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RESEARCH ARTICLE

EXTRACTION OF PECTIN FROM *CITRUS SINENSIS* FRUIT PEELS AND ITS EFFICIENCY AS A SUSPENDING AGENT

Edwin A Ubieko[1], Adaeze L Onugwu[2], John DN Ogbonna[2], Ebere Okoye[3], Callistus D Nwakile[3], Anthony A Attama[2]

[1]*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria.*
[2]*Department of Pharmaceutics, Faculty of Pharmaceutical Sciences University of Nigeria, Nssuka, Enugu State, Nigeria.*
[3]*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*

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***Address for Correspondence:**
Dr. Edwin A Ubieko, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria; Tel: 234-8133125184, E-mail: *edwinubieko@gmail.com*

Abstract

Aim and objective: The study's objective was to evaluate the performance of *Citrus sinensis* pectin, which had been extracted and used as a suspending agent during the production of fixed-dose artemether-lumefantrine oral powder for suspension.
Methods: The extracted pectin was used as an excipient to formulate an oral suspension powder of artemether and lumifantrine. Using analytical techniques including Fourier transform infrared (FTIR) and differential scanning calorimetry (DSC), the compatibility of the formulation's constituents, active medicinal ingredients, and extracted pectin was evaluated.
The formulated powder was tested for flow-ability, flow rate, sedimentation rate, re-dispersibility, dissolution rate, and short-term stability.
Results: In several of the batches, the extracted pectin displayed suspending characteristics at concentrations as high as 3.0% w/v. Hausner's and Carr's indices for the created artemether-lumefantrine granules for oral suspension were 30°C, 0.35 g/mL, 0.42 g/mL, 1.20 g/mL, and 15%, respectively. The flow rate was 5.46 g/min, and the suspension's particle size was 7.20 g. The viscosities ranged from 19.50 to 27.20 cP and 12.80 to 21.30 cP at 30 rpm and 60 rpm, respectively. After 80 and 90 minutes, respectively, the dosage forms of artemether and lumefantrine released their respective 75% w/w medications. Artemether; short-term stability was 91.7±0.01, 91.35±0.00, 90.13±0.02, 89.35±0.03 and 84.70±0.01 at days 0, 30, 60, 90, and 180, respectively. Lumefantrine; short-term stability was also 91.18±0.00, 91.17±0.03, 88.43±0.01, 82.75±0.00, and 81.77±0.02
Conclusion: According to this study, the artemether/lumefantrine oral suspension granules or powder made with the pectin extract had a great suspending effect.
Keywords: *Citrus sinensis*, extracted pectin, powder for suspension, stability study.

INTRODUCTION

Excipients have been characterized in a broad range of ways, including as inert substances utilized as drug diluents and transporters. Excipients have lately shown that they are far from inert and are capable of causing toxicities to patients as well as interacting with other chemicals in the formulation, resulting in unfavorable and hypersensitive responses. This definition poses a problem as a result. These responses may range in intensity from mild (like a rash) to fatal. The excipients in the same drug, such as preservatives and colorants, may differ across different brands. Excipients are included in the Consumer Medicines Information, and medical reference books may give further information regarding the safety of each excipient.
The English term "excipient" is derived from the Latin verb excipere, which might mean “different than” for example, anything used in pharmaceutical formulations but not an active medicinal component is referred to as an excipient. Excipients ought to be neutral in theory, but recent instances of adverse responses would appear to suggest otherwise. In addition to the active pharmaceutical component, pharmaceutical excipients are chemicals that are voluntarily employed in a medication delivery system and have completed the

requisite safety studies (API). An ideal excipient attributes include cost-effectiveness, pharmacological inertness, stability and reproducibility, absence of adverse drug-drug interactions (API). Even the most effective new medicinal component is only modestly helpful without a good delivery method[1]. There are several dosage forms for medications that are currently accessible, including tablets, capsules, oral liquids, topical creams and gels, transdermal patches, injectable goods, implants, eye products, nasal products, inhalers, and suppositories. Pharmaceutical excipients are chemicals used in a pharmaceutical dosage forms for functions beyond its immediate therapeutic effect. These aspects may also include manufacturing aid, protection, support, or increase of stability in addition to patient acceptability or bioavailability. They could also make it easier to identify the goods and increase its overall usability or safety while being kept or utilized[2]. Excipients, which may come in a variety of hundreds of different forms, make up around 90% of all pharmaceuticals. Industry analysts estimate that it accounts for €3 billion (nearly $4 billion), or 0.5% of the worldwide pharmaceutical market.

Excipients should not interact with other excipients or the active components and should not be poisonous or have any pharmacological effects. However, in actuality, hardly many excipients satisfy these requirements. Both chemicals left over from the production process (such as solvent residues) and the substances employed as excipients in the final dosage form may be harmful[3].

MATERIALS AND METHODS

Without additional purification, substances including sodium carboxylmethyl-cellulose, xanthan gum, citric acid, Polyethylene glycol 4000, talc (Merck, Germany), absolute ethanol, methanol, concentrated hydrochloric acid, methyl - 4 hydroxybenzoate, and propyl - 4 hydroxy benzoate were utilized (BDH, England). Distilled water (Madonna University Industerial Pharmacy laboratory, Elele, Rivers State.). *Citrus sinensis* fruits were taken from the Obla orchard in the settlements of Ajobe, Otukpo-Icho, and Otukpo in the Benue State.

At the Madonna University Industrial Pharmacy Laboratory in Elele, Rivers State, the pectin was isolated from the peels of *Citrus sinensis* fruit peels.

The powder samples of artemether and lumefantrine were a gift from Emzor Pharmaceuticals Ltd. in Lagos, Nigeria. The usage of all other reagents reflected their analytical grade.

Collection of *Citrus sinensis* fruits

The *Citrus sinensis* fruits were bought on November 25, 2019, from the Obla Orchard in the Nigerian settlements of Ajobe, Otukpo-Icho, and Otukpo LGA in Benue State. The *Citrus sinensis* fruits were validated, identified, and given the herbarium number UPHR0477 by Professor G.E. Omakhua of the Department of Forestry and Wild Life Management in the Faculty of Agriculture at the University of Port Harcourt.

Sample Preparation

To determine the degree of wholesomeness in the obtained fruits of *Citrus sinensis*, physical examination was performed. The peels, a soft, white material found within the fruit's skin, were taken off each fruit after it had been split into four pieces. The peels were subsequently divided into tiny pieces and thoroughly washed in clean water to get rid of the glycosides. The peels were then weighed using an electronic digital weighing scale, dried for 14 days in the shade, pulverised, and weighed before being kept in airtight containers to be extracted later[4,5].

Extraction of pectin from the prepared sample

Total 100 g of the produced sample was weighed, transferred, and correctly mixed into a 1000 mL beaker with 500 mL of distilled water and 2.5 mL of concentrated HCI in accordance with the procedure of Enkuahone (2018). The slurry was boiled for 45 minutes at a constant temperature of 120ºC. Whatman filter paper No. 1 was used to filter it, the cake was washed with around 250 mL of boiling water. To prevent the pectin extract from being damaged by heat, the combined filtrate was then chilled to approximately 28ºC. The filtrate was well stirred with a magnetic stirrer for approximately 30 minutes, allowing the pectin to float to the top, and then precipitated with absolute alcohol in a 1:2 ratio of pectin extract to absolute alcohol. Next, the gelatinous pectin flocculants were removed by skimming. The resulting pectin extract was refined by washing in twice as much fresh 100% ethanol. In order to remove the remaining HCl and universal salt from the extract, this was then squeezed on a muslin cloth. In order to preserve it for later use, the extracted pectin was dried in an oven set at 30–40°C[5].

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscope was used to collect the materials' FTIR spectra (Agilent Technology Cary 630 FTIR, California). The crystal was adequately cleaned with an organic solvent before being tested against the collecting backdrop. The sampling procedure was then started by choosing transmittance mode and turning on the transmitter. The alignment of the sample was tested after placing a pellet made of 15 mg of the extracted pectin, artemether, lumefantrine, and the formulation (which contains the extracted pectin as the suspending agent in addition to artemether, lumefantrine, and the formulation). Following the proper coding of each sample for identification, the machine was then set up for sampling. The “peak” was chosen and chosen for labeling after sampling. Spectra of the peaks were printed[5].

Differential scanning calorimetry (DSC)

For the sake of not harming the machinery, all of the samples were completely dried. A Tzero hermetic aluminum pan was used to weigh 5 mg of the sample (the extracted pectin), the unloaded medications (artemether and lumefantrine), and the pharmaceuticals (a mixture of artemether, lumefantrine, and the extracted pectin as the suspending agent). The pan was then placed in a blue holder, covered with its lid, and squeezed into place. To seal the pan and the lid, the handle was lowered. With an empty pan serving as a

guide, this process was repeated. After allocating and recording the slot numbers, the sealed pans were put in the sample tray. Using a differential scanning calorimeter (Netzsch 204 F1, Germany) outfitted with an auto-sampler, the reference pan was also inserted into a reference slot after being made aware of the slot number and kept secure for several runs. The power for the chiller was turned on. The software, TA Universal Analysis, was launched on the computer's desktop. The nitrogen gas tank, which was held at a pressure of 20 psi, was triggered at a sample purge flow of 50 mL min^{-1}. The program was then updated with each sample's name, sample weight, slot number, reference number, and data storage location. The machine was then turned on and adjusted to a temperature range of -80 to 400°C to begin the operation. At the end of the procedure, the cooling system was automatically turned off, but the nitrogen gas was not. And for the data transfer operation, files for the samples being processed were opened, and all the data was provided together with the temperature readings for the start, middle, and finish of the transition. The thermograph was then shown for each sample according to its prior coding[6].

Elemental analysis

Data from FTIR and ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) were used to determine the elements' identities.

Melting point determination of the extracted pectin powder

The melting point of the extracted pectin powder at room temperature was determined using the open capillary technique and a Stuart melting point instrument from Bibby Scientific Ltd. in the UK. A little amount of the powdered pectin from the extraction process was placed inside a sample capillary tube, which was then placed into the melting point equipment. The melting point was the temperature in degree Celsius at which the powder starts to melt within the tube. This conclusion was reached in three copies[6].

Formulation of the granules for oral suspension

Methyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate and the extracted pectin were accurately weighed at 6 mg, 300 mg, and 300 mg into a glass mortar that had been properly triturated for size reduction and mixed (M-Metlar Analytical Balance, Germany). After proper mixing, a drop of newly produced distilled water was added. In a second glass mortar, the proper trituration of 240 mg of artemether and 1440 mg of lumefantrine was completed. The identical glass mortar had 30 milligrams of citric acid that had been weighed. The contents of the two glass mortars were completely mixed and then passed through filter No. 1. Before going through sieve number two, the moist granules were dried in a desiccator for 24 hours. The glass mortar was used to combine the dry granules with 6 and 30 mg each of coconut and talc powder. After being well mixed, the granules were sealed in a sterile amber-colored plastic container and maintained in a desiccator for analysis. This procedure was performed for the various extracted pectin concentrations shown in Table 1. In each case, three duplicate formulations were employed[7].

Table 1: Composition of artemether/ lumefantrine granules containing the extracted pectin as the suspending agent.

Ingredient	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
Artemether (mg)	240	240	240	240	240	240	240	240	240	240
Lumefantrine (mg)	1440	1440	1440	1440	1440	1440	1440	1440	1440	1440
Pectin (mg)	300	600	900	1200	1500	1800	2100	2400	2700	3000
Citric acid (mg)	30	30	30	30	30	30	30	30	30	30
Methyl paraben (mg)	6	6	6	6	6	6	6	6	6	6
Propyl paraben (mg)	6	6	6	6	6	6	6	6	6	6
Coconut flavor (mg)	30	30	30	30	30	30	30	30	30	30
Talc (mg)	6	6	6	6	6	6	6	6	6	6
Total granule Weight	2058	2358	2658	2958	3258	3558	3858	4158	4458	4758

Analysis of granules for oral suspension.

Angle of repose and the flow rate

A modified static technique was used to calculate the inclination of repose of the artemether/lumefantrine granules for oral suspension using extracted pectin as the suspending agent. Total 50 g of the granules from each batch were poured into a plastic pipe that was 13.5 cm long, open at both ends, and had an internal diameter of 4 cm. This pipe was put on a sheet of paper on a level surface. The pipe was raised to allow the granules out, creating an h-shaped mound. The diameter of the mound's edge was carefully measured without being altered. Also timed was the granule's passage through the substantial plastic tube. The mean was obtained once these calculations had been completed in triplicate[8,9].

Bulk density

Each batch of the artemether/lumefantrine granules was divided into 20 g of powder, which was weighed, put into several 25 mL clean and dry glass measuring cylinders, and then set on a smooth, level surface. It was observed how much space the grains took up. The analysis was carried out in triplicate, and the densities were computed[6].

Tapped density

Using a digital electronic weighing scale, a 20 g sample of each batch was precisely weighed before being transferred into a dry, clean 25 mL measuring cylinder that was set down on a level, smooth surface. A consistent volume was produced by tapping the measuring cylinder repeatedly on the flat, smooth surface from a height of around 4 cm. Readings were obtained in triplicates[10].

Determination of true density of the granules

The real density of the granules was determined using n-hexane as a non-solvent liquid. W_1 was being weighed using a dry, empty 25 mL Pycnometer (Mettler, Germany). A 25 mL pycnometer was filled with precisely weighed 0.5 g of artemether/ lumefantrine granules. Along with its contents, the pycnometer was weighed, and the weight was recorded as W_2. N-hexane was put into the pycnometer after the granule was taken out, covered, and any spills were cleaned up. A further weigh-in and recording of the pycnometer as W_3 followed. After emptying the dry, empty pycnometer, 0.5 g of artemether/lumefantrine granules were weighed and put to it. To fill the vacant space, n-hexane was employed. W_4 was noted after the pycnometer and its contents were weighed. There were two separate analyses done[10].

Evaluation of the reconstituted suspension

Sedimentation rate/volume

The separate batches of the reconstituted artemether/lumefantrine powder for oral suspension, which included extracted pectin as the suspending agent, were put into 25 ml graduated glass measuring cylinders and left undisturbed on level, smooth surfaces. For five days, the individual batches' sedimentation rates were checked every 12 hours, then every 24 hours. Both the final sediment volume Vu and the initial suspension volume Vo were noticed and recorded. Below formula was used to compute the % sedimentation volume for the various batches of the reconstituted suspension[11].

$$F=\frac{Vu}{Vo} X\ 100$$

Where V_u is the final volume of the sediment, V_0 is the initial volume of the reconstituted suspension, and F is the sedimentation volume.

The mean was calculated after this determination was made in triplicates.

Flow rate of the reconstituted suspension

A 10ml glass pipette was used to pipette 10 ml of the reconstituted solution of artemether and lumifantrine. It was left to flow, and the duration of the flow was measured and recorded as the volume of pipette (mL) and flow time (min)[12]. For each batch, this determination was made in triplicate, and the mean results were calculated.

pH measurement

A digital pH meter was used to test the pH of the reconstituted suspensions of the different batches of artemether-lumefantrine granules every day for five days (Hanna instruments, USA). Results were recorded once the pH measurement was completed for each batch in triplicate.

Re-dispersibility study

Using newly produced distilled water, the artemether-lumefantrine suspension granules were reconstituted to a volume of 60 ml. For five days, the reconstituted suspensions were stored at room temperature in stoppered dispensing vials. Each time there were sediments at the bottom of the bottle, one dispensing bottle was grabbed and flipped upside down at an angle of 180°. It was noticed and kept track of how many rotations passed until re-dispersion happened[12].

Viscosity

At room temperature, the viscosities of numerous batches of the reconstituted suspension of artemether-lumefantrine and a commercial product were measured using a Brookfield Synchro electric viscometer, model LVF (spindle No 2). The results of these analyses were recorded and carried out three times[13].

Particle size

The particle size of the reconstituted artemether-lumefantrine suspension was assessed using a microscopic method. The suspension was spread out on a glass slide. The sizes were then measured using an eye-piece micrometer that had been calibrated. The marketing product's particle size was determined using the same procedure.

Organoleptic properties

The reconstituted suspensions of artemether-lumefantrine were compared to a commercial product in terms of their organoleptic properties, including color, taste, and odor. A note of the results was made. The judgment was printed three times.

Dissolution studies of the formulated artemether/ lumefantrine granules for oral suspension

Accurately measuring concentrated HCl (8.3 mL), 50 mL of methanol were added to a glass measuring cylinder. After being well mixed, the solution was allowed to cool. After that, the solution was poured into a 1000 mL glass measuring cylinder and filled to the proper level with methanol. A long glass stirring rod was used to gently swirl the fluid until it was well blended.

Plotting of calibration curve

Artemether

Total 200 mg of the pure artemether sample were dissolved in 50 mL of freshly made 0.1 M methanolic HCl to provide a stock solution with a concentration of 4 mg/mL. Additional dilutions to concentrations of 0.16, 0.64, 1.44, 2.56, 4.0, and 5.76 µg/mL were made from the stock solution. The maximum wavelength of a UV/Vis Spectrophotometer, 213 nm, was used to determine the absorbance for each concentration. 0.1M methanolic HCl was used as a blank. In triplicate, each concentration was calculated.

Lumefantrine

Total 50 mg of pure lumefantrine was accurately weighed, dissolved, and made up to 50 mL in newly prepared 0.1 M methanolic HCl to produce a stock solution with a concentration of 1 mg/mL. The stock solution was serially diluted to achieve concentrations of 0.04, 0.16, 0.36, 0.64, and 1.0 mg/mL. At maximum wave length 254 nm, absorbance was measured for each concentration using 0.1 M methanolic HCl as the blank. Three different determinations were conducted for each concentration[14].

Dissolution/release rate studies

Using the paddle approach, it was possible to gauge how quickly the produced artemether-lumefantrine granules would dissolve or release the drugs; artemether and lumefantrine. Total 900 mL of freshly prepared 0.1M methanolic HCl were added to the dissolving equipment, which was then allowed to equilibrate at 37±0.5°C. The powder for oral suspension containing 20 mg of artemether was

delivered to the dissolving compartments. The dissolving apparatus was set up to use the paddle method at a maximum value of 213 nm and a speed of 50 rpm. For up to two hours. A 5 mL of the solution was periodically removed to maintain the sink condition and replaced with the same volume of the dissolving media. In order to ascertain the drug content in the media, the Beer's plot was applied. Comparisons were made between the label and the percentage of the drug released. These were carried out twice for each batch, and the results were recorded. Lumefantrine's release rate was calculated using a similar technique. However, a powder for oral solution equivalent to 120 mg of lumefantrine and at a maximum wave length of 254 nm was used[15].

Short term stability studies of the formulated artemether/lumefantrine powder.

A 50 mL of 0.1M methanolic HCl was added to a 100 mL volumetric flask to dissolve the 120 mg of lumefantrine equivalent powder for oral suspension that had been carefully measured out. The mixture was stirred for 15 minutes at a speed of 1000 rpm using a magnetic stirrer. The resulting solution was diluted to a volume of 100ml using 0.1 M methanolic HCl. The solution was filtered using a Whatman's No. 1 filter paper after the proper mixing stage. The first 5 mL of the filtrate were discarded. The left over filtrate was diluted to 0.16 mg/mL for analysis using a UV/Vis spectrophotometer set to the preset maximum wave length of 254 nm. The absorbance was measured, and the actual lumefantrine concentration was calculated using the standard calibration curve. For the purpose of determining the artemether content, 20 mg of the powder was weighed into a 100 mL volumetric flask and dissolved in 50 mL of 0.1 M methanolic HCl. The same methods used to examine lumefantrine were used for artemether[16].

Data and statistical analysis.

Data analysis was done using social science statistical tools (SPSS Version 23). The results were expressed as mean SD. The changes in the means of the measured parameters were compared using one-way ANOVA.

RESULTS AND DISCUSSION

Yield of the pectin extracted from *C. sinensis* fruit peels.

C. sinensis fruit peels were subjected to an alcoholic extraction process in an acidic medium, and pectin extract was produced. According to Table 2, the yield was 8% w/w. This result marginally deviates from other papers' claims that the pectin output was between 12 and 14% w/w[17]. The different extraction conditions might be the cause of this little variation. The extraction was conducted for 45 minutes at 120ºC and pH 2.2. The extraction described in the literature, in contrast, was carried out at 95ºC and pH 1.5 for 105 min. The extracted pectin may have degraded due to the increased temperature of 120ºC. In addition, the yield may have been decreased due to the shorter extraction time and higher pH. Therefore, the ideal extraction conditions for a greater yield should be pH less than 2, temperature less than 100ºC, and an extraction time of close to 2 h[18,19]. The pH should also be lower than 2. With an increase in fruit maturity, the yield likewise declines[20].

Table 2: Yield of pectin extracted from *C. sinensis* fruit peels.

S.N.	*C. sinensis* fruit peels (g)	Pectin yield (%)
1	100	8.0
2	100	9.0
3	100	7.0
Mean	100	8.0
S.D	0.00	0.00

*S.D=Standard Deviation.

Thermal and/or non-thermal/ spectroscopic analysis

These analyses were done to see if there was any interaction between the pure drug samples and the excipient that would be used in the formulation. This interaction could be seen as an increase and/or decrease in major peaks and troughs, or it could be seen as a complete disappearance of major spectra.

Fourier transform infrared (FTIR) for the extracted pectin

It is possible to discriminate between organic, polymeric, and sometimes inorganic materials using analytical techniques like FTIR spectroscopy. Using infrared light, the FTIR analytical technique scans test samples to seek for chemical changes. FTIR analysis, a potent method for identifying

Chemicals and/or detecting contamination, generates a unique spectral fingerprint for each molecule or chemical structure in the sample[21,22]. A variation in the material's structure or the existence of contamination is clearly shown by a change in the identifiable pattern of absorption bands. The extracted pectin consists of hydroxyl groups that are carboxylic and alcoholic. The extracted pectin's spectra showed the peaks listed below: 1025 cm^{-1} for C=O, 1237.5 cm^{-1} for OH, 1319–1420 cm^{-1} for - OH –, 1638 cm^{-1} for C=C, 1990–2109 cm^{-1} for aromatic combination bands, 2818.5 cm^{-1} for methyl C-H, and 3287.5 cm^{-1} for polymeric OH stretch[23]; 872.2 cm^{-1}, 991.5 cm^{-1}, 1025 cm^{-1}, 1103.1 cm^{-1}, 1371.1 cm^{-1}, 1736.3 cm^{-1}, and 3350 cm^{-1} are the heights of the peaks of the extracted pectin plus artemether. The mid-IR region, which may be split into four sub-regions, includes the spectral values that correspond to this region;

1. Single bend sub-region 4000 – 2500 cm^{-1}
2. Triple bend sub-region 2500 – 2000 cm^{-1}
3. Double bend sub-region 2000 – 1500 cm^{-1}
4. Finger point sub-region 1500 – 600 cm^{-1}

Since each peak for the extracted pectin is greater than 5, this indicates that the molecules are complex[24]. The changes between the originals and the extracted pectin's FTIR and its interactions with the active medicinal substances are just a minor bend or shift. This finding suggests that there is minimal to no chemical interaction between the active medicinal components, and the extracted pectin, the excipient[25].

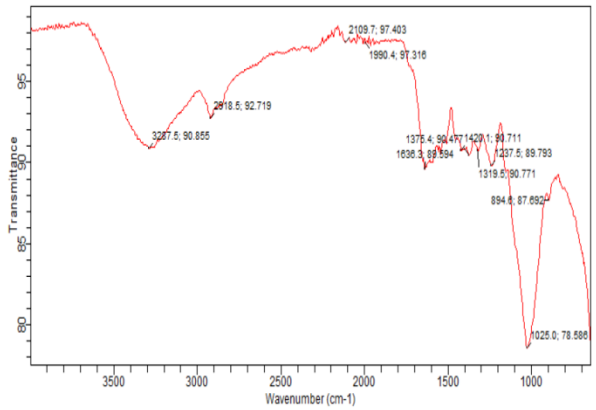

Figure 1: FTIR of the extracted pectin.

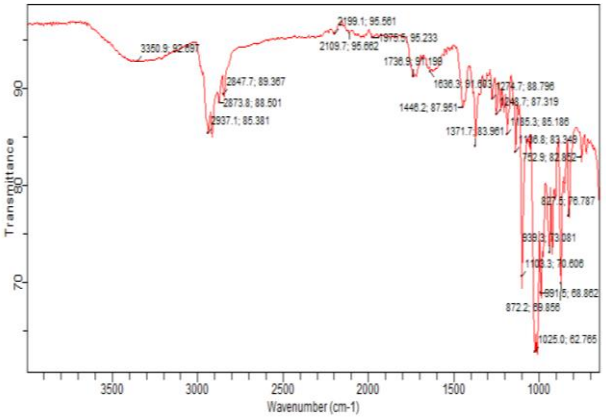

Figure 2: FTIR of the extracted pectin-artermether combination.

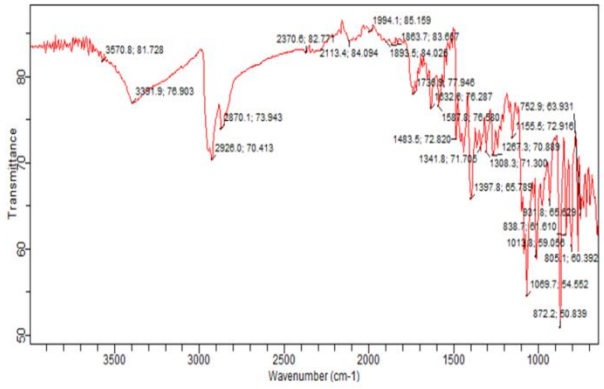

Figure 3: FTIR of the extracted

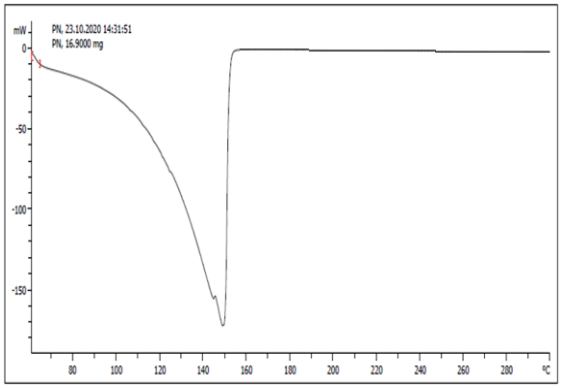

Figure 4: Thermograph of the extracted pectin.

Differential scanning calorimetry

Figure 4 to Figure 6 show the thermographs of the loaded formulations and the extracted pectin. Artemether, lumefantrine, and the extracted pectin all began to melt at 30, 75, and 30°C, respectively. These values fall below the excipients' and excipient-drug combination's respective melting points. Their instability at such high temperatures is shown by this finding. Within 24 hours after the excipient-drug combination, the drying process was completed in desiccators. The formulations were completed in an air-conditioned laboratory. For optimal stability, desiccators were used to store the powder that was formulated for oral suspension. According to these results, the extracted pectin has a high thermal stability and melts at a relatively high temperature of approximately 150°C, although it transitions to glass at a temperature of about 160°C, as shown in Figure 4. The melting point was lowered to around 115°C and the transition took place at about 125°C when the extracted pectin was mixed with lumefantrine. The melting point, however, remained at 150°C when artemether was added to the extracted pectin. Furthermore, neither the thermographs of the active pharmaceutical ingredients nor those of the mixture of active pharmaceutical ingredients and excipients changed. These results support prior reports[26] that the excipients and active medicinal components are chemically stable and compatible.

Elemental analysis of the extracted pectin

A larger amount of oxygen molecules (55.28±0.36) make up the isolated pectin, which encourages aerobic bacteria for quick breakdown. For the necessary strength, it additionally includes 5.00±0.20 of calcium and 39.74±0.31 carbon atoms. Organic compounds that are non-toxic, eco-friendly, biodegradable, and secure are mostly made up of these components. The calcium component contributes to the gelling ability of the substance, which has been extensively used as an excipient and delivery methods. These results match what have already been published[27].

Melting point determination of the extracted pectin

The extracted pectin powder's melting point was 151°C. The extracted pectin should be stable at temperatures lower than 151°C, according to the high melting temperature. This number is within the range of pectin's stated melting point, which is 150–154°C[28]. To avoid the product's melting point being excessively lowered when coupled with active medicinal substances, vigilance should be used[29].

Flow properties of artemether-lumefantrine granules formulated with the extracted pectin.

Micromeritic study data of pectin for bulk density and bulkiness, true density, total porosity, powder flow behavior is shown in Table 3. The bulkiness value indicated that the extracted pectin is a 'heavy' powder. The total porosity has been correlated with dissolution rate.

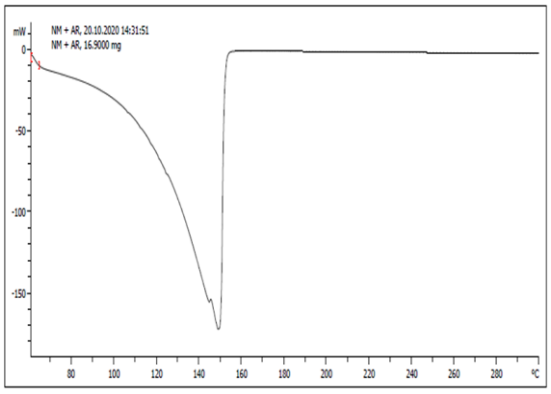

Figure 5: Thermograph of the extracted pectin-artemether combination.

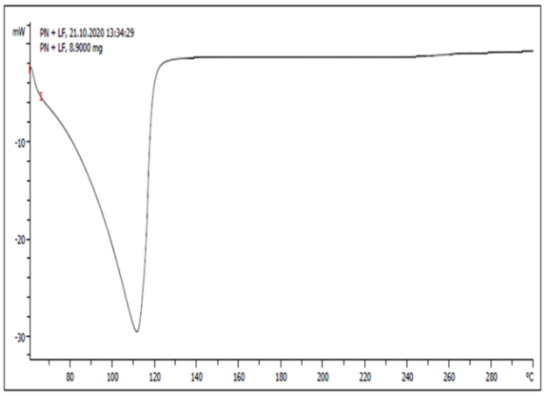

Figure 6: Thermograph of the extracted pectin-lumefantrine combination.

It was also found that the higher the porosity, the faster the rate of dissolution. Pectin exhibited good flow characteristics. The angle of repose for the extracted pectin is shown in Table 3 and its angle of repose was 30.00±0.04. This indicates a good flow into the final container, and the extracted pectin powder will not stick together. This finding means that the extracted pectin could produce good tablets with minimal tablet weight variation[11].

Table 3: Micromeritics properties of the extracted pectin powder.

Parameter	Result
Bulk density (g/mL)	0.35± 0.01
Tapped density (g/mL)	0.42±0.00
Angle of repose	30±0.04
Flow rate (g/min)	5.46± 0.01
Carr's index (%)	15±0.01
Hausner's quotient	1.2 ±0.00
Particle density (g/mL)	1.57±0.01

The results of the study of the bulk and tapped densities of the extracted pectin powder are shown in Table 3. The bulk densities of the extracted pectin powder were consistently lower than those of the tapped powder, indicating that the powder's volume was decreased during tapping. The calculated bulk density for the extracted pectin was 0.35±0.01 and the tapped density was 0.42±0.00 g/mL. The bulk densities didn't greatly alter (p>0.05). The tapping densities were similar as well. Table 3 displays the results of the examination of the Hausner's quotient and Carr's index of the extracted pectin powder. The extracted pectin's estimated Hausner's quotient was 1.20±0.01 and its Carr's index was 15.0±0.00%. These results show good flow-ability which translate into loose powder in the packaging containers and will eventually lead to production of tablets with limited tablet weight variation[11].

Flow rate of the extracted pectin powder

Table 3 displays the estimated extraction pectin flow rate. It was 5.46±0.01 g/min, which is a sign of excellent flow characteristics. As a result, it would be beneficial for creating tablets and suspension powder with appropriate hardness and friability features, excellent physical properties, and consistent weight and content.

Particle density

According to Table 3, the calculated particle density was 1.57±0.01 g/mL. This is the density of the tiny particles that make up the powder when there is no space, liquid, or air present[30,31].

Evaluation of the reconstituted suspension

The sedimentation volume/rate

As shown in Figure 7, the sedimentation volume of the artemether/lumefantrine solution decreased during the course of the five day storage period. The first sedimentation volume was 100%, confirming the suspension's stability. Different quantities of particles were observed during the course of the research when let to stand stationary. The rate of sedimentation decreases as storage duration and suspending agent concentration rise. The sedimentation volume or ratio may be related to the suspension's viscosity since the rate of sedimentation is inversely proportional to the viscosity of the dispersion medium[32]. When compared to suspensions with lower viscosities, drug particles remained suspended for a lot longer in the high viscosity solutions. How viscous the suspension is determined by the concentration of the suspending agent, in this case the extracted pectin. The rate of sedimentation is also influenced by the particle size of the insoluble components in the solution.

The propensity to sediment increases with increasing particle size. Smaller particles may be able to remain suspended for longer[33]. Based on these results, batches A6–A10 suspension were steady and followed this increasing sequence of results: A6–A7–A8–A9–A10. Batch A8's 4% w/v concentration of the extracted pectin provided the optimum suspension's best characteristics, such as a moderate sedimentation rate, elegance, pourability, and re-dispersion[34].

Re-dispersion of the reconstituted suspension

Figure 8 displays the findings of the re-dispersibility test of the reconstituted artemether-lumefantrine suspension. Re-dispersion issues exist in half of the formulation batches A1, A2, A3, A4, and A5.

This is probably caused by the formulation's low concentration of the extracted pectin used as the suspending agent. In contrast, formulations from batches A6, A7, A8, A9, and A10 were easily re-dispersed with only sporadic cakes forming.

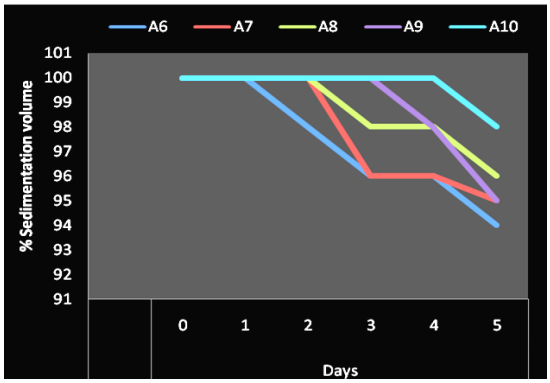

Figure 7: Percentage sedimentation volume.

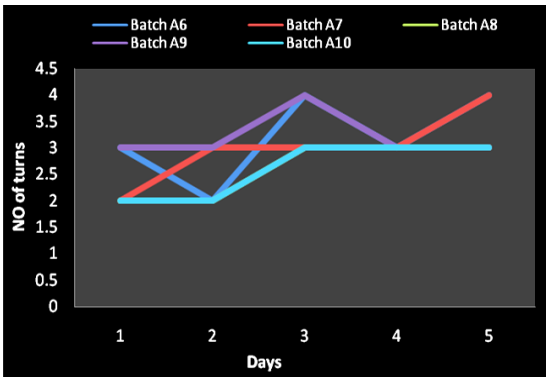

Figure 8: Re-dispersion of the formulation.

Higher extract pectin concentrations may be found in some batches. Caking may be caused by a densely packed bed lacking inter-particulate pores after settling, which makes it difficult for diluents to flow into the bed during agitation to help with re-dispersion. The capacity of a good suspension to redistribute its sediment upon gentle agitation is one of its qualities. Therefore, the suspending agent is more effective the less agitations are required to produce re-dispersion. The following is the sequence of re-dispersion: A6 > A7 > A8 > A9 > A10. After shaking the container, a good suspension is simple to re-dispense (low re-dispersion number), ensuring consistent dosage administration. According to the findings, formulation Batch A8 with a 4% w/v concentration is the most stable[13]. It displayed qualities that characterize an ideal suspension, such as a slow rate of sedimentation, elegance, pourability, and re-dispersibility[34].

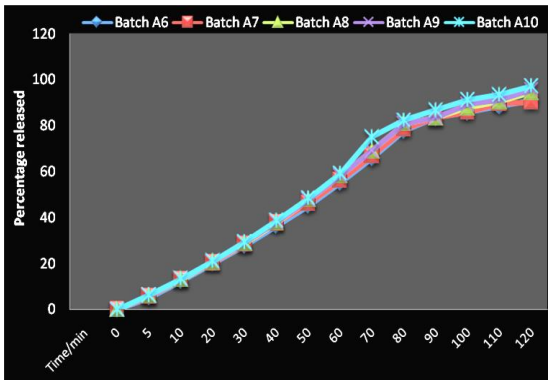

Figure 9: Artemether percentage cumulative release.

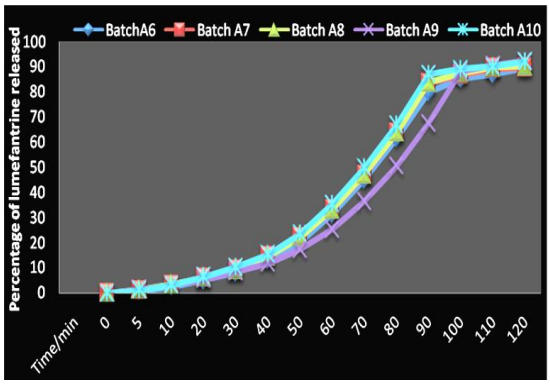

Figure 10: Lumefantrine percentage cumulative release.

Dissolution rate studies

The *in vitro* dissolution rate studies showed that none of the batches had 75% release after about 80 and 90 min. for artemether and lumefantrine respectively. Though the formulations are for immediate (conventional) release but to be administered every 12 h. Approximately100% of artemether and lumefantrine release was obtained after 120 min. These results are shown in Figure 9 and Figure 10. As the concentration of the suspending agent, the extracted pectin was increased; the drug release was continuously being increased, with few exceptions. When higher concentrations of the extracted pectin was used as suspending agent, flocculated suspensions were produced, and were easily re-dispersed on moderate agitation even after days. The release of artemether from the formulation was faster and higher than lumefantrine. For the release of lumefantrine, batch A9 had the least rate of release, though, reached the peak like other batches in terms of their release. These drug release results are in agreement with previous reports. Currently dissolution methods for fixed dose artemether-lumefantrine suspension is not in the official monographs. The available methods, using independent dissolution conditions for each active pharmaceutical ingredient and/or considering the relative effect of dissolution method variables described in literature by researchers demonstrated relative long dissolution time of about 120 min[34,35].

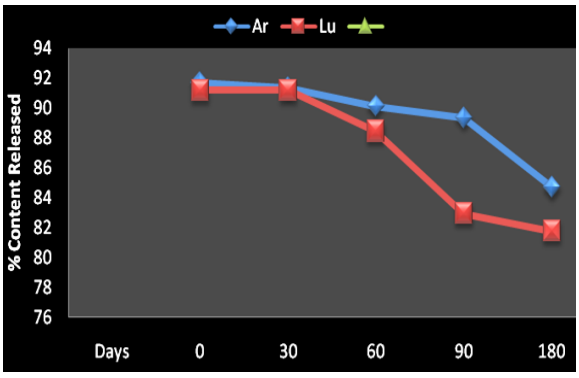

Figure 11: Short term stability study for artemether and lumefantrine.

Short term stability study of the formulated artemether-lumefantrine powder for oral suspension

It took 180 days to complete the short-term stability experiments for the compounded artemether/ lumefantrine, during which time the drug content was examined on days 0, 30, 60, 90, and 180. According to Figure 11, the formed granules' artemether content decreased from 91.70% at day 0 to 85.50% at day 180, while lumefantrine decreased from 91.18% at day 0 to 81.77% at day 180. Artemether and lumefantrine had a smaller and larger proportion of their drug components released, which may be related to their short and lengthy half-lives of 2-3 h and 2-6 days, respectively. This experiment employed the typical dissolving equipment, while the prior investigators used chromatography as previously described[16], and the values obtained are quite similar to those previously published but lower. However, the permitted range is officially set as 90 to 110% weighted[36].

Limitations of the study

The effect of the main constituent of the extracted pectin as a suspending agent/thickner in the formulation of artemether-lumefantrine suspension and comparing the suspension with that formulated with hybrids of the extracted pectin xanthan gum and sodium carboxylmethyl cellulose.

CONCLUSIONS

The drug content was examined at days 0, 30, 60, 90, and 180 as part of the 180-day short-term stability tests for the formulation artemether/lumefantrine. The artemether content in the formed granules decreased from 91.70% at day 0 to 85.50% at day 180, while lumefantrine decreased from 91.18% at day 0 to 81.77% at day 180. an As a result of lumefantrine's longer half-life of 2-6 days and artemether's shorter half-life of 2-3 hours, the percentage of real material released was greater for artemether. These values were reached, but lower than those previously published, and it's possible that this is because the procedures employed were different. In this study, the typical dissolving equipment was utilized, while the earlier investigators used chromatography as previously described[16]. The permitted range, however, is 90 to 110% weighted[37].

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AUTHOR'S CONTRIBUTION

Ubieko EA: writing, review and editing, methodology. **Onugwu AL:** formal analysis, data curation, conceptualization. **Ogbonna JDN:** writing, review, and editing, methodology, data curation. **Okoye E:** resources, review. **Nwakile CD:** writing, review and editing, data curation. **Attama AA:** acquisition, analysis. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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