

## IN-VITRO AND IN-VIVO EVALUATION OF MUCOADHESIVE PATCHES CONTAINING METOPROLOL SUCCINATE

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### ABSTRACT

Mucoadhesive Buccal patches of metoprolol Succinate were prepared by solvent casting method using chitosan, polyvinyl alcohol (PVA) and hydroxyethyl cellulose (HEC). Mucoadhesive properties and swelling index were determined for both plain and drug loaded patches. The results showed a remarkable increase in radial swelling after addition of drug to the plain patches. A decrease in the residence time was observed for PVA and Chitosan patches. A considerable decrease in release was observed for chitosan patches after the addition of water soluble excipient polyvinyl pyrrolidone (PVP). The *in-vivo* studies carried out on rabbits and evaluate % inhibition of Isoprenaline induced tachycardia. Ageing was done on the patches and the results showed there was no influence on the chemical stability of metoprolol, as reflected from the drug content result. Physical characteristics of the studied patches showed an increase in the residence time with storage accompanied with a decrease in drug release.

**Keywords:** Ageing, Buccal patches, Drug release, Metoprolol Succinate, Mucoadhesion.

### INTRODUCTION

Buccal mucosa is an attractive route for systemic delivery of drugs since it is relatively permeable with a rich blood supply. A drug can be easily applied and localized to the application site and can be removed from there if necessary<sup>1</sup>. Attempt has been made earlier to formulate various Mucoadhesive buccal devices, including tablets, films, patches, disks gels and ointments. Buccal patches are highly flexible and thus much more readily tolerated by the patient than tablets<sup>2</sup>. Patches also ensure more accurate dosing of the drug compared to gel and ointment. In the present study, the natural bioadhesive polymer chitosan was used for the preparation of buccal patches<sup>3,4</sup>. Chitosan is the N-deacetylated product of the polysaccharides chitin. Chitosan is an important ingredient in the pharmaceutical field due to its good biocompatibility, non-toxicity and its biodegradability<sup>5</sup>. Metoprolol Succinate is a  $\beta$ -adrenergic blocking agent, has been widely used in the treatment of hypertension, angina pectoris, and many others cardiovascular disorders<sup>6,7,8</sup>.

In this study, we attempted to formulate Mucoadhesive patches, which would release the drug in a sustained manner using chitosan, PVA and HEC as polymer. In addition, the effect of ageing on the Mucoadhesive characteristic and the *in vitro* release pattern of a selected patch were investigated.

### MATERIAL AND METHOD

#### Material

Metoprolol Succinate (Aarti drugs Pvt Ltd. Mumbai), Chitosan (Sigma Aldrich, Mumbai), polyvinyl alcohol (Qualigens fine chemicals, Mumbai), hydroxyethyl cellulose (Qualigens fine chemicals, Mumbai), poly vinyl pyrrolidone (Qualigens fine chemicals, Mumbai) were used. Other chemicals were of analytical grade.

#### Preparation of Mucoadhesive buccal patches

For chitosan patches 2% (m/V) chitosan was dissolved in 1.5% (V/V) acetic acid under constant stirring for 24 h. To improve elastic and film forming properties of the patches, PVP (1% m/V) was added. PVP was first dissolved in a small volume of distilled water, and then added to the prepared solution<sup>9</sup>.

For PVA patches 10% (m/V) was dissolved in hot water at 80 to 100°C and stirred for 12 h. For HEC patches, 1.5% (m/V) was dispersed in purified water under constant stirring. Glycerol as Plasticizer 5% (V/V) was added in all formulations. The resultant viscous solution was filtered and filtrate was left to stand until all air bubbles disappeared. The solution was poured into a clean, dry, glass petri dish and left to dry at room temperature. The dried films

were carefully removed from the petri dish, checked for any imperfection or bubbles and cut into 10mm diameter patches. The samples were packed in aluminium foil and stored in a glass container maintained at room temperature<sup>10</sup>.

Patches containing metoprolol succinate were prepared by dissolving the calculated amount of drug in 20 ml distilled water. The drug solution was added to the polymer solution under stirring (Table-1).

**Table 1: Composition of mucoadhesive buccal patches containing 2% (m/V) metoprolol Succinate.**

| Composition      | PVA | HEC | C-1 | C-2 |
|------------------|-----|-----|-----|-----|
| PVA (% m/V)      | 10  | -   | -   | -   |
| HEC (% m/V)      | -   | 1.5 | -   | -   |
| Chitosan (% m/V) | -   | -   | 2   | 2   |
| PVP (% m/V)      | -   | -   | -   | 1   |

### EVALUATION OF PATCHES

#### Mass uniformity and patch thickness

Measurement of mass and thickness was done on ten patches. The mean and standard deviation were calculated<sup>11</sup>.

#### Surface pH

Patches were left to swell for 1 h on the surface of agar plate, prepared by dissolving 2% (m/V) agar in warmed phosphate buffer of pH 6.8 under stirring and then set a side till gelling at room temperature. The surface pH was measured by means of a pH paper placed on the surface of the swollen patch. The mean of three readings was recorded<sup>12</sup>.

#### Folding endurance

Folding endurance was determined by repeatedly folding the patch at the same place till it broken or folded up to 300 times, which is considered to reveal good film properties. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance<sup>13</sup>.

#### Radial Swelling

Radial swelling was determined by diameter method. After determination of the original patch diameter, the patch was allowed to swell on the surface of an agar plate kept in an incubator maintained at 37°C. Measurement of the diameter of the swollen patch was done at one hour intervals for 6 h. Radial swelling was calculated from the following equation:

$$S_D (\%) = [(Dt - Do) / Do] \times 100$$

Where  $S_D$  (%) is the percent swelling,  $D_t$  is the diameter of the swollen patch after time  $t$ , and  $D_o$  is the original diameter of the patch at time zero<sup>14</sup>.

#### Residence time

The *in vitro* residence time was determined by a locally modified USP disintegration apparatus using phosphate buffer of pH 6.8 maintained at 37° C as medium. A segment of rabbit intestinal mucosa was glued to the surface of glass slab, vertically attached to the apparatus. The buccal patch was hydrated from one surface using 10  $\mu$ L isotonic phosphate buffer and then hydrated surface was brought into contact with the mucosal membrane. The glass slab was allowed to move up and down and then the time necessary for complete erosion or detachment of the patch from the mucosal surface was recorded<sup>15</sup>. (Mean of triplicate was determined).

#### Bioadhesion force

The tensile strength required to detach the bioadhesion patch from the mucosal surface was applied as a measure of the bioadhesion performance. The apparatus was locally assembled and mainly composed of two-arm balance. The left arm of the balance was replaced by a small platinum lamina vertically suspended through a wire. At the same side, a movable platform was maintained in the bottom in order to fix the mucosal membrane. For determination of bioadhesion force, the mucoadhesive patch was fixed to the platinum lamina using cyanoacrylate adhesive. A piece of rabbit intestinal mucosa was also glued to the platform. The patch surface was moistened with 10  $\mu$ L of phosphate buffer and left for 20 s for initial hydration. On the right pan, a constant weight of 5 g was added at 2 min interval, until the hydrated patch was brought into contact with the mucosal surface. The total weight required for complete detachment of the patch was recorded and the bioadhesion force was calculated per unit area of the patch as follows:

$$F = (W_w \times g) / A$$

where  $F$  is the bioadhesion force ( $\text{kg m}^{-1} \text{S}^{-2}$ ),  $W_w$  is the mass applied (g),  $g$  is the acceleration due to gravity ( $\text{cm s}^{-2}$ ),  $A$  is the surface of the patch ( $\text{cm}^2$ ). The bioadhesion force data reported represent the mean of three determinations<sup>16</sup>.

#### Content uniformity

The drug loaded patch was allowed to dissolved in 100mL phosphate buffer, pH 6.8 The amount of metoprolol succinate in the patch was measured spectrophotometrically at  $\lambda_{\text{max}}$  of 222 nm ( $n = 5$ ).

#### In vitro release study

The release study was carried out in a USP dissolution apparatus type 1, slightly modified in order to overcome the small volume of the dissolution medium. The dissolution medium was 50mL phosphate buffer, pH 6.8, maintained at 37± 0.5 °C and kept in a glass beaker fixed inside the dissolution flask. The patch was fixed to the central axis, which rotates at 50 rpm. Filtered samples (2 mL) were manually collected at intervals of 1,2,3,4,5,6,7 and 8 h. The samples were compensated with equal volume of phosphate buffer kept at the same temperature. The concentration of the release in the medium was assayed spectrophotometrically at 222 nm after suitable dilution with the dissolution medium when necessary<sup>17</sup>. The experiment was carried out in triplicate.

## PHARMACODYNAMIC STUDY

### Preparation of Animals

Healthy albino rabbits of either sex (2.0-2.5 kg) were selected for the study. Institution's animal ethics committee (IAEC) of IFTM Moradabad, U.P., India, permission was obtained prior to start the study. (Approval No.: 3/837ac/PH/10). Rabbits were anaesthetised by intraperitoneal administration of 30mg/kg of pentobarbitone sodium in sterile normal saline. Electrocardiograph electrodes were set subcutaneously. Lead I or Lead II was used for recording ECG on a physiograph. The chart speed was kept at 5mm/sec. Heart rate was determined by counting the "R-waves" of the ECG.

### Administration of Metoprolol Succinate (Intravenous, Oral, Buccal Patch)

Normal heart rate of the rabbit was recorded before administration of isoprenaline. Standard dose of isoprenaline (0.25 $\mu$ g/kg) were given at interval of 30 min and hear rate was recorded. Metoprolol Succinate in sterile saline at a dose of 200  $\mu$ g/kg (i.v.), 2 mg/kg (orally) and buccal patch was administered. Isoprenaline (0.25  $\mu$ g/kg i.v.) doses were administered during the study. The difference in heart rate before and after each isoprenaline injection was determined<sup>18</sup>. The experiment was performed in triplicate.

### Analysis of % inhibition of Isoprenaline induced tachycardia

The percentage inhibition of isoprenaline induced tachycardia was calculated by:

$$\% \text{ inhibition} = (HR_0 - HR / HR_0) \times 100$$

Where  $HR_0$  was number of heart beats increased by isoprenaline before Metoprolol Succinate administration and  $HR$  the number of heart beats increased by isoprenaline after Metoprolol Succinate administration.

### Ageing

Optimized drug loaded patches were subjected to accelerated stability testing. Patches were kept in an incubator maintained at 37± 0.5 °C and 75± 0.5 RH for 4 months. Changes in the appearance, residence time, release behavior and drug content of the stored buccal patches were investigated after 1, 2, 3 and 6 months. The data presented were the mean of three determinations<sup>19</sup>.

## RESULT

Physical Characteristics of plain patches containing individual polymer are shown in Table 2.

The patches were 10 mm in diameter, and 0.53 to 0.722 mm in thickness. The mass ranged from 79 to 210 mg. The surface pH of all formulations was nearer to neutral and hence no mucosal irritation was expected. The recorded folding endurance of the patch was > 300 times. Assessment of the swelling behavior was done by measuring radial swelling. HEC patches showed high radial swelling, followed by PVA and then chitosan ones; the recorded swelling values after 6 h were 40.82, 21.43 and 2.02 % respectively. For *in vitro* residence time, all patches, except chitosan, remained attached to the mucosal surface till complete erosion. PVA patches showed convenient duration for complete erosion (4.5 h), longer duration was recorded for HEC (9.5 h). Chitosan patches retained their integrity during the study time (12 h) without detachment. Maximum bioadhesion was recorded for PVA (482.70 x 10<sup>2</sup> kg m<sup>-1</sup>s<sup>-2</sup>), followed by the chitosan (76.82 x 10<sup>2</sup> kg m<sup>-1</sup>s<sup>-2</sup>), then HEC (53.34 x 10<sup>2</sup> kg m<sup>-1</sup>s<sup>-2</sup>).

**Table 2: Characteristics of plain mucoadhesive patches containing different concentration of Polymers.**

| Characteristics  | PVA         | HEC        | C-1        |
|--|-------------|------------|------------|
| Patch thickness (mm)   | 0.722±0.102 | 0.53±0.194 | 0.402±0.31 |
| Patch mass (mg)  | 210±0.24    | 155±0.04   | 79±0.08    |
| Surface pH   | ≈ 7         | ≈ 7        | ≈ 7        |
| Folding endurance  | > 300       | > 300      | > 300      |
| Radial swelling ( 6 h)   | 21.43±2.323 | 40.82±1.94 | 2.02±0.522 |
| Residence time (h)   | 4.5±0.201   | 9.5±0.411  | 12±0.202   |
| Bioadhesive force (x10 <sup>2</sup> ,kgm <sup>-1</sup> s <sup>-2</sup> ) | 482.70±4.2  | 53.34±0.7  | 76.82±0.72 |

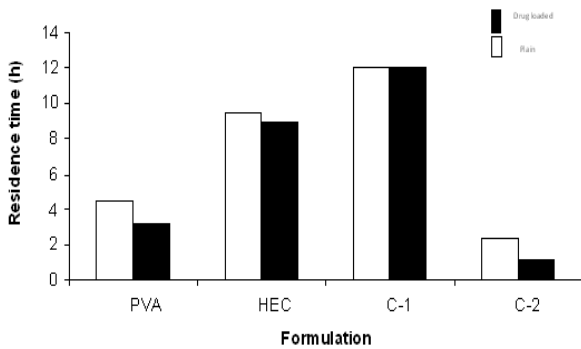
Properties of the drug loaded patches are shown in the Table 3. The patches were 10 mm in diameter, and 0.87 to 1.04 mm in thickness. The mass ranged from 95 to 132 mg. The patches were characterized by convenient surface pH, good film properties and remarkable radial swelling. Maximum swelling was shown by HEC;

the diameter progressed with time till a 68% increase after 6 h. PVA patch enlarge radially by 27% and chitosan containing patches exhibited relatively a lower increase in diameter within 6 h (10.2%, 17% and 24% for C<sub>1</sub> and C<sub>2</sub> respectively). The presence of PVP in chitosan patches seemed to increase the surface wettability and swelling of the patches.

**Table 3: Characteristics of mucoadhesive patch containing 2% (m/V) metoprolol Succinate.**

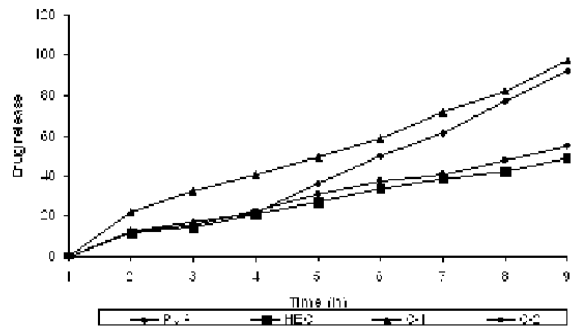
| Characteristics       | PVA       | HEC        | C-1        | C-2        |
|-----------------------|-----------|------------|------------|------------|
| Patch thickness (mm)  | 0.98±0.05 | 1.02±0.04  | 0.87±0.05  | 1.04±0.04  |
| Patch mass (mg)       | 95±0.19   | 132±0.51   | 97±0.89    | 112±0.19   |
| Surface pH            | ≈ 7       | ≈ 7        | ≈ 7        | ≈ 7        |
| Folding endurance     | > 300     | > 300      | > 300      | > 300      |
| Radial swelling (6 h) | 27.0±0.5  | 68.0±1.98  | 10.2±0.05  | 17±0.11    |
| Residence time (h)    | 3.2±0.88  | 9.0±0.12   | 12±0.0.82  | 1.1±0.52   |
| % Release after 1 h   | 12.5±0.22 | 11.76±1.98 | 22.42±1.21 | 12.42±0.42 |
| after 8 h             | 92.45±1.2 | 48.88±3.7  | 97.11±2.8  | 55.0±1.3   |

Values of the residence time differed from one polymer to the other. PVA and HEC patches resided on the surface until complete erosion after 3.2 and 9 h, respectively. C<sub>1</sub> patches remained to the surface during the time of the study (12 h) without erosion. However, the addition of PVP to chitosan caused patch dislodgement. The presence of metoprolol succinate, a water soluble drug, affected the residence time of the patch (Fig. 1).



**Fig. 1: The residence time of plain and drug loaded mucoadhesive patches.**

The release profile of metoprolol succinate patches is shown in Fig. 2. The extent of metoprolol succinate release within 1h from PVA and HEC patches was 12.5 and 11.76% respectively. In time, a marked rise in the release rate from PVA patches was observed compared to HEC patches; 50% metoprolol succinate was release within 4.2 h in the case of PVA patches compared to 8 h in case of HEC patches.



**Fig. 2: The release profile of metoprolol succinate**

Results in Table 2 reveal acceptable swelling and residence time for PVA patches. These characteristics make them good candidate for stability studies. PVA patches containing 10 mg metoprolol succinate were subjected to 6-months storage at 37± 0.5 °C and 75± 0.5 RH. Patches exhibit excellent drug content over the storage period. The folding endurance test revealed good flexibility and elastic properties. The effect of i.v., oral and buccal patch of metoprolol succinate on the isoprenaline induced tachycardia in albino rabbits was observed and found that metoprolol succinate at a dose of 200 µg/kg i.v., 2 mg/kg orally and buccal patch, produced a maximum of 74.02 ± 5.21 (5 min), 89.54 ± 1.24 (15 min) and 35.87 ± 3.21 (60 min) percent inhibition of isoprenaline induced tachycardia respectively. The inhibitory effect was gradually decreased and at the end of 8 hours, the effect was 7.8 ± 1.41, 41.25 ± 1.32 and 27.45 ± 0.51 for i.v., oral and buccal patch respectively. However, a delay in the residence time of the storage patches was noticed (Table 4).

**Table 4: Stability data of PVA patches, stored at 37± 0.5 °C and 75± 0.5 RH.**

| Characteristics    | Duration of storage (months) |           |            |            |            |            |            |
|--------------------|------------------------------|-----------|------------|------------|------------|------------|------------|
|                    | 0                            | 1         | 2          | 3          | 4          | 5          | 6          |
| Residence time (h) | 1.8±0.4                      | 1.8±0.6   | 2.0±0.11   | 2.3±0.3    | 2.6±1.2    | 2.8±0.9    | 3.1±0.71   |
| % Release          |                              |           |            |            |            |            |            |
| after 1 h          | 14.7±2.1                     | 11.62±1.1 | 11.54±0.35 | 11.01±0.07 | 10.87±2.1  | 10.21±0.66 | 9.81±0.53  |
| after 8 h          | 92.45±1.2                    | 90.89±1.2 | 87.24±0.24 | 82.2±0.87  | 78.78±0.98 | 75.67±0.87 | 72.53±1.01 |

The percent metoprolol released versus time demonstrates a decrease in the amount of drug released with time. Non-storage patches released 92.45% after 8 h, whereas patches stored for 6 months released 72.53% drug in the same period.

## DISCUSSION

No correlation was found between the bioadhesion force and residence time of the polymer. It seems that highly bioadhesive polymers do not necessarily reside longer on the mucosal surface. Surface charge density and chain flexibility are considered to be prerequisites for bioadhesion, whereas the residence time is primarily dependent on the dissolution rate of the polymer.

In the patches containing metoprolol succinate radial swelling was more as compare to the plain patches because the presence of drug would modify the way water is bound to or taken up by the polymer. In addition, the presence of a water soluble drug might improve the surface wetting of the matrix.

Relatively high swelling of HEC increased the gel layer thickness and consequently the diffusion path length, which in turn may be the cause of the slower drug release from HEC patches compared to PVA patches.

The decrease in release during storage may be a direct consequence of the reduced erosion rate of the patches.

**CONCLUSION**

It may be concluded that mucoadhesive patches are a promising drug delivery system for metoprolol succinate in maintaining drug level in blood. The non-ionic polymer, PVA, showed good mucoadhesive and swelling characteristics. Medicated PVA patches maintained a satisfactory residence time in the buccal cavity and released the drug for 8 h, which made them good candidate for stability studies. Ageing did not affect the elastic properties of the PVA patches but affected the extent of drug release; this may be attributed to changes in the crystal habit of the drug.

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