Exploitation of capsule system for colon targeted drug delivery of biopolymer-based microparticles: *in vivo* and *in vitro* applications

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**A B S T R A C T**

The current study was to improve and control aceclofenac delivery prepared as biopolymer-based microparticles for effective colon-targeted drug delivery using modified gelatin capsules (MGCs) at different time intervals developed in two batches (C1 and C2). Microparticles were formulated with extracted mucuna gum using liquid paraffin oil (AC.LPO) and soybean oil (AC.SO) and evaluated in vitro for physicochemical performance and in vivo in rats. Encapsulation efficiency ranges from 54.48 ± 0.21% to 82.83 ± 0.22% for AC.LPO and 52.64 ± 0.11% to 80.36 ± 0.22% for AC.SO. SEM showed oblong and irregular shapes with porous and cracked surfaces. DSC showed low enthalpy and a very broad endothermic peak depicting high amorphous property. Delayed drug release was observed in the upper gastrointestinal tract with sustained release depicted in the lower gastrointestinal tract (GIT) using 3 and 9-h batch C1 of MGCs. AC.SO exhibited significantly (p < 0.05) higher anti-inflammatory activity (86%) than AC.LPO (77%). Hence, aceclofenac colon delivery could be improved and controlled using biopolymer-based colon-targeted microparticles delivered with MGCs.

1. Introduction

Research has been ongoing to seek novel technologies that will enhance the delivery of new and existing chemical moieties. A biffittly designed novel drug delivery technology can be the main advancement for solving some of the issues associated with this challenge. Microparticles are formulated using the process of microencapsulation by entrapment of drug substances in a matrix or reservoir. This process improves low solubility, and physicochemical instability, control drug release patterns and enhances patients’ friendly drug delivery due to the decreased frequency of drug administration (Khan et al., 2013). In microencapsulation, the three states of matter (solids, liquids, and gases) can be encapsulated in a microscopic particle forming a very thin polymer coating of core material around the drug. The polymer (carrier) is a protective material that isolates the drug and prevents physical exposure. This pharmaceutical technique delivers a drug to a targeted site with specificity. It maintains the desired amount of a drug at the site of interest especially if modified. The encapsulant influences drug release mechanisms of which mucuna gum from *Mucuna sloanei* is not an exception.

*Mucuna sloanei* (Fabaceae) is found in some parts of the semi-arid sub-Saharan and tropical zones of Africa. The plants are used in herb-alism against several disease conditions which include urinary tract infection, elephantiasis neurological, and Parkinson’s disease (Cilia et al., 2014). The seeds are edible and contain natural gum. The seeds are used in food processing as a thickener, while the gum has pharmaceutical applications. In Pharmacy, gums have been employed as a binding agent in tablet technology, emulsifying agents, thickeners in food processing, and suspensions as film-forming agents and transitional colloids (Ugwu et al., 2022; Onwuka et al. 2021., Kaliaperumal et al., 2012; Haroldo et al., 2012; Meena et al., 2012). *Mucuna* gum has gelation properties and imparts a gummy texture which increases the use of the gum for industrial purposes, particularly in drug development. It is easily accessible, biocompatible, biodegradable, and so forth. Some applications of this gum have been evaluated: stabilizer for drug emulsions and suspensions (Darekar et al., 2013; Sanji et al., 2017),
tablet binder (Majekodudunmi et al., 2014), gel matrix for bioadhesive drug delivery (Attama et al., 2003), glibenclamide microspheres using incubation method of incorporation (Attama and Nwabuzue, 2007), and film former for tablet coating (Ojile et al., 2000), among others.

Aceclofenac (AC) is a nonsteroidal anti-inflammatory drug (NSAID) and a phenylacetic acid derivative. It is used extensively for its antipyretic, anti-inflammatory, and analgesic effects. The mode of action is by inhibiting the cyclooxygenase enzyme during prostaglandin synthesis (Caner and Sirri, 2018). It is used in the management of rheumatoid arthritis, ankylosing spondylitis, and colitis, among others. Aceclofenac is a member of class II of biopharmaceutical classification systems with poor solubility challenges. It has a poor aqueous solubility of about 60 μg/ml (Soni et al., 2008). It has a biological half-life of less than 4 h which results in multiple dosing frequencies of two to three times daily with a dose range of 100–200 mg to maintain the therapeutic effect (Santanu and Barik, 2015; Ugwu et al., 2022). It is chemically unstable in the presence of light, acidic, and alkaline media (Bhinge et al., 2008). More so, it suffers both compression and flow challenges, and gastrointestinal tract (GIT) side effects when taken orally (Zahradnik et al., 2010). According to a previous report AC was depicted as a good candidate for control release formulation, which enables reproducible drug absorption compared to conventional dosage forms (Lakshmana et al., 2009).

Hence, aceclofenac is associated with challenges such as poor dissolution, erratic absorption, instability, short half-life, and so forth. More so, microparticle drug delivery suffers some shortfalls such as erratic drug release, poor encapsulation, and so on. Conventional colonic delivery has equally undergone some limitations such as abnormal drug discharge, enzymatic drug degradation, irrational pH change, and so forth. The current study is to curtail some of the challenges facing conventional colonic drug delivery by adopting capsule system technology to enhance colonic delivery of biopolymer-based microparticles.

2. Materials and methods

2.1. Materials

The materials used in this study include aceclofenac, 99.98% (Jagmag Pharm, India), soybean oil (Solve, Nigeria Ltd, Nsukka), liquid paraffin oil (Labolo Chemie, India), Labrafil® (Gattefosse, France), Cremophor® RH 40 (BASF Ludwigshafen, Germany) and dialysis membrane (6000–8000 Da). The Mucuna sloanei seeds (Fabaceae) were obtained from the Ibagwa market in Nsukka, Enugu State, and the mucuna gum was prepared in the Drug Delivery Unit of the Department of Pharmaceutical Technology and Industrial Pharmacy, UNN.

2.2. Experimental animal

Wistar rats were procured from the Department of Veterinary Medicine, University of Nigeria, Nsukka (UNN), Enugu State, Nigeria. The animal experiment was conducted by the guidelines established by the Institutional Animal Care and Use Committee of the University of Nigeria Nsukka with authorization (DOR/UNN/18/00021), which adhered to the European community guidelines for the use of experimental animals.

2.3. Extraction of mucuna gum from Mucuna sloanei seeds

The Mucuna sloanei seeds were authenticated by a consultant taxonomist from the Department of Pharmacognosy and Environmental Medicine, UNN, and the voucher specimen was deposited in the herbarium of the Department. The mucuna gum from the seeds was extracted using the coacervation method with little modification (Attama and Nwabuzue, 2007). Briefly, the dry seeds were de-husked, and the cotyledons air-dried and pulverized in a hammer mill. The milled flour (396.23 g) was soaked in distilled water containing 1% sodium metabisulphite to prevent oxidation for 24 h in a powder: water ratio of 1:15 and was sieved through a muslin cloth. The filtrate was desolvated with absolute ethanol to precipitate the gum. It was thereafter dried in an oven (Gallenkamp, OV110, Germany) set at 40 °C to a constant weight, milled and sieved (aperture size of 0.25 mm). The gum was defatted with petroleum ether, dried, and then stored in an air-tight container. And some physicochemical properties were determined.

2.4. Formulation of aceclofenac-loaded microparticles

The microparticles were prepared by an emulsion cross-linking technique. An appropriate amount of aceclofenac and mucuna gum was weighed out at different drug: polymer ratios (1:1, 1:2, 2:1, 1:3, and 3:1) to prepare 100 mL dispersion of the gum using distilled water as shown in Table 1. A 1% w/w talc (anti-adherent) was incorporated and the dispersion was added dropwise with a syringe into a beaker containing 50 mL of liquid paraffin oil and 1% (w/w) of Labrafil® (emulsifier) and homogenized using an Ultra-Turrax homogenizer (IKA® T25 digital, Germany) at a speed of 10,000 rpm for 5 min to produce a dispersion. This was assembled on a magnetic stirrer set at 600 rpm without heat for 30 min and (15 mL) acetone was added followed by incorporation of 1 mL of 4% glutaraldehyde (cross-linker) solution prepared with acetone: HCl (0.01 mol/L) in ratio 7:3 after 1-h stirring, and further stirred for 4 h. After 4 h of stirring the microparticles were centrifuged (SM800B, Uniscope, England) for 5 min at 1500 rpm. The supernatant was decanted. The microparticles were washed with petroleum ether and harvested. Then, air-dried and stored in a desiccator for further characterization. These formulations were denoted as AC.LPO (microparticles produced with liquid paraffin oil). Following a similar procedure as above mentioned, second formulations were prepared and coded AC.SO (microparticles prepared with soybean oil). Then, the yields obtained and the % yields of the microparticles were calculated using Eq. (1).

\[
\text{Yield of microparticles} \% = \frac{\text{Weight of the microparticles}}{\text{Weight of the constituents}} \times 100
\] (1)

2.5. Preparation and evaluation of modified gelatin capsules (MGCs)

An earlier method was adopted with little change in the modification of the gelatin capsules (Abdul et al., 2011). In briefly, 25 mL of formaldehyde solution (37% v/v) was prepared and transferred into a desiccator and about ≤0.5 g of potassium permanganate was incorporated to produce a formalin vapour. The desiccator was closed for about 120 min to enable adequate vapour saturation of the chamber. Some caps and bodies of gelatin capsules were separately and placed on a wire mesh

Table 1. Composition of biopolymer-based aceclofenac microparticles.

| Batch | AC:MC (mg) | Labrafil (% w/w) | Talc (% w/w) |
|-------|------------|------------------|--------------|
| AC SO |            |                  |              |
| AC LS | 100:100    | 1                |              |
| AC L2 | 100:200    | 1                |              |
| AC L3 | 200:100    | 1                |              |
| AC L4 | 300:100    | 1                |              |
| AC L5 | 400:100    | 1                |              |
| AC L PO|          |                  |              |

Key: AC.L1-S and AC.S1-S: Microparticles formulated with liquid paraffin and soybean oil at different ratios (1:1, 1:2, 2:1, 1:3, and 3:1) respectively; AC: aceclofenac; MC: mucuna gum.
inside the desiccator and allowed to modify at different time intervals of 1, 3, 6, 9, and 12 h then removed and dried for about 30 min at 50 °C in an oven (Gallenkamp, OV110, Germany) to ensure complete formaldehyde and gelatin-reaction before air-drying for about 1 h to remove residual formaldehyde, while some caps were left unmodified (unexposed caps). Then evaluated for aequous resistance and swelling study test to determine the solubility dispersion resistance and swellability in different media, while the safety of the MGCs was determined according to previous techniques (Abdul et al., 2011; Chauhan and Shah, 2012).

More so, two optimized time intervals (3 and 9-h) of MGCs were selected based on the two properties (high aqueous dispersion resistance and swellability). These modified capsules were employed to investigate the most suitable device to deliver AC (aceclofenac) for colon-targeted drug delivery. From the optimally modified capsules (3 and 9-h), two techniques (batches C1 and C2) were developed (Figure 1). Batch C1 contains a modified capsule body and cap with a needle-pierced hole of about 0.5 mm diameter at both ends which were sealed off with a coating solution (5% w/v Eudragit® S 100 in methanol). Batch C2 constitutes a modified capsule body and unexposed cap which was coated with the same solution to avoid unintended leaking and release of the drugs via the unexposed modified cap. The coatings occurred after filling the devices with microparticles. The batches (C1 and C2) were air-dried at ambient temperature and allowed a curing time of one day before further application.

### 2.6. Characterization of the microparticles

#### 2.6.1. Encapsulation efficiency and drug loading

Acelclofenac contents of the microparticles were determined by the digestion method (Attama et al., 2009). An equivalent weight of 10 mg of each formulation was dispersed in 10 mL of methanol: phosphate buffer (pH, 7.4) admixed at a 1:9 ratio at ambient temperature for 24 h. The dispersion was then centrifuged (SM800B, Uniscope, England) at 6000 rpm for 30 min. The supernatant was assayed spectrophotometrically (Spectrumlab 752, Netherlands) for acelclofenac content at 290 nm (Ugwu et al., 2022). All samples were analyzed in triplicate. The amount of drug encapsulated in the microparticles was calculated with reference to a standard Beer’s plot of acelclofenac and the linearity or lack of fit of the method was determined. The drug load and encapsulation efficiency were obtained using Eqs. (2) and (3), respectively:

\[
\text{Drug loading} = \frac{\text{Amount of drug loaded}}{\text{Total quantity of the formulation}} \times 100
\]

\[
\text{Drug encapsulation efficiency} = \frac{\text{Amount of drug encapsulated}}{\text{Theoretical drug content}} \times 100
\]

#### 2.6.2. Scanning electron microscopy (SEM) and particle size determination

The surface characteristics of optimum microparticles were evaluated by SEM. The samples were mounted on a double-sided tape on aluminium stubs and were sputter-coated with gold using fine coat ion sputter and morphologies were recorded using a scanning electron microscope (JEOL, JSM-6100).

The particle sizes of the microparticles were determined by observing the particles with a light microscope observed with a Motic® camera and analyzed using Motic® images software (Moticam, Xiamen, China). The polydispersity index was calculated using ratios as a standard deviation to the mean particle size of the formulation (Agubata et al., 2015).

#### 2.6.3. Differential scanning calorimetry (DSC)

Melting transitions and changes in heat capacity of the microparticles formulations, pure acelclofenac, and mucuna gum were determined using a differential scanning calorimeter (Netzsch DSC 204 F1, Geratebau, GmbH, selb, Germany). About 3–5 mg of each sample was weighed into an aluminium pan, hermetically sealed and thermal characteristics were obtained within the range of 20–400 °C at a heating rate of 10 K/min under a 20 mL min nitrogen flux. The thermal properties were recorded.

#### 2.6.4. Swelling study of the microparticles

The swelling index of microparticles was determined according to a previous method with little modification (Ramesh et al., 2013). An equivalent amount of 50 mg of each microparticle was weighed into a dialysis membrane of known weight (previously soaked in simulated biorelevant media for 24 h) and then immersed in 100 mL of different simulated biorelevant media of varying pH values (phosphate buffer (pH, 7.4), simulated intestinal fluid (SIF, pH 6.8), and simulated gastric fluid (SGF, pH 1.2)), contained in a 250 mL beaker and set up on a magnetic stirrer at 50 rpm at 37 ± 0.1 °C. At predetermined time intervals of 30, 60, 90, and 120 min, the membranes were withdrawn from the beaker carefully and quickly molted and then weighed. The swelling index was then determined using Eq. (4).

\[
\text{Swelling index} = \frac{W_t - W_0}{W_0} \times 100
\]

Key: \( W_t \) is the weight of the sample determined at time \( t \) and \( W_0 \) is the weight of the sample determined before immersion of samples in the fluid.

#### 2.7. Dissolution study of acelclofenac biopolymer-based microparticles

In vitro drug release of the microparticles was evaluated using dialysis membrane and modified gelatin capsules in simulated gastric fluid (SGF, pH, 1.2), and phosphate buffer fluid (pH of 6.8 and 7.4) without enzyme. An equivalent of 10 mg of the drug from each formulation from the drug: polymer ratios 1:1, 1:2, 2:1, 1:3, and 3:1 was weighed into dialysis membrane tubing (6000–8000 DaMwt) with hermetically sealed ends and previously soaked in an appropriate medium. Then, it was lowered into a beaker mounted on a magnetic stirrer assembly set at 100 rpm and 37 ± 0.1 °C containing 900 mL of an SGF (pH, 1.2) for 2 h followed by a phosphate buffer of pH, 6.8 for 3 h and pH, 7.4 for 5 h, respectively with the aid of a central membrane. In SGF, 2 h were chosen to mimic the average gastric emptying time (Akhgari et al., 2005). After 2

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**Figure 1.** Schematic diagram of encapsulated microparticles in modified gelatin capsules of batches C1 and C2. Key: Blue arrows (pierced and sealed holes); green arrows (seals), a yellow arrow (unmodified cap with seal).
h, the dissolution medium was replaced with 900 mL of phosphate buffer, pH 6.8, and dissolution was allowed to run for 3 h to simulate average intestinal transit time (Ibekwe et al., 2004). After this, the medium was discarded and replaced with a third medium of phosphate buffer pH 7.4 to simulate the colon at the ileocecal pH (Ugwu et al., 2019). After 2, 5, 6, 7, 8 and 10 h time intervals, 5 mL aliquots of the dissolution medium were withdrawn. To each withdrawn sample 0.5 mL methanol was added and assayed at 290 nm using a UV spectrophotometer (Spectrumanal, 752s, Nethelands). After each withdrawal, 5 mL of the fresh medium kept at the same temperature was added to replace the withdrawn sample.

More so, an in vitro drug release study of aceclofenac microparticles filled in the two selected modified gelatin capsules (3 and 9-h) was carried out using two developed techniques, batch C1 (both modified cap and body with needle holes at both ends and then sealed with coating solution) and batch C2 (unexposed cap and modified body and coated with coating solution). This was done using formulations AC.L5 (AC.LPO) and AC.S3 (AC.SO) selected based on the highest drug encapsulation efficiency. An equivalent of 10 mg of drug in each microparticle was weighed into an optimized 3 and 9-h time intervals MGCs body. The body was covered with an appropriate cap (modified cap (batch C1) or unexposed cap (batch C2)) and then developed as batches C1 or C2. With the aid of a centralized membrane, each device was lowered into a beaker mounted on a magnetic stirrer assembly containing 900 mL of SGF (pH, 1.2) for 2 h, SIF (pH, 6.8) for 3 h and phosphate buffer (pH, 7.4) from 5 h, respectively and the setup allowed to run for 24 h. The % drug release was calculated and the mean of the duplicate determinations was used in data analysis. The cumulative percentage of drug release was plotted against each time interval. From the plot, the T50% (time to release 50% of the drug), T75% (time to release 75% of the drug) and T80% (time to release 80% of the drug) were determined.

2.8. Anti-inflammatory study

The animal experiment was conducted by the guidelines established by the Institutional Animal Care and Use Committee of the University of Nigeria Nsukka, which adhered to the European community guidelines for the use of experimental animals (86/609/EEC) (EECD, 1986). Before the commencement of the experiment, the Animal Ethics Committee of the Faculty of Pharmaceutical Science, University of Nigeria, Nsukka approved the research protocol for the use of experimental animals. An in vivo anti-inflammatory activity of aceclofenac-loaded microparticle prepared in liquid paraffin (AC.LPO) and soybean oil (AC.SO) was determined using the rat paw oedema test according to Ugwu et al. (2022) with little modification. The phlogistic agent employed in the study was fresh undiluted egg albumin which was used to induce oedema, a cardinal symptom of inflammatory. Adult female Wistar rats (100–150 g) were divided into six rats per group for each group: aceclofenac liquid (AC.LPO) and AC.S3 (AC.SO) selected based on the highest drug encapsulation efficiency. An equivalent of 10 mg of drug in each microparticle was weighed into an optimized 3 and 9-h time intervals MGCs body. The body was covered with an appropriate cap (modified cap (batch C1) or unexposed cap (batch C2)) and then developed as batches C1 or C2. With the aid of a centralized membrane, each device was lowered into a beaker mounted on a magnetic stirrer assembly containing 900 mL of SGF (pH, 1.2) for 2 h, SIF (pH, 6.8) for 3 h and phosphate buffer (pH, 7.4) from 5 h, respectively and the setup allowed to run for 24 h. The % drug release was calculated and the mean of the duplicate determinations was used in data analysis. The cumulative percentage of drug release was plotted against each time interval. From the plot, the T50% (time to release 50% of the drug), T75% (time to release 75% of the drug) and T80% (time to release 80% of the drug) were determined.

2.9. Histopathological study

Following the in vivo study, the animals were sacrificed by ether anaesthesia for a necropsy. The liver and kidney parts of the various groups of experimental rats were harvested and collected for histopathological assessment. They were fixed in 10% formal saline and dehydrated in ascending grades of ethanol. Thereafter, the tissues were cleared in chloroform overnight, infiltrated, and embedded in molten paraffin wax. The blocks were later trimmed and sectioned at 5–6 μm. The sections were deparaffinized in xylene, taken to the water, and subsequently stained for light microscopy. Photomicrographs of the organ sections were taken and observed to determine any pathological changes by using a motic camera fixed onto a light microscope (Bancroft and Gamble, 2002).

2.10. Statistical analysis

Data were analyzed using SPSS Version 16.0 (SPSS Inc. Chicago, IL, US) and one-way analysis of variance (ANOVA). Values were shown as average ±SD (standard deviation). Differences between averages were measured by a two-tailed student’s T-test and p < 0.05 was considered statistically important.

3. Results and discussion

3.1. Physicochemical property of the modified gelatin capsules and mucuna gum

The reaction between formaldehyde vapour and gelatin modified and rendered the exposed caps and bodies of gelatin capsules insoluble in both aqueous and physiological fluids. Potassium permanganate is incorporated to aid in releasing formalin vapour and hasten the reaction between formaldehyde and gelatin. The results of the modification of gelatin capsules at each time interval of 1, 3, 6, 9, and 12 h observed visually showed that there was no change between the unexposed and modified gelatin capsules (MGCs) in colour but with little reduction in the sizes of the latter. The modified gelatin capsules were well fitted when closed and odourless which is similar to the unexposed (unmodified) gelatin capsules. The MGCs were non-sticky when touched with wet fingers, unlike unexposed gelatin capsules that stick easily to the fingers. In different fluids such as distilled water (pH, 7.0), SGF (pH, 1.2), phosphate buffer (pH, 6.8) and (pH, 7.4), the unexposed gelatin capsules collapsed and dispersed in the medium within 15 min, unlike the MGCs that resisted dispersion in the media but rather became swollen after 24 h. This fluid resistance property occurred because the modification resulted in a decreased solubility of gelatin capsules due to the cross-linking of the amino groups in the gelatin molecular chain with the aldehyde groups of formaldehyde by Schiff's base condensation. This result was similar to a previous report (Mastiholmamath et al. 2007). While the 1 h (time interval) MGCs could not resist dispersion in any of the fluid as it dispersed similarly to the unexposed capsules, the 12 h MGCs swelling capacity was poor and disqualified (1 and 12 h MGCs) use in the further experiment. The indispensibility of other MGCs (3, 6, and 9-h) in
different media confirmed that modification took place and was time-dependent. Thus, 3 and 9-h MGCs were selected for further studies.

In the qualitative chemical test, a less intense yellow-coloured solution was observed when compared to the standard solution. The intensity of the colour is proportional to formaldehyde concentration. Formaldehyde reacts with acetylacetone to give a yellow complex, dimethylpyridine (Chauhan and Shah, 2012). The less intense colouration is an indication that the MGCs are free of formaldehyde toxicity and therefore, can be taken as a reliable packaging for oral drug delivery suitable for controlled release dosage form since it has been reported that 0.1% value of formaldehyde is orally tolerated (WHO, 2005; Gélbkea et al., 2019).

The physicochemical characteristics of extracted mucuna gum indicated that it is a light brown powder gum. It is an odourless and tasteless powder with a mixture of spherical and irregular-shaped particles. The loss on drying was found to be 51%. This indicates that it is hygroscopic. The pH of the mucuna gum in an aqueous medium was obtained to be 5.85. The major peaks were found within the range of 1404.8–1254.26 cm⁻¹, 3157.24–2925.6 cm⁻¹, and 3357.96–3439.02 cm⁻¹ representing functional groups carboxylic acid salts/phenol or tertiary alcohol, alkenes, and alcohol, respectively as detected by Fourier transform infrared spectroscopy (Figure not presented). DSC depicted amorphous material as shown in Figure 5.

### 3.2. Developed modified gelatin capsules

Batches C1 and C2 were developed on each of the optimal MGCs (3 and 9-h). Batch C1 contains both modified cap and body with needle-pierced holes at both ends which were sealed off together with the central joint of the capsule body and cap using coating solution (5% w/v Eudragit® S 100 in methanol), and batch C2 comprise unexposed cap and a modified body which was coated up to four times in the same coating solution. These two batches were developed to address some restrictions associated with conventional methods of colon-targeted drug delivery such as the erratic and untimely release of the drug, and the side effect of some drug substances (especially the NSAIDs) which may cause acidic irritation/bleeding of the gastrointestinal tract tissues. Efforts were made to maintain uniform weight within the developed MGCs and relatively coating thickness (75%) was achieved to synergistically confer protection and decrease the chances of untimely drug release from the developed techniques before the targeted site is reached as a result of any abnormal physiological changes, especially in pH.

### 3.3. Characterization of microparticles

#### 3.3.1. Encapsulation efficiency, loading capacity, and % yields of the microparticles

Microparticles formulated in liquid paraffin oil (AC.LPO) and soybean oil (AC.SO) had higher encapsulation efficiency and drug loading capacity approximate eight folds of the original weight at 30 min followed by AC.L4 (approximately seven folds of the original weight) at the same time in buffer (pH, 7.4). At 90 and 120 min, the dissolution or erosion of matrices was observed with a remarkable decrease and disappearance of bars. This may be depicted as an increased solubility ascribable to good hydration capacity of the matrix owing to the pH of the medium and increase in swelling time. This effect was observed in formulations AC.L1, AC.L2, and AC.L5 which led to a significant weight decrease (p < 0.05). In SIF (pH, 6.8), the swelling index decreased with concentration (μg %) with a regression coefficient of 0.9983 (−1) as shown in Figure 2.

#### 3.3.2. Swelling properties of the microparticles

The swelling tests of the formulations carried out in different simulated biorelevant media [phosphate buffer (pH, 7.4), SIF (pH, 6.8) and SFG (pH, 1.2)] are presented in Figure 3. The result showed that formulations AC.LPO (aceclofenac microparticle formulated in liquid paraffin oil); AC.S1-S5 (Acelofenac microparticle formulated in soybean oil); Y: (% yield); EE: (Encapsulation efficiency); LD: (loading capacity); PDI: (polydispersity index); SD (standard deviation). The pH of the mucuna gum in an aqueous medium was obtained to be 5.85. The major peaks were found within the range of 1404.8–1254.26 cm⁻¹, 3157.24–2925.6 cm⁻¹, and 3357.96–3439.02 cm⁻¹ representing functional groups carboxylic acid salts/phenol or tertiary alcohol, alkenes, and alcohol, respectively as detected by Fourier transform infrared spectroscopy (Figure not presented). DSC depicted amorphous material as shown in Figure 5.

#### Table 2. Yields, encapsulation efficiency, loading capacity, and polydispersity index of microparticles.

| Batch | Y (% w/w) | EE (% w/w ± SD) | LC (% w/w ± SD) | PDI |
|-------|-----------|-----------------|-----------------|-----|
| AC.L1 | 66.1      | 62.4 ± 0.12     | 20.5 ± 0.30     | 0.39|
| AC.L2 | 93.6      | 62.8 ± 0.11     | 20.6 ± 0.04     | 0.84|
| AC.L3 | 96.8      | 54.5 ± 0.21     | 17.8 ± 0.11     | 1.29|
| AC.L4 | 97.6      | 64.8 ± 0.05     | 22.3 ± 0.08     | 0.39|
| AC.L5 | 97.8      | 82.8 ± 0.22     | 28.9 ± 0.13     | 0.86|
| AC.S1 | 98.0      | 66.9 ± 0.07     | 22.9 ± 0.03     | 0.43|
| AC.S2 | 62.8      | 65.1 ± 0.11     | 22.4 ± 0.11     | 0.47|
| AC.S3 | 98.0      | 80.4 ± 0.22     | 28.1 ± 0.21     | 0.16|
| AC.S4 | 92.1      | 55.2 ± 0.23     | 17.4 ± 0.13     | 0.56|
| AC.S5 | 98.2      | 52.6 ± 0.11     | 17.2 ± 0.21     | 0.35|

Key: AC.L1-L5: (Acelofenac microparticle formulated in liquid paraffin oil); AC.S1-S5: (Acelofenac microparticle formulated in soybean oil); Y: (% yield); EE: (Encapsulation efficiency); LD: (loading capacity); PDI: (polydispersity index); SD (standard deviation).

Figure 2. A calibration curve of aceclofenac.
than three and a half folds of the original weight followed by AC.L2 (three folds of its original weight) at 120 min. Then in SGF (pH, 1.2), formulation AC.L4 had the highest swelling of about five-folds of the original weight at 60 min followed by AC.L2 (four folds of the original weight) at the same time. At 120 min, there was a significant decrease in the swelling index that led to another disappearance of bars (p < 0.05) due to dissolution or erosion on the matrices.

In the microparticles prepared in soybean oil (AC.SO) formulation AC.S4 (Figure 3b) had the highest swelling of approximately four folds of the original weight at 60 min, followed by AC.S3 (three folds of the original weight) at 30 min in pH 7.4. In SIF (pH, 6.8) formulation AC.S4 also had the highest swelling of about three folds of the original weight at 120 min followed by AC.S3 (more than twice its original weight) at 30 min. More so, in SGF (pH, 1.2), formulation AC.S3 had the highest swelling of approximately six-fold the original weight at 30 min, followed by AC.S4 (three times the original weight) at the same time. Hence, the high swelling property depicted the high swelling capacity of the material, which depends upon its water absorption or sorption.

Hence, AC.LPO showed the best swelling capacities (Figure 3a). The higher swelling index in a pH of 7.4 is an indication that the gum might be useful as a matrix former in colon-targeted drug delivery since the swelling has been detected as a primary mechanism in a diffusion-controlled drug release (Akpabio et al., 2011).

### 3.3.3. Scanning electron microscopy (SEM) and particle sizes

The SEM result as presented in Figure 4 showed oblong and irregular-shaped particles with porous and cracked surfaces which may be a result of the interconnectivity of internal phase droplets during the final stage of solvent evaporation which led to structural deformation. In the SEM, there was a simultaneous solidification of the carrier with the drug which led to the formation of pores and coarse microparticles. This was similar to a previous report (Miyazaki et al., 2006). These pores may act as drug passages during the drug release period from the inner part of the microparticles. More so, on these pores and cracks, some drugs might have been adsorbed on the surfaces which may contribute to the burst effect during drug release which at the same time would be useful to obtain a certain effective concentration immediately after oral administration (Tao et al., 2009).

The particle size of AC.LPO (microparticles in liquid paraffin oil) with formulations AC.L1-AC.L5 were within the range of 2–13.9 μm with the highest particle sizes occurring in formulations AC.L1 and AC.L4 and the smaller particle sizes observed in AC.L2 and AC.L5. In AC.SO (microparticles in soybean oil) formulations (AC.S1-AC.S5) particle size range of 2–99 μm was obtained with the highest particle size occurring in formulation AC.S5 and the smallest in AC.S1. Hence, it is necessary to note that one or more of these factors can influence the particle sizes of microparticles. These include stirring rate, type and concentration of polymer, homogenization process, drug load, and so forth (Ugwu et al., 2022). In AC.LPO (AC.L2 and AC.L5), an increase in the polymer concentration produced smaller microparticles. In the same batch, a formulation with a high drug load was found to generate a high particle size as observed in AC.L5. Similar results have also been reported (Barakat and Yassin, 2006; Joseph et al., 2002). On the other hand, in AC.SO (AC.S5) an increase in polymer concentration produced a large particle size. This may be due to an increase in the viscosity of the internal phase of the formulation. This

Figure 3. A swelling study of aceclofenac microparticles in liquid paraffin oil (a) and soybean oil (b) in different biorelevant fluids. Key: AC.L1, AC.L2, AC.L3, AC.L4, AC.L5 and AC.L1, AC.L2, AC.L3, AC.L4, AC.L5: (formulations of AC.LPO and AC.SO containing drug:polymer ratio 1:1, 1:2, 2:1, 1:3 and 3:1, respectively); A: (phosphate buffer pH 7.4), B: (SIF pH 6.8), C: (SGF pH 1.2).
has been reported previously that an increase in polymer concentration increased the sizes of SEM, microparticles, and nanoparticles (Patel et al., 2012; Momoh et al., 2019; Lung-Hsin et al., 2010).

The results of the polydispersity index (PDI) of the AC.LPO and AC.SO shown in Table 2 ranged between 0.29 ± 0.28–0.86 ± 0.43 and 0.15 ± 0.14–0.43 ± 0.41, respectively. Polydispersity is a measure of how each particle's size varies from one another which increases from 0–1. The value of zero (0) indicates monodispersity, whereas 1 shows high dispersity amongst particles (O bitte et al., 2017). The PDI index gives an idea of the particle size distributions. Thus, microparticles could be monodispersed or polydispersed. The lower the value (monodisperse) the more uniformity in size and the higher the stability of the formulation. The depicted average PDI of the microparticles is in good range since they fall within less than 1.

3.3.4. Differential scanning calorimetry

The DSC enables us to determine the thermotropic behaviour of a drug in a formulation in its solid state. The DSC results of aceclofenac, mucuna gum, and microparticles as presented in Figure 5 showed thermal endothermic peaks at 154.56, 81.53, and 81.06 °C, respectively. The DSC thermogram of AC indicated a sharp endothermic peak which depicted the crystalline nature and purity of the drug similar to a reported thermal property of AC (Mohsen et al., 2012). The gum showed very broad endothermal peaks which indicated high amorphous nature. The microparticle formulation showed a very broad endothermic peak with low enthalpy. This broad peak indicated that the drug underwent a phased transition from a remarkable crystallinity to an amorphous form. Formulations with low enthalpies indicated the formation of an imperfect crystal lattice of the drug. This is in agreement with an earlier report (Momoh et al., 2020).

3.4. In vitro release of aceclofenac from the microparticles using dialysis membrane

In vivo drug release of microparticles was studied in three different media; SGF (pH, 1.2) for 2 h, SIF (pH, 6.8) for 3 h, and phosphate buffer (pH, 7.4) till the end of the experiment using dialysis membrane. The result showed that the microparticles formulated in liquid paraffin oil (AC.LPO) with the drug: polymer ratios AC.L1, AC.L2, AC.L3, AC.L4, and AC.L5 had T50% (time to release 50% of the drug) of 5, 5, 7, 9, and 5 h and T75% (time to release 75% of the drug) of 0, 0, 0, 0, and 9 h, respectively (Figure 6). The highest drug release of 84% was observed in AC.L5 with a prolonged time (9 h) to release 75% aceclofenac (AC), while the lowest drug release of 61 and 67% was got in AC.L2 and AC.L4, respectively (Figure 6a). The high drug release may be a result of the good swellability and aqueous solubility of the gum which could be ascertained by the high erosion and dissolution observed in the swelling study that led to the disappearance of its bars in pH, 7.4. More so, the observed prolonged-release manner in some formulations may be related to the encapsulation carrier in response to pH. This best drug release observed in AC.L5 may be backed up by its good % yield (98%), and EE (%) of 83%.

All the microparticles except AC.L5 from formulation AC.LPO could not attain 75% drug release within the experimental period which may be a result of the strong attachment of the drug to the gum matrix and this was consistent with an earlier report (Attama and Nwabueze, 2007). More so, the high swellability of the hydrophilic matrix which formed the gel layer may become a barrier to drug release allowing release through diffusion or erosion. This effect had been detected in previous research (Maderuelo et al., 2011).

In AC.SO (aceclofenac microparticles formulated in soybean oil), results of the release study as presented in Figure 6b showed that AC.S1, AC.S2, AC.S3, AC.S4, and AC.S5 had T50% of 5, 7, 4, 7, and 7 h and T75% of 8, 9, 8, 9, and 8 h, respectively. A burst effect was observed in formulation AC.S3 in SGF (pH of 1.2). They all had high drug release (>80%) with the highest aceclofenac release observed in AC.S3 (98%). This microparticle which showed the highest drug release had significant (p < 0.05) encapsulation efficiency of 80%, particle size (8–10 μm), and good yield (98%). In AC.S3, a significant (p < 0.05) weight decrease was even observed during the swelling study which may imply that the encapsulated drug eroded the surface with drug diffusion from the
Figure 5. Differential scanning calorimetry (DSC) thermographs of aceclofenac (AC), mucuna gum (MC), and microparticles (MPs) in superposition.
matrix. The burst effect observed in formulation AC.S3 in SGF (pH of 1.2) may be a result of an unencapsulated drug on the periphery of the matrix with the encapsulated drug released in a prolonged style.

In the drug release of both microparticles AC.LPO and AC.SO, it was observed that AC.LPO formulations (especially AC.L5) produced more rigid microparticles with stronger binding effects and good swellability. It is worth to note that all except AC.L5 could not release up to 75% of the drug as their encapsulation efficiency was >75%, unlike in AC.SO that released up to 98% aceclofenac. This high drug release may be due to the presence of phospholipid (lecithin) as an emulgent in soybean oil with higher solubility. It has been similarly reported (Wang et al., 1997).

3.5. In vitro release of aceclofenac from the microparticles using developed MGCs

In vitro drug release of aceclofenac from microparticles was carried out using the developed techniques, batches C1 and C2 of modified gelatin capsules (MGCs) were done with optimum selected formulations.
AC.L5 (AC.LPO) and AC.S3 (AC.SO) as shown in Figure 7. The MGCs were rendered insoluble in the entire GI tract fluid to control and deliver the drug to the colon. A desirable colon-targeted drug delivery (CTDD) is expected to be sufficiently gastro-resistant and protective to delay drug release, followed by prolonging the drug delivery to the colon. This showed that formulation meant for CTDD should not be allowed to release at upper GIT such that the therapeutic efficacy of the medication should be achieved and maintained in the treatment of colon-related ailments and be prevented from irritating the stomach lining in case of acidic substance. The 3 and 9-h developed MGCs were selected due to their aqueous dispersion resistance and good swelling properties and were filled with optimum formulation AC.L5 (AC.LPO) or AC.S3 (AC.SO) selected based on their high drug encapsulation efficiency. The two techniques (batches C1 and C2) were developed on the 3 and 9-h MGCs with batch C1 comprising both modified body and cap which were pierced with a needle at both ends and sealed off with the coating solution, and batch C2 constituting the modified body and unexposed cap coated with the coating solution. Both batches (C1 and C2) were allowed a curing time of 24 h before the drug release test to enhance the rigidity and stability of the devices. In batch C1, drug release was observed to start after the swelling and expansion of the capsule shell followed by the ionization of the sealing coats based on the pH of the environment. In

![Graph A](image)

![Graph B](image)

Figure 7. Drug release of aceclofenac from AC.LPO and AC.SO microparticles using 3-h (A) and 9-h (B) modified gelatin capsules of batches C1 and C2. Key: AC.L-3A & AC.S-3B and AC.S-3A & AC.S-3B; AC.L9A & AC.S9B and AC.S9A & AC.S9B (microparticles from AC.LPO and AC.SO containing drug: polymer 3:1 (AC.L5) and 2:1 (AC.S3) using 3-h and 9-h batches C1 and C2, respectively).
batch C2, the drug release was also pH-dependent and occurred after the swelling and ionization of the coating material which led to the collapse and dispersion of the unexposed capsule shell thereby allowing drug release. In vitro drug release of the two developed capsule batches C1 and C2 as presented in (Figure 7), showed that there was no drug release in the SGF (pH, 1.2) unlike the in vitro drug release using a dialysis membrane (Figure 6). In the SIF (pH, 6.8), the MGCs were observed to start swelling and expanding with subsequent slight drug release at the end of 5 h. In the phosphate buffer (pH, 7.4) simulating the colon environment, the swelled and expanded MGCs allowed fluid penetration or ingestion into the formulations which initiated the drug release. This led to delayed and gradual sustained drug release. It has been proposed that fluid penetration possibly caused gel formation with a capacity to retard drug release (Ali et al., 2008).

On the other hand, the microparticles showed the time to release 50% (T50%) of AC at 10 h and 10 h for AC.LPO and, 9 h and 8 h for AC.SO, and time to release 80% (T80%) of AC content at 19 h and 12 h (AC.LPO); and 16 h and 12 h (AC.SO) using 3-h developed batches C1 and C2, respectively (Figure 7a). Batches C1 and C2 had a delayed time in the release of 50% and 80% of the drug content. Thus, a remarkable prolonged time difference of 19 h was observed in T80% of the drug (AC.L5, from AC.LPO) using 3-h batch C1 MGCs. This may be due to the nature of the developed device based on the less surface area or narrow pathway for drug release. In both microparticles, there was no variation in the time to release 50% and 80% acesofenac using 3-h batch C2.

In the drug release using 9-h MGCs, batches C1 and C2 in Figure 7b showed the time to release 50% (T50%) of AC at 12 h and 10 h (AC.LPO); and 15 h and11 h (AC.SO) and time to release the 80% drug content (T80%) at 0 h and 19 h (AC.LPO); and 23 h and 17 h (AC.SO), respectively. The AC released via 9-h developed batch C1 MGCs had remarkably prolonged T50% of 15 h and T80% of 23 h in AC.SO this may also be due to the nature of the developed device with less surface area/release pathway. In AC.L5 (from AC.LPO) up to 80% drug release was not observed using batch C1 which could also be a result of the nature of the technique in conjunction with the robustness of the microparticles. Subsequently, in batch C2, T80% was achieved in 19 h and this fact could be supported by its high encapsulation efficiency being >80%. In the 9-h batch, C2 MGCs, the faster release of the drug observed was due to larger surface areas obtained after the ionization of the coat which caused the collapse and dispersion of the unexposed capsule cap leading to microparticle hydration, swelling, dissolution, and drug diffusion.

Thus, in the use of 3 and 9-h MGCs, there was a significant (p < 0.05) difference in T50% and T80% of 8–19 h and 12–23 h, respectively. The implication was observed to be a result of an increase in the modification time of the capsule which provided more cross-linking between gelatin and formaldehyde generating tight methylene bridges with a stronger covalent bond since the cross-linking period increased. A similar effect has equally been reported (Hoffman et al., 2015). This prolonged drug release found generally in the use of both 3 and 9-h developed modified gelatin capsules is of immense advantage in drug delivery.

### 3.6. Anti-inflammatory activity of the microparticles

Egg albumin-induced rat paw oedema is an animal model for screening anti-inflammatory drugs and it assesses the anti-inflammatory effect of anti-inflammatory products. Egg albumin is a natural agent used to induce the release of inflammatory and pro-inflammatory mediators such as prostaglandins, leukotrienes, histamine, and bradykinin. The course of acute inflammation is biphasic. The first phase includes the release of serotonin and histamine from mast cells and kinins after the injection of a phlogistic agent (egg albumin) in the first 1–2 h (Shukla et al., 2009), while the second phase is mediated by the release of prostaglandins, cyclooxygenase and lipooxygenase products in 2–3 h. The second phase is sensitive to both steroidal and non-steroidal anti-inflammatory drugs (Barung et al., 2021). Two important types of inflammatory mediators, prostaglandins (especially prostaglandin E2) are the main initiators of the cardinal signs of acute inflammation, and leukotriene B4 is a mediator of leukocyte activation in the inflammatory event (Ayoola et al., 2009). Non-steroidal anti-inflammatory drug (NSAID) inhibits cyclooxygenase leading to blockage of the synthesis of prostaglandins.

The result of the anti-inflammatory study of the microparticles presented in Figure 8 showed that formulations AC.LPO and AC.SO had 34–51% and 41–69% oedema inhibition 1 h post drug administration with their maximum oedema inhibition of 77 and 86% both at 8 h, respectively. The AC.SO exhibited a higher anti-inflammatory activity than AC.LPO. More so, a remarkable higher oedema inhibition was observed in the microparticles groups when compared to the pure drug, commercial product, negative control, and placebo. The highest anti-inflammatory activity observed in AC.SO may be a result of the higher aqueous solubility of the microparticles since they contain biodegradable mucuna gum and soybean oil. More so, the presence of lecithin, a natural emulsifier found in soybean oil may have improved the drug's bioavailability. This activity enhancement may be attributed to improved solubility and dissolution of the drug by the natural gum and emulsifier. Subsequently, the drug has the activity to block histamine and 5-HT, two mediators that are released by egg albumin and prostaglandins synthesis (Beg et al., 2011).

### 3.7. Histopathological study

The histopathological profiles of the liver and kidney sections of the Wistar rats are shown in Figure 9. The histoarchitecture of different groups of Wistar rats that received the microparticles was compared to three different controls; control 1 (as C1, a group that received neither inflammatory agent nor treatment), and control 2 (as C2, a group that received an inflammatory agent but no treatment) as shown in Figure 9a. Control 1 showed the normal architecture of structural units of the hepatic lobules, formed by cords of hepatocytes separated by hepatic sinusoids (white arrows) and was remarkably different from control 2. The C2 showed central venous and sinusoidal congestion due to inflammation as indicated with a white arrow. There was no significant histologic change in liver sections of rats that received the microparticles, especially AC.SO. Therefore, histopathological evaluation of the liver tissues of formulations AC.LPO and AC.SO had normal hepatocytes distributed in a radial pattern away from the central veins with no visible changes except mild congestion of the central veins.

Photomicrograph sections of the kidney in the control groups: control 1 (KC1, a group that received neither inflammatory agent nor treatment),

![Figure 8](https://example.com/fig8.png)  
**Figure 8.** Anti-inflammatory activity of the acesofenac microparticles. Key: AC.LPO and AC.SO as microparticles formulated in liquid paraffin oil, and soybean oil, respectively; AC.P: pure acesofenac; AC.COM: acesofenac commercial brand.
and control 2 (KC2, a group that received an inflammatory agent but untreated), showed that the glomerulus (GL) and renal tubules (white arrows) which had no signiﬁcant histologic change in all the groups (Figure 9b). These served as a baseline for comparing all the kidney architectural sections. The histopathological study of groups that received AC.LPO and AC.SO microparticles showed that the microscopic architecture of the kidneys in all the groups had a similar appearance as the standard with the renal corpuscles having the normal size of urinary space and tubular structures with no sign of congestion, although the glomeruli showed signiﬁcant hypercellularity (Figure 9b).

Hence, the histoarchitecture of the liver showed no histological change unlike the kidney section with higher cellularity or hypercellularity observed in both microparticles. This may or not be a physiological change or well-being and could imply that both the microparticles have a cellular regeneration activity though further research is required.

4. Conclusion

Modified gelatin capsules (MGCs) were successfully developed to deliver aceclofenac-based biopolymer microparticles to improve and control colonic drug delivery. The exploited capsule system controlled and delayed drug release in the proximal part of the stomach but released at the distal part. Batch C1 technique was observed to exhibit longer prolonged drug release (p < 0.05) than batch C2 which was a result of the nature of the developed device based on the narrow pathway created for drug release. Aceclofenac-based biopolymer microparticles formulated with soybean (AC.SO) demonstrated the best microparticles based on the evaluated properties. Hence, the potential use of the developed MGCs to deliver aceclofenac-based biopolymer microparticles can be a good alternative to improve and control drug delivery for easy targeting to the colon than conventional colonic drug delivery. Thus, further clinical research is entertained for in vivo attestation.
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