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Formulation, Characterisation and Evaluation of Sustained Release Microcapsules of Gemifloxacin

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Abstract: The aim of the present study is to develop, optimize, characterize and evaluate the gemifloxacin microcapsules prepared using cellulose acetate phthalate (CAP) and hydroxyl propyl methyl celluose (HPMC) as polymers. In the present study the sustained release gemifloxacin microcapsule formulations were successfully prepared by o/o emulsification-solvent evaporation technique using different concentrations of polymer and span 60 as emulsifying agent. Six formulations F1 to F6 were prepared and were evaluated for particle size, drug content and subjected for in-vitro dissolution studies, based on the drug release data, the formulation having higher drug release that is F6 was selected as the optimized formulation. It was observed that particle size of the microcapsules decreased with the increasing concentration of emulsifying agent and decreasing concentration of polymer. Entrapment efficiency and dissolution rate also increased with sustained release of drug. The optimised formulation F6 was characterized for particle size analysis by optical microscopy, surface morphology by scanning electron microscopy(SEM), drug excipients compatabilty by fourier transform infra-red spectrophometer(FTIR), and physical change of drug by differential scanning calorimeter(DSC). The percentage yield and entrapment efficiency of all the formulations was also determined. The surface of the optimized formulation was smooth, spherical and wavy. DSC studies indicated that the drug changed its physical form in the presence of combination of polymers. The drug release of optimized formulation is higher compared with the drug release of pure drug. The data obtained from the dissolution profiles were subjected for different release kinetics models and regression coefficients. The drug release profile follows Higuchi order release kinetics. It was found that gemifloxacin microcapsules sustained the release of gemifloxacin. All results are reported.
Keywords: Microcapsules, Gemifloxacin, CAP, HPMC, Span 60, Emulsification-solvent evaporation, sustained release.

Introduction

Sustained and novel delivery envisages optimized drug in the sense that the therapeutic efficacy of a drug is optimized, which also implies nil or minimum side effects. It is expected that the 21st century would witness great changes in the area of drug delivery. The products may be more potent as well as safer. Target specific dosage delivery is likely to overcome much of the criticism of conventional dosage forms. The cumulative outcome could be summarized as optimized drug delivery that encompasses greater potency and greater effectiveness, lesser side effects and toxicity, better stability, low cost hence greater accessibility, ease of administration and best patient compliance (Jain N K., 2001)[1]. The efficacy of a drug in a specific application requires the maintenance of appropriate drug blood level concentration during a prolonged period of time. However the conventional administration of drugs, gives a poor control of the concentration of these

substances in plasma because of variations in the concentration of the bioactive product, once a specific dose has been administered. The conventional dosage systems can give rise to alternative periods of inefficacy or toxicity. These difficulties have been called for the development of new administration techniques for bioactive compounds, directed towards attaining the steady state plasma concentration.

Conventional drug delivery system achieves as well as maintains the drug concentration within the therapeutically effective range needed for treatment only when taken several times a day. This results in a significant fluctuation in drug level. Oral delivery of drugs is far by most preferable route of drug delivery due to the ease of administration, patient compliance and flexibility in formulation. From immediate release to site specific delivery, oral dosage forms have really progressed. In conventional dosage form, there is a little or no control over the drug release from the dosage forms, an effective concentration at the target site can be achieved by intermittent administration of drug, which results in constantly changing, unpredictable and often sub- or supratherapeutic drug concentrations[2]. Sustained release, prolonged action, sustained release, extended release, depot dosage forms are terms used to identify drug delivery systems that are designed to achieve prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose. Conventional pharmaceutical dosage forms give up drug to surrounding tissues or fluids at a time with varying rates that are highest initially and decline continuously thereafter. The primary consideration or objective in clinically treating pathological or physiological disorders is the attainment and maintenance of a predetermined plasma drug concentration (Minimum effective concentration) in the body for the said amount of time[3].

Sustained release systems are designed to achieve therapeutically effective concentrations of drug in the systemic circulation over an extended period of time thus achieving a better patient compliance and allowing a reduction of both the total dose of drug administered and the incidence of adverse side effects. In recent years, newer drug delivery systems have been designed and evaluated in order to overcome the limitation of conventional drug therapy[4]. Sustained release technology has emerged as an important new field in the development of pharmaceutical dosage form. Introduction of sustained release (SR) has given a new platform for novel drug delivery system (NDDS). Sustained release systems include any drug delivery system that achieves slow release of drug over an extended period of time. More precisely, sustained drug delivery can be defined as a "Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable effects"[5].

Micro-encapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsules. In a relatively simplistic form, a microcapsule is a small sphere with a uniform wall around it. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating, or membrane. Most microcapsules have diameters between a few micrometers and a few millimeters. The technique of microencapsulation depends on the physical and chemical properties of the material to be encapsulated**.**[6] These micro-capsules have a number of benefits such as converting liquids to solids, separating reactive compounds, providing environmental protection, improved material handling properties. Active materials are then encapsulated in micron-sized capsules of barrier polymers[7,8].

Microencapsulation processes are usually categorized into two groupings: chemical processes and mechanical or physical processes. These labels can, however, be somewhat misleading, as some processes classified as mechanical might involve or even rely upon a chemical reaction, and some chemical techniques rely solely on physical events[9,10]. A clearer indication as to which category an encapsulation method belongs is whether or not the capsules are produced in a tank or reactor containing liquid, as in chemical processes, as opposed to mechanical or physical processes, which employ a gas phase as part of the encapsulation and rely chiefly on commercially available devices and equipment to generate microcapsules. There are various techniques available for the encapsulation of core materials[11,12]. The objective of this study was to prepare microcapsules of gemifloxacin and evaluate the same. The results are discussed.

Materials and Methods

Gemifloxacin was obtained as a gift sample from Suven Nishtaa, Cellulose acetate phthalate, Hydroxy propyl methyl cellulose, Ethanol, Acetone, Dichloromethane, Liquid paraffin, Sorbitan monooleate, n-hexane from Sd fine chemicals limited. All the other ingredients used were of analytical grade.

Preformulation Studies with the Drug

The preformulation studies with the Gemifloxacin obtained were performed using conventional and reported techniques. The UV-Visible spectrum, solubility, flow properties, drug crystallinity were determined.

O/O Emulsification solvent evaporation method

- Required quantity of CAP was dissolved in appropriate quantity of acetone in a beaker and required quantity of Drug and HPMC was dissolved in appropriate quantity of mixture of ethanol and DCM in another beaker and mixed.
- In another beaker required quantities of liquid paraffin(continuous phase) and sorbitan monooleate (emulsifying agent)were added and kept for stirring on magnetic stirrer.
- Drug and polymers solution was added to liquid paraffin drop wise with the help of syringe and the o/o emulsion is formed and it is allowed for stirring on magnetic stirrer for 12hrs at 1000rpm.
- Then it was filtered and washed 3 times with 50ml of n-hexane. And micro capsules were recovered by vacuum filtration[13-16].
- Six formulations were prepared with different ratios of drug and polymer and using different concentrations of emulsifying agent.

Table 1: Different formulations of gemifloxacin microcapsules

Ingredients	F1	F2	F3	F4	F5	F6
Drug	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg
CAP	1000 mg	1000 mg	1000 mg	1000 mg	1000 mg	1000 mg
HPMC	600 mg	400 mg	200 mg	200 mg	200 mg	200 mg
Liquid paraffin	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml
Sorbitan monooleate	1ml	1ml	1ml	1.5ml	2ml	2.5ml
Acetone	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
Ethanol	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
DCM	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml
N-hexane	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Characterisation of microcapsules[17-20]

Particle size analysis:

The mean particle size was determined by using optical microscope. In this method particle size was determined by using stage micro meter and the average particle size was determined in this method. The eye piece was adjusted and the stage micrometer was adjusted according to the eyepiece. Calibration factor was calculated by the following formula:

Calibration factor = stage micrometer/eye piece

A minute quantity of gemifloxacin microcapsules was spread on a clean glass slide. Then particle size of the micro capsules were measured from which average particle size was calculated which was then multiplied with the obtained calibration factor. In this way, the average particle size was calculated for all the five batches.

Percentage yield:

Percentage practical yield is calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of gemifloxacin microcapsules recovered from each batch in relation to the sum of starting material. The percentage yield of prepared gemifloxacin microcapsules was determined by using the formula:

Percentage yield = (practical yield/theoretical yield)x100

Assay of drug:

10mg of each batch of microcapsules were dissolved in 5ml of methanol in a test tube, shaken well and were left open overnight for the evaporation of methanol. Then 10ml of methanol was added to the remnants in the test tube, stirred well, filtered and then analysed for the drug content in UV spectroscopy at 271nm.

Drug entrapment efficiency:

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula:

PDE=(practical drug content/theoretical drug content)x100

Scanning electron microscopy (SEM):

In order to examine the particle surface morphology and shape, Scanning Electron Microscopy (SEM) was used. Dry gemifloxacin microcapsules was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with gold layer 20 nm thick. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 20 kV.

Fourier transform Infra-red spectrophometer (FT-IR studies):

The FT IR spectra of drug, CAP, HPMC and gemifloxacin microcapsules were obtained. Sample about 5 mg was mixed thoroughly with 100 mg potassium bromide IR powder and compacted under vacuum a pressure of about 12 Psi for 3 minutes. The resultant disc was mounted in a suitable holder in Perkin Elmer IR spectrophotometer and the IR spectrum was recorded from 4000 cm^{-1} to 625 cm^{-1} in a scan time of 12 minutes.

Differential scanning calorimetry (DSC):

Thermal properties of the powder samples were investigated with a DSC. Approximately 10 mg of sample was analyzed in an open aluminum pan, and heated at scanning rate of 10°C/min between 0°C and 400°C. Magnesia was used as the standard reference material.

In vitro drug release studies:

In-vitro dissolution studies of samples were carried out using USP apparatus II paddle method. Accurately weighed drug, gemifloxacin microcapsule sand, gemifloxacin marketed tablet were added to 500 ml of 6.8pH phosphate buffer saline media in separate baskets at 37 + 0.5°C and stirred at 100 rpm. An aliquot of 10ml was withdrawn at different time intervals. The solid particles were prevented from pipetting by withdrawing the sample through a pipette fitted with a cotton plug. An equal volume of fresh dissolution medium was immediately replaced. The filtered samples were assayed.

In-vitro release kinetics: (Harris shoaib et al., 2006)[21, 22]:

To analyze the *in vitro* release data various kinetic models were use to describe the release kinetics. The zero order rate Eq. (2) describes the systems where the drug release rate is independent of its concentration. The first order Eq. (3) describes the release from system where release rate is concentration dependent. Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion. The results of in vitro release profile obtained for all the formulations were plotted in modes of data treatment as follows:

1. Zero - order kinetic model – Cumulative % drug released versus time.
2. First – order kinetic model – Log cumulative percent drug remaining versus time.
3. Higuchi's model – Cumulative percent drug released versus square root of time.
4. Korsmeyer equation / Peppa's model – Log cumulative percent drug released versus log time.
5. Hixson crowell model.

Results and Discussions

Table 2: Preformulation Data

Physical property	Drug-Gemifloxacin
Appearance	White to off white
Shape	Amorphous
Water solubility	Freely soluble
Melting range	235-237ºc
Λmax	271nm

Characterisation of Microcapsules

Table 3: Particle size analysis

Formulation	Average particle size (microns)
F1	287±0.32
F2	256±0.5
F3	225±0.9
F4	193±0.68
F5	169±0.13
F6	**140±0.1**

From the above results it is observed that from F1 to F6, particle size is decreased due to the influence of polymer concentration and increase in emulsifying agent. Particle size range from 287±0.32µm for f1 and found to be least for F6 140±0.1µm.

Table 4: Percentage yield

Formulations	Percentage yield
F1	68.12
F2	73.56
F3	75.65
F4	80.69
F5	82.35
F6	**85.62**

The results of percentage drug entrapment efficiency are shown in the above table. From the results it can be inferred that there is a proper distribution of gemifloxacin in the microcapsules. The percentage entrapment efficiency was found to be 59.2% to 79.8%. Percentage yield was found to be highest for F6 formulation and least for F1 formulation.

Table 5: Drug Entrapment Efficiency

Formulation	Entrapment efficiency
F1	59.2
F2	63.5
F3	68.9
F4	70.1
F5	72.5
F6	79.8

Scanning electron microscopy

Surface morphology of the microcapsules was examined by SEM. Microcapsules were smooth, spherical and in nature. Microcapsules were obtained in the micro range of 65.3 to 105µm. SEM pictures are shown in figure 1.

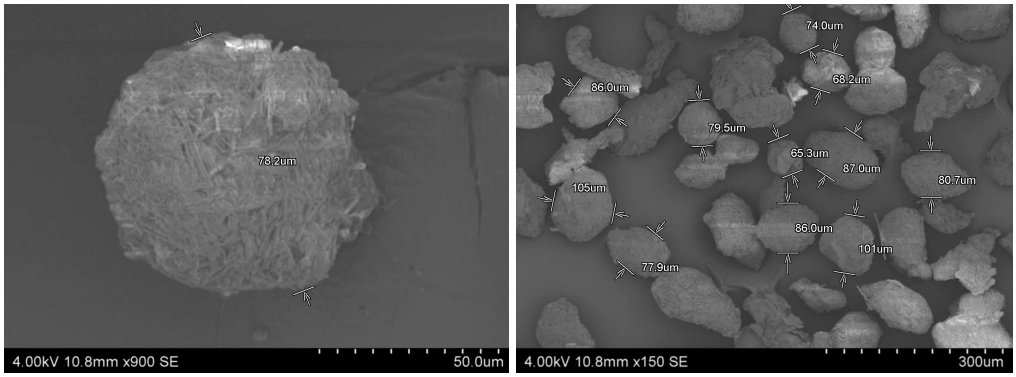

Fig 1: Pictograms of optimized microcapsules

FTIR studies

FTIR has been used to access the interaction between the drug and the polymers CAP and HPMC. The FTIR spectra of drug and polymers are compared with that of the spectra of GFX microcapsules.From the graph of IR spectrum,it is concluded that there is no shift in peaks in the formulation F6.The peaks of drug,polymer and the formulation F6 observed as the same.Thus we can confirm that there is no drug excipient incompatabilty. Hence,drug and excipients are compatible.

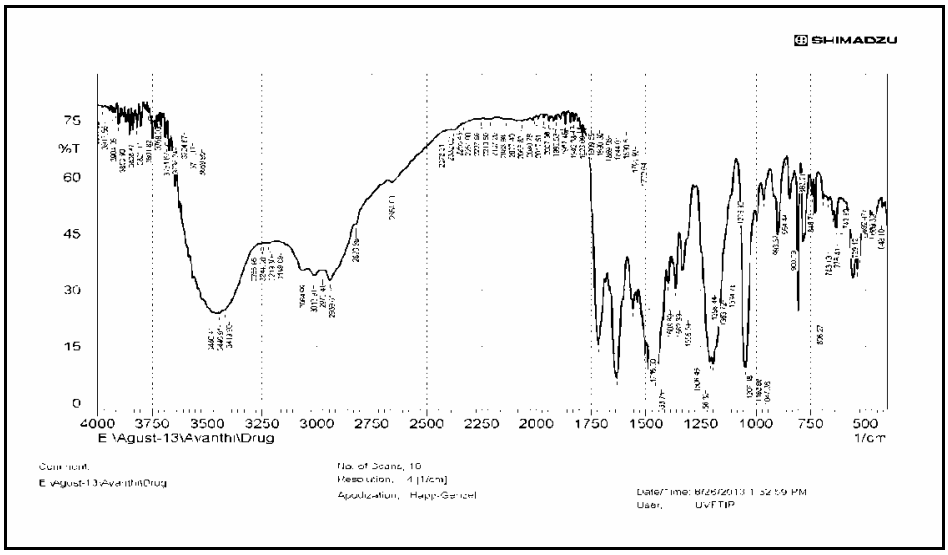

Fig 2: FTIR of drug

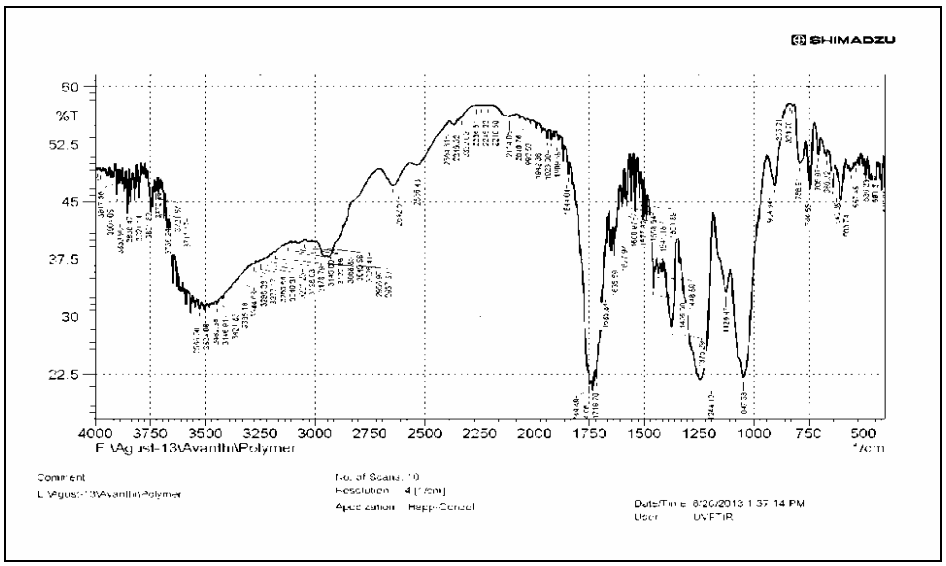

Fig 3: FTIR of CAP

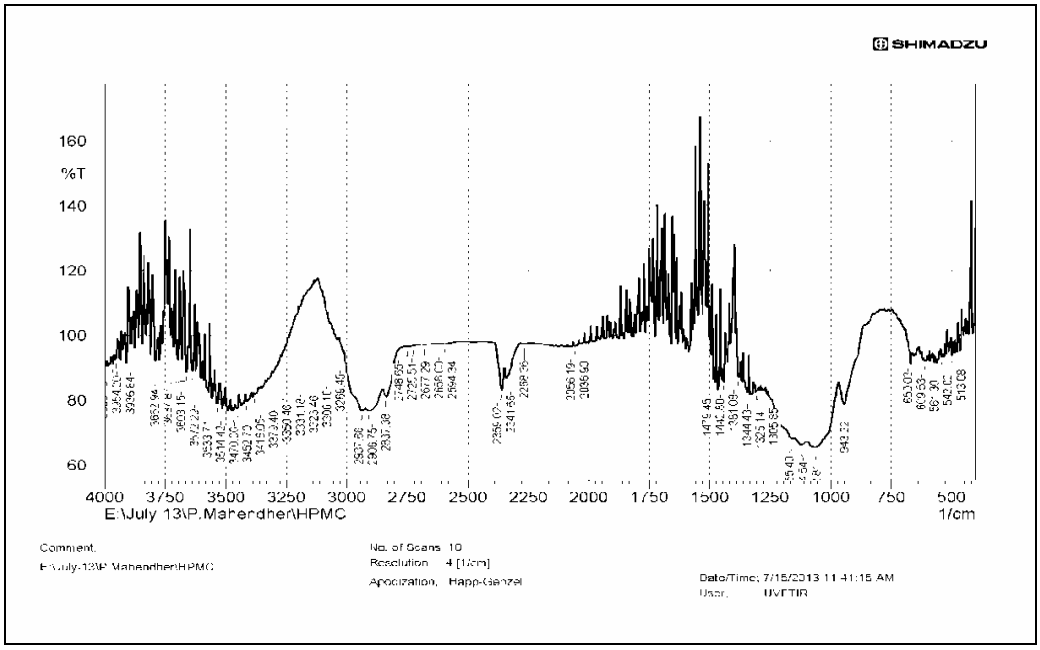

Fig 4: FTIR of HPMC

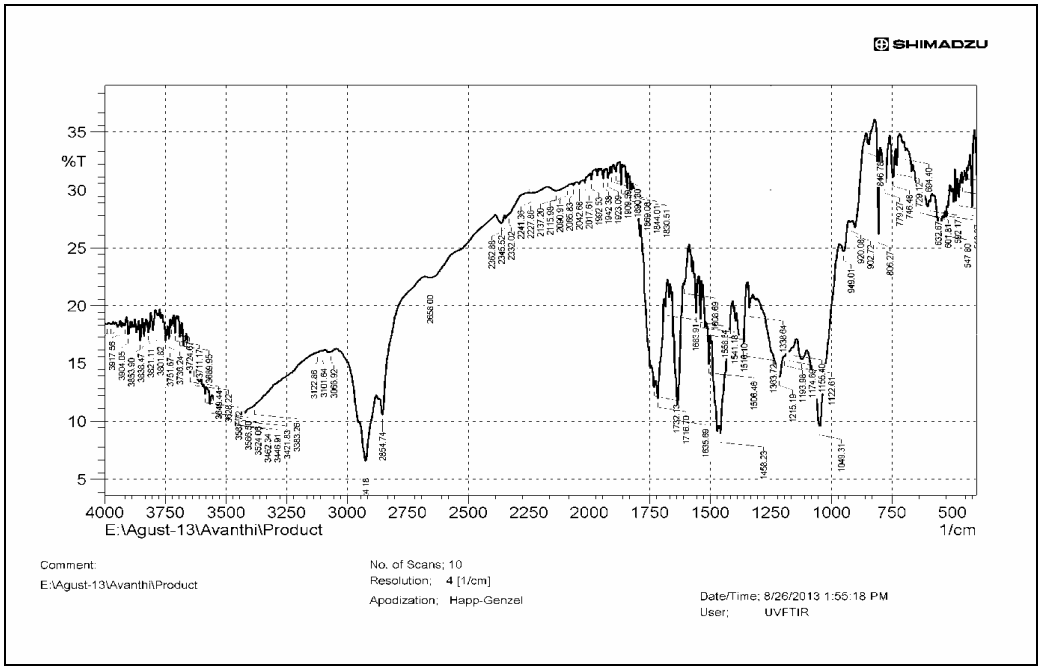

Fig 5: FTIR of microcapsules

Differential scanning calorimetry

DSC studies were performed to understand the nature of the encapsulated drug. DSC analysis was performed on gemifloxacin(drug) and gemifloxacin microcapsules(product). From the dsc results it was observed that the thermogram of characteristic peak of drug is changed in the formulation. Hence it indicates the physical nature of drug is changed from amorphous form to crystalline in the formulation. DSC results are shown in the figure 6 and 7.

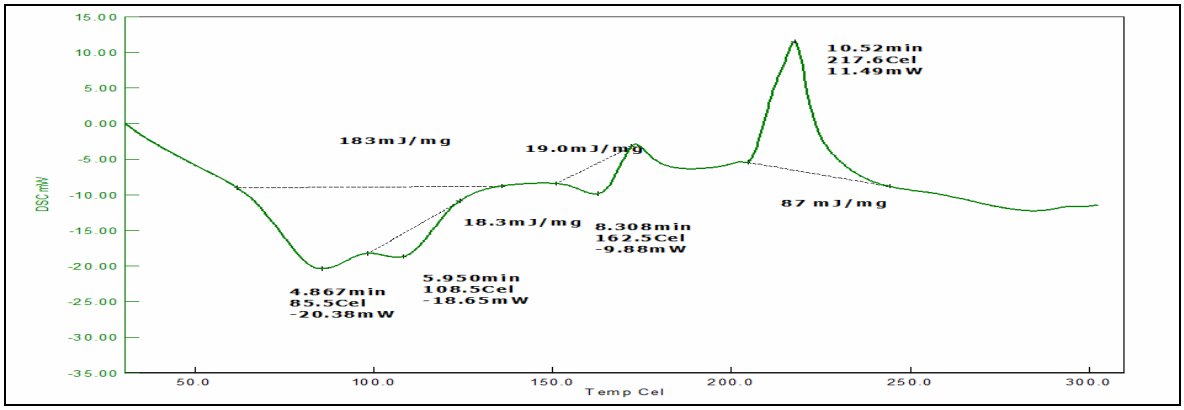

Fig 6: DSC of drug

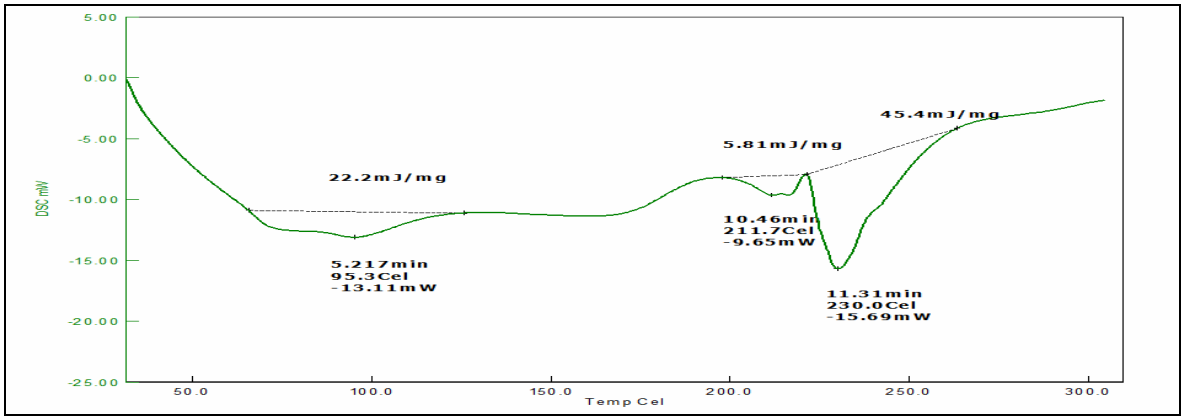

Fig 7: DSC of optimized microcapsule

In-vitro drug release studies

In-vitro drug release from gemifloxacin microcapsules were performed in USP dissolution apparatus for 5 hours.

Table 6: Dissolution Profile of Gemifloxacin microcapsules

TIME(hrs)	F1	F2	F3	F4	F5	**F6**
0	0	0	0	0	0	**0**
1	52.68	55.26	59.58	60.08	62.16	**65.12**
2	55.89	60.56	63.49	65.96	73.56	**78.72**
3	60.77	68.74	70.26	73.45	80.56	**83.23**
4	64.088	70.14	72.06	75.23	83.68	**89.46**
5	64.088	70.14	72.06	75.23	83.68	**89.46**

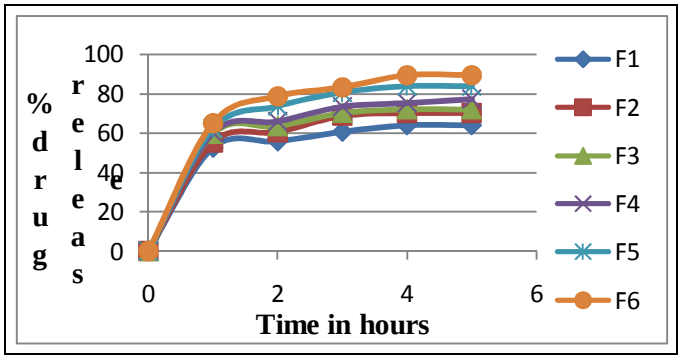

Fig 8: Comparision of invitro drug release profile of F1 to F6

F6 fomulation has shown maximum % drug release of 89.56 at 5 hours. Hence it is taken as optimised and preferred formulation. F6 formulation was characterised for solid state characterization by DSC, surface morphology by SEM and for drug excipient compatability studies by FTIR studies. Minimum % drug release of 64.088 was observed with F1 formulation.

Table 7: Comparison of dissolution profile of optimised formulation and pure drug

Time	Pure Drug	Formulation
0	0	0
15	25.98	26.68
30	34.78	32.46
45	41.36	40.97
60	49.62	65.12
120		78.72
180		83.23
240		89.46
360		89.46

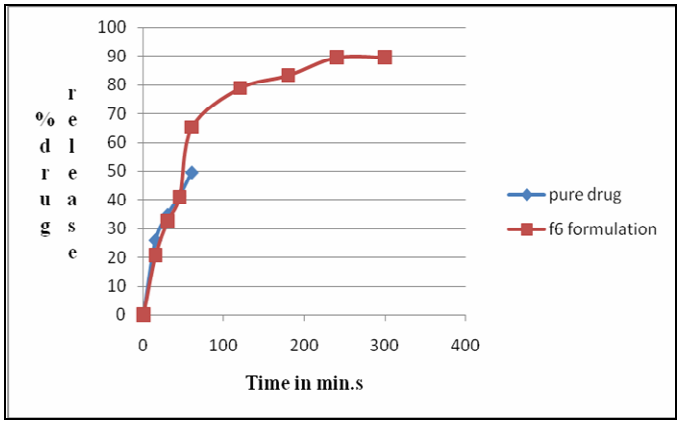

Fig 9: Comparison of dissolution profile of optimized formulation and pure drug

Pure drug has shown the % drug release of 49.62 and optimized formulation has shown the % drug release of 89.46 at 5 hours microcapsules has shown the sustained release.

Invitro release kinetics:

Table 8: Invitro release kinetics

-	Release Kineitcs				
	Zero	**Higuchi**	**Peppas**	**First**	**Hixson Crowell**
Slope	14.995	40.242	0.203	-0.010	0.448
Correlation	0.8167	0.9502	0.9827	-0.9425	0.9046
R^2	0.6670	0.9030	0.9658	0.8883	0.8182

Conclusion

Gemifloxacin sustained release microcapsules were prepared by o/o emulsification solvent evaporation method employing CAP and HPMC as polymers and sorbitan monooleate as emulsifying agent with micron in size and without any drug-excipient interactions and increased dissolution rate. Finally it is concluded that prepared microcapsules has sustained release property.

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