# ARTICULO ORIGINAL

# Development of a Single Combined Microencapsulated Formulation of Allopurinol and Nimesulide and Investigation of Their Release Behaviours

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## ABSTRACT

The aim of this study was to develop a single combined once-daily sustained release microencapsulated dosage form of Allopurinol and Nimesulide using Ethyl cellulose as release controlling factor and to evaluate drug release parameters as per various release kinetic models. In order to achieve required sustained release profile, microparticles were prepared using coacervation thermal change technique. The formulated microparticles were also characterized by physical and chemical parameters and results were found in acceptable limits. Different dissolution models were applied to drug release data in order to evaluate release mechanisms and kinetics. The drug release data fit well to the Higuchi expression. Drug release mechanism was found as a complex anomalous one.

## INTRODUCTION

Chronic diseases are increasing drastically now a days. This situation requires the use of a number of drugs and for a longer period simultaneously, which causes an increase in patient non-compliance. This problem proves to be serious for drugs with short biological half lives because they must be taken more frequently. To solve such problems one method is to design a dosage form which releases drug gradually. In this regard, microencapsulation has been used as one of the techniques to design a formulation for delivering the drug in a controlled manner [1]. Microencapsulation ia a technique that involves the coating of some active substance by some suitable polymer resulting in fine free flowing tiny particles [2].

Allopurinol (1H-pyrazolo [3,4-d]pyrimidin-4-ol) is a commonly used drug in the treatment of chronic gout or hyperuricaemia associated with leukaemia, radiotherapy, anti-neoplastic agents and treatment with diuretics conditions [1]. Allopurinol is a structural isomer of hypoxanthine (a naturally occurring purine in the body) and acts to inhibit xanthine oxidase In the presence of xanthine oxidase, Allopurinol will be converted to Allopurinolxanthine,

after that the formation of uric acid from xanthine and hypoxanthine will be inhibited. This enzyme is responsible for the successive oxidation of hypoxanthine and xanthine resulting in the production of uric acid, the product of human purine metabolism [2-4]. Direct use of this drug can have several side effects on the skin or digestive system and which appear in the form of fevering, shivering and vomiting [5]. The microencapsulation of this drug can reduce any side effects largely.

Nimesulide is described chemically as *N*-(4-nitro-2-phenoxyphenyl) methane sulphonamide classified as a nonsteroidal anti-inflammatory drug (NSAID). It is a unique nonsteroidal anti-inflammatory (NSAID) agent having specific affinity to inhibit cyclooxygenase-2 enzyme [6]. It is effective in reducing pain associated with osteoarthritis, rheumatoid, and other degenerative joints disorders, low back pain, dysmenorrhoea, gynaecological condition, thrombophlebitis, dental pain and inflammations etc. [7,8]. It a slightly acidic and because of this character it causes G.I irritation, so microencapsulation technique can be used to prevent GI irritation.

These two drugs should be prescribed in combination in gout as an uricosoric agent (Allopurinol) and an NSAID (Nimesulide). Due to above mentioned side effects of Allopurinol and Nimesulide, they were formulated into a single stable oral dosage form to increase the patient compliance. Both drugs were microencapsulated separately into ethylcellulose, evaluated for any chemical interaction, determined their entrapment efficiency of specified amount of microparticles and filled in a hard gelatin capsule shell of appropriate size after mixing drug release study.

## 1. MATERIAL AND METHODS

### 1.1 Material

Allopurinol active as a kind gift GSK Pvt. (Ltd.) Karachi Pakistan, Nimesulide active as a gift from Pharm-Evo Pharma Pvt. (Ltd) Karachi, Pakistan, Ethyl cellulose from Sigma (USA). The chemicals such as cyclohexane, liquid paraffin (heavy and light) and n-hexane of analytical grade supplied by Merck (Germany).

### 1.2 Preparation of microparticles

Coacervation thermal change technique was selected for this study keeping in mind the physico-chemical properties of both drugs e.g. melting points of Allopurinol and nimesulide are 300 °C and 145 °C, respectively. A weighed amount (1 g) of ethyl cellulose 22 cp as a polymer was dissolved in specific amount of cyclohexane (30 ml) as a solvent by heating to 70-80 °C with vigorous stirring with the help of magnetic stirrer at 150 RPM. In this solution, weighed amount (1 g) of drug was dispersed. Vigorous stirring and high temperature was maintained throughout the process. The temperature was then slowly reduced using cold water bath to induce phase separation. The product obtained was washed thrice with n-hexane (100 ml) at room temperature, air-dried following oven drying at 45 °C

and passed through sieve no. 80 to separate individual microcapsules [9]. After determination of entrapment efficiency, they were filled in to capsules shell of appropriate size (000) for in-vitro release study so that each capsule should contain 300 mg Allopurinol and 200 mg Nimesulide.

### 1.3 *In vitro* drug release of microparticles

In vitro drug release of various microparticles filled capsules was determined using automatic apparatus I USP (Rotating basket, Pharma Test, Germany) in 900 ml of phosphate buffer (pH 6.8). 5 ml of sample was collected at 0; 0.25; 0.5; 1.0; 1.5; 2.0; 3.0; 4.0; 6.0; 8.0 and 10 hours with an automated fraction collector after filtering through 10 μm Sinter filters. All samples were analyzed as such at 404 nm for Nimesulide and at 250 nm for Allopurinol using a UV-spectrophotometer (Shimadzu 1601, Japan). Percentage drug release at different sampling intervals was calculated.

### 1.4 Determination of drug loading, encapsulation efficiency and microparticles yield

For the determination of amount of Allopurinol in the microcapsules, a known amount of microcapsules is taken and polymer solvent (Methylene chloride) is added. In this way, the wall polymer was dissolved and drug sediment. The solution was filtered, what is left on the filter paper was separated, and again polymer solvent was added. This is done several times and what is left on the filter paper is the amount of the drug. It is weighed anti the drug for a known amount of microcapsule material is calculated.

Average Nimesulide content was measured by extracting a sample of 20 mg of microparticles using methanol. After filtration and appropriate dilution, the concentration was determined using the spectrophotometer (Shamidzo Japan 1601). Percentage drug loading was calculated using the following equation:

Loading (%) = (Weight of drug/Weight of microparticle) x 100

The encapsulation efficiency was determined by the following equation:

Encapsulation efficiency (%) = (Calculated drug content/Theoretical drug content) x 100

The yield % of the produced microparticles was calculated for each batch by dividing the weight of microparticles (M) by the total expected weight of drug and polymer ($M_0$):

Yield % = (M/$M_0$) x 100

Each determination was performed in triplicate [9].

### 1.5 Particle size analysis

Particle size was determined by sieve method. The mean particle size was calculated

after sieving as follows [10];

$$d_{ave} = \sum nd / \sum n$$

Where $d_{ave}$ is the arithmetic mean diameter of microparticles, 'n' is percentage weight fraction retained on smaller sieve and 'd' is the arithmetic mean size of sieve opening.

## 1.6 Flow properties

Angle of repose of different formulations was measured according to the fixed funnel standing cone method and was given by [9]:

$$\theta = \tan^{-1} hr^{-1}$$

Where $\theta$ is the repose angle, r is the radius and h is the height.

Bulk density was measured by tapping method [9]. Kawakita equation was used to calculate the packing rate (b) according to the following equation [9]:

$$n/c = (1/ab) + (n/ab)$$

$$C = (Vo-Vn)/ Vo$$

Where a and b are constants representing the proportion of consolidation at the closest packing attained and packing rate, respectively, n is the number of taps, Vo and Vn are the powder bed volumes at initial and tapped state, respectively.

Compressibility index (Ci) or carr's index values of microparticles was computed according to the following equation [9]:

$$Carr\% = [(tapped\ density - fluff\ density) / tapped\ density] \times 100$$

Hausner ratios of microparticles were determined by comparing the tapped density to the fluff density using the equation [9].

$$Hausner's\ ratio = tapped\ density / fluff\ density$$

### 1.7 Kinetic treatment of release data

The obtained dissolution data were fitted to zero order, first order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell to determine the mechanism of drugs release from the prepared microparticles [9].

### 1.8 X-ray diffraction studies

The physical nature of the drug substances in microparticles was determined using the

X-ray diffraction method (Philips PW 1830). Powdered samples of Nimesulide, Allopurinol, ethylcellulose and their microparticles were examined for comparison.

### 1.9 FTIR spectroscopy studies

FTIR spectra of the drug substances, polymer, and microparticles were obtained using a (model FTIR-8400 S Shimadzu, Japan) FTIR spectrophotometer.

## 2 RESULTS AND DISCUSSION

The coacervation thermal change technique was applied to prepare Nimesulide and Allopurinol microparticles. Cyclohexane (at high temperature) was used as a solvent for ethyl cellulose, whereas Nimesulide and Allopurinol are cyclohexane insoluble drugs. Ethylcellulose was used as a wall-forming material because of its safety, stability, hydrophobicity and compact film forming nature among water insoluble polymers [11].

The microparticles of Allopurinol were white, free flowing and spherical whereas the microparticles of Allopurinol were light yellow, free flowing and spherical as observed under scanning electron microscope (Figure 1). The mean particle size varied between 126-137 µm for different formulations of Allopurinol-Nimesulide-Ethylcellulose microparticles. The entrapment efficiency (Table 1) of the microparticles was 93% and 94% for Allopurinol and Nimesulide, respectively. The rest of micromeritic characteristics are given in table 1. Weight variation of capsules was with in the limits.

**Table 1**: Characterization of microparticles

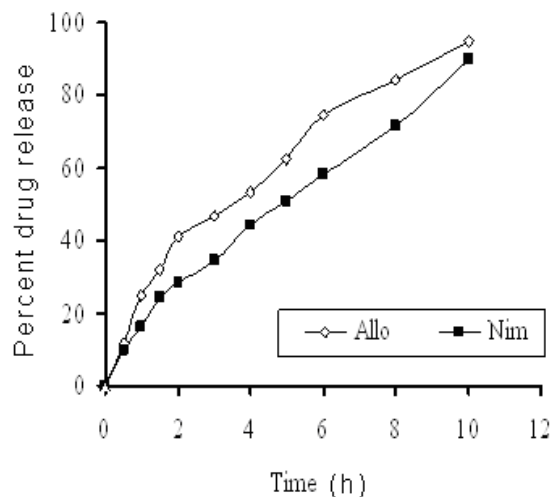| Formulations | Allopurinol | Nimesulide |
|---|---|---|
| Bulk density (g/ml) | 0.173 | 0.263 |
| Taped density (g/ml) | 0.203 | 0.302 |
| Hausner's ratio | 1.176 | 1.15 |
| Compressibility index (%) | 15 | 13 |
| Angle of repose | 22.47$^{\circ}$ | 21.67$^{\circ}$ |
| Packing rate (C) | 0.15 | 0.13 |
| Yield (%) | 96 | 96 |
| Entrapment efficiency (%) | 93 | 94 |
| Particle size (µm) | 137 | 126 |

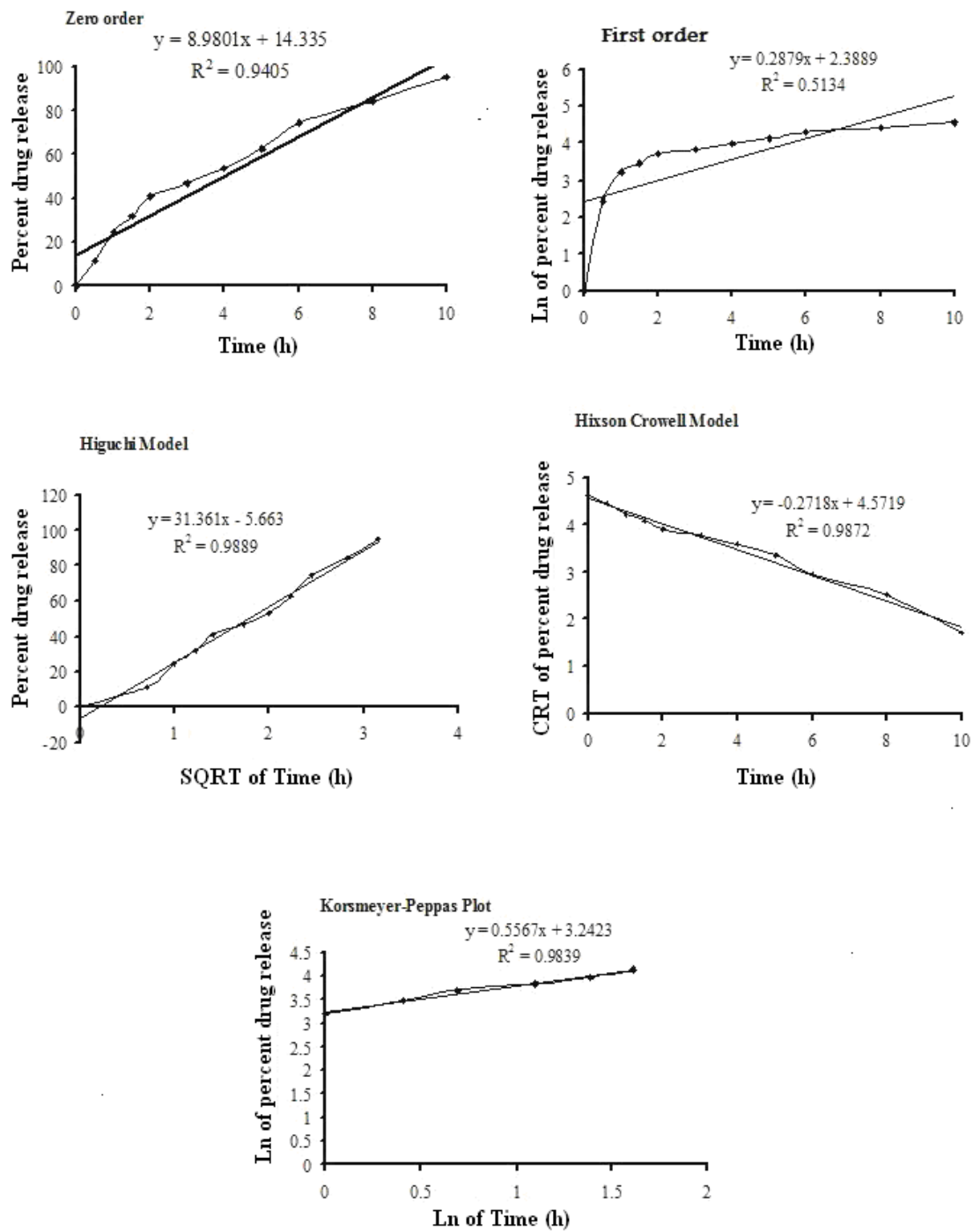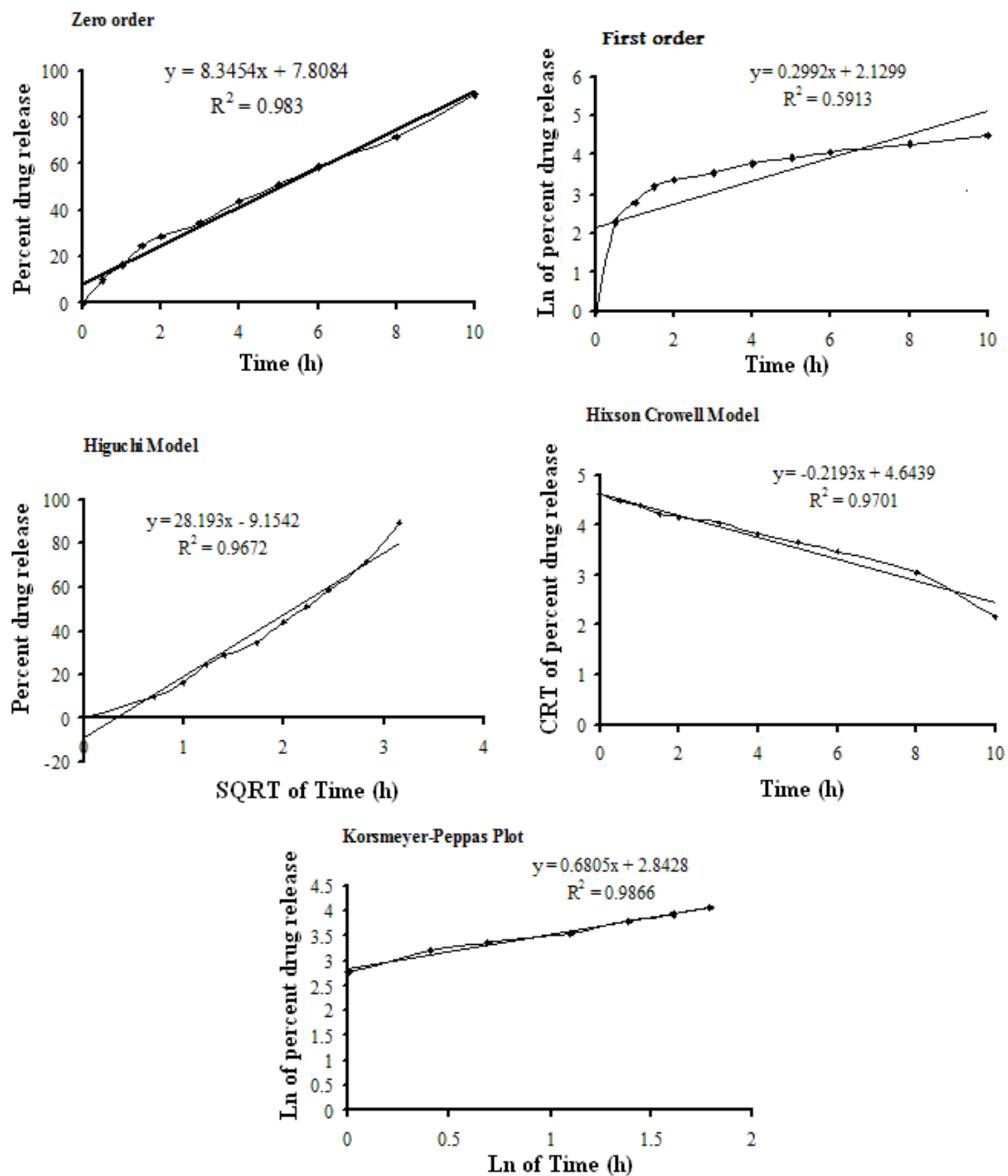**Figure 1:** Scanning electron microscope of microparticles
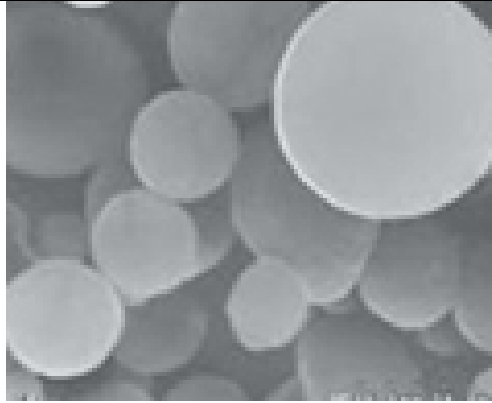
### 2.1 Drug release kinetics

As shown in Figure 2, 3 & 4, various plots are drawn in accordance with various release kinetic models, are giving linear relationship. In Zero order plot (Figure 2), the $R^2$ value is 0.9405, 0.983 and first order (Figure 3) giving 0.5134 and 0.5913 (Table 2) for Allopurinol and Nimesulide respectively describing the relationship between drug release rate and concentration of drug and we can see it that drug release is independent of drug concentration. The best fitting was found in Higuchi's model (Table 2) ($R^2 = 0.9889$, $R^2 = 9672$ for Allopurinol and Nimesulide, respectively) indicating the release of drug from microparticles as a square root of time dependent process based on Fickian diffusion.

**Figure 2:** Dissolution profile of Allopurinol and Nimesulide in microparticles

**Figure 3:** Plots of different kinetic models for allopurinol

**Zero order**

$y = 8.9801x + 14.335$
$R^2 = 0.9405$

**First order**

$y = 0.2879x + 2.3889$
$R^2 = 0.5134$

**Higuchi Model**

$y = 31.361x - 5.663$
$R^2 = 0.9889$

**Hixson Crowell Model**

$y = -0.2718x + 4.5719$
$R^2 = 0.9872$

**Korsmeyer-Peppas Plot**

$y = 0.5567x + 3.2423$
$R^2 = 0.9839$

**Figure 4**: Plots of different kinetic models for nimesulide



The dissolution release data was also plotted according to Hixson Crowell cube root model (Table 2). Applicability of data ($R^2 = 0.9872$, $R^2 = 0.9701$ for Allopurinol and Nimesulide, respectively) shows a change in surface area and diameter of microparticles with the progressive dissolution of as a function of time.

**Table 2:** Goodness of Fit (using $R^2$) for mathematical models used for determining drug release rate

| Models | Parameters | Microparticles | |
|---|---|---|---|
| | | Allopurinol | Nimesulide |
| Zero Order | Y-equation | 8.980x + 14.335 | 8.345x + 7.8084 |
| | $R^2$ | 0.9405 | 0.983 |
| First Order | Y-equation | 0.288x + 2.388 | 0.299x + 2.129 |
| | $R^2$ | 0.5134 | 0.5913 |
| Higuchi | Y-equation | 31.361x - 5.663 | 28.193x - 9.1542 |
| | $R^2$ | 0.9889 | 0.9672 |
| Hixson-Crowell | Y-equation | -0.272x + 4.572 | -0.219x + 4.644 |
| | $R^2$ | 0.9872 | 0.9701 |
| Korsmeyer-peppa | Y-equation | 0.557x + 3.242 | 0.681x + 2.843 |
| | $r^2$ | 0.9839 | 0.9866 |
| | n (release exponent) | 0.55 | 0.68 |

## 2.2 Mechanism of drug release

By incorporating the first 60% of dissolution release data, mechanism of release can be indicated according to Korsmeyer where "n" is the release exponent, indicative of mechanism of drug release. Fickian diffusional release and a case-II relaxational release are the limits of this phenomenon. Fickian diffusional release occurs due to the molecular diffusion of the drug because of chemical potential gradient. Case-II relaxational release occurs due to stresses and state-transition in hydrophilic glassy polymers which swell in water or biological fluids. This term also includes polymer disentanglement and erosion [12]. The value of the release exponent 'n' in Allopurinol and Nimesulide sustained release microparticles obtained as 0.55 and 0.68 (for Allopurinol and Nimesulide respectively) shows anomalous or non-Fickian diffusion.

And from the XRD and FTIR studies (Figure 5 and 6) it is clear that the slight changes may be due to polymorphism of the drug and not due to strong interaction between the drug and polymer which confirms the stability of formulation.

**Figure 5.** XRD of A (active nimesulide), B (active allopurinol), C (ethylcellulose), D (microparticles of nimesulide), E (microparticles of allopurinol).
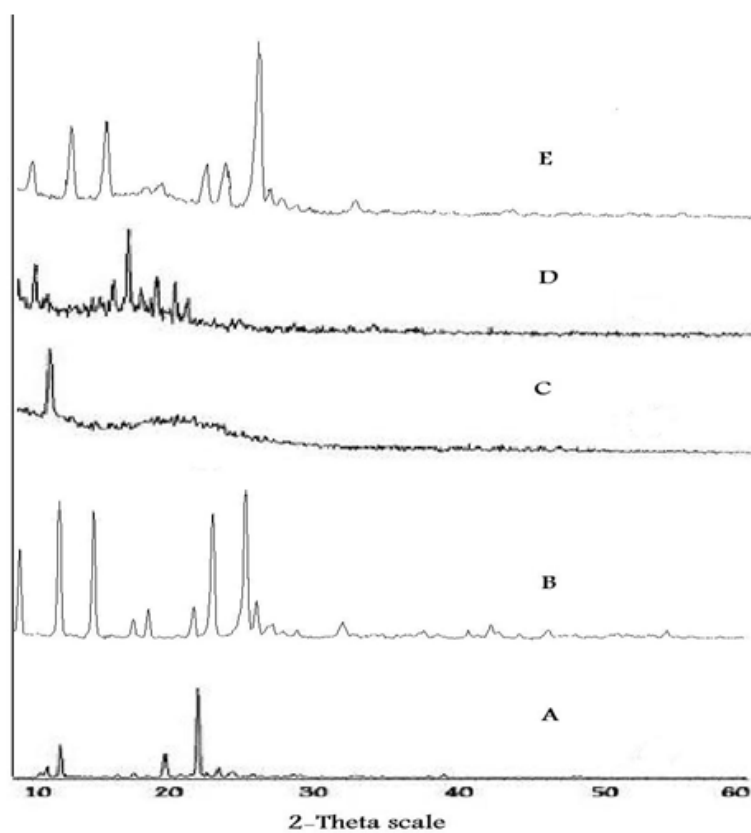


**Figure 6.** FTIR of A (active nimesulide), B (active allopurinol), C (ethylcellulose), D (microparticles of nimesulide), E (microparticles of allopurinol).
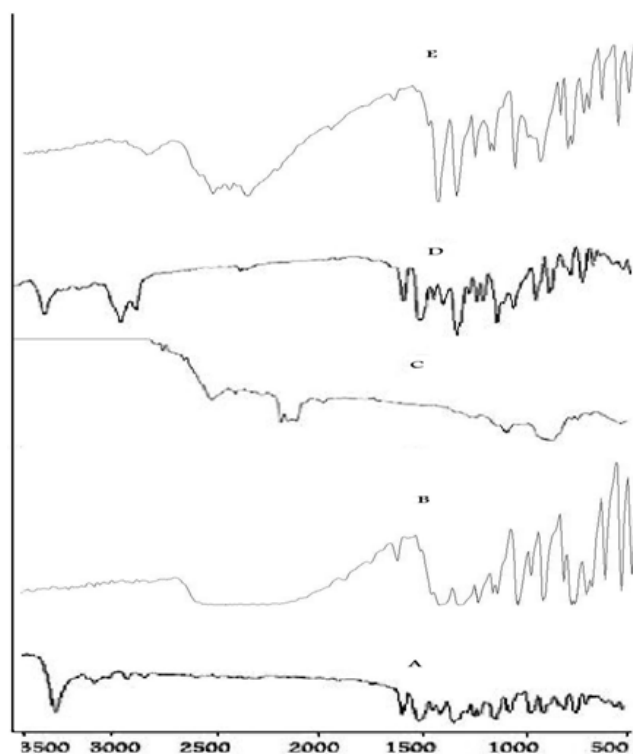
## 3. CONCLUSION

Allopurinol-Nimesulide sustained release microencapsulated single dosage form was prepared successfully using ethylcellulose as polymer to retard release and achieve required dissolution profile. Drug release kinetics of this formulation correspond best to Higuchi's model and drug release mechanism as per "n" value of Korsmeyer & Peppas (Power law) was found to be anomalous diffusion.

## ACKNOWLEDGEMENT

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