# Formulation and Evaluation allopurinol microencapsulation by double emulsion solvent diffusion method

**Sisode Sagar Rajendra[1], Shivangni Rathore[2], Dr. Revathi A. Gupta[3]**

1PG Scholar, Institute of Pharmacy, Dr. A.P. J. Abdul Kalam University, Indore.

2Associate Professor, Institute of Pharmacy, Dr. A.P. J. Abdul Kalam University, Indore.

3Professor and Principal, Institute of Pharmacy, Dr. A.P. J. Abdul Kalam University, Indore.

## ABSTRACT
The objective of the present study was to develop This study reports the microcapsulation of Allopurinol by the double emulsion solvent diffusion method and the release of the drug from the microcapsules. This sustained release of microcapsules is additionally influenced by the formulation of latest biodegradable polymer Ethyl cellulose, hence drug release pattern. the ready microcapsule were subjected to numerous pre and post formulation studies. Prepared microcapsule were evaluated for the particalsize ,percentage yield, entrapment efficiency,estimatin of drug content and in vitro drug release studies. Result of the present study indicate that allopurinol microcapsule can be successfully designed to develop sustained drug delivery , that reduces the dosing frequency and their by we can increase the patient compliance.

**Keyword** : Allopurinol, Ethyl Cellulose , Microcapsules, double emulsion solvent diffusion.

## I. INTRODUCTION
Microencapsulation is a useful method which, prolongs the duration of the drug effect significantly and improves patient compliance. Eventually the total dose and few adverse reactions may be reduce since a steady plasma concentration is maintained.[1]

Allopurinol is used to treat Gout andTumor lysis syndrome,Inflammatory bowel disease. Its work its active metabolite, oxypurinol, inhibits the enzyme xanthine oxidase, blocking the conversion of the oxypurines hypoxanthine and xanthine to uric acid. Elevated concentrations of oxypurine and oxypurine inhibition of xanthine oxidase through negative feedback results in a decrease in the concentrations of uric acid in the serum and urine.

Microencapsulation ids difined the application of a thin coating to individual core materials that have an arbitararypartical size range between 5 and 5000 um. Microencapsulation is wiedlyused in the pharmaceutical and other sciences to mask tasted or ordors,prolong release , impart stability to drug molecules, improve bioavailabiliy ,and as multi particulate dosage form to produce controlled or targeted drug delivery. It is therefore, a rapidly expanding technology for achieving sustained –release  dosage form.[1]

Double-emulsion droplets have found widespread applications in various engineering and biomedical fields because of their capability in encapsulating different components in each layer. The conventional double-emulsion method is the two-stage stirring emulsification method, which suffers from poor monodispersity and low encapsulation efficiency. With recent advances in micro fabrication, some novel methods for fabricating double-emulsion droplets have been developed, including micro fluidic emulsification (double-T-junction micro channel, double-cross-shaped micro channel and several three-dimensional micro channels), membrane emulsification and coaxial electro spraying.[2]

## Materials And Method
**Materials:** Allopurinol was obtained as a gift sample from Piramal healthcare, limited,Pithampur,Dhar (M.P).Ethylcellulose,SdFine-chem. Acetronitril, SdFine-chem. Dichlomethane, HiMedia Laboratories. Span 80 SdFine-chem.FromSdFine-chem. limited (Mumbai).

## II. EXPERIMENTALS
### 2.1 Identification of drug

### 2.1.1 By UV Spectroscopy

For the determination of λ max, Stock solution of drug was prepared by dissolving 100mg of drug in 0.1M HCL and make up the volume to 100ml (conc.1000 μg/ml). 10ml of stock solution was diluted to 100ml of 0.1M HCL and then 10ml of this solution was diluted to 100ml with 0.1M HCL. The resulting solution was examined in the range of 230nm to 360nmby UV-visible spectrophotometer[3].The resulting solution was showed maximum absorptions shown in figure 5.1.

### 2.1.2 By melting point determination

Melting point of Allopurinol was determined by using open capillary method. The capillary was filled with small amount of drug powder and and it was placed along with thermometer in melting point apparatus. The temperature was noted using thermometer[4]. The average of three values was considered as the melting point of drug as shown in table 5.3.

### 2.1.3 By Fourier transform infrared spectroscopy analysis

Samples of 1-2 mg of drug alone, each excipient alone, physical mixtures of allopurinol with the investigated excipients (1:1, w/w) prepared by physical and perfect mixing and solid dispersion were scanned from 4,000-400 cm-1. The spectrophotometer was of shimadzu[5].The results suggested that there was no interaction of between allopurinol and excipients. The IR Spectra of pure drug, carrier and formulations are shown in figure 5.2,5.3,5.4,5.5.

### 2.2 Preparation of standard Calibration curve of allopurinol

Standard calibration curve of allopuinol was prepared by taking accurately weighed 100ml of allopurinol and dissolved in 100 ml of 0.1 N sodium hydroxide then make up the volume upto1000 ml with 0.1 N sodium hydroxide. Then 1,2,3,4,5,6,7,8,9, and 10 ml of these solution was taken in 10ml volumetric flask and make up the volume with 0.1 N sodium hydroxide upto 10ml. The dilutions were analysed by UV spectrophotometer at 258nm and absorbance was noted[6]. The standard curve was plotted with absorbance values against drug concentration as shown in figure 5.6.

### 2.3 Solubility study

Drug was added to 10ml of different solvents. The solutions were sonicated for 1 hr at room temperature.The solutions obtained were filtered through a filter paper and the filtrate was diluted with distilled water[7]. The diluted solutions were measured spectrophotometrically at a λmax of 258 nm using the same medium as a blank and the resulting solubility is shown in the table 5.4.

### 2.4 Method of preparation micropencapsulation

The drug The drug Allopurinol and the polymer, ehtylcellulosel were mixed and weighed amounts of ethylcellulose and drug were dissolved in mixture of acetonitrile and dichloromethane. The initial w/o emulsion ws formed by adding of deionised water to the drug –polymer solution with constant stirring at 500 rpm for 5min. then slowly added to light liquid paraffin containing span 80 as a surfactant with constant stirring for 2hrs.the n-hexane was added and the stirring was further for 1hrs [8]

**Composition of optimized microcapsule system**

| Formulation Code | Drug (in mg) | Ethylcellulose (in gm) | Acetrinitril (ml) | dichloromethane (in ml) |
|---|---|---|---|---|
| F1 | 500 | 2 | 10 | 10 |
| F2 | 1000 | 2 | 10 | 10 |
| F3 | 1.2 | 2 | 10 | 10 |

| F4 | 1.3 | 2 | 10 | 10 |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |

## III. EVALUATION OF MICROENCAPSULATION

**3.1 Partical size analysis:** Determination of average particle size of the allopurinol microcapsules was carried out by the optical microscopy methods. A minute quantity of microcapsules were dispersed in liquid paraffin and then spread on clean glass slide and average size if microcapsules were determinedin each batch.

**3.1. Bulk characterisation of microencapsulation.**
Bulk characterisations of liquisolid system were estimated by Bulk density, Tapped density,
Carr's index, and Hausner's ratio. The flow property was determined by Angle of repose.
These properties were determined by using the following equations [8,9]

Bulk Density = Mass (g)/ bulk volume
Tapped density= Mass (g)/tapped volume
Carr's index= Tapped density- bulk density/ tapped density X 100
Hausner's ratio= tapped density/ bulk density
Angle of repose= Tan0=h/r.
The bulk characterisation and flow properties of microcapsules are shown in table

**3.2. Determination of saturation solubility of microencapsulation**
Solubility study was performed according to method reported by Higuchi and Connors. Theliquisolid compact system F1,F2,F3,F4 were added in 10 ml distilled water taken instoppered conical flask and were shaken for 24 hrs at 370C 1 in orbital shaker. Twomlaliquots were withdrawn at 1 hr intervals and filtered through whatman filter paper. Thefiltered solution were analysed spectrophotometrically at nm against blank. Thesaturation solubility of liquisolid system is shown in fig 4.

**3.3. Drug content of microencapsulation**
An amount equivalent to 10 mg of allopurinol was weighed from each resultant microencapsule and in 50 mL 0.1 N sodium hydroxide using a 100 mL volumetric flask and then was stirred for 10 min. The volume obtained was completed to 100 mL with 0.1 N sodium hydroxide and shaken well. 2ml from the previous solution were taken and were completed to 10ml with 0.1N sodium hydroxide[11]. The absorbance was measured using a UV spectrophotometer at 258nm ,using 0.1N sodium hydroxide as a blank.The drug content ofvarious formulations are shown in table 6.1.

**3.4. Fourier transform infrared (FTIR) spectroscopy**
The characteristic peak attributable to various functional groups present in the molecule of drug was assigned to establish the identity of drug . The results suggested that there was no interaction of between allopurinol and excipients. The IR Spectra of spure drug, carrier and formulations are shown in figure 5.2, 5.3, 5.4, 5.5.

**3.4. Scanning electron microscope (SEM) studies;**
Sem photographs were taken for tha pectin microcapsulation prepared by double emulsion solvent diffusion technique and are depicted in .the sample was allopurinol on an ethylcellulose study using double adhesive carbon tape. microcapsules.were coated using poloron e5100 sem ,coating system .scanning was done using JEOL JSM 5600 electron microscopy ltd,Cambridge;uk. The micrographs were recorded at ht 15k v accelerating voltage using leo 435vp[12]

A. **In vitro dissolution of allopurinol tablet from microcapsule**
B. The rotating basket dissolution apparatus was used for the determination of dissolution rates of allopurinol microcapsule. An accurately weighed amount of each solid dispersion equivalent to 100 mg of allopurinol tablet was placed into the basket of the dissolution test apparatus. The basket was rotated at 50 rpm in 900 mL of the dissolution medium (0.1 N HCl) and maintained at a constant temperature (37 ± 0.5°C). Each of 5 mL, were withdrawn from the dissolution medium at time intervals of 5, 15, 30, 45 and 60. The same volume of 0.1 N HCl was used to replace the withdrawn samples. The samples were suitably filtered, diluted, and measured spectrophotometrically

at 258 nm.Thein vitro release of various          formulations are shown in figure 6.2, 6.3.

# IV.    RESULT AND DISCUSSION

4.1. UV Spectroscopy
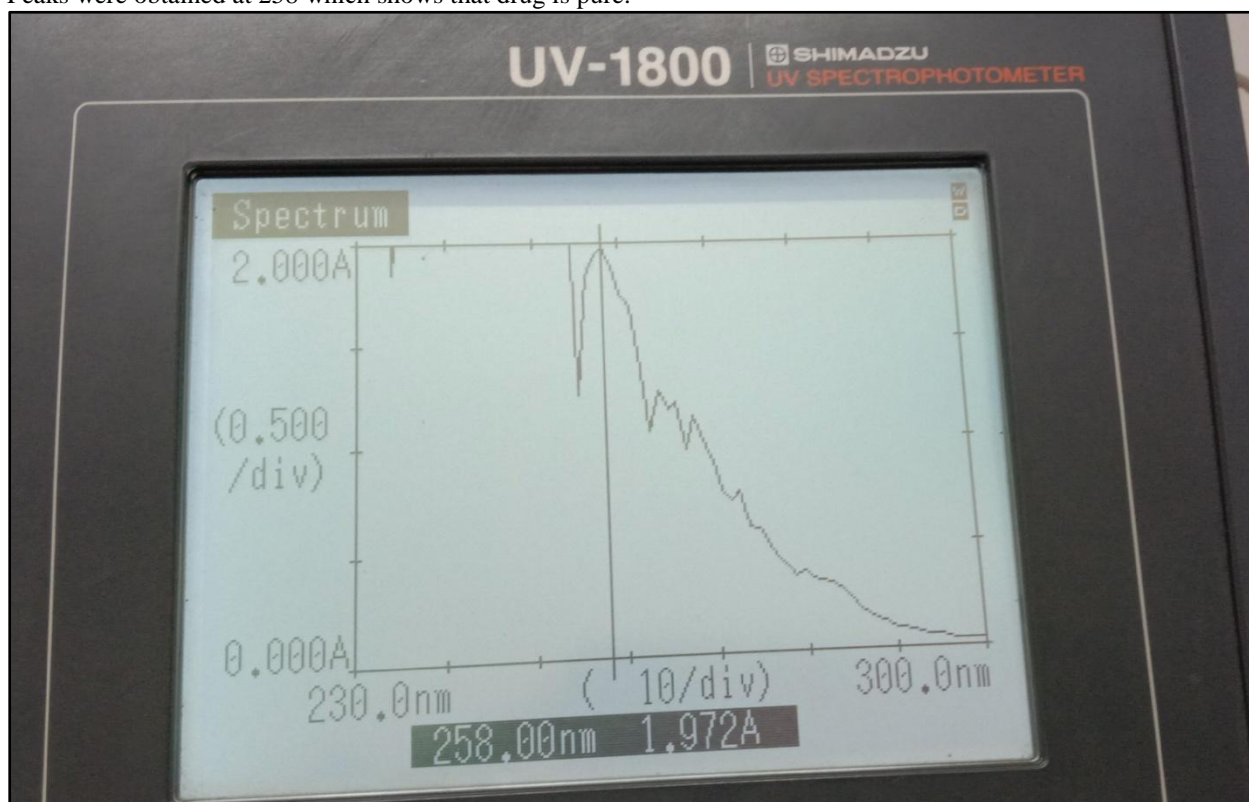Peaks were obtained at 258 which shows that drug is pure.



Fig.1. Lamda max of allopurinol

## A.    Melting point determination

The melting point of drug sample was determined by using melting point apparatus. The melting point was found between the range of  344-355°C.

**Table 5.3 Melting Point**

| S.No. | Melting Point | Average |
|-------|---------------|---------|
| 1. | 344-355℃ | 344℃-355℃ |
| 2. | 340-352℃ | |
| 3. | 348-360℃ | |

**4.3. Standard curve of allopurinol**          Standard calibration curve of allopuinol was prepared by taking accurately weighed 100ml

of allopurinol and dissolved in 100 ml of 0.1 N sodium hydroxide then make up the volume upto1000 ml with 0.1 N sodium hydroxide. Then 1,2,3,4,5,6,7,8,9, and 10 ml of these solution was taken in 10ml volumetric flask and make up the volume with 0.1 N sodium hydroxide upto 10ml.

The dilutions were analysed by UV spectrophotometer at 250nm and absorbance was noted. The standard curve was plotted with absorbance values against drug concentration as shown in figure 5.6.

## Calibration Graph

$y = 0.080x + 0.071$
$R^2 = 0.998$
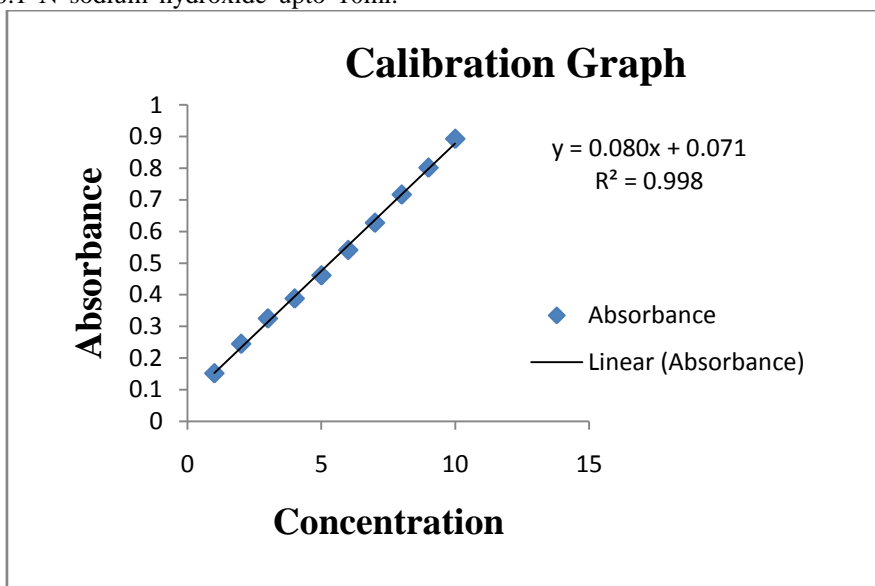
**Calibration curve of Allopurinol in 0.1N NaOH**

### 4.4. Solubilty studies

Drug was added to 10ml of different solvents. The solutions were sonicated for 1 hr at room temperature.The solutions obtained were filtered through a filter paper and the filtrate was diluted with distilled water. The diluted solutions were measured spectrophotometrically at a λmax of 250 nm using the same medium as a blank and the resulting solubility is shown in the table 5.4.

| S.No. | Solvents | Solubility mg/ml |
|-------|------------|------------------|
| 1 | Methanol | 0.612 |
| 2 | Chloroform | 0.750 |
| 3 | Octanol | 0.375 |
| 4 | Ethanol | 0.575 |
| 5 | Water | 0.316 |
| 6 | NaOH | 0.911 |

### 4.5. Bulk characterization and flow properties of liquisolid compact systems
The flow ability of the formulation can be shown in the Table no.4

| Formulations | Bulk density(g/cm3) | Tapped density(g/cm3 | Hausner's ratio | Carr's ratio% | Angle of repose |
|---|---|---|---|---|---|
| F1 | 0.34 | 0.58 | 1.70 | 41.37 | $26.4^0$ |
| F2 | 0.51 | 0.71 | 1.39 | 28.16 | $30.06^0$ |
| F3 | 0.70 | 0.95 | 1.35 | 26.31 | $25.4^0$ |
| F4 | 0.33 | 0.55 | 1.66 | 40.0 | $26.95^0$ |

**Determination of saturation solubility**

Solubility study was performed according to method reported by Higuchi and Connors. Toevaluate the increase in solubility of allopurinol in solid dispersion F1,F2,F3,F4 were added 10 ml distilled water taken in stoppered conical flask and were shaken for 8 hrs at37°C in incubator shaker. And solution were kept for 24 hrs, after shaker to achieveequilibrium, two ml aliquots were withdrawn at 1 hr intervals and filtered through whatmanfilter paper. The filtered were solution analysed spectrophotometrically at 258 nm against blank.
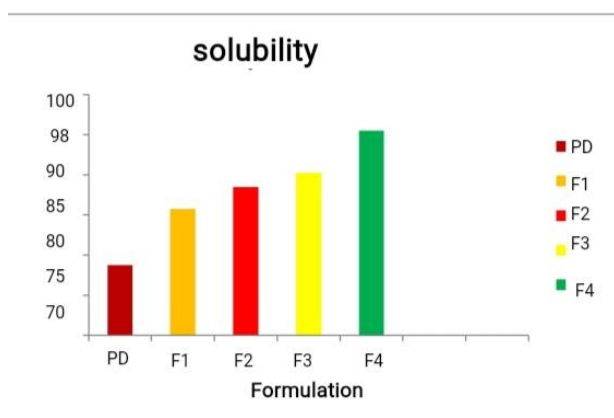


**A.      Drug content microencapsule**

The drug content estimation was performed to ensure uniform distribution of drug. The drugcontent of solid dispersion of Allopurinol was performed for all the prepared formulations.The result indicates that the drug content in all the formulations was found uniform between 87% to 96% which was analysed spectrophotometrically at λmax 250nm. The drug content ofvarious formulations are shown in table 6.1

**Table 6.6Drug content of various formulation**

| FormulationCode | %DrugContent |
|---|---|
| F1 | 93.09% |
| F2 | 95.20% |
| F3 | 97.30% |
| F4 | 99.60% |

The drug content of all the formulations were found in the range 93.09% to 99.60%. These values are within the acceptable range

**Fourier transforms infrared spectroscopy (FTIR)**

The characteristic peak attributable to various functional groups present in the molecule of drug was assigned to establish the identity of drug . The results suggested that there was no interaction of between allopurinol and excipients. The IR Spectra of spure drug, carrier and formulations are shown in figure 5.2, 5.3, 5.4, 5.5.


**5 FTIR of Allopurinol**


**5.1 FTIR of ethycellulose**
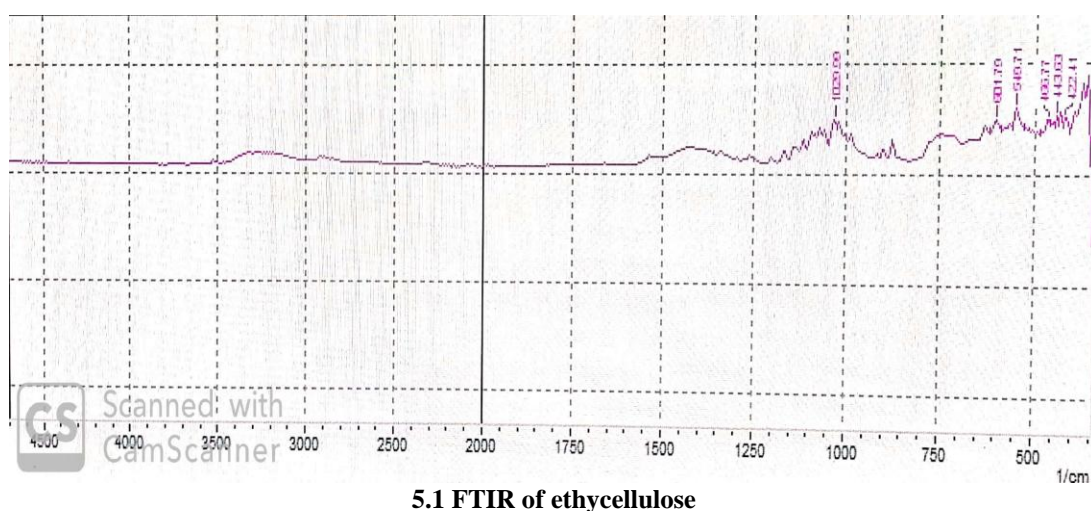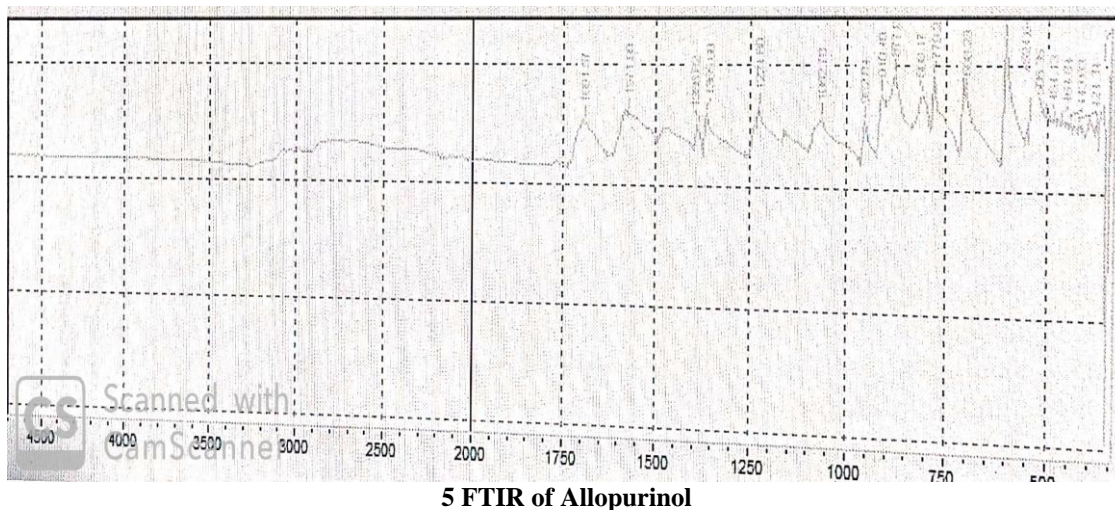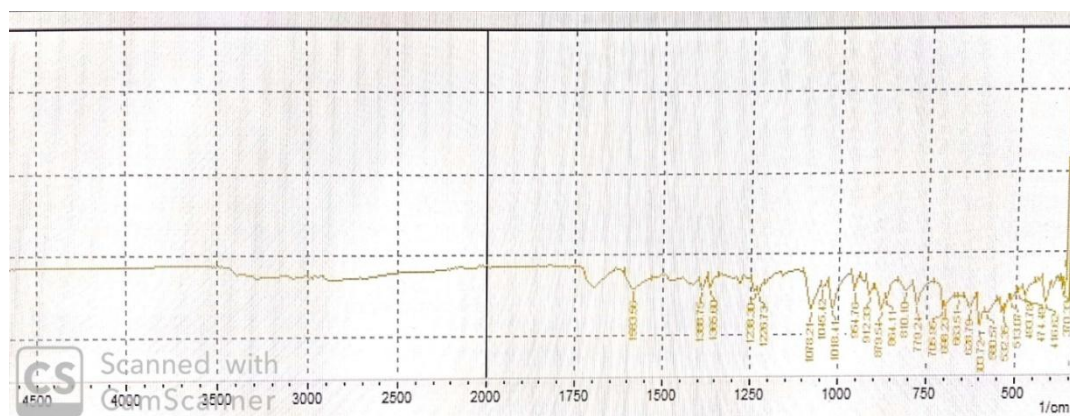
**FTIR of Formulation 3 (Allopurinol + ethylcellulose**

**Scanning electron microscope (SEM) studies;**

Sem photographs were taken for tha pectin microcapsulation prepared by double emulsion solvent diffusion technique and are depicted in .the sample was allopurinol on an ethylcellulose study using double adhesive carbon tape. microcapsules.were coated using poloron e5100 sem ,coating system .scanning was done using JEOL JSM 5600 electron microscopy ltd,Cambridge;uk. The micrographs were recorded at ht 15k v accelerating voltage using leo 435vp

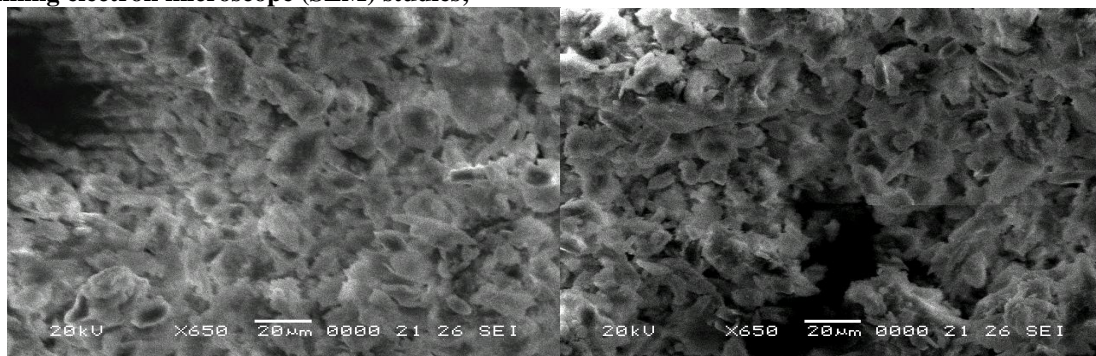**Scanning electron microscope (SEM) studies;**



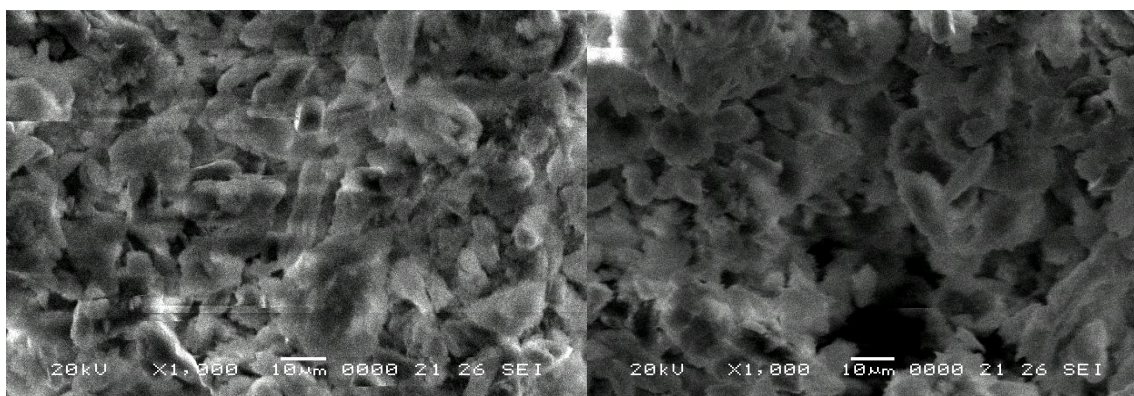**FIG 1.5 Scanning electron microscopy formulation F1 , F2**



**FIG 1.6 Scanning electron microscopy formulation F3,F4**

The in vitro drug release profile of pure drug Allopurinol, solid dispersion in dissolution medium are shown in figure (8.1, 8.2).microcapsule of Allopurinol showed a significant increase in the drug release as compared with marketed tablet Allopurinol. In the formulations F1 and F2 showing 96.18% and 95.6% drug release, F3 and F4 showing 89.43%and 97.31% drug release, and marketed tablet showing 95.66% and drug release respectively. All the formulation showed improved drug release rate as compared to pure Allopurinol. The in vitro release of various formulations are shown in figure 6.2, 6.3

The rotating basket dissolution apparatus was used for the determination of dissolution rates of allopurinol microcapsule. An accurately weighed amount of each solid dispersion equivalent to 100 mg of allopurinol tablet was placed into the basket of the dissolution test apparatus. The basket was rotated at 50 rpm in 900 mL of the dissolution medium (0.1 N HCl) and maintained at a constant temperature (37 ± 0.5°C). Each of 5 mL, were withdrawn from the dissolution medium at time intervals of 5, 15, 30, 45 and 60. The same volume of 0.1 N HCl was used to replace the withdrawn samples. The samples were suitably filtered, diluted, and measured spectrophotometrically at 258 nm.Thein vitro release of various formulations are shown in figure 6.2, 6.

### C. In vitro dissolution of allopurinol tablet from microcapsule
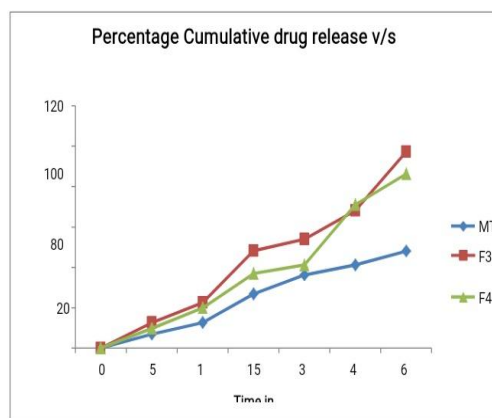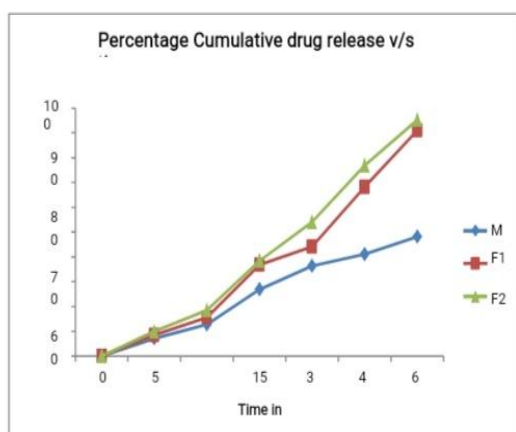


**2 Comparison of drug release profile of market preparation Allopurinol & F1,F2,F3 Batch**

**Partical size analysis**

| size of range in micrometer | Mean of size range (d) in micrometer | No.of partical in each size range (n) | nd | $nd^2$ | $nd^3$ | $nd^4$ |
|---|---|---|---|---|---|---|
| 0.05-0.10 | 0.1 | 55 | 6.5 | 4.95 | 1.485 | 0.445 |
| 0.10-0.15 | 0.125 | 30 | 4.375 | 0.5468 | 0.06835 | 0.008543 |
| 0.15-0.20 | 0.175 | 5 | 0.875 | 0.1531 | 0.02579 | 0.004688 |
| 0.20-0.25 | 0.225 | 5 | 1.125 | 0.2531 | 0.05694 | 0.00128 |

| | | $\sum n = 95$ | $\sum nd = 20.675$ um | $\sum nd = 3.903$ um | $\sum nd^3 = 4.26629$ um | $\sum nd^4 = 0.371042$ um |
|---|---|---|---|---|---|---|

**Equation**

$$\sum nd^2 \div \sum nd = 3.903 \div 20.675 = 0.1869 \text{ um}$$
$$\sum nd^3 \div \sum nd = 3.96629 \div 20.675 = 0.191839 \text{ um}$$
$$\sum nd^4 \div \sum nd = 0.371042 \div 20.675 = 0.194908 \text{ um}$$

Particle size analysis was perform ad calculate this equation for each formulation the minimum = **0.191839 um** maximum **0.194908 um** and average **0.1869um** of the particle size was calculated by using the sample equation of prepared microcapsule was randomly selected and their size increased compare by drug particle size was determined using an optical microscope.

**Acknowledgement**

# V. CONCLUSION

- Formulation of microcapsule of allopurinol for the prolonged release of drug.
- Formulation F2 and F3 were found to be best among all other formulation.
- It was used in improve the solubility.
- It was used for controlled and targeted release.
- It was also used for masking of taste or odors
- Allopurinol can be used as an Gout, Tumor lysis syndrome,Inflammatory bowel disease.

# REFERENCES

[1]. Venkatesan P. and Manavalan R., "Microencapsulation: A Vital Technique In Noval Drug Delivery System",Journal of Pharmaceutical Sciences and Reserch,Vol.1,Issue 4,2009,26-35

[2]. Bansode S.S.,Banarjee S.K.,G Gaikwad D. D. and Jadhav S .L., "Microencapsulation", International Journal of Pharmaceutical Sciences Review and Research,Vol.1,Issue 2, Mar-Apr. 2010,38-43

[3]. Hamid M.,Qazi H.J.,Waseen S.and zhong F., "Microencapsulation Can Be a Novel Tool in Wheat Flour with Micronutriwnts Fortification :Current Trends and Future Application",Czech Journal Food Sciences , Vol. 31,2013,527-540.

[4]. Mishra D.K., Jain A.K. and Jain P.K.,Various Techniques of Microencapsulation , International Journal of Pharmaceutical and Chemical Sciences, Vol.2,Issue2,Apr-jun 2013,962-977.

[5]. Tiwari S., Goel A., Jha K. K. and Sharma A., "Microencapsulation Techniques and Its Applicaton",The Pharma Research,A Journal Vol.3,2010,112,116.

[6]. Dubey R. and Rao K. U. Bhasker, "Microencapsulation Technology and Applications", Defense Science Journal, Vol. 59, Issue 1, Jan. 2009, 82-95.

[7]. Mutiniprasanna P., "Microencapsulation", International Journal of Pharma and Bio

[8]. Sciences, Vol. 3, Issue 2, Jan.-Mar. 2012, 509.

[9]. Umer H. and Nigam H., "Microencapsulation: Process, Techniques and Application's , Journal of Research in Pharmaceutical and Biomedical Sciences, Vol. 2, Issue 2, Apr.-Jun. 2011, 474-481

[10]. Naga M. and Banji D. "Microencapsulation", International Journal of Pharmaceutica1 sciences Review and Research, Volume 5, Issue 2, Nov.-Dec.2010, 58-62.

[11]. Lachman L, Liberman H.A., Kanig, l. 1990, The theory and practice of industrial pharmacy, 3rd edn, Varghese publishing house, Mumbai,430-453.

[12]. Mathiowiz, E., 2009, Encyclopedia of controlled drug delivery, vol-2,john wiley & sons. Delhi, 493-512.

[13]. Belghumwar, V. S., Kawatikwar, P. S, Phutane, G. P.,& Belghumwar A. V. Design & evaluation of

[14]. Microcapsules containing ciprofloxacin hydrochloride for sustained release, Indian Drugs,2008, 45 ( 7 ), 553-557.

[15]. Omi, S., Katami, K., Taguchi, T., Kaneko, K., Iso, M.: Synthesis of uniform PMMA microspheres employing modified SPG(Shirasu porous glass) emulsification

technique. J. Appl. Polym.Sci. 57, 1013–1024 (1995)

[16]. Sun, G., Zhang, Z.: Mechanical strength of microcapsules made of different wall

[17]. Su, J., Ren, L., Wang, L.: Preparation and mechanical properties of thermal energy storage microcapsules. Colloid Polym. Sci.284, 224–228 (2005)

[18]. Arfsten, J., Bradtmoller, C., Kampen, I., Kwade,A.Compressive testing of single yeast cells in liquid environment using a nanoindentation system. J. Mater. Res. 23, 3153–3160 (2008)

[19]. Rahman, A., Dickinson, M., Farid, M.: Microindentation of microencapsulated phase change materials. Adv. Mater. Res. 275,85–88 (2011)

[20]. Singh, J. and Robinson, D.H., Drug Develop. Ind. Pharm., 1988, 14, 545.
Saravanam, G., Bhasker, K., Srinivasa, R.G. and Dhanaraju, M.D., J. Microencapsulation, 2003, 20, 289.
Mandol, T.K., Shekleton, M., Onyebueke, E., Washington, L. and Penson, T., J. Microencapsulation, 1996, 13, 545.

[21]. Mandol, T.K. and Tenjarla, S., Int. J. Pharm., 1996, 137, 187

[22]. Lutton, J.D., Mathew, A., Levere, R.D. and Abraham, N.G., Amer. Indian J. Pharm. Sci., 2007, 69 (2): 244-250

[23]. Mandol, T.K. and Tenjarla, S., Int. J. Pharm., 1996, 137, 187.

[24]. Lee, J.H., Park, T.G. and Choi, H.K., Int. J. Pharm., 2000, 196, 75.

[25]. Hayton, W.L. and Chen, T., J. Pharm. Sci., 1982, 71, 820.

[26]. Pavan S. Narkhede*, Arvind R. Umarkar, IJPBS |Volume 1| Issue 3 |2011|372-376

[27]. Lachman L, Liberman H.A., Kanig, l. 1990, The theory and practice of industrial pharmacy, 3rd edn, Varghese publishing house, Mumbai,430-453.

materials. Int. J. Pharm. 242, 307–311 (2002)

[28]. Mathiowiz, E., 2009, Encyclopedia of controlled drug delivery,vol-2,john wiley & sons. Delhi, 493-512.

[29]. Belghumwar, V. S., Kawatikwar, P. S, Phutane, G.Belghumwar A. V. Design & evaluation of microcapsules containing ciprofloxacin hydrochloride for sustained release, Indian Drugs,2008, 45 ( 7 ), 553-557.