

# **Influence of polymer ratio and surfactants on controlled drug release from cellulosic microsponges**

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## **Abstract**

Microsponge refers to a highly cross-linked particle system with a capacity to adsorb (like a dry sponge) pharmaceutical materials. There are various methods available to prepare microsponge formulations, in this study we used quasi emulsion-solvent diffusion method with a combination of hydrophobic (ethyl cellulose) and hydrophilic polymers (hydroxypropyl methylcellulose) mediated via Tween 80 and polyvinyl alcohol. Various ratios and amounts of the polymers and surfactants were used to prepare microsponge formulations using ketoprofen as a model drug and extensively characterised. Our results, for the first time, indicate successful and optimised formulation with desired pharmaceutical characteristics using a combination of hydrophobic and hydrophilic polymers.

**Keywords:** Microsponges; hydroxypropyl methylcellulose; ethyl cellulose; tween 80; polyvinyl alcohol; dissolution; drug release

## 1. Introduction

Controlling the drug release is crucial for an effective delivery at the site of action following oral administration. Controlled release delivery systems have the ability to maintain a constant plasma drug concentration over a long period of time, thus minimizing the side effects associated with the conventional dosage forms (Osmani et al., 2015). However, attainment at the site of action is often challenged by the poor drug solubility, degradation, and low bioavailability and bio-distribution (Akram et al., 2016). One way of addressing low solubility and poor bioavailability issue is to encapsulate the drug in a polymeric matrix that allows for a precise and controlled drug release at a constant rate for a long period of time (Gómez-Gaete et al., 2017; Soppimath et al., 2001). In recent years, polymeric particulate systems including microparticles, microsponges and nanoparticles have gained tremendous attention because of their diverse and tunable properties (Floyd et al., 2015; Nidhi et al., 2016; Osmani et al., 2015). Since micro- and nano-carriers are routinely fabricated with biodegradable and biocompatible polymers, the formulations are completely removed from the body after delivering the drugs at the site of action (Gómez-Gaete et al., 2017). Microcarriers, more specifically the microspheres, are spherical particles containing the active pharmaceutical ingredient in the core and surrounded by a polymeric layer, which usually controls the drug release from the microspheres (Lakshmi et al., 2012). Any defect in the outer polymer layer may compromise the drug release properties of microspheres, and the drug may leach out before even it reaches the target site. Conventional microspheres are often prepared by the emulsion-solvent evaporation method in which the solvent is gradually evaporated, thus leaving a non-porous core and an unacceptable residual solvent (Dixit et al., 2015; Hong et al., 2005). On the other hand, porous microspheres offer tunable properties in terms of surface area, porosity, better cell adherence, drug loading and release kinetics (Hossain et al., 2015).

Recently, another type of porous microspheres, dubbed as microsponges, have been investigated for their ability to control the drug release rates. Microsponges have interconnecting pores within a non-collapsible sponge like spherical structure and have the ability to encapsulate a large variety of hydrophilic and hydrophobic compounds as well as macromolecules (Roh et al., 2016; Vikrant and Jessy, 2007; Zhang et al., 2016). Microsponges are widely studied in topical preparations because of their larger size, which usually ranges between 5 to 300 micron (Bothiraja et al., 2014). The larger size of these porous microspheres allows for retention at the skin surface, thus aids in delivery of various drugs for topical ailments (Li et al., 2013; Pandit et al., 2016; Patel et al., 2016; Rizkalla et al., 2011; V. Kadam et al., 2016; Zhang et al., 2016). More recently, the scope of microsponges has been extended from topical delivery to controlled release formulations intended for oral delivery (Arya and Pathak, 2014; Gupta et al., 2015a; Gupta et al., 2015b; Osmani et al., 2015; Sareen et al., 2014; Srivastava et al., 2012).

The most popular strategy to formulate microsphere is the quasi emulsion-solvent diffusion method, which requires water insoluble polymers to be dissolved in a water immiscible organic solvent and subsequently emulsified with an aqueous phase using a

hydrophilic surfactant. The organic solvent is then diffused out slowly under constant agitation, thus leaving behind spherical polymeric spheres with internal and external pores. A number of factors can contribute in the formation of pores, namely displacement of organic solvent (Rizkalla et al., 2011), drug to polymer ratio and the stirring rates (Nokhodchi et al., 2007), and the use of surfactants and inorganic salts as pore forming agents (Cai et al., 2013). Among various hydrophobic polymers, Polyvinyl alcohol (PVA), Polystyrene, poly(2-hydroxyethyl methacrylate) (pHEMA), ethyl cellulose (EC) and Eudragit RS 100 are widely used as a matrix for the microsponges (Jadhav et al., 2013).

Here we report the fabrication of EC and hydroxypropyl methylcellulose (HPMC) microsponges loaded with a model drug, namely ketoprofen. To the best of our knowledge, this is the first study reporting the use of combination of hydrophobic and hydrophilic polymers as matrix components for microsphere formulation. Additionally, the influence of two surfactants, namely polyvinyl alcohol and Tween 80 was investigated on the microsphere production and drug loading capacity. The microsponges were subjected to solid-state characterisation using a variety of analytical methods, micromeritic investigations, and the drug release properties. Varying HPMC concentration resulted in highly porous and spherical microspheres with enhanced efficiency in terms of drug release properties.

## **2. Material and methods**

### **2.1. Materials**

Ketoprofen, ethyl cellulose, and polyvinyl alcohol (30,000 – 70,000 MW) were purchased from Sigma-Aldrich. HPMC K200M was a kind gift from Colorcon Limited (Dartford Kent, UK). Tween-80 was sourced from Merck Inc. Dichloromethane (DCM) and ethanol (EtOH) were purchased from Sigma-Aldrich, and were used as it is without further purification. Double distilled water was used throughout the experiments.

### **2.2. Quantitative drug analysis**

A validated UV spectroscopy was adopted to quantify ketoprofen content in the microsponges at a maximum wavelength of 238 nm. Initially, a stock solution (500 µg/mL) of ketoprofen was prepared in phosphate buffer (6.8 pH). A calibration curve was plotted using a series of standard solutions of ketoprofen in the concentration range of 2 – 50 µg/mL. A linear regression was applied to obtain linear equation ( $y = 0.0364x + 0.0231$ ) with  $R^2$  value of 0.999. The accuracy ranged between 99% and 102%, while the precision (CV) was <5% for the assay in the given range of concentrations. The inter- and intra-day variations were also checked.

### **2.3. Preparation of microsponges**

Ketoprofen-loaded microsponges were prepared using quasi emulsion-solvent diffusion method with slight modifications (Li et al., 2013). A varying proportion of ethyl cellulose (EC)

and hydroxypropyl methylcellulose (HPMC K200M) was dissolved in 5 mL of organic phase consisted of DCM and EtOH at 4:1 v/v ratio (Table 1). This polymeric organic phase was designated as the internal phase. External phase consisted of different concentrations of polyvinyl alcohol (PVA) or Tween-80 as emulsifier in 100 mL double distilled water. The internal phase was added slowly to the external phase under constant stirring at 5000 rpm using a high-speed homogenizer (Heidolph SilentCrusher M, Germany). The stirring was continued for 4 h at room temperature for evaporation of dichloromethane and ethanol from the mixture. The prepared microsponges were then filtered through a filter paper of 0.45  $\mu\text{m}$  pore size (Whatman® Cellulose filter paper, Sigma-Aldrich), washed with double distilled water, and dried in hot air oven at 40 °C for 24 hours to obtain a constant weight. This also ensured that any residual organic solvent was completely removed from the microsponges. The dried microsponges were carefully weighed and stored in a glass vial and then placed in a desiccator until further used.

#### 2.4. Production yield

The production yield for all microsphere formulations was calculated gravimetrically using the following equation;

$$\text{production yield (\%)} = \frac{M_{\text{Micro}}}{M_{\text{RM}}} \times 100$$

Where  $M_{\text{Micro}}$  is the weight of formulated microsponges and  $M_{\text{RM}}$  is the initial weight of the raw materials (polymers and drug). Production yield was calculated in triplicate and the mean  $\pm$  standard deviation values are reported.

#### 2.5. Drug entrapment efficiency

A 10 mg of ketoprofen equivalent quantity of microsponges were taken in a test tube and crushed with the help of a glass rod. To this tube, 1 mL of ethanol was added and vortexed for 20 minutes to extract the drug. Then phosphate buffer of pH 6.8 was added to make up 10 mL volume and subjected to vortex for another 10 minutes. The insoluble residue was separated by centrifuging the sample at 3000 rpm for 10 minutes. The supernatant layer was taken and quantified spectrophotometrically at 238 nm after appropriate dilution. The procedure was repeated three times and mean along with standard deviation values are reported.

**Table 1: Composition of microsponges**

FOMULATIONS	DRUG (mg)	EC:HPMC ratio	TWEEN 80 (%)	PVA (%)	WATER (q.s. mL)
F1	250	9:1	2	0	100
F2	250	9:1	0	0.1	100
F3	250	9:1	5	0	100
F4	250	9:1	0	0.25	100
F5	250	9:1	10	0	100
F6	250	9:1	0	0.5	100
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F7	250	8:2	2	0	100
F8	250	8:2	0	0.1	100
F9	250	8:2	5	0	100
F10	250	8:2	0	0.25	100
F11	250	8:2	10	0	100
F12	250	8:2	0	0.5	100
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F13	250	7:3	2	0	100
F14	250	7:3	0	0.1	100
F15	250	7:3	5	0	100
F16	250	7:3	0	0.25	100
F17	250	7:3	10	0	100
F18	250	7:3	0	0.5	100

## **2.6. In vitro characterisation**

### **2.6.1. Micromeritics of microsponges**

The porosity and its descriptive parameters (total intrusion volume, bulk and apparent densities, pore area, pore diameter and porosity) were determined using mercury intrusion porosimetry (AutoPore IV 9500, Micromeritics, USA). Blank and drug loaded microsponges were submerged under a pool of mercury in a calibrated cell within a vacuum chamber. Mercury intrusion volume was measured by gradually increasing the cell pressure, which allowed the mercury to forcefully penetrate the pores of the microsponges. The total

intrusion volume was then calculated from the apparent mercury volume remained in the cell. Micromeritic properties were determined using various equations as described previously (Orlu et al., 2006).

### 2.6.2. *Thermal analysis*

To study thermal behavior, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were conducted. DSC thermograms of ketoprofen, unloaded micro-sponges and drug-loaded formulations were recorded using DSC 1 Star (Mettler-Toledo, Leicester, UK). Whilst Thermobalance Mettler TG50 (Mettler-Toledo Ltd., Leicester, UK) was used for TGA. An accurately weighed quantity (4-8 mg) of samples was sealed and pierced in flat-bottomed aluminum crucibles and heated at a rate of 10 °C/min, from 25-150 °C for DSC and 25-500 °C for TGA, under continuous dry purge of liquid nitrogen flowing at a rate of 50 mL/min.

### 2.6.3. *X-ray Diffraction (XRD)*

X-ray diffraction patterns were recorded using X-ray diffraction system (PANalytical X'Pert Powder) between 5 to 50° at 2 $\theta$  angle. The XRD instrument was equipped with a CuK $\alpha$  radiation source (1.5406 Å) with a generator voltage and current of 30 kV and 10 mA, respectively.

### 2.6.4. *Fourier Transform Infra-red Spectroscopy (FT-IR)*

FT-IR spectra of ketoprofen, EC, HPMC, Tween80, PVA and microsp sponge formulations were recorded over wavelength range of 4000 to 500 cm<sup>-1</sup> at a resolution of 4 cm<sup>-1</sup> using an ATR FT-IR spectrometer (Alpha Bruker).

### 2.6.5. *Scanning Electron Microscopy (SEM)*

Formulations were evaluated for morphology by a JEOL model JSM-6060LV scanning electron microscopy (Japan). Samples were lightly spread over specimen stubs, and a sputter coater (SC7620) was used for coating the surface of the material with AuPd for high quality images. Samples were carefully prepared to ensure particles are not crushed. Images were captured at multiple magnifications.

## 2.7. *In vitro* dissolution studies

*In vitro* drug dissolution from microsponges was evaluated using a six vessels USP type II dissolution apparatus (Curio 2020+ Paddle Apparatus). A 10 mg drug equivalent sample of microsponges was placed in 500 mL of phosphate buffer (pH 6.8) maintained at 37 $\pm$  0.5°C with a paddle rotation set at 50 rpm. A sample of 5 mL was withdrawn at a predetermined time interval for a total time of 8 hours. The dissolution media was replenished with preheated fresh phosphate buffer after each sampling in order to maintain the sink conditions. The samples were analysed in triplicate using a UV visible spectrophotometer

(UV-160 IPC, Shimadzu) at set wavelength of 238 nm and the percentage drug release was calculated based on a series of standard solutions.

## 2.8. Kinetic data analysis

Kinetic analysis of dissolution data was performed on DDSolver, an add-in extension for Microsoft® Excel® 2013. Different kinetic models were applied such as zero-order, first-order, Higuchi and Korsmeyer-Peppas to study the drug release mechanism.

## 3. Results and Discussion

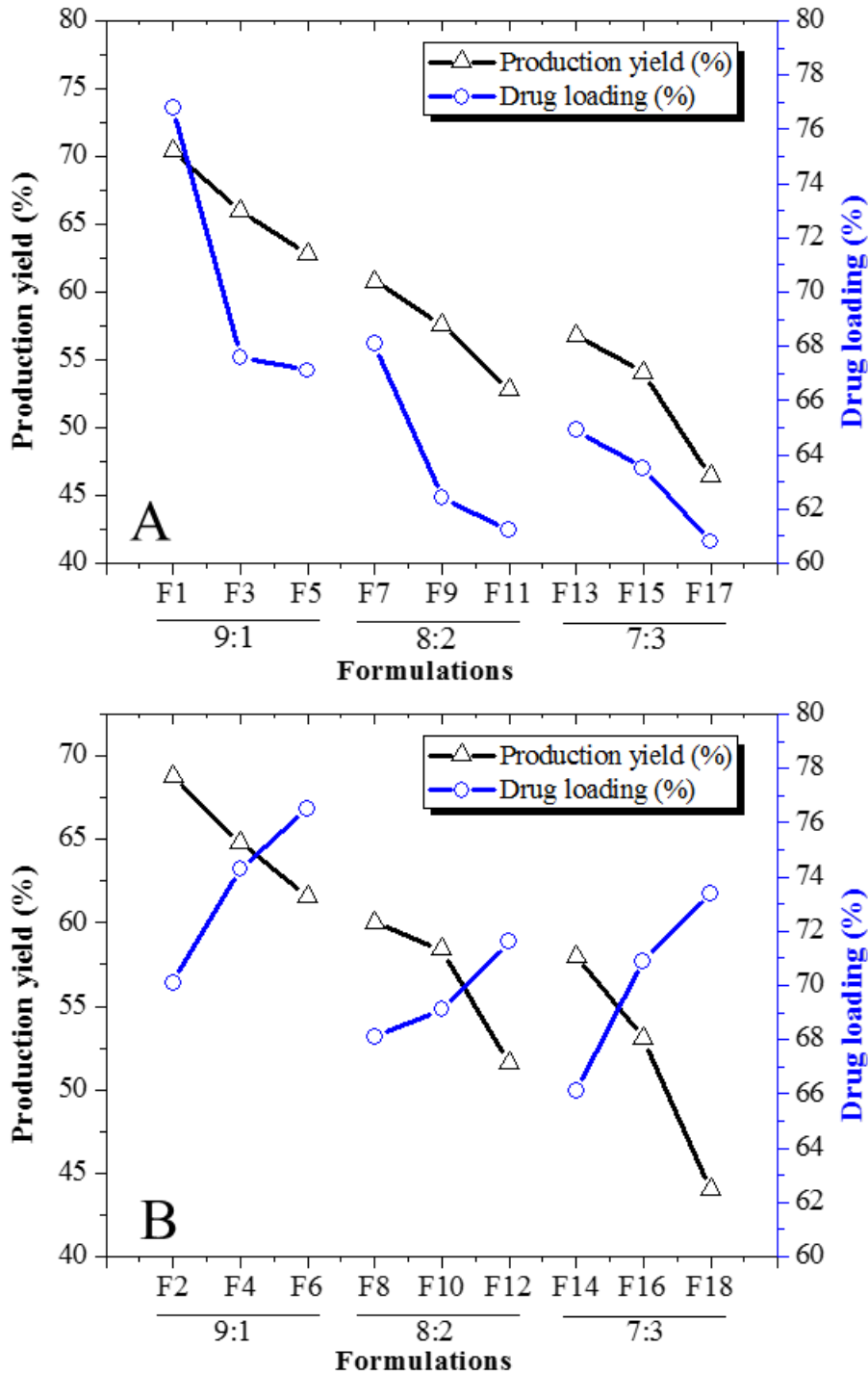
### 3.1. Influence of polymers and surfactants concentration on production yield and drug loading

Ketoprofen loaded microsponges were prepared using quasi emulsion-solvent diffusion method. In order to elucidate the loading capacity and the production yield, a varying concentration of polymers and surfactants were used (Table 1). This study was carried out using two different polymers, namely EC and HPMC as the matrix forming agents, whilst Tween 80 and PVA were used as surfactants for emulsion stability and for the pore formation. It is an established fact that drug to polymer ratio influence microsp sponge production and surface properties as demonstrated in published studies (Nokhodchi et al., 2007; Osmani et al., 2015). Therefore, to remain in the scope of this study, drug quantity was fixed while other factors such as polymer and surfactant contents were systematically altered in order to investigate a possible relationship. From the values of production yield and the loading capacity (Table 2), a relationship between polymer ratio and surfactants concentration can be seen in Fig. 1. Polymer (HPMC) and surfactants (Tween 80 and PVA) influenced the production yield and the drug loading capacity of prepared microsponges.

From Fig. 1A, it was evident that as the concentration of HPMC and Tween 80 was increased, microsponges yield decreased with decreasing amount of drug trapped inside the porous particles. The production yield for F1, F3 and F5 (9:1; EC:HPMC ratio) was 70.4%, 62% and 60.8%, respectively. The production yield for F7, F9 and F11 (8:2; EC:HPMC ratio) was 60.6%, 57.6% and 52.8%, respectively, whilst same for F13, F15 and F17 (7:3; EC:HPMC ratio) was 56.8%, 54.1% and 46.4%, respectively. The drug loading capacity was also decreased from 76.8% to 60.8% with increasing HPMC and Tween 80 contents.

Similarly, upon increasing HPMC and PVA content (Fig. 1B), the production yield gradually decreased from 68.8% to 61.6% for F2, F4 and F6 formulations (9:1; EC: HPMC ratio), from 60% to 51.6% for F8, F10 and F12 formulations (8:2; EC:HPMC ratio), and from 58% to 44% for F14, F16 and F18 formulations (7:3; EC:HPMC ratio). More interestingly, the drug loading capacity improved with increasing concentration of PVA in the formulations. Yet an overall decreased drug loading was observed with an increase in the HPMC content.





**Fig. 1.** Production yield and drug loading efficiency of microsponges: **(A)** Tween 80 as surfactant and, **(B)** PVA as surfactant.

**Table 2: Kinetic analysis of drug release from micro-sponges**

Formulation	Production yield (%)	Entrapment efficiency (%)	Zero order ( $R^2$ )	First order ( $R^2$ )	Higuchi ( $R^2$ )	Korsmeyer-Peppas
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						(R <sup>2</sup> )	(n)
F1	70.4±3.5	76.8±3.8	0.992	0.961	0.854	0.994	0.95
F2	68.8±3.4	70.1±3.5	0.986	0.974	0.884	0.997	0.86
F3	66.0±3.0	67.6±2.3	0.807	0.957	0.986	0.997	0.58
F4	64.8±2.9	76.5±1.8	0.467	0.714	0.953	0.962	0.45
F5	62.8±2.1	67.1±3.4	0.982	0.952	0.839	0.982	0.97
F6	61.6±3.1	74.3±2.7	0.549	0.901	0.988	0.991	0.47
F7	60.8±2.0	68.1±3.4	0.286	0.631	0.953	0.984	0.41
F8	60.0±3	71.6±3.6	0.920	0.977	0.944	0.992	0.69
F9	57.6±2.8	62.4±1.1	0.780	0.905	0.965	0.974	0.57
F10	58.4±1.9	68.1±1.4	0.163	0.559	0.879	0.924	0.39
F11	52.8±2.6	61.2±1.1	0.816	0.916	0.971	0.984	0.59
F12	51.6±1.5	69.1±3.4	0.853	0.959	0.972	0.993	0.61
F13	56.8±2.8	64.9±2.3	0.882	0.988	0.966	0.997	0.64
F14	58.0±2.9	66.1±1.3	0.983	0.962	0.856	0.985	0.93
F15	54.1±1.7	63.5±3.1	0.769	0.876	0.966	0.972	0.56
F16	53.1±0.7	73.4±3.7	0.985	0.971	0.862	0.988	0.91
F17	46.4±2.3	60.8±3.0	0.951	0.899	0.741	0.972	1.33
F18	44.0±2.2	70.9±2.5	0.989	0.997	0.876	0.996	0.88

The fact that we achieved lower yield of microsponges with higher HPMC contents, could stem from the fact that HPMC is a hydrophilic polymer, and by the time dichloromethane diffused out, some of the HPMC could have been dissolved in the aqueous phase, thereby decreasing an overall content. A low drug loading capacity in case of Tween 80 as emulsifier indicates a possibility of drug being leached out from the porous microsponges during solidification process because of larger pore size (see section 3.2.1). However, increasing amount of PVA might have increased the viscosity of internal phase by arranging themselves in layers around the emulsion droplets. This conformation may have restricted the escape of ketoprofen until the droplets converted into microsponges. With this conformation, one can easily judge that the diffusion of dichloromethane would also be slow, thus leaving more precise and smaller pores in microsponges, as confirmed by the mercury porosimetry and SEM images (see sections 3.2.1 and 3.2.2).

## 3.2. Characterisation of microsponges

### 3.2.1. Pore structure of microsponges

The quasi-emulsion diffusion method of microsphere preparation is known to produce particles with high porosity (KAWASHIMA et al., 1992). The pore structure of selected formulations of microsponges was determined using mercury intrusion-extrusion curves and presented in Table 3. According to intrusion-extrusion curves (data not shown), majority of pores were spherical type as can be seen from the SEM images (see section 3.2.2). From the results presented in Table 3, it is evident that the prepared microsponges were highly porous with a porosity of 66.11% for microsponges prepared with PVA as surfactant,

and 77.10% for microsponges prepared with tween 80 as surfactant. Interestingly, the porosity was slightly lower when the ketoprofen was loaded in the microsponges, thus endorsing the pore confinement or entrapment of drug in the porous particles. Since loading of drug could possibly decrease the intrusion volume, pore diameter and pore area, a decrease in these values is sufficient to validate drug loading in the microsponges. On the other hand, bulk and apparent densities were increased, which could be attributed to the drug loading. Similar results were reported previously where microsponges prepared with PVA as surfactant resulted in porous particles with 60%-70% porosity (Orlu et al., 2006).

**Table 3.** Total intrusion volume, average pore diameter, bulk and apparent densities, total pore area and porosity of microsphere formulations ( $n=3$ ).

Formulation	Total Intrusion volume (mL/g)	Average Pore diameter ( $\mu\text{m}$ )	Bulk density (g/mL)	Apparent density (g/mL)	Total pore area ( $\text{m}^2/\text{g}$ )	Porosity (%)
Blank microsphere (PVA)	1.33 $\pm$ 0.11	0.48 $\pm$ 0.02	0.40 $\pm$ 0.04	1.18 $\pm$ 0.10	29.33 $\pm$ 0.55	66.11 $\pm$ 3.87
Ketoprofen-loaded microsphere (PVA)	1.19 $\pm$ 0.09	0.33 $\pm$ 0.08	0.48 $\pm$ 0.03	1.25 $\pm$ 0.09	24.88 $\pm$ 0.61	61.60 $\pm$ 2.56
Blank microsphere (Tween 80)	1.99 $\pm$ 0.15	0.65 $\pm$ 0.01	0.30 $\pm$ 0.01	1.31 $\pm$ 0.15	77.88 $\pm$ 0.50	77.10 $\pm$ 1.40
Ketoprofen-loaded microsphere (Tween 80)	1.66 $\pm$ 0.18	0.51 $\pm$ 0.03	0.39 $\pm$ 0.04	1.32 $\pm$ 0.07	49.22 $\pm$ 0.25	70.45 $\pm$ 2.11

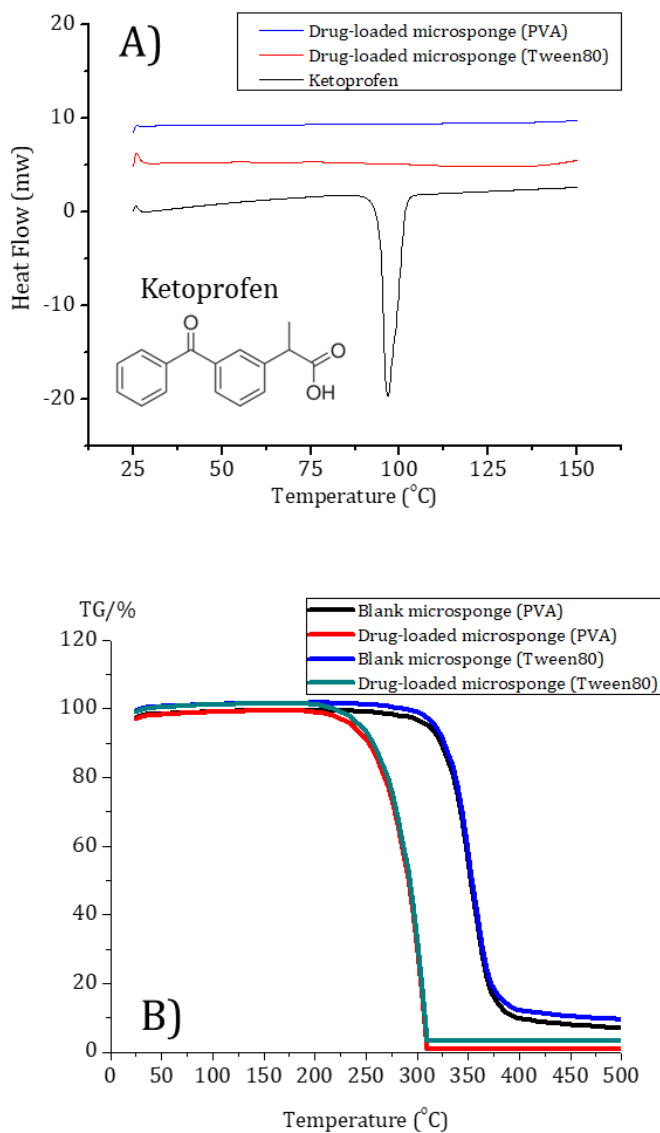
### 3.2.2. Solid-state characterization

A combination of analytical tools including DSC, TGA, XRD, FT-IR and SEM were used to elucidate the thermal properties, crystalline state of the drug, possible drug and polymer interactions and the surface morphology, respectively for the drug loaded microsponges. Such information is crucial in designing precise and stable controlled release formulations.

DSC thermograms were recorded in order to understand the thermal behavior of drug loaded microsponges, and are presented in Fig. 2. A single sharp melting endotherm appeared at 96° C confirmed the crystalline nature of ketoprofen. Ketoprofen loaded microsponges were subjected to DSC analysis in order to investigate the state of ketoprofen. The absence of melting endotherm in the formulations indicated a complete transformation of ketoprofen from crystalline to amorphous state inside the pores, as noted in Fig. 2A.

Thermal stability is crucial for solid dosage forms as the storage conditions may vary, in terms of temperature, during logistics. Thermograms recorded from TGA showed thermal stability of drug-loaded microsponges up to 200°C, which is far beyond the storage conditions in various pharmaceutical setups. However, after reaching 200°C, the thermal stability was compromised and a 100% loss was observed above 300°C, as exemplified in Fig. 2B. In case of blank microsponges, thermal instability was more prominent once the

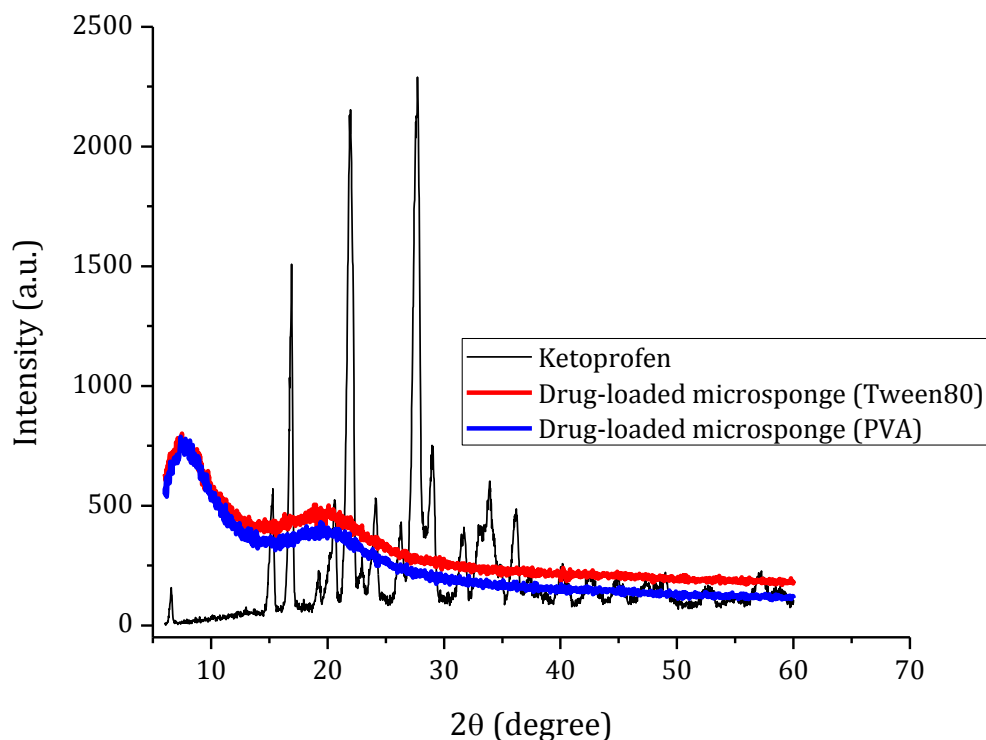
furnace temperature reached 350°C, endorsing the versatility of EC and HPMC based microsponges.



**Fig. 2.** Thermal analysis of microsponges A) DSC of ketoprofen & drug-loaded microsponges and B) TGA of blank & drug-loaded microsponges.

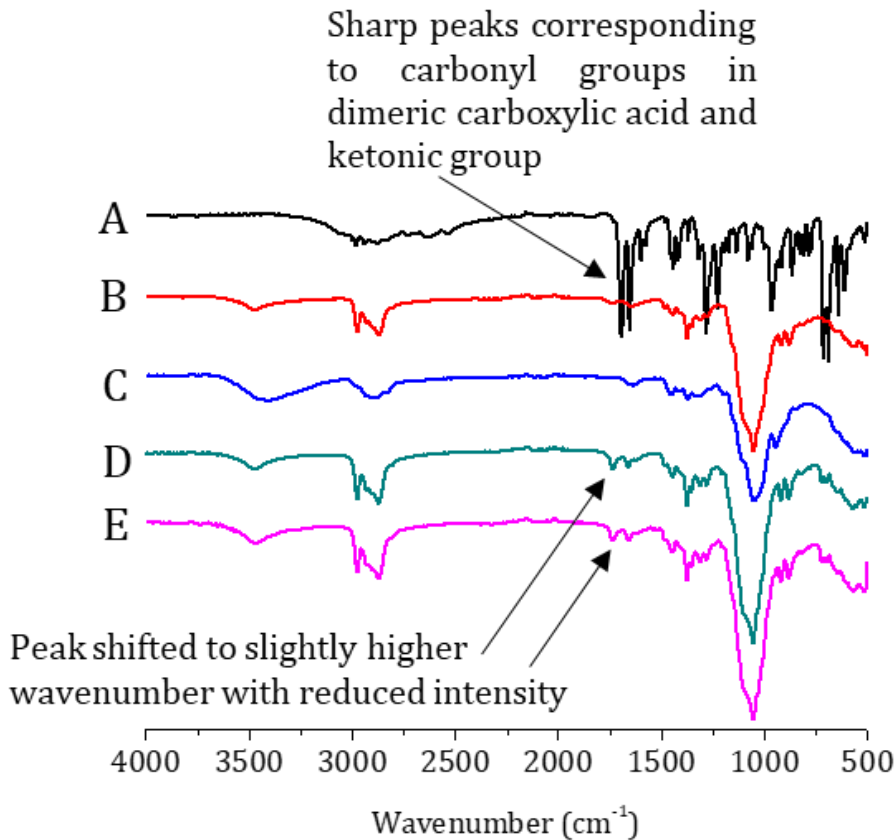
The results of XRD for pure ketoprofen and selected drug-loaded microsponges are presented in Fig. 3. The XRD pattern of ketoprofen showed sharp peaks at 16°, 23° and 28° at the 2-theta scale, corresponding to the crystalline nature of the pure drug. However, no sharp peaks were detected in drug-loaded microsponges, thus endorsing a complete pore confinement of ketoprofen predominantly in amorphous state.

The formulations were also stored under stressed conditions for three month, and no significant difference was observed in DSC and XRD patterns (Data not shown). This confirmed the stability of formulations over the studied time length.



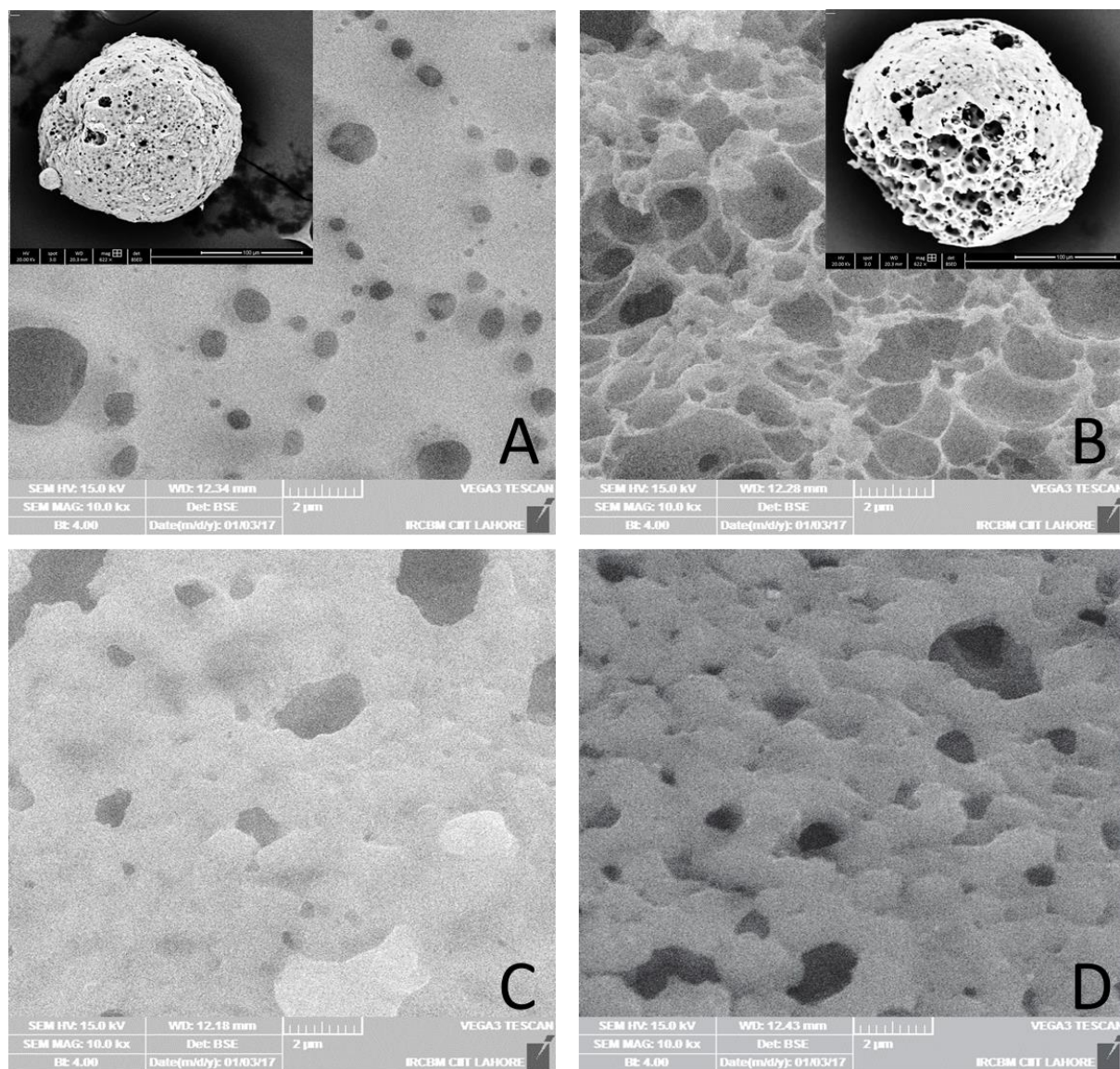
**Fig. 3.** XRD patterns of ketoprofen-loaded microsponges

Infrared spectroscopy is an important tool to investigate possible drug and polymer interactions or degradation of the drug during formulation process. The spectra of ketoprofen, EC, HPMC and drug loaded microsponges prepared with two different surfactants are presented in Fig. 4. Ketoprofen is a crystalline drug having two distinct peaks, one appearing at  $1692\text{ cm}^{-1}$  for carbonyl group in the dimeric carboxylic acid, while the other appearing at  $1650\text{ cm}^{-1}$  due to stretching vibration of carbonyl group in the ketonic group. The results obtained in this study are in line with previously reported data (Kazarian and Martirosyan, 2002; Khan et al., 2015; Manna et al., 2007). In case of microsponges, the peak shifted to higher wavenumbers ( $1692\text{ cm}^{-1}$  to  $1720\text{ cm}^{-1}$ ), which is attributed to breakage of dimeric form of ketoprofen (Manna et al., 2007). On the other hand, the attenuation of peak intensity also points out towards pore confinement of the drug, where the drug is majorly present in an amorphous form, as confirmed by the thermal and XRD analysis.



**Fig. 4.** FT-IR analysis of A) ketoprofen, B) EC, C) HPMC, D) drug-loaded microsponge prepared with Tween 80 and E) drug-loaded microsponge prepared with PVA.

The surface morphology and topography of microsponges were investigated using scanning electron microscopy and the SEM images are presented in Fig. 5. The microsponge particles were spherical in shape with highly porous surface as can be seen from the SEM images. Interestingly, microsponges prepared with PVA as surfactant had smooth surface with tiny pores or have relatively smaller pore diameter as compared with the microsponges prepared with Tween 80 as surfactant. The surface was spongier in case of Tween 80 based microsponges. The porosity was also higher in Tween 80 based microsponges as confirmed by the SEM and mercury intrusion porosimetry. The pores in the drug-loaded microsponges appeared to be filled with ketoprofen with a very few ketoprofen crystals observed visually in SEM images.



**Fig. 5.** SEM images of microsponges A) 10k-X magnification of PVA based microsp sponge, B) 10K-X magnification of Tween80 based microsp sponge, C) 10k-X magnification of PVA based drug-loaded microsp sponge and D) 10k-X magnification of Tween80 based drug-loaded microsp sponge. Left small image shows blank microsp sponge (PVA) and right small image shows blank microsp sponge (Tween80).

### 3.3. *In vitro* dissolution and kinetics of drug release

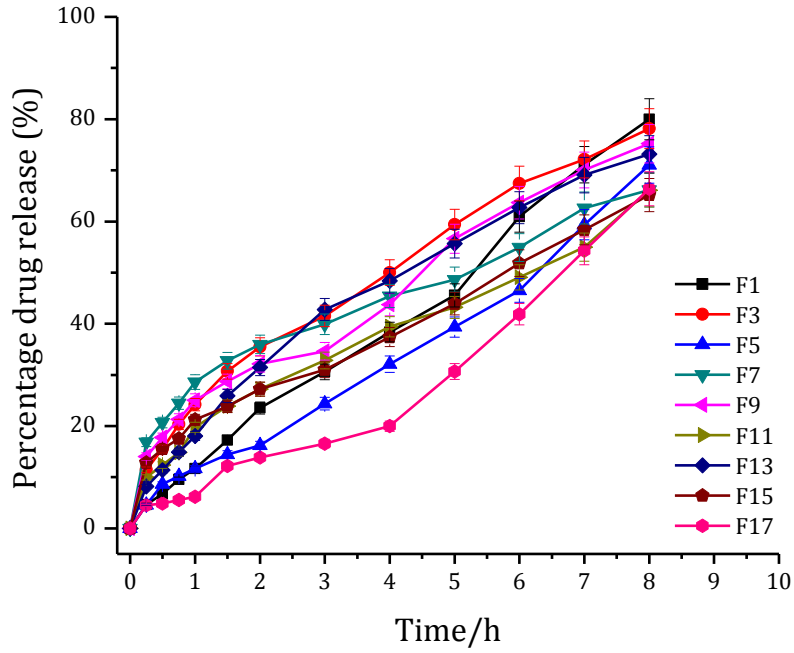
Dissolution studies were conducted with the aim to elucidate the release behavior of ketoprofen from microsponges. It is noteworthy that perfect sink conditions existed throughout the dissolution run and the concentration of ketoprofen in the dissolution medium was always lower than its solubility (Fini et al., 1995). Figures 6 and 7 represent dissolution profiles of ketoprofen-loaded microsponges prepared with two different



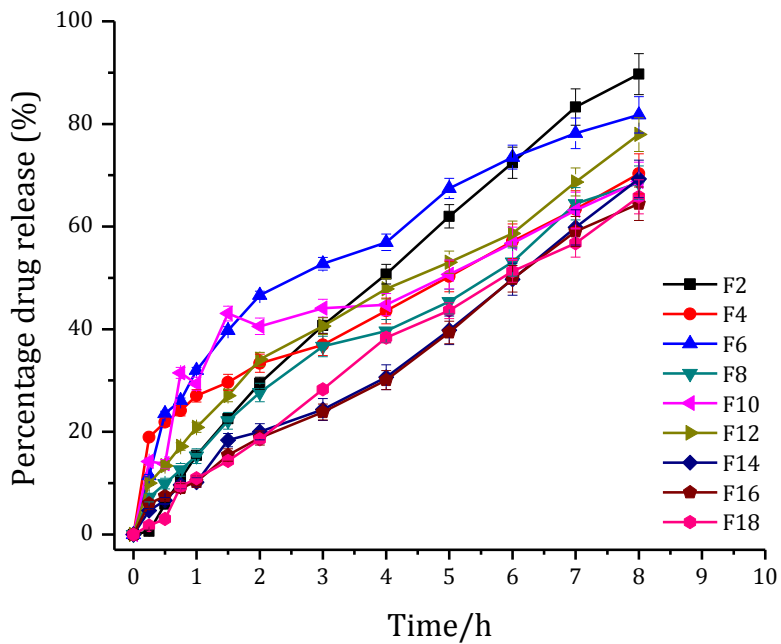
surfactants and at various polymeric ratios. It was observed that dissolution of ketoprofen was influenced by the type and concentration of surfactant as well as the polymeric ratio. During the 8h dissolution run, the cumulative ketoprofen release from microsponges was observed to be ranged from 79.98% ( $\pm$  3.99%) to 65.17% ( $\pm$  3.31%) in case of tween 80 as surfactant, whilst microsponges prepared with PVA as surfactant released ketoprofen from 89.69% ( $\pm$  4.48%) to 64.45% ( $\pm$  3.22%). It was noteworthy that no burst release was observed in all ketoprofen-loaded microsponges, rather a more precise and controlled drug release was achieved over the course of dissolution run. This also confirmed the pore confinement of the drug with less drug being adsorbed on the microsp sponge surface; thereby a more controlled drug release was observed.

In order to understand the drug release mechanism from formulated microsponges, drug release data were fitted to various kinetic models. The best-fit data in terms of  $R^2$  values are presented in Table 2, and the best-fit model selection was based on highest  $R^2$  value. For microsponges prepared with different EC to HPMC ratio and Tween 80 or PVA as surfactants, Krosmeier-Peppas model was more appropriate. In Krosmeier-Peppas model,  $n$  value is an empirical parameter characterizing the release mechanism from the matrix system (Peppas, 1985). An  $n$  value equals 0.5 refers to a Fickian diffusion controlled release mechanism, while  $n$  value equals 1 refers to a non-Fickian diffusion controlled release (case II transport), and the system approaches zero-order drug release. On the other hand, if  $n$  value is between 0.5 and 1, it is assumed that the release mechanism is governed by both Fickian diffusion and case II transport (Perrie and Rades, 2012). In our study, polymer ratio and the choice of surfactant influenced the ketoprofen release and majority of formulations released the drug by both Fickian diffusion and the case II transport because the  $n$  values were ranged from 0.45 to 0.97. This also confirmed that the porosity of microsponges is important in Fickian type diffusion process and our results are in agreement with previously reported study (Nokhodchi et al., 2007). It is noteworthy that formulation F17 (made from 10% Tween 80 at 7:3 EC to HPMC) showed an anomalous release mechanism because the  $n$  value was more than 1.





**Fig. 6.** Dissolution profile of ketoprofen-loaded microsponges prepared using Tween 80 as surfactant.



**Fig. 7.** Dissolution profile of ketoprofen-loaded microsponges prepared using PVA as a surfactant.

#### 4. Conclusion

In this manuscript, we report the first successful microsphere formulation strategies while utilizing hydrophobic and hydrophilic polymers as matrix components in the presence of two non-ionic surface active polymers (PVA and Tween 80). The resulting microspheres were thoroughly characterized and *in vitro* drug release mechanism was studied. Interestingly, altering the amount of polymers and surfactants had significant impact on efficiency and performance of the microsphere formulations. The results indicate, microsphere fabrication with EC:HPMC ratio of 9:1 in the presence of 2% Tween 80 (F1) resulted in the highest yield, maximum drug loading capacity, and sustained release via Fickian diffusion mechanism. The model studied drug (ketoprofen) is an anionic drug, success of this study has warranted the investigation of various other classes of active compounds based on charge, size and hydrophobicity and is underway with this new formulation.

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#### Conflict of interest

Declared none.

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