Original Article

Effect of Ethyl Cellulose Content on Release Profile and Pharmacodynamics of Fenoprofen Microparticles

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ABSTRACT

Introduction: Fenoprofen calcium (FC) is a non-steroidal anti-inflammatory drug and due to its short half-life, high absorption, extensive metabolism and adverse effect, sustained release formulation of FC is desired. **Objective:** this study was to develop sustained release microcapsules of FC to obtain better drug delivery. **Material and Methods:** Different formulae of FC microcapsules were prepared by o/w emulsion solvent evaporation method using Ethyl cellulose (EC) in three different ratios. The processed microcapsules were evaluated *in-vitro* for production yield, entrapment efficiency, micromeritic properties and thermal characteristics and *in-vivo* for its pharmacodynamics. **Results:** *In-vitro* release studies of formulae H1 and H3 showed regression coefficient (r) value in the Higuchi diffusion model of 0.967 and 0.946 respectively, suggesting diffusion mechanism release from these forms. While marketed capsules and H2 microcapsules showed regression coefficient value in the zero-order model (0.985 and 0.985 respectively), suggesting non-linear release. *In-vivo,* the carrageenan-induced hind paw edema was induced in rats; the area under the inhibition of inflammation percentage–time curve ($AUC0-_{24}$) showed that formula H3 possesses the highest therapeutic efficiency comparing with marketed capsules followed by formula H1 and finally H2. Moreover, in hot plate test in mice, there were highly significant increases in the mean reaction time in groups H2 and H3 over the marketed capsule at 12h interval ($p<0.001$). **Conclusion:** FC loaded EC microcapsule H3 produced a sustained and effective drug release over a prolonged timeframe that led to greater therapeutic efficacy.

Key words: Ethylcellulose, Microencapsulation, Fenoprofen calcium, Sustained release, Pharmacodynamics, Microparticles.

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INTRODUCTION

Patients experiencing chronic diseases and its complications are on the rise. These situations necessitate prolonged therapy resulting in non-compliance.[1] This problem tends to be severe for drugs with short biological half –lives like FC as these drugs are taken more frequently. FC is a Non-steroidal anti-inflammatory drug (NSAID) and highly absorbed (85%) on oral administration. It is used for symptomatic relief for some chronic diseases as osteoarthritis, rheumatoid arthritis and for weak to moderate pain. It should be noted that FC is extensively metabolized through first pass effect and its problematic issue is rapid elimination from the plasma (2-3h).[2] Because of its short half-life, extensive metabolism, high absorption and adverse effect, sustained release FC formulation is desired.

For a Drug delivery system (DDS), to be an ideal one, there are two main requirements: First, minimize dosing all over the treatment duration and second, the active ingredient being directly sent to the spot of action, so it minimizes side effects.[3]

Modified release DDS is dosage form where the active drugs are directed to a target at a rate and duration designed to

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accomplish therapeutic and/or convenience objectives. One of the main modified DDS is sustained/extended release dosage form that provides continuous release of their ingredients at a predetermined/prolonged time.[4]

Microencapsulation means utilizing a thin coating to separate core materials. Techniques for incorporating drugs into polymeric microcapsules have acquired substantial interest. This is attributed to their competence to accomplish a sustained / controlled drug release. The function of microcapsules into diversity therapeutic applications had led to the layout of many different microencapsulation methods, each specifically engineered in view of the requirements of each drug's properties and production setup.[5,6] Microencapsulation can be benefited in modifying and retarding drug release and improving its absorption. In pharmaceutical sustained release formulations, the advantage of microcapsules lies in the wide distribution throughout the Gastrointestinal tract (GIT). This potentially reduces side effects interrelated to confined build-up of the irritating drugs against the mucosa of the GIT.[7,8]

Many different microencapsulation techniques and coating materials can be used. The Emulsion solvent evaporation (ESE) process has been utilized effectively in the formulation of drug microcapsules using different biocompatible polymers.[9] EC is a biodegradable, biocompatible, tasteless, odorless, colorless, non-caloric, hydrophobic polymer. The Drug release (DR) from EC depends largely on the pores presented in the hydrophobic microcapsule. Although EC is water insoluble, it can take up water. This is accredited to its hydrogen bonding potential with water.[5] EC is the utmost utilized polymer in the preparation of microcapsules for sustained drug delivery because it's biocompatible, versatile and lower cost.[10] Generally, the single emulsion procedure is most suitable for water-insoluble drugs as steroids, whereas the double emulsion one is considered ideal to encapsulate water-soluble drugs as peptides. The ESE procedure is an economical process that does not prerequisite expensive chemical agents and instruments.[11]

FC conventional oral dosage form can't meet the ideal DDS prerequisites sufficiently. Therefore, this study planned to prepare and evaluate FC microcapsules using EC as coating polymer by O/W ESE process. The formulated FC loaded EC microcapsules have been *in-vitro* dissolution and *in-vivo* assayed.

MATERIALS AND METHODS

Fenoprofen calcium (FC) was received as a gift sample from Western Pharmaceuticals, Al-Obour, Egypt. Ethylcellulose (EC) purchased from Alpha Chemika, Mumbai, India. Ethyl acetate, acetone and ethanol were purchased from El-Nasr for Pharmaceutical Chemicals Co. Egypt. All the chemicals utilized in this investigation were of analytical grade.

Preparation of FC Microcapsules

Microcapsules were formulated based on o/w ESE by using EC as polymer. The different microcapsules were set by dissolving the EC polymer (In different ratios) and dispersing drug in ethyl-acetate and acetone (Oil phase). This solution was poured gradually in 500 ml solution of distilled water (Aqueous phase) containing 0.4 gm tween 80, as emulsifying agent, with nonstop stirring on a magnetic stirrer. The resultant mixture was emulsified 5 hr. Fine droplets immediately formed from the EC polymer and dispersed drug solution. By solvent evaporation these droplets hardened into rigid microcapsules. The microcapsules were collected by filtration, washed by distilled water to eliminate the excess oil and dried in room temperature and characterized.[12]

Determination of the Production Yields

The production yields of FC microcapsules were calculated via dividing the actual yield of microcapsules formulated over the theoretical yield (weight of polymer + drug) and multiplied by 100 according to following equation.[13]

$$\text{Percentage yield} = \frac{(\text{actual yield})}{(\text{weight of polymer} + \text{drug})} \times 100$$

Evaluation of the Entrapment Efficiency (EE)

A quantity of the microcapsules formed equivalent to 5 mg drug was dissolved in ethanol and 0.2 M Phosphate buffer PB (pH 6.8) solution using mortar by pestle and vortex for 5 min. The sample was then filtered with Whattman filter paper to obtain clear solution. The absorbance was measured after suitable dilutions with ethanol and 0.2 M PB (pH 6.8) solution at 272 nm with using ethanol and 0.2 M PB (pH 6.8) solution as a blank. All analysis was done in triplicate, then the average ± SD was calculated using Microsoft office, 2010.[14]

$$\text{Entrapment \%} = \frac{\text{actual content}}{\text{theoretical content}} \times 100$$

Fourier Transforms Infrared Spectrophotometry (FTIR)

The samples of FC powder and EC alone and the formulated microcapsules were ground and utterly mixed with spectral grade potassium bromide (KBr). The KBr discs were ready by compressing the powders. The scanning range was from 4000-400 cm^{-1}. IR spectra were

achieved using an IR spectrophotometer. This was performed in the faculty of pharmacy, Cairo University.

Calorimetric Differential Scanning (DSC)

DCS scans of FC powder and EC alone and the made microcapsules were done by DSC. The thermal behavior was studied by heating nearly 2mg of samples in a covered aluminum pans under nitrogen gas flow (30 ml. min^{-1}) over the temperature range of (0- 400°C) and heating rate of (10°C min^{-1}). This was executed in the faculty of pharmacy, Cairo University.

Densities of Microcapsules

Both bulk density (Db) and tapped density (Dt) were determined. One gram of microcapsules was put in measuring cylinder (10 ml capacity). The initial volume was estimated. The cylinder was tapped until no more difference in volume was observed. The following equations were utilized to calculate Db and Dt.[15]

Db = Wt/bulk volume = Wt /Vb

Dt = Wt /tap volume – Wt /Vt

Hausner ratio

It is the ratio between tapped density and bulk density. It provides an idea about the flow characters of powder particles.[16]

Hausner ratio = Dt/Db

Compressibility Percent (Carr's index)

The bulk density, size and shape, surface area, moisture content and cohesiveness of materials are indirectly measured by compressibility index.[16] The compressibility percent of a material can be estimated as.

Compressibility %= (Dt-Db/Dt) ×100

Angle of Repose

It was measured by passing the solid microcapsules through a fixed height funnel which was kept at a set height in all tests. Both radius (*r*) and height (*h*) of the cone formed were determined. The angle of repose was estimated from the next equation.[16]

Tan θ = h/r

In-vitro Release Study of FC Microcapsules

Dissolution studies were done by USP dissolution, apparatus II (Paddle type). These studies were done using a USP dissolution tester (Rotating paddle apparatus) at 50 rpm. It was performed at 37 ± 0.5°C in 750 mL of 0.1 N HCl (pH 1.2). The duration was 2 h and after that, in PB (pH = 6.8) for a period of 10 h after changing the *p*H from 1.2 to 6.8 via the addition of 250 ml of 0.20M sodium phosphate.[17] Marketed capsules and its equivalent in formulated microcapsules (H1, H2 and H3) were tied at the bottom of the paddle using tea bags. Five-milliliter samples were withdrawn after 0.5, 1, 1.5, 2, 2.5, 4, 6 and 8 h, the volume was preserved in the vessel by means of new dissolution medium. The samples analyzed for FC content were measured for the absorbance at predetermined λ_{max} 272 nm. Five milliliters of PB and ethanol solution (1:1) was put into each of the withdrawn samples then these samples were exposed to spectrophotometric analysis at the predetermined λ_{max} utilizing phosphate buffer and ethanol solution (1:1) as blank. The cumulative percentage which released was calculated.[18]

The experiments were run in triplicates and the mean ±SD was calculated using Microsoft office excel 2010.

Data Analysis[19,20]

Three kinetic models, the zero-order release equation (1), Higuchi equation (2) and first order equation (3), were used to manage the *in vitro* data to discover the equation with the best fit.

Q = k1t (1);

Q = k2 (t)0.5 (2);

Q = 100(1 - e-k3t) (3).

Where Q is the release percentage at time t, the k1, k2 and k3 are the rate constants of zero order, Higuchi and first order model, respectively.

Pharmacodynamics evaluation of FC Microcapsules

A total of 55 Laboratory animals (25 male Wistar rats weighing 150-200 g and 30 male albino mice weighing 25-30 g), were purchased from laboratory animal house (Faculty of veterinary medicine faculty, Zagazig University) and were used for the comparative pharmacological studies.

These animals stayed in approved plastic cages, with metal mesh lids, at a temperature of 22 ± 1°C and were exposed to a 12-h light dark cycle. They had free entrance to food and water and kept for 1 week to get familiar with the laboratory conditions.

All animals were set apart on the tail with a permanent pen for distinguishing proof. The animals were fasted from food for overnight, maintained in room temperature and each type of animals divided into five groups (Control, standard and 3 Tests).

Each animal was treated with the selected formulae that were picked by the total order. The dose given to each animal of standard and tested groups was equivalent to 100mg/kg body weight.[21]

Carrageenan-Induced Hind Paw Edema in Rats for Assessment of Anti-Inflammatory Activity

Rat hind paw edema method assesses the acute anti-inflammatory activity of the formulated microcapsules by injection of an irritant (Phlogistic agent) into the

tissues of the plantar surface of the hind paw of the rat.[22] The experiment was executed using one dose level of the standard and test groups. The inflammation was made in rat paws through the injection of 0.1 ml carrageenan suspension (1% in 0.9% NaCl solution) in the hind paw sub-plantar tissue.

At the beginning of the test, the thickness of the paws (Baseline) of all test subjects was measured by a Vernier caliper. The first group was considered as a negative control group, injected in the right hind paw sub-plantar tissue by 0.1 ml o carrageenan suspension and served as carrageenan negative control group. While the second group, the positive control group received a marketed FC product dose orally. In the test groups, the three microcapsules were orally administered using the same dose of FC given to the standard group. Meanwhile the left paw was set as a reference. The measurement was recorded at 1, 2, 3, 6, 8, 12 and 24 h after the medicated formulations administration.[23] The criteria of comparison were the percent change in paw swelling (% oedema) in contrast to the baseline measurement. Inhibition % of the induced edema was considered as an indicator to compare the anti-inflammatory activity with the negative control:

$$\%\ \text{oedema} = \frac{\text{test paw thickness} - \text{initial paw thickness}}{\text{initial paw thickness}} \times 100$$ [24]

$$\%\ \text{inhibition of inflammation} = \frac{\text{control \% edema} - \text{test \% edema}}{\text{control \% edema}} \times 100$$ [24,25]

Hot Plate Method in Mice for Assessment of Analgesic Activity

The paws of albino mice are very sensitive to heat at a temperature that is not harmful to the skin. The mice respond to heat by jumping, the paws withdrawal and/or the paws licking ("Hot plate method," 1991). The hot plate method was adopted to evaluate the analgesic activity of the formulated microcapsules. This trial was performed using one dose level of the standard (received a marketed FC product) and test groups. A certain amount equivalent to (100 mg/kg of FC) of the prepared microcapsules and marketed capsules as a suspension in 0.1% sodium lauryl sulphate solution was taken orally to the mice. Each mouse was put in two-liter beaker placed over a hot plate thermostatically controlled at 55 ± 0.5°C. When the animals lift and lick their paws or attempt to jump out of the beaker considering the pain threshold is reached. The time taken for the mice to respond in this manner was gotten utilizing a stopwatch and was a reaction time. Recordings were taken after administration at 1, 2, 4, 6, 8 and 12h.[26]

Statistical Analysis

The experimental findings were expressed as mean ± SD. Statistical analysis was done by one-way analysis of variance ANOVA followed by post hoc test. A level for $p<0.05$ was thought to be statistically significant.

Ethics Approval

This work was approved by the Faculty of Pharmacy Ethics Committee – Suez Canal University, Ismailia, Egypt under registration number 201810RA1.

RESULTS AND DISCUSSION

Production Yield and Entrapment Efficiency (EE) of FC Microcapsules

The yield of microcapsules was observed to be in the range of 71.11–87.33 % of total solid substance used during the microcapsules formulation as displayed in the Table 1. The reduction in the drug (FC) to polymer (EC) ratio decreased the production yield of FC microcapsules. This might be because of an amount of the polymer and drug was stuck on the exterior of stirrer and an amount of the microcapsules adhered to the wall of the container during the microcapsules collection from the external phase. This might explain the low yield in a bit of the batches, this was in agreement with Khairnar *et al.* who explained the reason for the low yield in some of the batches might be that during the collection of NTG microspheres from the external phase, some of the precipitated polymer was stuck on the surface of stirrer and some of the microspheres were adhered to the wall of the container.[27]

Regarding the percent of FC encapsulated into the polymer, the results discovered that the quantity of EC polymer employed affected the encapsulation of FC significantly as the active principle to the polymer ratio decreased the percent of drug entrapped increases. Table 1 shows the percent of FC entrapped within the formulated different microcapsules ranged from 82.57% ± 2.35 to 59.69 %± 0.57.

When the polymer amount increased, the EE increased. This is a result of the increase in the organic phase saturation concentration with the viscosity at a lower drug to polymer ratio that aids in maximizing encapsulation with a homogeneous matrix. As polymer concentration increased matrix forming ability or the binding capability of polymer with active principle also increased. This might be accredited to the maximum quantity of medication get captured in polymeric center producing higher encapsulation in recovered microparticles in lower drug to polymer ratio than that in the higher ratio.[28] Maji *et al.*[29] observed that the active constituent entrapment

efficiency (%) of ethyl cellulose microparticles enclosing metformin HCl prepared by solvent-evaporation technique increased with increasing polymer content, when speed of stirring and concentration of the surfactant were constant, as increasing polymer contents facilitated better coating onto the drug particles.

Fourier Transform Infrared Spectroscopy (FTIR)

FC, EC and microcapsules formulated (H1, H2 and H3) were exposed to IR analysis to evaluate whether there is an interaction between the drug and polymer (EC). The characteristic *v* OH stretching, *v* O-C=0 asymmetric and symmetric stretching and *v* O-C-O stretching of pure drug was observed at 3596 cm-1, 1558 cm-1 and 1690 cm-1 (Figure 1A). The characteristic peaks confirmed the structure of FC.[30] The characteristic peaks of FC and EC polymer which included v C-H stretching bands at 2977 cm-1 and 2923 cm-1and v C-H bending at 1379 cm-1 were also present (Figure 1A and B). No significant alteration in the type of peaks in loaded microcapsules denied any strong FC-EC interaction when FC was encapsulated into EC coats by ESE. From this spectral analysis, the OH and carbonyl groups were affected by possible host/guest hydrogen bond formation. In fact, when a carbonyl group and/ or hydroxyl groups were connected to a hydroxylic compound by hydrogen bonds, the stretching band moved to lower frequency due to a weakening of the carbonyl and hydroxyl radical double bond.[31] This appears clearly in the spectrum of formula H1 (Figure 1C) where there are a clear shift and fall in the intensity of the characteristic v-OH stretching of the hydrate. While, with the increase of EC in microcapsules formulated, the characteristic peaks of FC and EC appear clearer in H3 than H2 (Figure 1 D and E) indicating non-significant polymer and drug interaction. Khairnar *et al*.[27] have found that the spectrum of microparticles showed that all characteristic peaks are at the same values as in that of the pure NTG which confirmed the drug. No significant alteration in the type of peaks denied any strong NTG-EC interaction when NTG was encapsulated into EC coats. These findings stated that in different spectra of microcapsules no new peaks appeared which indicate that no chemical bonds were created in the formulated microcapsules.

Thermal Analysis

Calorimetric Differential Scanning (DSC)

DSC was carried out to investigate the effect of formulations on thermal behavior of FC. DSC thermograms of unprocessed FC shows an endothermic peak at approximately 94.42°C corresponding to a loss of water accompanied by collapse of the crystal structure to a glass, which is in good agreement with the thermal behavior of FC reported in the literature at approximately 94°C.[30] On the other hand, a diffuse endotherm occurs between 50°C and 120°C, correspond to EC was observed in thermograms of the prepared FC microcapsules. Moreover, the peaks of FC unprocessed material disappeared in DSC thermograms of H1and, H3 microcapsules, that perhaps due to uniformly dispersed drug at the molecular level in the microcapsules (Figure 2). These results were favorably compared with the findings reported by Gupta *et al*.[32] who stated that

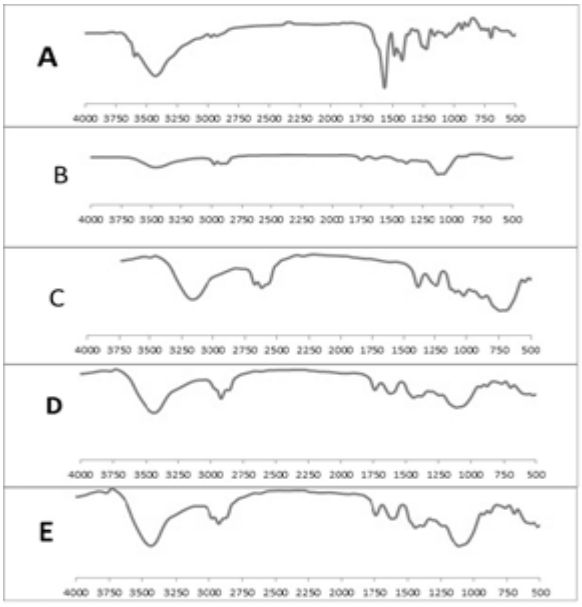

Figure 1: FTIR Spectra of: (A) FC, (B) EC, (C) Formula H1, (D) Formula H2 and (E) Formula H3.

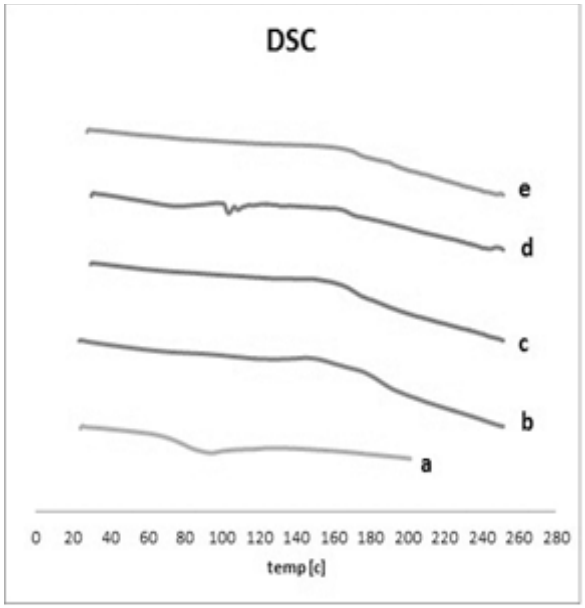

Figure 2: DSC thermograms of: (A) FC, (B) EC, (C) Formula H1, (D) Formula H2 and (E) Formula H3.

DSC profile of captopril exhibited a sharp endothermic peak at 111°C, which matches the melting point of the drug, while DSC profile of captopril loaded ethyl cellulose microcapsules did not exhibit endothermic peak at 111°C.

In another study, Vohra and Patil[33] conducted DSC study on pure drug (Stavudine), empty and drug-loaded microcapsules. Stavudine exhibits a sharp endothermic peak at 172.88°C corresponding to its melting point. The thermogram of microcapsules did not show any drug peak, the authors attributed the absence of drug endothermic peak to uniform dispersion of the drug in the microcapsules.

It was also noted that DSC Thermograms of microspheres H2 showed a slight endotherm at approximately 103.68°C corresponding to the melting endotherm of FC, this may be due to the surface particles of FC on EC microcapsules. This agreed with Dubernet *et al*.[34] who found a slight endothermic peak of ibuprofen in ethyl cellulose loaded microcapsules.

It may be concluded that thermal behavior of FC microcapsules suggested that the drug was compatible and was entrapped in the EC microcapsules.

Micromeritics Properties of FC Microcapsule

Flow characteristics of FC microcapsules were evaluated in terms of angle of repose, tapped density, bulk density and compressibility index. It was found the angles of repose were below 20oC for all microcapsules indicating free flowing nature of microcapsules. Formulation H3 showed the best value, while formula H1 showed the least flowability among the formulations, as displayed in the Table 1. Where the values of the bulk densities of formulated microcapsules ranged from 0.186 g/ml (H3) to 0.240 g/ml (H1). In tapped density, the densities ranged from 0.209 g/ml (H3) to 0.337 g/ml (H1) indicated that the best ratio belonged to H3 which was 1.125, while the worst ratio was 1.404 which belonged to H1. The maximum compressibility percent for the tested FC microcapsules was 28.80 for H1 and the least value was 11.181 for H3. So, from the previous data, the microencapsulation technique employed in this research produced particles with flowability enhancement over the marketed FC, that is considered relatively good flowability. Bansode *et al*.[35] have evaluated the telmisartan microcapsules micromeritic properties in the angle of repose, tapped density, bulk density and compressibility index. It was found the angle of repose in all microcapsules were below 400, indicating free flowing nature of microcapsules.

In-vitro Drug Release

The dissolution studies of marketed FC capsules and FC formulated microcapsules (H1, H2 and H3) were performed in acidic medium (0.1 N HCl, pH 1.2) for 2h and followed by 6 h in PB solution (pH 6.8). FC being a weak acidic drug of pKa 4.5, the pure drug dissolved better in pH6.8 than in pH1.2. The dissolution in pH 6.8 was decreased when EC formulated microcapsules were used. The *in vitro* release profiles of formulation H1, formulation H2 and formulation H3 comparing to marketed FC capsules are displayed in Figure 3. It shows the plot of cumulative percent of drug released as a function of time for different formulations.

The cumulative percentage drug released after 8 h was 77.13%, 51.22%, 30.01% and 40.52% for marketed FC capsules, H1, H2 and H3, respectively, which indicates that the formulated microcapsules produced a prolonged drug release over an extended timeframe. From the *in vitro* drug release profiles, the drug release from microcapsules decreased with the increase in coat material as observed in microcapsules H1 in comparison with microcapsules (H3). As the coat-core ratio increases from 1:1 to1: 2, the *in vitro* drug release decrease, refer to Figure 3. The reason for this could be longer diffusion pathway of the active ingredient in the higher ratio, so the release is retarded. This agrees with Yadav *et al*.[36] who prepared Aceclofenac microcapsules with EC and have concluded that EC slows the drug release from prepared microcapsules.

The regression coefficient (r) values for marketed FC capsules and formulations H1 to H3 are tabulated in the Table 2. The model that gave higher '*r*' value indicated the most suitable model. The regression coefficient '*r*' values were found to be highest in the zero-order model in marketed FC capsules and formulation H2 (0.985273 and 0.984572 respectively) than any other model, this can be entitled to the saturation of elimination system because of the high concentration, while H1 and H3 microcapsules showed highest regression coefficient '*r*' value in the Higuchi diffusion model (0.967984 and 0.945691 respectively), suggesting the drug release by diffusion. The order of release rate observed with all microcapsules was H1>H3>H2. Incorporation of varying concentrations of EC (H1, H2 and H3) controlled drug release. This might be credited to decreased penetration of the solvent molecules in the existence of the hydrophobic polymer, causing lower diffusion of the drug from the matrix as indicated by penetration theory.[37]

Pharmacodynamic Studies of FC Microcapsules

Anti-Inflammatory Activity using Carrageenan-Induced Hind Paw Edema in Rats

The anti-inflammatory effect of formulated microcapsules (H1, H2 and H3) that contain 100 mg/kg equivalent FC was monitored and contrasted with the

Table1: Micromeritics Properties of FC Microcapsules and Commercial FC.							
Formula	**Yield % ±SD**	**Entrapment efficiency ±SD %**	**Angle of repose ±SD**	**Hausne ratio ±SD**	**Compressibility index ±SD**	**Bulk density ±SD**	**Tapped density ±SD**
H1	87.33%±0.12	59.69±0.57	19.97± 0.325	1.404±0.007	28.80±0.402	0.240±0.003	0.337±0.006
H2	74.67%±0.1	61.06±0.38	17.016± 0.144	1.23±0.0035	18.883±0.230	0.209±0.002	0.258±0.003
H3	71.11%±0.2	82.57±2.35	15.73±0.21	1.125±0.001	11.181±0.121	0.186±0.002	0.209±0.002
Market FC	100	100	23.5±0.5	1.480±0.006	32.43±0.305	0.162±0.001	0.240±0.003

Table 2: *In vitro* Release Kinetics Studies of FC Microcapsules and Commercial FC.			
Formulation	Regression coefficient (r) values		
	Zero order	First order	Higuchi
Commercial FC	0.985273	0.940804	0.968214
H1	0.960056	0.805711	0.967984
H2	0.984572	0.711208	0.974779
H3	0.930258	0.768802	0.945691

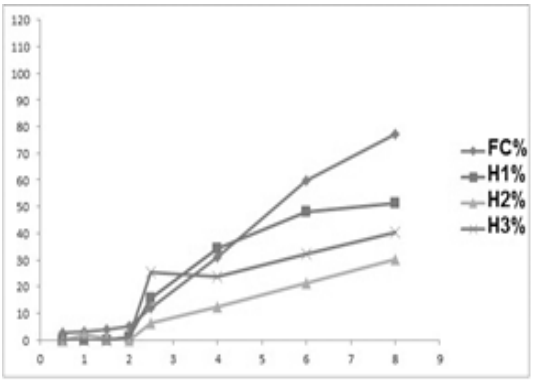

Figure 3: *In vitro* Dissolution Profile of Commercial FC and FC Microcapsules (H1 to H3) in 0.1N HCl (pH 1.2) for 2 h, then in Phosphate Buffer (pH 6.8) for 6h.

marketed FC capsules that served as a positive control. The mean percentage change in rat paw volume (% edema) and the percentage inhibition of inflammation post consuming the all formulae and the marketed FC capsules were valued and the pharmacodynamics parameters as in maximum percentage of inhibition (% Inh_{max}), T_{max} and ($AUC_{0\text{-}24h}$) for the mean value of each tested group were calculated and all these numbers are displayed in Table 3. The percentages of inhibition of inflammation-time profiles of all formulae were quite different.

It was found that marketed FC capsules (Positive control) showed the maximum percent of inhibition after 2h of injection of the inflammatory agent (75.29%) followed by a decline in percentage afterward. However, for H1, H2 and H3, were 68.24%, 38.82% and 40% respectively and followed by a rise in the percent of inhibition afterward. Formula H1 showed the maximum percent of inhibition (71.26%) after 3 h with maintaining a percent of inhibition above 60 % until 6h after the inflammatory agent was introduced. On the other hand, H2 showed the most inhibition at 24 h and its percent of inhibition never exceeded 65%, while H3 showed at 12h a maximum inhibition of edema (80.36%). It was detected a fall in the % of inhibition of carrageenan-induced rat paw edema observed after 6 h of marketed FC capsules post administration, while formulation H3 showed an increase in the percentage of inhibition 6 h after consuming the inflammatory agent.

Moreover, latter formula showed higher percentages of inhibition of the rat paw edema induced by carrageenan after both 12 and 24h. This can be inferred to be because of prolonged release effect of EC microcapsules and in turn, indicates its better anti-inflammatory effect 12h post-administration compared to marketed FC capsules.

Concerning the area under the inhibition of inflammation percentage –time curve ($AUC_{0\text{-}24}$) using the trapezoidal rule as a measurement of therapeutic effectiveness, formula H3 possesses the highest therapeutic effect as compared with marketed FC capsules followed by formula H1 and finally H2. The T_{max} value of formulation H3 showed a higher value at 12h compared to T_{max} value of marketed FC capsules at 2h.

This result points that H3 possesses a slower onset of action and strongest anti-inflammatory effect 12h after administration compared to marketed FC capsules. These finding states that the microcapsule H3 expresses

Table 3: Anti-inflammatory Activity of Commercial FC and the Tested Formula of FC Microcapsules using Carrageenan-Induced Hind Paw Edema in Rats as Percent of Edema Inhibition.

Time (h)	Commercial FC	H1	H2	H3
1	50.51 % ±0.14	50.51%±0.23	13.13 %±o.09	24.24 %±0.21
2	75.29 % ± 0.7	68.24 %±0.7	38.82 %±0.4	40.00 %±0.4
3	58.62 %±0.13	71.26 %±0.12	49.43 %±0.16	33.33 %±0.21
6	40.28 %±0.12	64.17 %±0.16	62.50 %±0.29	52.78 %±0.11
8	20.78 %±0.15	48.05 %±0.27	61.04 %±0.12	70.13 %±0.18
12	16.07 %±0.13	58.93 %±0.32	23.21 %±0.21	80.36 %±0.31
24	10.71 %±0.16	50.00 %±0.55	64.29 %±0.45	50.00 %±0.40
$AUC_{(0\text{-}24h)}$ (%inh)	573.68	1312.02	1055.04	1403.98
Inh. max (%)	75.29%	71.26%	64.29%	80.36%
T_{max} (h)	2	3	24	12

a sustained effect, which can be recognized as EC based microencapsulated DDS.[38]

One-way ANOVA followed by post-hoc test was executed to determine the significant variance between the percentage of inhibition of edema of the tested formulae and marketed FC capsules using Microsoft excel 2010® software. The results revealed a significant variance between the percentage inhibition of edema of prepared formulations (H1, H2 and H3) and marketed FC Capsules at all-time intervals till 12h (p<0.05) and no significance at 24h. This is a result to the release of edema after 24h. In case of marketed FC capsules, there was a significant variation between the percentage change in rat paw volume of the marketed product in contrast with the negative control group from 1h interval till 6h interval (p<0.05).

While there was a highly significant difference between the percentage change in rat paw volume of H1 and H3 compared to the negative control group from 2h interval till 12h interval (p<0.001). Compared to the positive (Marketed FC capsules) group, H1 and H2 showed a highly significant difference in the percentage change in rat paw volume after 6h interval till 12h (p<0.001).

In case of H2 there is a highly significant difference among the percentage change in the volume of rat paw of H2 compared to the control group from 2h interval till 8h interval (p<0.001) and a highly significant difference among the rat paw volume change percent of H2 and with the marketed product group after 6h interval till 8h interval (p<0.001). These results prove that the formulae H1 and H3 provide an anti-inflammatory action that is more effective than the marketed drug and a sustained action for at least 12h.

Hot Plate Test (The Analgesic Effect)

The analgesic activity of FC prepared in form of microcapsules was studied by hot plate method compared with the marketed FC capsules that served as standard. The Mean reaction time (MRT) in mice was calculated and the outcomes are presented in Table 4.

It is observed that, standard group which treated with FC capsules produced the maximum increase in MRT (41 sec) at 6 h. While, the group treated with microcapsule H1 produced the maximum increase in MRT (39 sec) at 4 h, the group treated with microcapsules H2 produced the maximum increase in MRT (38.7 sec) at 8 h and the group treated with microcapsule H3 produced a maximum increase in MRT (44 sec) at 6 h.

The ANOVA analysis concerning the analgesic effect of FC microcapsules and marketed FC capsules using hot plate method was done and showed that marketed FC capsules and all the examined formulations significantly increase MRT compared to negative control (p<0.05). Where marketed FC Capsules significantly increase MRT compared to control from 1h interval till 6h interval then became insignificant after that.

In case of the formulated microcapsules, H1 showed a highly significant increase in MRT in relation to control (p<0.001) from 1h interval till 4h interval as the marketed FC Capsules. The results of MRT in H2 and H3 were significant compared with that of control from 2h interval and lasted till 12 h interval (p<0.05).

Moreover, these two formulae in comparing to marketed FC capsules there was a significant increase in MRT at an 8h interval (p<0.05) and a highly significant rise in mean reaction time at a 12h interval (p< 0.001). These findings confirmed the sustained analgesic action of H2 and formula H3 formulations.

The longer the MRT, the longer the extent of analgesic activity of FC microcapsules (H2 and H3), this may be resulted from the slow and prolonged release of entrapped drug in microcapsules in contrast with the marketed product as the drug release retarded by

Table 4: Analgesic Activity of Commercial FC and the Tested Formula of FC Microcapsules using Hot Plate Test as Mean Reaction Time in sec.

Time	Control	Commercial FC	H1	H2	H3
1	15.7	31.3	32.3	22.8	25.8
2	13.2	40.3	26.3	28.8	29.0
4	15.2	34.5	39.0	30.5	31.3
6	13.7	41.0	33.5	34.3	44.0
8	15.3	35.8	31.0	38.7	38.5
12	14.8	18.8	20.3	33.8	33.7
Mean reaction time$_{max}$	15.7	41	39	38.7	44
T_{max}	1	6	4	8	6

the polymer coat. After carrying out the analgesic test, the studied formulation H2 and formulation H3 have a decent and sustained analgesic effect. After carrying out pharmacodynamic studies, H3 possessed a highest sustained action in both analgesic and anti-inflammatory..

CONCLUSION

Development of FC loaded ethyl cellulose microcapsules represents a promising sustained release drug delivery system that offers prolonged and uniform drug release. The formulation H3 (1:2 drug to polymer ratio) is the most fit one for extended drug delivery among other formulations. The current data proved that formulation H3 provided the best formulation in physical, *in-vitro* and *in-vivo* results. This formula showed best entrapment efficiency, highest followability and no interaction between the EC coat and FC as presented in the FTIR, DSC. In terms *in-vitro* and *in-vivo* evaluation H3 proved an effective and drug release sustained over 12h timeframe suggesting decreasing the frequency of daily dosing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

FC: Fenoprofen calcium; **EC:** Ethyl cellulose; **DDS:** Drug delivery system; **NSAID:** Non-steroidail anti-inflammatory drug; **GIT:** Gastrointestinal tract; **DR:** Drug release; **ESE:** Emulsion solvent evaporation; **KBr:** Potassium Bromide; **FTIR:** Fourier Transmission Infrared; **DSC:** Differential scanning calorimetry; **Db:** Bulk density; **Dt:** Tapped density; **Wt:** Weight; **Vb:** Bulk volume; **Vt:** Tapped volume; **PB:** Phosphate buffer; **USP:** United States Pharmacopeia; **HCl:** Hydrochloric acid; **NaCl:** Sodium chloride; **NTG:** Nateglinide; **EE:** Entrapment efficiency; **MRT:** Mean reaction time; **SD:** Standard deviation.

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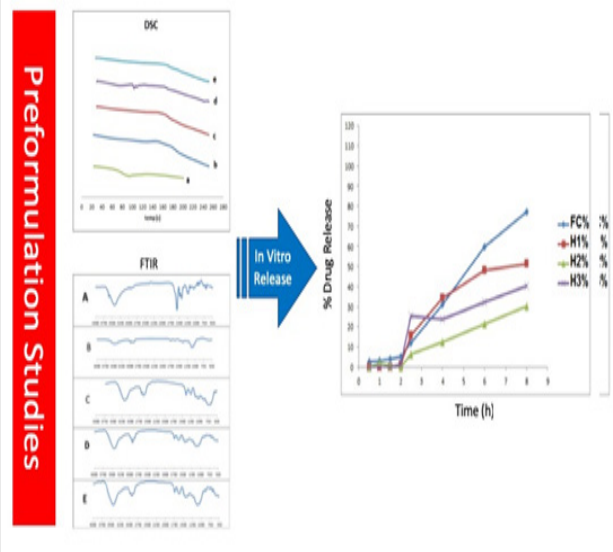
SUMMARY

Fenoprofen Calcium is a non-steroidal anti-inflammatory drug and due to its short half-life, high absorption, extensive metabolism and adverse effect, sustained release formulation of FC is desired. This study was to develop sustained release microcapsules of FC to obtained better drug delivery. The formulated microcapsules have shown a good yield, higher entrapment efficiency with the increase of polymer content, better flow properties than unformulated FC and FTIR and DSC assay results shown no new peaks in IR spectra or DSC thermograms, thus no chemical interaction between the FC and EC. The formulated FC loaded EC microcapsules have been *in vitro* dissolution and *in vivo* assayed. *In vitro* release studies of formulae H1 and H3 showed regression coefficient '*r*' value in the Higuchi diffusion model (0.967 and 0.946 respectively), suggesting the drug release from these forms follow diffusion mechanism, while marketed capsules and H2 microcapsules showed regression coefficient '*r*' value in the zero-order model (0.985 and 0.985 respectively), suggesting non-linear release. *In vivo*, Carrageenan-induced hind paw edema in rats, the area under the inhibition of inflammation percentage–time curve (AUC_{0-24}) showed that formula H3 possesses the highest therapeutic efficiency comparing with marketed capsules followed by formula H1 and finally H2. Moreover, in hot plate test in mice, a highly significant increase in mean reaction time in groups H2 and H3 over marketed capsule at 12h interval ($p<0.001$). The current data proved that formulation H3 (1:2 drug to polymer ratio) provided the best formulation in physical, *in vitro* and *in vivo* results. FC loaded EC microcapsule H3 produced a sustained and effective drug release over a prolonged timeframe that will lead to greater therapeutic efficacy.

PICTORIAL ABSTRACT



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