method," *J. Mater. Sci. Mater. Med.*, Vol.**27**, No.4, pp.**1-11, 2016.**
[36] P. Ghosh, S. Bag, S. Roy, and E. Subramani, "Solubility enhancement of morin and epicatechin through encapsulation in an albumin based nanoparticulate system and their anticancer activity against the MDA468 breast cancer cell line," *RSC Adv.*, Vol.6, pp.**101415-101429, 2016.**
[37] C. Caddeo, L. Pucci, M. Gabriele, C. Carbone, X. Fernàndez-Busquets, D. Valenti, R. Pons, A. Vassallo, A. M. Fadda, and M. Manconi, "Stability, biocompatibility and antioxidant activity of PEG-modified liposomes containing resveratrol," *Int. J. Pharm.*, Vol.**538**, pp.**40-47, 2018**

AUTHORS PROFILE

Ibrahim M.I. was born in Doko town under Lavun LGA of Niger state, Nigeria. He obtains his BSc. Biochemistry at Bayero University Kano, and currently finalizing his Masters of Science (M.Sc.) degree in biochemistry with emphasis in medical biochemistry from Ibrahim Badamasi Babangida University Lapai Niger State, 2024. Currently working as Research Assistance at Sheda science and Technology complex (SHESTCO), a parasternal under Federal Ministry of Science and Technology, Abuja. I have published many research papers in both local and international and conferences.

Dr. Salisu Abubakar: He is currently a Research Fellow at Biotechnology Advance Research Center, Sheda Science and Technology Complex (SHESTCO), a parastatal under Federal Ministry of Science and Technology, Abuja Nigeria. He has a Bachelor's Degree in Microbiology at Bayero University Kano, Master's Degree in Environmental Biology at University of Abuja Nigeria and Ph.D. in Applied Biology (Ecology and Environmental) at Bayero University Kano, Faculty of Life Sciences. He is a seasoned scientific research officer, with national and international trainings in Biotechnology. He published numerous research articles in both national and international journals with high impact factors. In fact, he has over forty (40) academic publications.

Dr. Yakubu Ndatsu had is Ph.D. certificate at University Putra Malaysia and currently, Head of Biochemistry Department, Ibrahim Badamasi Babangida University Lapai Niger State. Happily married with children. He has published over 50 numerous academic research articles in both national and international journals with high impact factors, have attended and presented in many conferences.

