CHAPTER 4

DEVELOPMENT OF p (HEMA) DRUG LOADED NANOMEMBRANES AND THEIR CHARACTERISATION

4.1 INTRODUCTION

In this chapter, the production technique to develop p (HEMA) and p (HEMA) drug loaded nanomembranes using electrospinning equipment and their characterization have been reported. p (HEMA) consists of poly (2-Hydroxy ethylmethacrylate) which is a hydrogel polymer in nature. Ketoprofen has been used as drug which is loaded in to the nanomembranes.

4.2 PREPARATION OF P (HEMA) ELECTROSPINNING SOLUTION

For the preparation of p (HEMA) electrospinning solution, solvents of formic acid and ethanol have been used in 1:1 ratio. In this research work p(HEMA) solutions of four different concentrations 6 wt%, 8 wt%, 12 wt% and 13 wt% have been prepared using magnetic stirrer at 25°C celsius and the solution has been stirred for 2 hours. Since, the concentration of the solution directly influences the stirring speed, four different stirring speeds have been maintained for the above p (HEMA) solutions such as 1050 rpm for 6wt%, 850 rpm for 8wt%, 650 rpm for 12wt% and 350 rpm for 13 wt%. The stirring speed has been optimized on the basis of pellet wobbling. The p (HEMA) polymer solutions have been placed in the sonicater and sonicated for 1 hour so as to get uniform viscous solutions.
4.3 DEVELOPMENT OF P (HEMA) NANOMEMBRANES USING ELECTROSPINNING EQUIPMENT

The schematic diagram of electrospinning equipment has been shown in Figure 4.1. The positive electrode has been connected to a syringe metal tip, while the negative electrode has been connected to a rotating drum collector which has been wrapped by aluminum foil and being used for ground earthing through high voltage generator. Two milliliter of p (HEMA) spinning solution concentration of 6 wt%, 8 wt%, 12 wt% and 13 wt% have been poured into a disposable plastic syringe and it has been mounted in the equipment which is operated along with infusion pump. The infusion pump pushes the spinning solution from the syringe through syringe tip at the rate of 0.5-1.5 milliliter/hour. A voltage range of 5-15 kilovolt was applied to the syringe tip by high voltage generator. The generator helps to supply high voltage to the capillary tip.

Figure 4.1 Electro spinning equipment
The electric field induces a charge on the surface of the liquid due to the increased surface tension of the fluid solution. Mutual charge repulsion and the contraction of the surface charges to the counter electrode cause a force directly opposite to the surface tension. Further, increasing the electric field, a critical value is attained at which the repulsive electrostatic force overcomes the surface tension of the liquid, the charged jet is ejected from the tip of the jet which intend to form of the taylor cone. Meanwhile, the solvents formic acid and ethanol from the jet polymer evaporates, leaving behind a charged p (HEMA) electrospun nanomembrane. p(HEMA) electrospun nanomembrane has been collected in a rotating drum collector which was placed at a distance of 12 centimeter from the syringe tip, rotates at a speed of one thousand revolution per minute. p (HEMA) nanomembrane has been uniformly deposited on the drum due to continuous rotation. The developed p (HEMA) nanomembrane samples have been separated manually from the aluminum foil sheet.

4.4 OPTIMIZATION OF PROCESS PARAMETERS AND SOLUTION CONCENTRATION

Three important parameters such as solution parameters, process parameters and ambient parameters are influence the transformation of polymer solutions into nanomembrane through electrospinning. The solution parameters include concentration, viscosity, and surface tension. The process parameters include solution flow rate, high voltage at the capillary tip, and the distance between the tip and the collector. The ambient parameters include such as solution temperature, humidity, and air velocity in the electrospinning chamber. Amongst these above many parameters, polymer solution concentration and process parameters have the most significant influence in the electrospinning process and the resultant fiber diameter and morphology. The resultant fiber diameter and fibre morphology achieved in
electrospinning determines several properties of the electrospun nanomembrane. Hence, parameters such as solution concentration in the range of 6 – 13 wt%, and process parameters such as high voltage of 5 – 15 kilovolt and tip to collector distance of 8 – 12 centimeter and flow rate of 0.5 – 1.5 millilitre/hour have been tried for field trials to produce the p(HEMA) nanomembrane. Beyond the above mentioned range of the process and polymer solution parameters, more droplets have been seen from the syringe tip and this is not an ideal condition for the electrospinning process.

Optimisation of process parameter and polymer solution concentration is an important one since this will significantly affect the electrospinnability and the fibre formation capability. The influence of solution concentration and process parameters on the fibre formation ability for the production of p(HEMA) nanomembrane were shown in Table 4.1

From the Table 4.1 it was observed that, the polymer solution concentration has maximum influence on the fibre formation capability. At 6 and 13 wt% of p(HEMA) solution concentration more number of beads and powder like structure could be found on the nanomembrane morphology even at higher range of process parameters. In case of 8 wt% solution concentration and highest process parameters breaking fibres are formed. The uniform fibre formation could be obtained at 12 wt% solution concentration. Hence, at 15 kilovolt power supply, 1.5 millilitre/hour flow rate and tip to collector distance of 12 centimeter and 12 wt% solution concentration have been optimised for the production of p(HEMA) nanomembranes.
Table 4.1  Influence of p (HEMA) polymer solution concentration and electrospinning process parameters on fibre formation ability

<table>
<thead>
<tr>
<th>S.No</th>
<th>Polymer Solution Concentration (wt%)</th>
<th>Flow rate (milliliter/hour)</th>
<th>Voltage (kilovolt)</th>
<th>Tip to collector distance in cm</th>
<th>Fibre formation capability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>6</td>
<td>0.5</td>
<td>5</td>
<td>8</td>
<td>Fibres are not formed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>10</td>
<td>10</td>
<td>Beads only formed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>15</td>
<td>12</td>
<td>Beads on fibres</td>
</tr>
<tr>
<td>2.</td>
<td>8</td>
<td>0.5</td>
<td>5</td>
<td>8</td>
<td>Fibres are not formed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>10</td>
<td>10</td>
<td>Beads on fibres</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>15</td>
<td>12</td>
<td>Fibres are formed with breaks</td>
</tr>
<tr>
<td>3.</td>
<td>12</td>
<td>0.5</td>
<td>5</td>
<td>8</td>
<td>No fibre formation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>10</td>
<td>10</td>
<td>Beads on fibres</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>15</td>
<td>12</td>
<td>Uniform fibre formation</td>
</tr>
<tr>
<td>4.</td>
<td>13</td>
<td>0.5</td>
<td>5</td>
<td>8</td>
<td>Fibres are not formed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>10</td>
<td>10</td>
<td>Powder like structure are formed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>15</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

4.5 DEVELOPMENT OF P (HEMA) DRUG LOADED NANOMEMBRANES

For the preparation of drug loaded electrospinning solution, the solvent of distilled water has been used. 0.5 gram of ketoprofen drug was dissolved in distilled water. The Ketoprofen drug solutions were prepared under constant stirring using magnetic stirrer in a room temperature for 1 hour. The Ketoprofen drug solution was sonicated for one hour and it was found uniform after the sonication. The ketoprofen drug solution was
incorporated in optimised solution concentration of 12 wt%. The p (HEMA) drug loaded solution has been prepared in 50:50 ratio. In the second phase of this work, p (HEMA) ketoprofen drug loaded solution was subjected to electrospinning process at optimized process parameters (15kv, 12cm and 1.5 millilitre/ hour) for the production of p (HEMA) drug loaded nanomembrane.

4.6 RESULTS AND DISCUSSION

To assess the characteristic of the above developed p (HEMA) drug free and drug loaded nanomembrane, the various test methods such as morphological study, chemical structure analysis, drug release study, swelling capacity and anti bacterial activity were tested.

4.6.1 Morphological Structure

Electrospun drug free p(HEMA) nanomembrane characteristics is influenced by various process parameters such as the applied voltage, solution flow rate, distance between capillary tip to collector and especially the properties of the polymer solutions including solution concentration, surface tension and the nature of the solvents. Amongst these parameters, the solution concentration is one of the important factor which has the maximum effect on the process of fibre formation and the average diameter. Hence, the influence of polymer solution concentration of 6, 8, 12 and 13 wt% on p (HEMA) nanomembranes morphology characteristics were studied through scanning electron microscope (SEM) and results were discussed.

It was observed from the Figure 4.2, at 6 wt% p (HEMA) solution concentration, higher beads are found in nanomembrane. At a lower solution concentration, the higher amount of solvent molecules and fewer chain entanglements will mean that surface tension has a dominant influence along the electrospinning jet causing beads to form along the fiber. p (HEMA)
nanomembranes produced with 8 wt% concentration fibres are formed with breaking on the nanomembrane surface. At 12 wt% solution concentration, fibres are formed uniform due to higher amount of polymer chains entanglement in the solution, the charges on the electrospinning jet will be able to fully stretch the solution with the solvent molecules distributed among the polymer chains. More number of beads have been found in the p (HEMA) nanomembrane produced with 13 wt% concentration this is due to higher concentration which make it very difficult to pump the solution through the syringe tip. Moreover, when the solution concentration is too high, the solution dries at the tip of the needle before electrospinning can be initiated.

Figure 4.2 Influence of p (HEMA) polymer concentration on morphological structure of nanomembranes a) 6 wt% b) 8 wt% c) 12 wt% d) 13 wt%
One of the most important quantities related with electrospinning is the fiber diameter. The p(HEMA) polymer solution concentration has significant influence on the average fibre diameter. One of the most significant parameters influencing the fiber diameter is the solution concentration.

The average fibre diameter of p (HEMA) nanomembranes produced with 6, 8 and 12 wt% were shown in Figure 4.3 since at 13 wt% concentration more number of beads were formed on the nanomembrane morphology, it is difficult to measure the average fibre diameter. It was observed from the Figure 4.3 that, when the p (HEMA) polymer solution concentration was increased from 6% to 12 %, the average fibre diameter of nanomembranes has been increased proportionately from 150 nm to 400 nm. There is no significant difference in average fibre diameter of p (HEMA) nanomembranes at 8% and 12 wt% polymer concentration where as at 6 wt% and 8 wt% nominal difference has been noticed. Hence the average fibre diameter increases proportionately with the increases in the polymer solution concentration. This is due to the greater resistance of the solution to be stretched by the charges on the polymer solution jet. Hence, the concentration of polymer solution plays a vital role on morphology and average fibre diameter of nanomembranes.
The morphological characteristics of drug free p (HEMA) nanomembranes and drug loaded nanomembranes produced from the 12 wt% polymer solution have been compared as shown in Figure 4.4.

![SEM morphologies of (a) Drug-free and (b) Drug-loaded electrospun 12 wt% concentration of p (HEMA) nanomembrane.](image)

Both drug-free and drug-loaded p (HEMA) nanomembranes appeared smooth surface and round shape. The drug crystals were detected on the p(HEMA) drug loaded nanomembrane surface. The SEM photographs show that ketoprofen loaded nanomembrane was dispersed homogeneously in the nanomembrane. It was clear that, the drug in the p(HEMA) solutions affect the morphology of the drug loaded by increased diameter of the drug loaded nanomembrane. However with the incorporation of drug in the sample the average diameter of the fiber shifted from 400nm to 600nm.

### 4.6.2 Chemical group analysis

Figure 4.5 shows that, the FT - IR spectra of p (HEMA) nanomembrane produced with three different concentrations (6, 8 and 12 wt %) and p(HEMA) with ketaprofen drug loaded nanomembrane. The FT-IR spectrum of the p(HEMA) electro-spun nanomembrane showed that all the
peaks corresponding to p(HEMA). S1 shows carbonyl absorption in alkyl aldehydes is 1740 [cm\(^{-1}\)] - 1730 [cm\(^{-1}\)].

![FTIR spectra of p(HEMA) nanomembranes and p(HEMA) nanomembrane loaded with Ketoprofen drug](image)

Figure 4.5 The FTIR spectra of p(HEMA) nanomembranes and p(HEMA) nanomembrane loaded with Ketoprofen drug (S1 – 6 wt%, S2 – 8 wt%, S3 – 12 wt% and S4 – Drug loaded nanomembrane)

Aldehydes conjugated with aromatic rings absorb in the 1710-1690 [cm\(^{-1}\)] region. The aliphatic C-H stretching vibrations are highly characteristic and occur between 2900 and 2700 [cm\(^{-1}\)]. S2 & S3 nanomebranes show the CH\(_3\) asymmetric stretching vibration occurs at 2975 – 2950 [cm\(^{-1}\)] while the CH\(_2\) absorption occurs at about 2930 [cm\(^{-1}\)]. The symmetric CH\(_3\) vibration occurs at 2885-2865[cm\(^{-1}\)] while the CH\(_2\) absorption occurs at about 2870 – 2840 [cm\(^{-1}\)]. The observed broad and strong band around 1725 – 1700 [cm\(^{-1}\)] was attributed to C=O stretching vibrations. The appearance (S4) of new peaks along with changes in existing peaks (and/or their disappearance) in IR spectra of drug loaded complex is a direct indication of the complexation of p (HEMA)/ ketoprofen drug.
4.6.3 In-vitro Drug Release

The determination of the release characteristics of the ketaprofen from drug loaded p (HEMA) nanomembrane was carried out by the total immersion method. Acetate buffer was chosen to stimulate human skin pH condition of 4.5. The experiment was carried out in acetate buffer with varying pH (1.2, 4.5, and 7.5) at a temperature of 37°C.

Figure 4.6 Drug release profile of ketaprofen drug
Figure 4.7 Drug release profile of p (HEMA) drug loaded nanomembrane

The cumulative release of pure ketaprofen drug in three different pH medium, pH 1.2, 4.5, and 7.5, were shown in Figure 4.6. Pure drug ketaprofen dissolution was measured spectrophotometrically. The drug release was completed approximately after 20 minutes in pH 7.5 medium. On the other hand, drug release of 78% was observed in pH 4.5 and 54% was measured for pH 1.2. The drug dissolution was faster in neutral medium than acidic medium.

The cumulative release of ketaprofen drug from p (HEMA) nanomembrane in different pH medium was shown in Figure 4.7. All the cumulative results were given for 180 minutes solute release time. The drug release from p (HEMA) nanomembrane follows the same release trend as in the previous dissolution study. It was observed that the drug release was completed about 87% in pH 7.5. The second release was monitored in pH 4.5 and the last ketaprofen drug release was measured in high acidic media which is pH 1.2. The cumulative release occurred as 72% in pH 4.5 and 54% in pH 1.2.
4.6.4 Swelling Capacity

The swelling capacity of the nanomembrane plays an important role in the antibacterial activity, wound healing capacity, and for biomedical application due to their high water/solvent holding capacity. Drug can further absorb a slight to moderate amount of the wound exudates by swelling which helps in fast healing of the wound.

Figure 4.8 shows the degree of swelling (%) drug-loaded p(HEMA) nanomembrane and p(HEMA) drug loaded cast film after immersion in an acetate buffer solution at 37 °C for 24 hours. The degree of swelling of the p(HEMA) nanomembrane was greater than that of the p(HEMA) drug loaded cast film due to the result of the highly porous nature of the electro-spun nanomembrane.

![Figure 4.8 Degree of swelling (%) drug-loaded p(HEMA) nanomembrane and p(HEMA) drug loaded cast film](image)

4.6.5 Antibacterial Activity

The antibacterial activity of ketaprofen drug-loaded p(HEMA) nanomembrane was assessed against typical pathogenic bacteria P. aeruginosa MTCC 2297, S. aureus ATCC 933 and E. coli (IP-406006) to evaluate their wound dressing application potentials. The activity of drug-free p(HEMA)
nanomembrane against these bacteria was used as control. As reported in Fig 4.8, after 24 hours of contact intervals, drug loaded specimen gave a 30 mm diameter zone for *Pseudomonas aeruginosa*, a 24 mm diameter zone for *Staphylococcus aureus* and a 28 mm diameter zone for *Escherichia coli* (the diameter of specimen is 15 mm), respectively.

![Image](a) (b) (c)

**Figure 4.9** *In-vitro* testing of drug loaded p(HEMA) electro spun nanomembrane for antimicrobial activity against:
- a) *Pseudomonas aeruginosa*,
- b) *S. aureus* and
- c) *E. coli*

### 4.7 CONCLUSIONS

p (HEMA) nanomembranes of five different samples were developed using electrospinning equipment with different process parameters and solution parameters. The process parameters and solution parameters used for the production of p (HEMA) electrospun nanomembrane were optimized. The optimized parameters for the production of p (HEMA) nanomembranes are solution concentration 12 wt%, applied voltage 15Kv and solution flowrate 1.5 millilitre/hour. Ketoprofen drug loaded p (HEMA) nanomembrane has been developed using the optimized parameters. Characteristics of p (HEMA) drug free and drug loaded nanomembrane were evaluated by the various test methods such as morphological study, chemical structure analysis, drug release study, swelling capacity and anti bacterial activity.
From the morphology study, it was observed that, the polymer solution concentration play a vital role in the fibre formation ability and average fibre diameter. As the concentration of the p(HEMA) solution increases the fibre formation ability also increases, and also the fibre diameter of the nanomembrane samples also increased. The SEM photographs confirms that ketoprofen loaded nanomembrane was dispersed homogeneously in the nanomembrane. From the FT-IR spectra study it was found that all the chemical structure were present in the drug free and drug loaded nanomembrane. Antibacterial activity study shows that drug loaded nanomembrane retained its biological functionality even after it has been subjected to a high electrical voltage. The cumulative release of ketoprofen drug was higher in acidic medium than alkali medium. This nanomembranes show higher swelling capacity than cast film. Hence, the p(HEMA) drug loaded electrospun nanomembrane have the great potential in drug delivery.