



## Diclofenac sodium-loaded chitosan microparticles: formulation, characterization and targeted drug delivery properties

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### ABSTRACT

An investigation into the suitability of medium-molecular-weight chitosan microparticles for enhanced oral delivery of diclofenac sodium is presented. Diclofenac sodium -loaded chitosan microparticles were formulated by simple emulsification-coacervation technique using glutaraldehyde saturated toluene (GST) as the cross-linking agent at different cross-linking times. The microparticles were characterized with respect to morphology, particle size, yield and loading efficiency. The swelling properties was assessed in both simulated gastric fluid (SGF) without pepsin (pH 1.2) and simulated intestinal fluid (SIF) without pancreatin (pH 7.4) while their *in vitro* release of diclofenacsodium from the microparticles was studied in 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4). Results indicated that discrete, spherical, and free flowing microparticles of size range  $324.50 \pm 29.82 \mu\text{m}$  to  $658.23 \pm 8.34 \mu\text{m}$  were obtained. Microparticles subjected to cross-linking times of 1, 2, and 4 h showed greater swelling in SGF than in SIF, whereas those subjected to cross-linking times of 6 and 10 h showed good swelling characteristics in SIF. It was further observed that microparticles cross-linked for 1 h gave the highest entrapment efficiency of  $66.40 \pm 9.07 \%$ , whereas those subjected to a cross-linking time of 10 h exhibited a low loading efficiency and delayed the release of the incorporated drug the most for up to 10 h, yielding 92.34 % and 66.58 % drug release in phosphate buffer and 0.1 N HCl, respectively. Drug release showed a biphasic pattern, in all cases, characterized by an initial phase of rapid and higher release followed by a more gradual release for the rest of the release period. The release pattern of the drug was kinetically analysed and found to follow the Higuchi square root model. This implies that a formulation of diclofenac sodium-loaded medium-molecular-weight

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Ofokansi and Kenekchukwu

chitosan microparticles could offer a better and more reliable approach of delivering diclofenac sodium by the oral route.

**Keywords:** Medium-molecular-weight chitosan, diclofenac sodium-loaded microparticles, gastrointestinal side effects, oral delivery.

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## INTRODUCTION

Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microparticles form an important part of such novel drug delivery systems [1 – 3]. Microspheres refer to the micro-particulate polymer-based drug delivery system with an average particle size larger than 1  $\mu\text{m}$  [4]. Micro-particulate carrier systems made from naturally occurring biodegradable polymers have attracted considerable attention for several years [5]. They have been extensively studied for use as drug delivery systems, where they have been shown to protect sensitive macromolecules from enzymatic and acid degradation, and allow controlled release and tissue targeting of the formulated drug [6 – 17]. Because of short residence time of microspheres at the site of absorption, bioadhesive microspheres were developed to serve as a means for providing an intimate contact of the drug delivery system with the absorbing membranes [18 – 21]. The advantages of bioadhesive microspheres include efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site [22 – 25]. Many polymers have been used in the formulation of microspheres [26]. Polymers such as chitosan, gelatin, polylactic acids and their

derivatives have all been extensively studied for their ability to form microspheres [27 – 29]. Microspheres prepared with chitosan (a cationic polymer obtained by deacetylation of chitin) have been found to be highly bioadhesive (mucoadhesive) and biodegradable and have been used for the controlled release of many drugs [30 – 35]. Biodegradable microspheres break down completely into harmless metabolites that are easily eliminated through natural body functions [4].

A trend in non-steroidal anti-inflammatory drugs (NSAIDs) development has been to improve therapeutic efficacy and reduce the severity of gastrointestinal (GI) side effects through altering dosage forms by modifying release of the formulations to optimize drug delivery. One such approach is using polymeric microspheres as carriers of drugs [36]. Many NSAIDs have been formulated into microspheres using biodegradable and non-biodegradable polymers, and various methods for oral, parenteral and topical applications have been devised [37 – 39]. Studies have indicated that the NSAIDs entrapped into microspheres were superior to the conventional formulations with respect to bioavailability and pharmacodynamic properties [37 – 39]. More so, chitosan is the most widely studied polymer for the formulation of NSAID-loaded microspheres for oral use [36, 40]. Diclofenac is a non-steroidal anti-inflammatory drug (NSAID), which is indicated for the relief of signs and

Ofokansi and Kenechukwu

symptoms of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and also for relief of migraines and menstrual pain. Diclofenac is used commonly to treat mild to moderate post-operative or post-traumatic pain, particularly when inflammation is also present. Diclofenac is the most potent NSAID on a molar basis, and is among the better tolerated NSAIDs [3, 41-43]. Diclofenac sodium (DS) is a sodium salt of an aminophenyl acetic acid which is rapidly absorbed after oral administration, but following the administration by this route, common side effects are observed, such as gastritis, peptic ulcer and bleeding. The exact mechanism of action of diclofenac is not entirely known, but it is thought that the primary action responsible for its anti-inflammatory, antipyretic and analgesic effects is through inhibition of prostaglandins synthesis by non-selective inhibition of cyclooxygenase (COX) isoenzymes. Cyclooxygenase catalyzes the formation of prostaglandins and thromboxane from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A2). Prostaglandins act as messenger molecules in the process of inflammation. The resultant decrease in prostaglandins in the epithelium of the stomach makes it more sensitive to corrosion by gastric acid. This is also the main side effect of diclofenac [3]. To address this problem, researchers have formulated diclofenac into microspheres using biodegradable and non-biodegradable polymers and by various methods [3, 41 – 43]. It was reported that diclofenac-loaded microspheres reduced the GI toxic effects, exhibited sustained action and of course increased patient compliance [3, 41 – 43]. From a technological point of view, the molecular weight of the polymer is one of the critical parameters in the preparation of polymeric microspheres. Microspheres made with a mixture of high molecular weight and low molecular weight chitosan (1:2 w/w) showed a good drug content and encapsulation efficiency irrespective of polymer/drug ratio [44]. Although chitosan microspheres have been employed for oral delivery

of diclofenac sodium [43], a review of the literature has not revealed any study on diclofenac sodium-loaded microspheres prepared with medium-molecular-weight chitosan by glutaraldehyde cross-linking method using glutaraldehyde saturated toluene (GST) as the cross-linking agent. Thus, the objective of this study was to evaluate, *in vitro*, diclofenac sodium-loaded microspheres prepared by glutaraldehyde cross-linking technique using medium-molecular-weight chitosan with a view to enhancing the oral delivery of diclofenac sodium through entrapment into these microspheres. This way, a reduction in the gastro-erosive side effects of diclofenac sodium would be achieved.

## MATERIALS AND METHODS

Diclofenac sodium (Medrel Pharmaceuticals, Pvt, India), medium-molecular-weight chitosan (Sigma-Aldrich, USA), acetone, concentrated hydrochloric acid, glutaraldehyde, sodium chloride and glycine (BDH, England), sodium hydroxide, span 80 and toluene (Merck, Germany), monobasic potassium phosphate (Sigma Chemical Co., USA), petroleum ether (Fluka, Germany), and liquid paraffin (May and Baker, England) were used as procured from the manufacturers without further purification. All other reagents were analytical grade and used as such. Distilled water was obtained from an all-glass still.

### Preparation of glutaraldehyde saturated toluene (GST)

Glutaraldehyde (100 ml) and toluene (100 ml) were placed in a beaker and stirred at 1000 rpm for 1 h using a magnetic stirrer (Remi Instruments, Mumbai, India). Then, the solvent mixture was kept overnight for stabilization after which the upper toluene layer saturated with glutaraldehyde was decanted and used as glutaraldehyde saturated toluene (GST).

Ofokansi and Kenekchukwu

### Preparation of the chitosan microparticles

The microparticles were prepared by simple emulsification phase separation technique. Chitosan was used as a polymer and was cross-linked using glutaraldehyde saturated toluene (GST) as per the method described by Thanoo *et al*[45]. All the batch formulations contained diclofenac sodium and chitosan in the ratio of 1:2 and a fixed volume of GST (2.5 ml). Briefly, chitosan (2.0 g) was dissolved in 100 ml of 5 % v/v aqueous acetic acid solution. One gram of diclofenac sodium was dispersed in the polymer solution. The resultant mixture was extruded through a syringe into 125 ml of liquid paraffin (heavy and light, 1:1 ratio) containing 0.5 % v/v Span 80, which was stirred at 2000 rpm using a paddle stirrer (Remi Instruments, Mumbai, India). GST (2.5 ml) was added at once and stirring was continued for 1 hour. The cross-linking time was varied from 1 to 10 hours whereas the cross-linking agent, stirring speed and the polymer-to-drug ratio were kept constant. Five batches of the microparticles were formulated at cross-linking times of 1, 2, 4, 6, and 10 hours and were denoted as GST<sub>1</sub>, GST<sub>2</sub>, GST<sub>4</sub>, GST<sub>6</sub> and GST<sub>10</sub> respectively. A 2 ml-volume of glycine solution (4 %) was added to capture any free aldehyde group and quench the cross-linking reaction after each cross-linking time. Microparticles thus obtained were filtered and washed several times with petroleum ether (20 – 40 °C boiling point) to remove any traces of oil adhering to the microparticles. They were finally washed with acetone to remove excess of GST. The microparticles were then dried at the ambient temperature of 28 ± 2 °C for 24 hours.

### Determination of percentage yield

The dried microparticles were weighed to obtain the yield of microparticles formulated per batch. The percentage (%) yield was calculated using the formula:

$$\text{Percentage (\%)} \text{ yield} =$$



Eqn. 1

where:

W<sub>1</sub>= Weight of the microparticles formulatedW<sub>2</sub>= Weight of the drug addedW<sub>3</sub>= Weight of the polymer and Span 80.

### Particle size analysis and morphological characterization

The particle size of the microparticles was determined by computerized image analysis on a photomicroscope (Lieca, Germany). Samples from each of the batches were dispersed in a mixture of liquid paraffin and Span 80 and mounted on a slide and observed under a light microscope. With the aid of software in the microscope, the projected diameters of the particles corresponding to the particle sizes of the microparticles were determined and the mean calculated. The particle morphologies were also observed and captured by the photomicroscope.

### Swelling studies

The degree of swelling of the microparticles was investigated in simulated gastric fluid without pepsin (pH 1.2) and simulated intestinal fluid without pancreatin (pH 7.4). A dialysis membrane 9 cm long was activated by immersion in 50 ml of distilled water regulated at 90 °C for 1 hour and thereafter washed with distilled water. Approximately 300 mg of the microparticles were placed in the activated dialysis membrane which was tied at both ends and immersed in a beaker placed on a thermostated bath maintained at 37 ± 1 °C. At 5 min intervals, the membrane was removed from each medium, dried

Ofokansi and Kenechukwu

with filter paper and weighed. The degree of swelling was calculated using the formula:

$$H(\%) = \frac{(M_2 - M_1)}{M_1} \times 100$$

Eqn.2

where  $M_1$  = initial weight of microparticles (g), and  
 $M_2$  = final weight of microparticles (g).

### Determination of entrapment efficiency of the microparticles

A quantity (100 mg) of the microparticles was placed in a beaker containing 100 ml of phosphate buffer (pH 7.4). The dispersion was vortexed repeatedly to break up the microparticles and cause them to discharge their contents completely. The solution was then filtered and analyzed spectrophotometrically at a wavelength of 276 nm using a Uv-Vis spectrometer (Jenway 6405). The drug concentration in each batch of the microparticles was calculated from a Beers' plot previously determined for diclofenac sodium. An average of three determinations was taken as the mean drug content for each batch of microparticles. The drug entrapment efficiency was calculated using the following formula:

Encapsulation efficiency (%) =

$$\frac{\text{Drug in microparticles}}{\text{Total drug}} \times 100$$

Eqn. 3

### In vitro drug release studies

The USP XXVII paddle method was adopted in this study [46], which was performed in 500 ml of freshly prepared SIF (pH 7.4) and SGF (pH 1.2) maintained at  $37 \pm 1$  °C. A known quantity (300 mg) of each batch of the microparticles was placed in the appropriate chamber of the release apparatus and agitated at 50 rpm. At predetermined time intervals, 1 ml aliquots of the release medium were withdrawn, appropriately diluted and assayed spectrophotometrically at 276 nm. At every interval, 1 ml of fresh release medium was added to replace the sample that was withdrawn. The concentrations of the withdrawn samples were calculated with reference to the standard Beers' plot. Three replicate release studies were performed in each case and the mean values were taken.

### Statistical and data analyses

Statistical and data analyses were performed using the student's t-test with  $p \leq 0.05$  as the minimal level of significance.

## RESULTS AND DISCUSSION

Diclofenac sodium-loaded microparticles of medium-molecular-weight chitosan were prepared by simple emulsification phase separation technique using glutaraldehyde saturated toluene at different cross-linking times. The results of the percentage recovery of the microparticles are shown in Table 1, and in all cases, the yield was high. There was a good correlation between the cross-linking time and the microparticle yield (as well as percentage yield). As the cross-linking time increased the percentage yield increased resulting in microparticles prepared with a cross-linking time of 1 h having the lowest percentage yield (78.04 %) whereas those produced with a cross-linking time of 10 h gave the highest percentage yield (98.31%).

**Table 1:** Formulation and physical properties of diclofenac sodium -loaded chitosan microparticles.

Batch code	Cross-linking time (h)	Yield %	Loading efficiency <sup>a,b</sup>	Size (µm) <sup>a,c</sup>
GST <sub>1</sub>	1	78.04	66.40 ± 9.07	658.23 ± 8.34
GST <sub>2</sub>	2	89.62	51.94 ± 2.46	602.12 ± 10.69
GST <sub>4</sub>	4	90.03	42.61 ± 1.03	474.36 ± 19.16
GST <sub>6</sub>	6	94.30	32.39 ± 7.90	435.97 ± 7.63
GST <sub>10</sub>	10	98.31	20.87 ± 3.24	324.50 ± 29.82

<sup>a</sup> Mean ± SD, <sup>b</sup> n=3, <sup>c</sup> n=50.

Ofokansi and Kenekchukwu

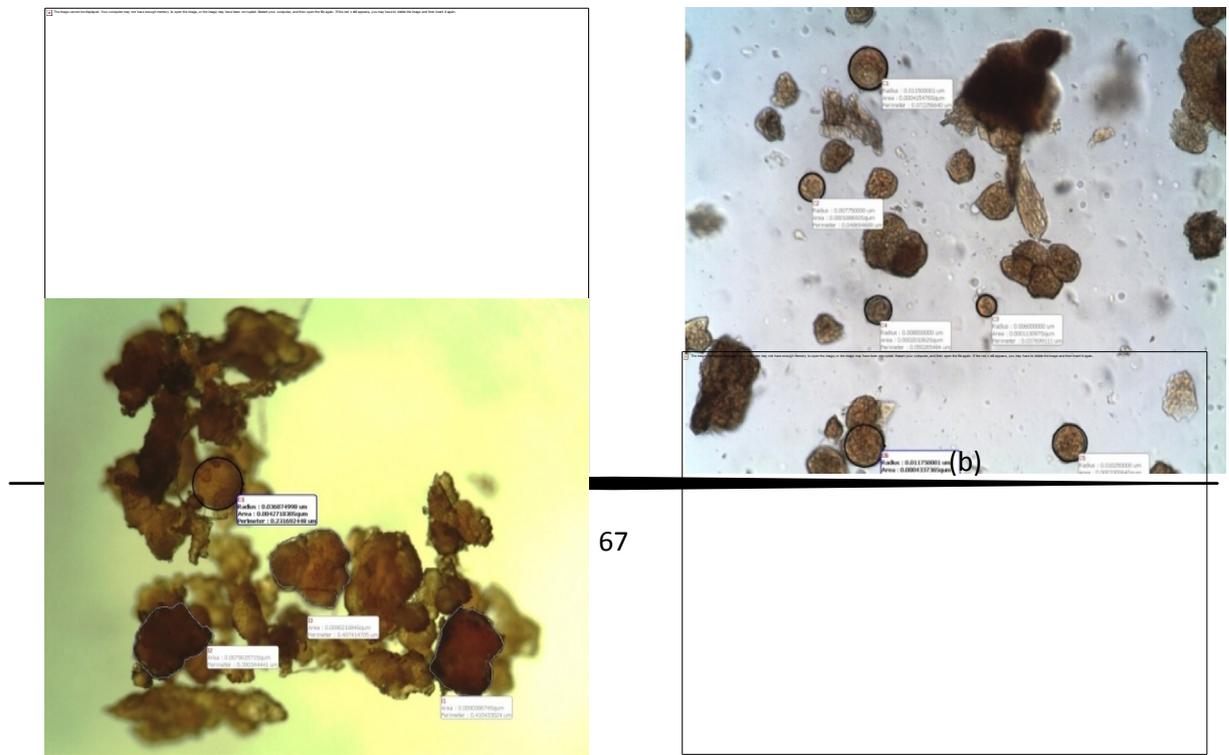
The particle size distribution of the microparticles is also presented in Table 1. The mean particle size (n = 50) of the microparticles ranged from  $324.50 \pm 29.82 \mu\text{m}$  to  $658.23 \pm 8.34 \mu\text{m}$ . Microparticles formulated with a cross-linking time of 10 h had the smallest particles size while microspheres prepared with a crosslinking time of 1 h possessed the largest mean particle size. The photomicrographs of the microparticles are depicted in Fig 1.

Brownish discrete spherical microparticles were obtained at low cross-linking time whereas increase in cross-linking time produced irregular microparticles, which may be attributed to partial hydration of the microspheres. The sizes of the microparticles were all within the micrometer range, indicating that the production process was able to achieve the intended end point. Particle size of microparticles is a very important parameter, since it affects drug release and pharmacokinetics [47]. For microparticles engineered for parenteral administration, large particles will find it difficult to pass through the syringe. However, the microparticles evaluated in this study are intended for oral administration and particle size will influence only the rate of drug release and subsequent pharmacokinetics.

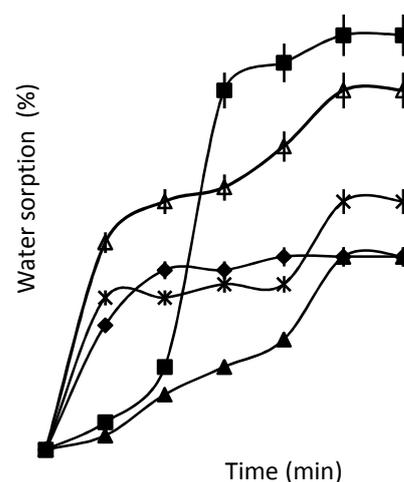
(C)

(e)

1.



In SIF, however, the highest water sorption (28.23 %) was shown by microparticles formulated with a cross-linking time of 10 h and this was closely followed by microparticles cross-linked for 6 h, which recorded 24.23 % water sorption. Water absorption and the rate of water absorption by the microparticles followed the order in the microparticle batches: GST<sub>10</sub>>GST<sub>6</sub>>GST<sub>1</sub>>GST<sub>4</sub>>GST<sub>2</sub> in SIF while the order in SGF is: GST<sub>1</sub>>GST<sub>4</sub>>GST<sub>6</sub>>GST<sub>2</sub>>GST<sub>10</sub>. The water sorption behaviour in the two media may be an indication that the chitosan microparticles had a pH-dependent swelling. Factors that influence swelling of swellable polymers prepared by cross-linking technique include the cross-linking time and the concentration of the cross-linking agent. Since the concentration of the cross-linking agent was kept constant, the prevailing factor was therefore, the cross-linking time. The differences observed in the amount and rate of water absorption in SIF and SGF may be attributable to the cross-linking of the chitosan microparticles. Chitosan is a known naturally occurring cationic polymer [26]. Cross-linked chitosan is relatively stable in acidic medium but rapidly swells and releases its encapsulated drug in an alkaline medium [9, 12, 23, 25, 29, 34, 35, 40, 43, 45]. By implication, encapsulation of diclofenac sodium in GST cross-linked chitosan microparticles can decrease the amount of drug released in the stomach thereby decreasing its corrosive effects on the gastric mucosa and further enhance its targeting to the intestine, where the desired sustained release effects would be achieved. This would be better achieved at a higher cross-linking time. If cross-linking time was lower, hydrolysis would lead to the erosion of the microparticles with the resultant release of the drug at sites unfavourable for its absorption, and the consequent gastrointestinal adverse effects of the drug. In addition, cross-linking through the hydroxyl groups of chitosan led to greater swelling, which favoured drug release. The results show that microparticles formulated with a cross-linking time of 2 h had the lowest water sorption capacity in

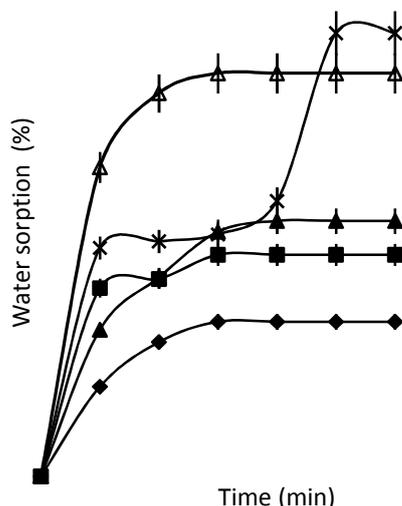


SIF. This may be the optimal cross-linking time required to produce microparticles for sustained release dosage form. Conversely, batch GST<sub>10</sub> containing microparticles cross-linked for 10 h sustained the release of diclofenac sodium to the highest degree.

Table 1 shows that the entrapment efficiency of all batches of the microparticles was in the range of  $20.87 \pm 3.24$  % to  $66.40 \pm 9.07$  % with microparticles cross-linked for 1 h recording the highest drug entrapment. The general pattern was that drug entrapment decreased with increasing cross-linking time. Microparticles formulated with a cross-linking time of 1 h entrapped greater amounts ( $p \leq 0.05$ ) of diclofenac sodium in comparison with those cross-linked for 10 h. The drug entrapment efficiency is an important variable for assessing the drug loading capacity of microparticles and their drug release profiles, thus suggesting the amount of drug that would be available at the absorption site. This parameter is dependent on the process of preparation, physicochemical properties of the drug, and formulation variables [5, 31, 40 – 45]. The drug entrapment efficiency was observed to be dependent on the cross-linking time. Microparticles prepared with a cross-linking time of 1 h had higher mean drug content and thus higher loading efficiency compared to the drug content of microparticles produced with a cross-linking time of 10 h (Table 1). This was the optimum cross-linking time for the chitosan microparticles using the fixed

proportion of the cross-linking agent (GST). In addition, the wide variation in the drug contents of the different batches of the microparticles could be a consequence of the varying degrees of drug sedimentation and the relative partitioning of diclofenac sodium between the dispersed and continuous phases of the emulsion prior to cross-linking of the polymer.

**Figure 2.** Swelling profiles of the microparticles inSGF. —◆— batch GST 10, —■— batch GST 15, —▲— batch GST 20, —x— batch GST 25, —△— batch GST 30



**Figure 3:** Swelling profiles of the microparticles in SIF. —◆— batch GST 10, —■— batch GST 15, —▲— batch GST 20, —x— batch GST 25, —△— batch GST 30

The release profiles of diclofenac sodium from the microparticles in phosphate buffer (pH 7.4) and 0.1 N HCl (pH 1.2) are graphically represented in Figs. 4 and 5. There was a sustained release of the drug from the microparticles. However, drug release was higher in phosphate buffer than in 0.1 N HCl. There

was an initial rapid release of diclofenac sodium from the microparticles within 30 min and this was followed by a much slower release over the next 570 min in all the batches. A characteristic feature of the release profile of diclofenac sodium from the microparticles in phosphate buffer is the biphasic pattern of release. Drug release in phosphate buffer was high and more sustained from microparticles crosslinked for 10 h where up to 92.34 % of the drug was released within 10 h. In 0.1 N HCl, the release of diclofenac sodium from the microparticles was lower than in phosphate buffer. Drug release from the microparticles in phosphate buffer and 0.1 N HCl followed the order: GST<sub>10</sub>>GST<sub>6</sub>>GST<sub>4</sub>>GST<sub>2</sub>>GST<sub>1</sub>. It is discernible from Figs. 4 and 5 that the percentage drug released is highly dependent on the pH of the release media and the cross-linking time. The rapid release of diclofenac sodium from the microparticles was possibly due to a burst effect caused by the leaching out of the untrapped drug adhering to the surface of the microparticles after the initial rapid hydration and swelling. Burst release resulting in biphasic release pattern may be utilized in therapeutic design of dosage forms. This has severally been reported for chitosan-based microparticles [25, 30, 33, 34, 40, 45]. There was a lot of peripheral attachment of the drug as a result of expulsion during microparticle drying (elastic contraction as seen in gels) or drug migration as a result of solvent drag during drying. In this case, however, this may be an advantage because it would lead to a high initial blood concentration of the drug and a gradual release of the remaining drug. In arthritis and chronic pain management, the objective is always to instantly alleviate pain and inflammatory conditions. This is possible if a bolus dose of diclofenac sodium is administered. The bolus dose, when required, would be provided by the initial burst as seen in all the microparticle formulations. All the batches of the formulation also had the tendency to sustain the release of diclofenac sodium. The high and rapid release of diclofenac sodium from the

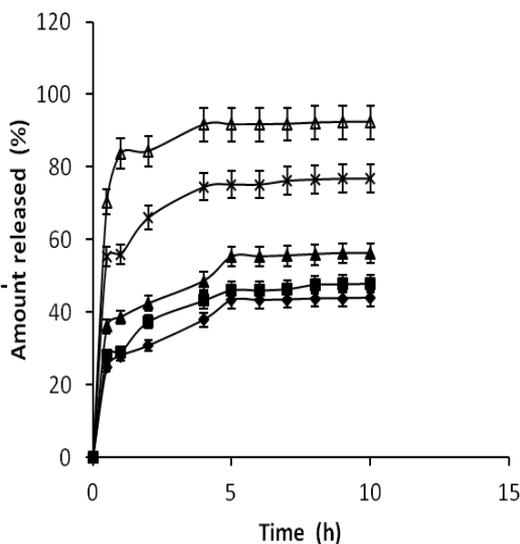
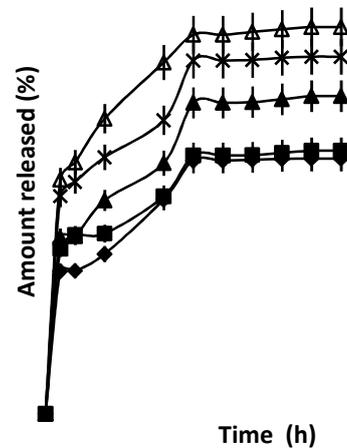
Ofokansi and Kenekchukwu

microparticles, in addition to the burst effect, may also be a result of the high rate of hydration and swelling of the microparticles in the media, which, in turn, could be attributable to the properties of the polymer (chitosan) used in preparing the microparticles. The subsequent slow release phase could be a consequence of the decreasing residual amount of drug in the microparticles and the build-up of drug concentration in the dissolution medium in the course of time. This indicates that once the drug adhering to the microparticles surface has leached, the drug release becomes diffusion-controlled [48]. Moreover, chitosan possesses bioadhesive properties, and crosslinked chitosan microparticles could be used in drug targeting [26]. This is an added advantage since the transit time of the dosage form would be prolonged in the intestine for maximum absorption of the active ingredient in addition to avoiding the adverse effects of the drug in the stomach. This technique has been employed in the delivery of diclofenac sodium [43]. The diclofenac sodium-loaded microparticles showed a controlled release. Once the microparticles are in artificial gastric fluid (pH 1.2), diclofenac sodium is converted to its ionized form, which is very poorly soluble in water. Conversely, when the microparticles were immersed in phosphate buffer (pH 7.4), drug reconversion into the soluble salt form depended on dissolution speed. Further kinetic analysis of the release data was performed on the diclofenac sodium-loaded chitosan microparticles. The criterion was based on a goodness-of-fit test. The result of the different parameters derivable from the Higuchi release model [48] showed that the microparticles obeyed

the Higuchi membrane diffusion-controlled model

better in phosphate buffer than in 0.1 N HCl and thus exhibited diffusion-controlled release characteristics.

**Figure 4.** Release profiles of diclofenac sodium from the microparticles in phosphate buffered saline (pH 7.4). —◆— batch GST 1, —■— batch GST 2, —▲— batch GST 4, —×— batch GST 6, —△— batch GST 10



**Figure 5.** Release profiles of diclofenac sodium from the microparticles in 0.1 N HCl (pH 1.2). —◆— batch GST 1, —■— batch GST 2, —▲— batch GST 4, —×— batch GST 6, —△— batch GST 10

## CONCLUSION

Diclofenac sodium-loaded microparticles were successfully prepared using medium-molecular-weight chitosan by the emulsification solvent evaporation technique using glutaraldehyde saturated toluene as the cross-linking agent. *In vitro* studies undertaken with the microparticles provided a basis to establish that medium-molecular-weight chitosan could be used to control the release of diclofenac sodium and reduce its gastrointestinal side effects and the dosing frequency. Thus, this study has shown that entrapment of diclofenac sodium in medium-molecular-weight chitosan microparticles could be used to improve the oral delivery of the drug and potentially reduce the gastro-erosive adverse effects of the entrapped drug after oral administration. Further studies would seek to evaluate these formulations by employing inflammation models in experimental animals.

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Ofokansi and Kenekukwu

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Ofokansi and Kenechukwu