DEVELOPMENT AND EVALUATION OF EXTENDED RELEASE MATRIX TABLETS OF RISPERIDONE, OLANZAPINE AND PROCHLORPERAZINE MALEATE (ANTIPSYCHOTIC DRUGS)

PhD Thesis

BY
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DEPARTMENT OF PHARMACY
UNIVERSITY OF PESHAWAR
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A thesis submitted to the department of Pharmacy, University of Peshawar in partial fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) in Pharmacy

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UNIVERSITY OF PESHAWAR
June, 2010
DEDICATION

To my father late Haji Tabar Ali Shah and my mother late Nawaba Bibi, whose foresight, love, zeal to educate and moral support have brought me to the position where I am.
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>List of Tables ..................................................................................</td>
<td>i</td>
</tr>
<tr>
<td></td>
<td>List of Figures ................................................................................</td>
<td>iii</td>
</tr>
<tr>
<td></td>
<td>List of Abbreviations .....................................................................</td>
<td>vii</td>
</tr>
<tr>
<td></td>
<td>Acknowledgments .............................................................................</td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td>Abstract .........................................................................................</td>
<td>ix</td>
</tr>
<tr>
<td>Chapter 1:</td>
<td>General Introduction .........................................................................</td>
<td>1</td>
</tr>
<tr>
<td>1.1</td>
<td>Drug delivery systems .......................................................................</td>
<td>1</td>
</tr>
<tr>
<td>1.1.1</td>
<td>Extended release matrix systems ..................................................</td>
<td>1</td>
</tr>
<tr>
<td>1.1.2</td>
<td>Tablets technology .........................................................................</td>
<td>3</td>
</tr>
<tr>
<td>1.1.3</td>
<td>Granulation/size enlargement .....................................................</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Drug dissolution and release mechanisms ......................................</td>
<td>4</td>
</tr>
<tr>
<td>1.3</td>
<td>Model polymers used for extending drug release period .................</td>
<td>6</td>
</tr>
<tr>
<td>1.3.1</td>
<td>Hydroxypropylmethylcellulose ....................................................</td>
<td>6</td>
</tr>
<tr>
<td>1.3.2</td>
<td>Ethylcellulose (Ethocel) ................................................................</td>
<td>7</td>
</tr>
<tr>
<td>1.3.3</td>
<td>Combination of other polymers with HPMC .....................................</td>
<td>8</td>
</tr>
<tr>
<td>1.4</td>
<td>Other factors affecting drug release ..........................................</td>
<td>8</td>
</tr>
<tr>
<td>1.4.1</td>
<td>Polymer: Drug Ratio ........................................................................</td>
<td>8</td>
</tr>
<tr>
<td>1.4.2</td>
<td>Drug solubility ..............................................................................</td>
<td>8</td>
</tr>
<tr>
<td>1.4.3</td>
<td>Drug particle size ..........................................................................</td>
<td>9</td>
</tr>
<tr>
<td>1.4.4</td>
<td>Process variables affecting drug release ....................................</td>
<td>9</td>
</tr>
<tr>
<td>1.4.4.1</td>
<td>Compression force/hardness of tablets .......................................</td>
<td>9</td>
</tr>
<tr>
<td>1.4.4.2</td>
<td>Tablet shape ..................................................................................</td>
<td>9</td>
</tr>
<tr>
<td>1.4.4.3</td>
<td>Tablet size ....................................................................................</td>
<td>10</td>
</tr>
<tr>
<td>1.5</td>
<td>Model drugs ....................................................................................</td>
<td>10</td>
</tr>
<tr>
<td>1.6</td>
<td>Motivation behind the current work ..............................................</td>
<td>16</td>
</tr>
<tr>
<td>1.7</td>
<td>Objectives, hypothesis and aims ..................................................</td>
<td>16</td>
</tr>
<tr>
<td>1.7.1</td>
<td>Objectives ......................................................................................</td>
<td>16</td>
</tr>
<tr>
<td>1.7.2</td>
<td>Hypothesis ......................................................................................</td>
<td>17</td>
</tr>
<tr>
<td>1.7.3</td>
<td>Aims .................................................................................................</td>
<td>17</td>
</tr>
</tbody>
</table>
## Chapter 4: Extended Release Tablets of Olanzapine

### 4.1 Introduction

### 4.2 Materials and methods

#### 4.2.1 Materials

#### 4.2.2 Methods

- **4.2.2.1 Tablets manufacture**
- **4.2.2.2 In-vitro evaluation**
- **4.2.2.3 In-vivo evaluation**
- **4.2.2.4 In-vitro and In-vivo correlation**

### 4.3 Results

- **4.3.1 In-vitro evaluation**
  - **4.3.1.1 Physicochemical evaluation of powders-mix and granules**
  - **4.3.1.2 Physicochemical evaluation of tablets**
  - **4.3.1.3 Drug dissolution**
  - **4.3.1.4 Selection of the optimized Test tablet**
  - **4.3.1.5 Reproducibility and accelerated stability study**

- **4.3.2 In-vivo evaluation**

- **4.3.3 In-vitro and In-vivo correlation**

### 4.4 Discussion

### 4.5 Conclusion

## Chapter 5: Extended Release Tablets of Prochlorperazine Maleate

### 5.1 Introduction

### 5.2 Materials and methods

#### 5.2.1 Materials

#### 5.2.2 Methods

- **5.2.2.1 Tablets manufacture**
- **5.2.2.2 In-vitro evaluation**
- **5.2.2.3 In-vivo evaluation**
- **5.2.2.4 In-vitro and In-vivo correlation**

### 5.3 Results

- **5.3.1 In-vitro evaluation**
  - **5.3.1.1 Physicochemical evaluation of powders-mix and granules**
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Classification of modified drug delivery systems (MDDSs) based on their sophistication and mechanism of drug release</td>
<td>2</td>
</tr>
<tr>
<td>Table 2.1</td>
<td>Composition of designed extended release tablets of risperidone, olanzapine and prochlorperazine maleate</td>
<td>21</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Kinetic models used for drug release analysis</td>
<td>27</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Interpretation of release exponent “n” in Power law for release mechanism of different geometries (Ritger and Peppas, 1987a; Ritger and Peppas, 1987b)</td>
<td>28</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Study design for pharmacokinetics and bioavailability of risperidone, olanzapine and prochlorperazine</td>
<td>30</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Physicochemical characteristics of powders-mix and granules prepared for manufacture of extended release tablets of risperidone (Mean ± SD, n = 3)</td>
<td>44</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Physicochemical characteristics of extended release tablets of risperidone for its selected formulation F3 (Mean ± SD, n = 10)</td>
<td>45</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Effect of formulation (F1, F2 and F3), dissolution media (pH-1.2 and pH-6.8) and tablet hardness (9 kg, 12 kg and 15 kg) on release kinetics of risperidone from its extended release tablets</td>
<td>46</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>Difference factor $f_1$ and similarity factor $f_2$ calculated for 9 kg, 12 kg and 15 kg hard extended release tablets of risperidone, while comparing their dissolution profiles in pH-6.8 with dissolution profiles in pH-1.2</td>
<td>52</td>
</tr>
<tr>
<td>Table 3.5</td>
<td>Stability indicating parameters (drug content, weight variation, friability, hardness and appearance) for the optimized extended release tablets of risperidone. (Mean ± SD, n = 10)</td>
<td>53</td>
</tr>
<tr>
<td>Table 3.6</td>
<td>Pharmacokinetic parameters for active moiety, risperidone and 9-hydroxyrisperidone, following oral administration of once a day 4 mg Reference or once a day 4 mg Test tablets of risperidone to rabbits (Mean ± SEM, n = 6)</td>
<td>56</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Physicochemical characteristics of powders-mix and granules prepared for manufacture of extended release tablets of olanzapine (Mean ± SD, n = 3)</td>
<td>73</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Physicochemical characteristics of extended release tablets of olanzapine for its selected formulation F3 (Mean ± SD, n = 10)</td>
<td>74</td>
</tr>
<tr>
<td>Table 4.3:</td>
<td>Effect of formulation (F1, F2 and F3), dissolution media (pH-1.5 and pH-6.8) and tablet hardness (9 kg, 12 kg and 15 kg) on release kinetics of olanzapine from its extended release tablets.</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Table 4.4:</td>
<td>Difference factor $f_1$ and similarity factor $f_2$ calculated for 9 kg, 12 kg and 15 kg hard extended release tablets of olanzapine, while comparing their dissolution profiles in pH-6.8 with dissolution profiles in pH 1.5.</td>
<td></td>
</tr>
<tr>
<td>Table 4.5:</td>
<td>Stability indicating parameters (drug content, weight variation, friability, hardness and appearance) for the optimized extended release tablets of olanzapine. (Mean ± SD).</td>
<td></td>
</tr>
<tr>
<td>Table 4.6:</td>
<td>Pharmacokinetic parameters for olanzapine, following oral administration of once a day 10 mg Reference and once a day 10 mg Test tablets of olanzapine to two separate groups of rabbits (Mean ± SEM, n = 6).</td>
<td></td>
</tr>
<tr>
<td>Table 5.1:</td>
<td>Physicochemical characteristics of powders-mix and granules prepared for manufacture of extended release tablets of prochlorperazine maleate (Mean ± SD, n = 3).</td>
<td></td>
</tr>
<tr>
<td>Table 5.2:</td>
<td>Physicochemical characteristics of extended release tablets of prochlorperazine maleate for its selected formulation F3 (Mean ± SD, n = 10).</td>
<td></td>
</tr>
<tr>
<td>Table 5.3:</td>
<td>Effect of formulation (F1, F2 and F3), dissolution media (pH-1.2, pH-6.8) and tablet hardness (9 kg, 12 kg and 15 kg) on release kinetics of prochlorperazine maleate from its extended release tablets.</td>
<td></td>
</tr>
<tr>
<td>Table 5.4:</td>
<td>Difference factor $f_1$ and similarity factor $f_2$ calculated for 9 kg, 12 kg and 15 kg hard extended release optimized tablets of prochlorperazine maleate, while comparing their dissolution profiles in pH-6.8 with dissolution profiles in pH 1.2.</td>
<td></td>
</tr>
<tr>
<td>Table 5.5:</td>
<td>Stability indicating parameters (drug content, weight variation, friability, hardness and appearance) for the optimized extended release tablets of prochlorperazine maleate (Mean ± SD, n = 3).</td>
<td></td>
</tr>
<tr>
<td>Table 5.6:</td>
<td>Pharmacokinetic parameters of prochlorperazine following oral administration of 5 mg Reference tablets three times a day and 15 mg Test tablets administered once a day to two separate groups of rabbits (Mean ± SEM, n=6).</td>
<td></td>
</tr>
<tr>
<td>Figure No.</td>
<td>Title</td>
<td>Page No.</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------------------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Comparative release profiles of risperidone from 9 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.2. (Mean ± SD, n = 6)</td>
<td>47</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Comparative release profiles of risperidone from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.2. (Mean ± SD, n = 6)</td>
<td>47</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Comparative release profiles of risperidone from 15 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.2. (Mean ± SD, n = 6)</td>
<td>48</td>
</tr>
<tr>
<td>Figure 3.4</td>
<td>Comparative release profiles of risperidone from 9 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6)</td>
<td>48</td>
</tr>
<tr>
<td>Figure 3.5</td>
<td>Comparative release profiles of risperidone from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6)</td>
<td>49</td>
</tr>
<tr>
<td>Figure 3.6</td>
<td>Comparative release profiles of risperidone from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6)</td>
<td>49</td>
</tr>
<tr>
<td>Figure 3.7</td>
<td>Effect of formulation (F1, F2 and F3); hardness (9 kg, 12 kg and 15 kg) and dissolution media (pH 1.2 and pH 6.8) on the drug release rates (K values) from risperidone extended release tablets (Mean ± SD, n = 6)</td>
<td>51</td>
</tr>
<tr>
<td>Figure 3.8</td>
<td>A representative chromatogram of 9-hydroxyrisperidone (labeled as 9-OH) and risperidone (labeled as Rsp) using a standard solution consisting of a mixture of 50 ng/mL 9-hydroxyrisperidone and 50 ng/mL risperidone</td>
<td>57</td>
</tr>
<tr>
<td>Figure 3.9</td>
<td>A representative chromatogram of extracted blank serum</td>
<td>57</td>
</tr>
<tr>
<td>Figure 3.10</td>
<td>A representative chromatogram of 9-hydroxyrisperidone (labeled as 9-OH) and risperidone (labeled as Rsp) extracted from a sample of rabbit serum withdrawn 6 hours after administration of Test tablet</td>
<td>58</td>
</tr>
</tbody>
</table>
Figure 3.11: A representative chromatogram of 9-hydroxyrisperidine (labeled as 9-OH) and risperidone (labeled as Rsp) extracted from a sample of rabbit serum spiked with 30 ng/mL of 9-hydroxyrisperidine and 30 ng/mL of risperidone..........................................................58

Figure 3.12: Comparative serum concentration-time profiles of active moiety-1 (Reference tablet) and active moiety-2 (Test tablet), following oral administration to rabbits (Mean ± SD, n = 6). Note: Active moiety represents the combined concentrations of risperidone and 9-hydroxyrisperidine .............................................................................59

Figure 3.13: Comparative serum concentration-time profiles of risperidone and 9-OH-risperidone following oral administration of Reference tablets to rabbits. (Mean ± SD, n = 6).................................................................59

Figure 3.14: Comparative serum concentration-time profiles of risperidone and 9-OH-risperidone following oral administration of Test tablets to rabbits. (Mean ± SD, n = 6) ..................................................................60

Figure 3.15: *In-vitro* and *In-vivo* correlation of risperidone Test tablet. Percent of drug absorbed is plotted against percent of drug released at times 1, 2, 4, 6, 8, 12 and 24 hours.................................................................61

Figure 4.1: Comparative release profiles of olanzapine from 9 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.5. (Mean ± SD, n = 6)...........76

Figure 4.2: Comparative release profiles of olanzapine from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.5. (Mean ± SD, n = 6)...........76

Figure 4.3: Comparative release profiles of olanzapine from 15 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.5. (Mean ± SD, n = 6)...........77

Figure 4.4: Comparative release profiles of olanzapine from 9 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6)...........77

Figure 4.5: Comparative release profiles of olanzapine from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, N = 6)...........78
Figure 4.6: Comparative release profiles of olanzapine from 15 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6)........78

Figure 4.7: Effect of formulation (F1, F2 and F3); hardness (9 kg, 12 kg and 15 kg) and dissolution media (pH 1.5 and pH 6.8) on the drug release rates (K values) from olanzapine extended release tablets (Mean ± SD, n = 6)..............................................................................................79

Figure 4.8: A representative chromatogram of olanzapine (labeled as Oln) using a standard solution of 50 ng/mL olanzapine .........................83

Figure 4.9: A representative chromatogram of extracted blank serum.........................83

Figure 4.10: A representative chromatogram of olanzapine (labeled as Oln) extracted from a sample of rabbit serum withdrawn 4 hours after administration of Test tablet ..................................................................................84

Figure 4.11: A representative chromatogram of olanzapine (labeled as Oln) extracted from a sample of rabbit serum spiked with 40 ng/mL olanzapine .............................................................................................84

Figure 4.12: Comparative serum concentration-time profiles of olanzapine-1 and olanzapine-2, following oral administration of Reference and Test tablets respectively to rabbits (Mean ± SD, n = 6).........................85

Figure 4.13: Percent of drug absorbed plotted against percent of drug released at times 1, 2, 4, 6, 8, 12 and 24 hours to show the In-vitro In-vivo correlation of olanzapine Test tablet.........................................................86

Figure 5.1: Comparative release profiles of prochlorperazine maleate from 9 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 1.2 (Mean ± SD, n = 6)........................................................................................................100

Figure 5.2: Comparative release profiles of prochlorperazine maleate from 12 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 1.2 (Mean ± SD, n = 6)........................................................................................................100

Figure 5.3: Comparative release profiles of prochlorperazine maleate from 15 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 1.2 (Mean ± SD, n = 6)........................................................................................................101
Figure 5.4: Comparative release profiles of prochlorperazine maleate from 9 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 6.8 (Mean ± SD, n = 6)..............................................................................................101

Figure 5.5: Comparative release profiles of prochlorperazine maleate from 12 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 6.8 (Mean ± SD, n = 6)..............................................................................................102

Figure 5.6: Comparative release profiles of prochlorperazine maleate from 12 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 6.8 (Mean ± SD, n = 6)..............................................................................................102

Figure 5.7: Effect of formulation (F1, F2 and F3); hardness (9 kg, 12 kg and 15 kg) and dissolution media (pH 1.2 and pH 6.8) on the drug release rates (K values) from prochlorperazine maleate extended release tablets (Mean ± SD, n = 6)...........................................................................................................103

Figure 5.8: A representative chromatogram of prochlorperazine (labeled as PCZ) from a standard solution containing 60 ng/mL of prochlorperazine maleate.................................................................108

Figure 5.9: A representative chromatogram of prochlorperazine (labeled as PCZ) from a standard solution containing 60 ng/mL of prochlorperazine maleate.................................................................108

Figure 5.10: A representative chromatogram of prochlorperazine (labeled as PCZ) extracted from a sample of rabbit serum withdrawn 4 hours after administration of Test tablet.................................................................109

Figure 5.11: A representative chromatogram of prochlorperazine (labeled as PCZ) extracted from a sample of rabbit serum spiked with 60 ng/mL prochlorperazine maleate .................................................................109

Figure 5.12: Comparative serum concentration-time profiles of prochlorperazine-1 and prochlorperazine-2, following oral administration of Reference and Test tablets respectively to rabbits (Mean ± SD, n = 6)...........................................................................................................110

Figure 5.13: Percent of drug absorbed plotted against percent of drug released at times 1, 2, 4, 6, 8, 12 and 24 hours to show the In-vitro In-vivo correlation of prochlorperazine Test tablet.......................................................................111
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-\infty}</td>
<td>Area under curve up to infinite time</td>
</tr>
<tr>
<td>AUC_t</td>
<td>Area under curve up to time ‘t’</td>
</tr>
<tr>
<td>AUMC</td>
<td>Area under movement curve</td>
</tr>
<tr>
<td>Cl_total</td>
<td>Total clearance</td>
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<td>C_{max}</td>
<td>Maximum plasma concentration</td>
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<td>ER</td>
<td>Extended release</td>
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<td>Millimolar</td>
</tr>
<tr>
<td>MRT</td>
<td>Mean residence time</td>
</tr>
<tr>
<td>MDDSs</td>
<td>Modified drug delivery systems</td>
</tr>
<tr>
<td>Oln</td>
<td>Olanzapine</td>
</tr>
<tr>
<td>Pk</td>
<td>Pharmacokinetic</td>
</tr>
<tr>
<td>PRZ</td>
<td>Prochlorperazine</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>Rsp</td>
<td>Risperidone</td>
</tr>
<tr>
<td>9-OH</td>
<td>9-hydroxyrisperidone</td>
</tr>
<tr>
<td>t_{1/2}</td>
<td>Half life</td>
</tr>
<tr>
<td>T_{max}</td>
<td>Time to reach maximum concentration</td>
</tr>
<tr>
<td>US FDA</td>
<td>United States Food and drug administration</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>Vd</td>
<td>Volume of distribution</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

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ABSTRACT

Antipsychotic drugs are widely used in the short term management of acute psychotic, manic and psycho depressive disorders and long period treatment of chronic psychotic disorders, including schizophrenia, psycho effective disorders and delusional disorders. However, these drugs produce serious side effects ranging from the most troublesome (Intense sedation, dry mouth and somnolence) to dangerous (Parkinsonism, dyskinesia and akathesia). The management of these side effects has become an important part of treatment plans as the frequency and intensity of the side effects play a major role in the effectiveness and tolerability of a particular antipsychotic agent. Non-compliance to antipsychotic medication is the primary issue directly linked to long term clinical outcomes.

The development of modified drug delivery systems (MDDSs) have improved patient compliance, reduced side effects and optimized the dosage schedule without compromising their therapeutic efficacy. As a result of reduction in side effects of antipsychotics, some MDDSs have been developed. In this respect, Quetiapine
fumarate (Seroquel XR®) and Paliperidone (Invega®) extended release tablets of antipsychotic drugs are offering improved treatment and tolerability profiles.

As oral route for administration of drug is mostly preferred and tablet is the most popular dosage form, therefore, extended release tablets of risperidone, olanzapine and prochlorperazine maleate were developed. Binary mixtures of the commonly recommended Methocel® K100 LV-CR (hydrophilic) and Ethocel® Standard 7FP Premium (hydrophobic) were used to prepare tablets by flow bound dry granulation-slugging method. Combination of the two polymers successfully extended the release period up to 24 hours. The release period was extended regularly as the amount of Ethocel® Standard 7FP Premium was sequentially increased from 30% to 60%. The inclusion of Methocel® K100 LV-CR helped in maintaining drugs knotted in its viscous gel layer, while presence of Ethocel® caused slow hydration & erosion of the matrices leading to extended drug release period.

pH independent drug release with zero order kinetics was an important achievement in the present study. Hardness of tablets did not influence the release kinetics. The two polymers played a role of functional copartners. The matrix tablets containing 30% Methocel® and 60% Ethocel® (F3) with 12kg hardness were selected for further studies. The optimized matrix tablets of the model drugs exhibited an acceptable level of stability under accelerated storage conditions.

Bioavailability studies of the optimized tablets of risperidone, olanzapine and prochlorperazine were conducted in rabbit’s serum using HPLC based validated methods. Measured serum concentrations of the drugs were used in calculation of the various pharmacokinetic parameters, including peak concentration (Cₘₐₓ), peak time (Tₘₐₓ), area under curve up to 24 hours (AUC₀-2₄), area under curve up to infinite time (AUC₀-∞), mean residence time (MRT₀-₄₈), half life (t₁/₂), volume of distribution (Vd), elimination rate constant (Kₑₑ) and total clearance (Cl_total) for the Test-extended release and Reference-conventional tablets using PK WinNonlin software. Optimum levels of the drugs serum concentrations (Cₘₐₓ) from the Test tablets were observed as compared to Reference tablets. Significantly prolonged peak time (tₘₓ) of the Test tablets indicated smooth and extended absorption phase of the drugs.

A good correlation between the In-vitro drug release and In-vivo drug absorption was achieved in case of each model drug. The area under curves (AUCs)
of Test extended release tablets and Reference-conventional tablets were not significantly different (p < 0.05), indicating their bioequivalence. The bioavailability data generated in the present study indicated that the absorption of risperidone, olanzapine and prochlorperazine maleate from gastro intestinal tract (GIT) were dependent on their release rate. A good level of In-vitro In-vivo correlation of the all three drugs showed successful use of the dissolution process, binary mixtures of the model polymers and rabbits as model animals. Further studies on binary mixtures of the Methocel® and Ethocel® may ensure their utility in formulation of extended release tablets of other similarly low dose water insoluble drugs. Extensive preclinical studies and clinical trials of the presently developed tablet formulations need to be conducted to determine improvement in safety profiles of the model drugs.
1.1: **DRUG DELIVERY SYSTEMS**

The aim of developing any drug delivery system (DDS) is to achieve a safe and effective drug concentration in body tissues. Traditional drug delivery systems (TDDSs) are characterized by rapid and unrestrained drug release kinetics, usually leading to abrupt increase of drug concentration in body tissues followed by a similar decrease.

1.1.1: **Extended release matrix systems**

Extended release (ER) dosage forms are designed in such a manner as to allow the enclosed drug available over an extended period of time after its administration. Various terms including sustained release, prolonged release and controlled release are also employed to distinguish such a modified drug delivery systems. A typical controlled release system is designed to keep concentration of drug in the blood or other target tissues at an optimum level over an extended period of time. In practical terms, an oral controlled release system shall allow a reduction in dosing frequency as compared to when the same drug is presented as a traditional dosage form (Qiu and Zhang, 2000).

The modified drug delivery systems (MDDSs) including extended release, evolved over a period of time. They are particularly designed to exert control on drug release to further enhance drug’s efficacy, safety and compliance. The MDDSs can also enhance the value of the company's current products, allowing it to charge a premium for the extra benefits. Foundations of MDDSs were laid in 1952, with the introduction of the first sustained-release capsule of Dexedrine. The MDDSs have been classified on the basis of their sophistication and mechanism of drug release (Leary et al, 2006; Hirlekar et al, 2008) as shown in Table 1.1.
Table 1.1: Classification of modified drug delivery systems (MDDSs) based on their sophistication and mechanism of drug release.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description/Mechanism of drug release</th>
</tr>
</thead>
</table>
| Rate-preprogrammed DDSs             | Polymer membrane permeation  
|                                     | Polymer matrix diffusion  
|                                     | Microreservoir partition                                                   |
| Physical-activated DDSs              | Osmotic pressure activated  
|                                     | Hydrodynamic pressure activated  
|                                     | Hydration activated  
|                                     | Vapor pressure activated  
|                                     | Mechanically activated  
|                                     | Magnetically activated  
|                                     | Ultrasound activated  
|                                     | Electrically activated                                                   |
| Chemically activated DDSs            | pH activated  
|                                     | Ion activated  
|                                     | Hydrolysis activated                                                     |
| Biochemically activated DDSs         | Enzyme activated  
|                                     | Biochemical activated                                                     |
| Site targeted DDSs                   | Passive targeting  
|                                     | Active targeting                                                        |

The term matrix indicates a three dimensional network composed of drug(s), polymer(s) and other excipientns. Because of simplicity; ease in manufacturing, scale-up and process validation; stability and low costs, matrix preparation has become a popular approach (Takka et al, 2001; Vendelli et al, 1998). Drugs are usually embedded in hydrophilic or hydrophobic matrices to exert control on their release (Kydonieus, 1992; Wise, 2000).

Now-a-days, controlled-release product development has become much easier than before because of availability of advanced technology for matrix fabrication. These advancements have made it feasible to design delivery of a wide variety of drugs (with different physical, chemical and biological characteristics) as desired. All this has resulted in a large number of patents and commercial products (Manthena et al, 2004) available in the market.

Oral drug delivery has become the major segment of the total drug delivery market. It is growing day by day because of being a favorite route for drug administration. Now-a-days, hydrophilic matrices are receiving preference in formulation of extended release oral products (Tiwari and Rajabi-Siahboomi, 2008).
1.1.2: Tablets technology

A large number of developments in the field of pharmaceutical ingredients and tablet machines have made tablet manufacturing a science and the tablets a most favorite dosage form (Rasenak and Muller, 2002). Tablets dosage form has become popular because of advantages including ease in manufacturing, convenience in administration, and accuracy in dosage, stability and safety. Wet granulation, dry granulation and direct compression are the widely used methods of tablet manufacture (Shangraw, 1989; Rudnic and Schwartz, 2005). Direct compression is limited by tendency of segregation of actives ingredients from excipinetns because of significant difference in their densities (Rubinstein, 1998). The dry state of the material during mixing may also induce static charges, which further complicates the problem; leading to variations in drug contents.

1.1.3: Granulation/size enlargement

The powder must have a large number of essential characteristics, including optimum levels of flow, cohesiveness, compressibility, moisture content and lubrication before being compressed into tablets. As most of such materials don’t possess such qualities, that is why granulation gets a role to introduce such qualities.

Wet granulation, performed in the presence of water or any other suitable liquid, is an expensive process. Other discrediting points of this technique include loss of material during processing, incompatibility between components and chances of decomposition of heat labile and moisture sensitive drugs. In this way dry granulation gets a favor, used when the formulation ingredients possess inherent cohesive qualities (Rudnic and Schwartz, 2005).
1.2: DRUG DISSOLUTION AND RELEASE MECHANISMS

*In-vitro* drug dissolution is a vital component of tablet development and quality assessment (Sood and Panchagnula, 1999; Adams et al, 2001). Release and further dissolution of the drug from the solid dosage form is a rate limiting step in drug absorption. Drug dissolution gets an essential role in checking batch to batch consistency in tablet production.

In new product development, dissolution testing with the aid of drug release modeling helps in the selection of excipients, optimization of the manufacturing process and formulation of test products with desired dissolution characteristics. (US FDA, 1997). The dissolution testing may also be used to find out long term stability of a dosage form and to find out the impact of post-approval changes in the manufacturing process. Under certain circumstances, it can be used as a substitution parameter in testing of bioequivalence.

**Drug release kinetics**

Factors affecting drug release kinetics include polymer, drug/polymer ratio, swelling of polymer, erosion of polymer, dissolution or diffusion characteristics of drug, drug distribution inside the matrix and geometry (cylinder or sphere) of the system (Conte et al, 1988; Colombo et al, 1999).

Upon contact of body fluids or water by tablets, the polymer swells and the drug dissolve. As soon as the penetrated solvent concentration exceeds a threshold value, polymeric chains start unfolding, leading to glassy-rubbery polymeric transition and a gel like layer surrounding dry core of the matrix begins to appear (Ju et al, 1995; Kararli et al, 1990). Such a transition cause molecular rearrangement of polymeric chains that tend to reach a new equilibrium condition as the old one is altered by the presence of the penetrated solvent (Grassi et al, 1998). The glassy-rubbery transition cause a large scale increase in mobility of the polymer chains, the network meshes enlarge and the dissolved drug diffuse through the gel layer. Drug concentration in the matrix can affects drug release kinetics to a large extent. Lee (1986, 1984a; 1984b) demonstrated that different release kinetics can be achieved by properly selecting uniform and sigmoidal steps or parabolic drug distribution. Dissolution of the drug present in the matrix/release environment can lead to a burst
effect in release profiles followed by a slower release in case of uniform drug distribution in a tablet (Huang and Brazel, 2001).

The mechanism of drug release from hydrophilic matrix tablets is based on the dissolution of the drug, diffusion of the drug through the hydrated layer of the matrix and erosion of the outer hydrated polymer present on the surface of the matrix. In general, when the matrix tablet gets contact with an aqueous media, the surface of the tablet becomes wetted and the polymer hydrates to form a jelly-like mass usually referred to as the 'gel layer'. This process is also termed as the glassy-to-rubbery state transition of the polymer (Ju et al, 1995; Kararli et al, 1990; Linder et al, 1996). The core of the tablet remains essentially dry at this stage. In the case of a highly soluble, large dose drug, this incident may lead to preliminary burst release because of the presence of drug on the surface of the matrix tablet. As more water penetrates, the gel layer (rubbery state) grows with time into the core of the matrix, increasing the thickness of the gel layer and providing a diffusion barrier to drug release (Rajabi-Siahboomi et al, 1996). Altogether, as the outer layer gets completely hydrated, the polymer chains become fully relaxed and remain unable to maintain the integrity of the gel layer, leading to disentanglement and erosion from the surface of the matrix. Water continues to penetrate into the tablet core through the gel layer, until it has been entirely eroded.

Water soluble drugs are usually released by a mix process of diffusion and erosion. Erosion is the major release mechanism for insoluble drugs, apart from their dose (Li et al, 2005; Rajabi-Siahboomi, 2000). Quick hydration of polymer and rapid gel layer formation on tablet surface are essential characteristics of an extended release system to prevent premature drug release. Small sized particles of hydrophilic matrices ensure rapid hydration and consistent gel layer formation on the surface of tablets (Dow, 2008).

The kinetics of drug release is dependent on many factors, major ones being water solubility of the drug and swelling & erosion properties of the polymer. The water soluble drugs are released mainly by diffusion with a partial input from erosion while the anomalous diffusion results from the relaxation of the macromolecular polymer chains (Melia, 1990).
Typically, zero-order release is desirable in order to match the drug input rate with the drug elimination rate, thereby maintaining the steady state plasma drug concentrations. Occasionally bi-modal drug release is desirable to compensate variations in drug absorption rate throughout GIT (Shah, 1988). The release of water soluble moieties typically follows the first order release kinetics while the water insoluble drugs are released primarily through matrix erosion and therefore exhibit time independent or zero-order release kinetics (Colombo et al, 1999, Colombo et al, 1996, Vazquez et al, 1992; Ranga-Rao et al, 1990).

### 1.3 MODEL POLYMERS USED FOR EXTENDING DRUG RELEASE PERIOD

Type and concentration of polymers are the two major factors influencing the rate and mechanism of drug release. Two major types of polymers (hydrophilic and hydrophobic) are usually used in the preparation of matrix systems (Venkatraman et al, 2000). Hydrophilic polymers include hydroxypropylmethylcellulose (HPMC), sodium alginate, and carbopols while ethylcellulose, fatty acids and fatty alcohols are the examples of hydrophobic polymers. In line with the scope of the present work, the hydroxypropylmethylcellulose and ethylcellulose will be discussed in detail.

#### 1.3.1: Hydroxypropylmethylcellulose

Hydroxypropylmethylcellulose (HPMC) is the most widely used release rate controlling polymer in hydrophilic matrices for extended release (ER) oral delivery systems. It provides robust versatile formulations and the possibility of simplified production (Tiwari and Rajabi-Siahboomi, 2008; Rajabi-Siahboomi and Jordan, 2000).

Different versions of commercially available HPMC are available from various manufacturers. They are mostly distinguished by relative proportions of the hydroxypropoxyl and methoxyl substitutions. Increasing the proportion of hydrophilic hydroxypropyl groups results in immediate hydration with a ranking order of Methocel®K > Methocel®E > Methocel®F. Generally, a rapid hydrating grade Methocel®K gets preference, particularly in case of water soluble drugs, where a rapid rate of hydration is essential to avoid burst release. Most often, a delayed or inadequate hydration of polymer lead to burst release and dose dumping (Dow Pharmaceutical Excipients, 1998).
For a fixed polymer level, viscosity of the selected grade of HPMC greatly influences the mechanical and diffusional properties of the matrix. Nellore et al (1998) suggested that the highly viscous gel layers of Methocel®K resulted in slower release of metoprolol by providing a more tortuous and resistant barrier to diffusion.

Faster release of adinazolam mesilate occurred from the formulation using K100LV as compared to Methocel K4M, K15M, and K100M (Sung et al. 1996). The K4M formulation showed a slightly higher drug release rate than that of K15M and K100M. The release profiles from the K15M and K100M were nearly same, so the viscosity of 15000 cps was suggested as the limit, above which the release rate remains unaffected.

1.3.2: Ethylcellulose (Ethocel)

Ethylcellulose is an ethyl ether of cellulose \{\(C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5\), where \(n\) can very to provide a wide variety of molecular weights\}. They are inert, bland, noncaloric hydrophobic polymers. They are commercially available in granular and fine particulate forms. These polymers have been extensively used as pharmaceutical vehicles in a number of dosage forms. In tablet technology, Ethocel ethers have been used as binders (Desai et al, 2001; Chowhan, 1980) and as hydrophobic coatings for tablets and granules (Sadeghi et al, 2001). Modified release tablet formulations may be produced using ethylcellulose as matrix former (Kulvanich et al, 2002; Pollock and Shesky, 1996). Fine particle ethylcellulose (FPEC) has shown a better efficiency as a release retarding matrix former (Khan and Meidan, 2007; Agrawal et al, 2003). They are also employed in preparing microcapsules and microspheres (Desai et al, 2001).

The direct compression of matrix-type tablets using nonsolvated ethylcellulose as the matrix-forming polymer has been studied thoroughly (Pather et al, 1998; Pollock and Sheskey, 1996; Shaikh et al, 1987a; Shaikh et al, 1987b). The lower viscosity grades (e.g. Ethocel standard Premium; 7cps, 10cps and 100cps) are more compressible than the higher viscosity grades of ethylcellulose, resulting in harder tablets and slower release (Upadrashta et. al, 1993).
1.3.3: Combination of other polymers with HPMC

Highly water-soluble drugs embedded in hydrophilic HPMC based matrices gets a chance of immediate burst release. Mixing of hydrophobic polymers with HPMC can partially or wholly eradicate the problem. They resist instant hydration of the matrix leading to slow drug dissolution and diffusion and elimination of initial burst release. Hydrophobic polymers include ethylcellulose (e.g., Ethocel® 7FP Premium), cellulose acetate, methacrylic acid copolymers and polyvinyl acetate (Dias et al, 2006; Tatavarti and Huag, 2006).

1.4: OTHER FACTORS AFFECTING DRUG RELEASE

Developing a matrix tablet formulation makes it mandatory to consider a number of factors effecting drug release profiles (Hogan, 1989; Gao and Meury, 1996; Levina, 2004; Hardy et al, 2006), some important ones are given below.

1.4.1: Polymer : Drug Ratio

Increase in polymer: drug ratio exhibited a significant reduction in release rate of diclofenac from HPMC based formulated tablets (Velasco et al, 1999). The investigators suggested that longer diffusional path and highly viscous gel was responsible for reducing the drug release rate. Release rate of metoprolol, a water soluble drug, decreased with increase in concentration of HPMC (Rekhi et al. (1999). By varying the polymer level (Methocel® K4M 10-40%), different release profiles for metoprolol were achieved by Nellore et al (1998). Sung et al (1996) observed a wide range of drug release rates with changes in HPMC: lactose ratio.

1.4.2: Drug solubility

Drug solubility is another important factor affecting the drug release kinetics of HPMC based hydrophilic matrices. This property of drug influences the choice of polymer viscosity and polymer chemistry grade. The use of a right viscosity grade polymer is essential in designing matrices for diffusion, diffusion and erosion, or erosion mechanisms of drug release. Depending on drug solubility, it may be crucial to mix different viscosity grades of HPMC to affect an in-between viscosity and attain desired release kinetics (Dow, 2006)
14.3: Drug particle size

Particle size of drugs greatly influence their release from matrices, particularly affecting release of moderately soluble drugs. Hogan (1989) observed a significant influence of particle size on drug release kinetics at a low Drug: HPMC ratio and a big particle size of drug (> 250 µM). Velasco et al., (1999), showed that for a given effective surface area, diclofenac particle size significantly influenced its release rate from HPMC tablets. The smallest particle size of drug dissolved more readily upon penetration of dissolution medium through the matrix resulting in rapid drug diffusion. The larger particle size dissolved less readily and therefore release was delayed until erosion of the matrix surface. Ford et al (1995) have also observed dependence of release on particle size in case of indomethacin (less soluble drug).

1.4.4: Process variables affecting drug release

1.4.4.1: Compression force/hardness of tablets

The compression force employed in tableting significantly affects the tablet hardness but does not influence the drug release kinetics (Velasco et al, 1999). Variation in compression force can be closely related to a change in porosity of the tablets. However, as the porosity of the hydrated matrix is independent of the primary porosity, the compression force seems to have little influence on drug release. Changes in compression force did not affect the drug release kinetics from HPMC based matrix tablets once critical hardness was reached (Rekhi et al, 1999). Increased dissolution rates were observed when the tablets were found to be extremely soft, and this phenomenon was attributed to a lack of powder compaction.

1.4.4.2: Tablet shape

Rekhi et al. (1999) have demonstrated influence of the size and shape of the matrix tablet undergoing diffusion and erosion on the drug dissolution rate. 20-30% increase in dissolution rate was observed at each time point during modification of the surface area of the metoprolol tartrate tablets (formulated with Methocel® K100LV) from the standard concave shape (0.568 sq. in.) to caplet shape (0.747 sq. in.). So it was suggested that tablet matrices shall be as much spherical as possible to generate a lowest release rate.
Varying the aspect ratio (radius/height) of the HPMC based tablets is an easy and successful tool for modifying the drug release kinetics (Siepman et al, 1999). Higher release rate were observed for flat shape cylinders (ratio=20) than regular shaped cylinders (ratio 2) and almost rod shaped cylinders (ratio 0.2) in case of tablets with same volume. The results were accredited to difference in surface areas of tablets.

1.4.4.3: Tablet size

Siepman et al (1999) experienced a significant impact of tablet size on the drug release rate in case of tablets with same aspect ratio and drug concentration. Within 24 hours, 99.8%, 83.1% and 50.9% drug was released from small sized, medium sized and large sized tablets respectively. It was suggested that the rapid drug release from smaller tablets was because of greater surface area per unit volume and decrease drug release rate from bulky tablets was due to existence of longer pathways for diffusion of drug.

1.5: MODEL DRUGS

The antipsychotic drugs are being used mainly for treatment of schizophrenia. They are also effective in some other psychoses and agitated states (Potter and Hollister, 2004). Applications include the short-term treatment of acute psychotic, manic and psychotic-depressive disorders as well as agitated states in delirium and dementia and the long-term treatment of chronic psychotic disorders including schizophrenia, schizoaffective disorder and delusional disorders. Antipsychotic agents are divided into two main categories: typical or First generation antipsychotics (FGAs) and atypical or Second generation antipsychotics (SGAs). Phenothiazine (e.g. promethazine, prochlorperazine); Thioxanthenes (e.g. thiothixene) and Butyrophenones (e.g. haloperidol) are examples of FGAs. The SGAs include risperidone, olanzapine and quetiapine. Although the SGAs have largely replaced FGAs in clinical practice (Leslie and Rosenheck, 2002; Gill et al. 2005), but it is still considered debatable that whether SGAs are more effective than FGAs and whether some SGAs are more effective than others (Geddes et al, 2000; Rosenheck et al, 2003; Jones et al, 2006; Rosenheck, 2006). Rosenheck (2006) has reported that the perphenazine, an FGA is more cost effective than the SGAs for the average individual with chronic schizophrenia.
Extra pyramidal symptoms, including tarditive dyskinesia and Parkinsonism were considered as the most common adverse drug reactions caused by FGAs. However, the post-marketing studies of antipsychotics suggest that SGAs may not be remarkably different from the FGAs and that they are also associated with EPS. No remarkable difference between FGAs and SGAs were observed with regards to the occurrence of tarditive dyskinesia (Lee et al, 2005) or parkinsonism (Rochon, 2005) during expanded geriatric studies. Similarly, in individuals with severe mental illness, use of the both FGAs and SGAs were associated with a high risk for tarditive dyskinesia (De Leon et al, 2007).

Metabolic complications were frequently reported with use of FGAs, though the findings was largely ignored (De Leon, 2008). Low potency FGAs including phenothiazine, may cause weight gain as much as by risperidone and olanzapine (Allison et al., 1999). Phenothiazine may also cause direct increases in lipid levels as olanzapine; quetiapine and clozapine do (De Leon et al, 2007; Meyer and Kora, 2004).

The association of FGAs, particularly phenothiazine with increased chances of sudden cardiac deaths has been well established (Harrison and Krishnan, 2002). Users of both SGAs and FGAs have a comparable dose related high risk of sudden cardiac death (Ray et al, 2009).

Modified drug delivery systems (MDDS) of antipsychotics offer advantages over conventional formulations in proviso of convenience, side effects and efficacy. Use of these MDDS can help in enhanced patient satisfaction and compliance with treatment, thereby improving patient prognosis. Formulations that deliver sustained levels of medications, such as Paliperidone (Invega®) extended release tablets or long-acting risperidone I/M injections, are important advances in the treatment of patients with psychiatric illness such as schizophrenia since they have the potential to impact favorably on efficacy and tolerability and thus, can help to improve compliance and long-term treatment prognosis (Keith, 2006).

Risperidone and olanzapine from the SGAs group were chosen as the model drugs because of their frequent use in psychiatry. Prochlorperazine maleate belonging to FGAs was selected as another model drug based on its landmarked success in
prevention and treatment of nausea and vomiting linked with chemotherapy, surgery and migraine. In addition, all of these drugs have some common features including side effects and water insolubility. A brief account of these model drugs is given below.

**Risperidone**

Risperidone, a benzisoxazole derivative (molecular formula: C\textsubscript{23}H\textsubscript{27}FN\textsubscript{4}O\textsubscript{2}; molecular weight: 410.49) is a potent 5HT\textsubscript{2} and moderate D\textsubscript{2} receptors antagonist (Schotte et al, 1996; He and Richardson, 1995; Janssen et al, 1988). It is a second generation antipsychotic (SGA), widely used in the clinical management of schizophrenia, the mixed and manic states of bipolar disorder and irritability in children with autism (Potter and Hollister, 2004). The drug has been developed by Janssen-Cilag. Originally it was registered under the trade name of ‘Risperdal’ in USA. Janssen's patent for Risperdal expired on December 29, 2007 and its exclusive marketing rights expired on June 29, 2008 (based on pediatric extension). Dose of 4-8 mg per day is recommended for getting maximum efficacy with minimum adverse drug reactions (Williams, 2001).

Risperidone is rapidly and completely absorbed orally and extensively metabolized in the liver to several metabolites, 9-hydroxyrisperidone being the major one (Heykants et al, 1994; Meuldermanns et al, 1994; Mannens et al, 1993). 9-hydroxyrisperidone appears to be pharmacologically as much potent as the parent compound (Beijsterveldt et al, 1994; Huang et al, 1993; Mannens et al, 1993). The serum concentration of active moiety is, thus the sum of serum concentrations of risperidone and 9-hydroxyrisperidone (Huang et al, 1993).

Risperidone shows large interindividual variations in pharmacokinetics (Zhou et al, 2006; Cho and Lee, 2006) In one study, samples of blood collected from 506 healthy human male subjects receiving single dose 2mg Risperdal\textsuperscript{®} tablets orally showed the following results: half life (t\textsubscript{1/2}), time of maximum concentration (T\textsubscript{max}), level of maximum concentration (C\textsubscript{max}) and area under curve (AUC\textsubscript{0-\infty}) for active moiety were found as 13.03 ± 2.87 hours, 1.44 ± 0.77 hours, 12.5 ± 4.07 ng/mL and 187.13 ± 40.46 ng.h/mL respectively while the same parameters were 4.99 ± 2.74 hours, 1.17 ± 0.39 hours, 8.96 ± 4.31 ng/mL and 58.93 ± 46.88 ng.h/mL respectively for
risperidone and 17.86 ± 4.96 hours, 7.46 ± 4.34 hours, 5.61 ± 2.47 ng/mL & 137.88 ± 32.30 ng. Hour/mL respectively for 9-hydroxyrisperidone (Cho and Lee, 2006). In another multidose clinical pharmacokinetic study, 23 Chinese psychotic patients received 2 mg risperidone tablets twice daily. Risperidone was rapidly absorbed (T\text{max} was 1.6 hour) and its t\text{1/2} in plasma was short (3.2 hour). 9-hydroxy-risperidone was quickly metabolized from the parent drug with a mean T\text{max} of 2.5 hours. 9-hydroxyrisperidone had a long half-life of 24.7 hours. The AUC\text{ss}\text{0-12h} of risperidone and 9-hydroxyrisperidone were determined as 443.2 ± 397.4 and 1327.2 ± 402.3 µg.hour/Liters respectively. Total clearance (CL/F) and volume of distribution (V/F) of risperidone were determined as 8.7 ± 6.2L/h and 34.1 ± 24.3Litres respectively (Zhou et al, 2006). Risperidone has a volume of distribution of 1.1 L/kg in human beings (Huang et al, 1993).

Risperidone is insoluble in water but dissolves readily in dilute acid solutions. Risperidone is freely soluble in dichloromethane, soluble in methanol and sparingly soluble in ethanol. It is available as a tablet in strengths of 0.25, 0.5, 1, 2, 3 and 4 mg; an oral solution (30 mL) in strength of 1 mg/mL and ampoules (Depot injections) in strengths of 25 mg, 37.5 mg and 50 mg with a trade name ‘Risperdal Consta’ The long acting injections are administered once biweekly. It is also available as wafers with trade names of ‘Risperdal M-Tabs’ and ‘Risperdal Quicklets’.

Risperidone cause moderate weight gain (Venina et al, 2002; Baldwin and mayers, 2003). Risperidone is worst of all SGAs for dose related extra pyramidal side effects in the dosage range of 8 to 16 mg per day (Lemmens et al, 1999).

Olanzapine

Olanzapine, a thiobenzodiazepine derivative (molecular formula: C\text{17}H\text{20}N\text{4}S; molecular weight: 312.43) is the most widely used antipsychotic drug (Raga et al, 2004). It is a potent 5HT\text{2} and D\text{2} receptor antagonist. It was originally patented by Eli Lilly and Company under the trade name of ‘Zyprexa’ and has been classified as second generation antipsychotic (Fulton and Goa, 1997). It is extensively used in the clinical management of schizophrenia, depressive episodes associated with bipolar disorder, acute manic episodes and maintenance treatment for bipolar disorder. Eli Lilly and Company’s patent for olanzapine proper expires in 2011. It has a low
propensity to cause extra pyramidal side effects (EPS) and a sustained increase in prolactin level (Lieberman et al, 2003). Olanzapine induce a significant weight gain (Strassnig et al, 2009; Thase et al, 2007; Robinson et al, 2006). Its use has been linked to increased onset of diabetes (Guo et al, 2007; Gianfrancesco, 2006; Lambert et al, 2006).

Olanzapine is practically insoluble in water, soluble in methanol and acidic solutions and highly soluble in dichloromethane. It is available as a tablet in strengths of 2.5 mg; 5 mg; 7.5 mg; 10mg; 15 mg and 20 mg. Orally disintegrating wafers (Zydis) are also available in market in different strengths including 5 mg, 10 mg, 15 mg and 20 mg. These wafers dissolve on tongue and absorb rapidly. It is also available as a rapid-acting intramuscular injection for short-term acute treatment. The drug is safe in therapeutic doses (Nemerof, 1997; Beasely Jr et al, 1997; Fulton and Goa, 1997). Perry et al (1997) observed that the clinical response to olanzapine among schizophrenic patients was directly related to its blood concentration and a minimum effective concentration of 9 ng/mL plasma was suggested by him. The concentrations of drug in serum showed a tendency to increase with the administered daily oral dose (Aravagiri et al. 1997). Samples of plasma from several patients receiving 10 to 20 mg olanzapine daily contained 8 to 31 ng/mL of drug (Aravagiri et al, 1997). In another study, following administration of 2.5 to 17.5 mg olanzapine tablets daily, the serum concentrations of 5 to 50 ng/mL was noted by Catlow et al (1995). A therapeutic range of 5 to 75 ng/mL has been proposed by Xue et al (1998). Robertson (1998) analyzed 1655 serum samples on olanzapine and found mean concentrations of 36 ng/mL. Toxicity symptoms were observed at blood concentrations above 70 ng/mL. The pharmacokinetics of olanzapine may be significantly influenced by carbamazepine, an agent often used concomitantly in the treatment of maniac psychotic disorders (Lucas et al, 1998).

Pharmacokinetics of olanzapine was studied following 5 mg olanzapine tablets administered to 15 healthy human (11 male and 4 female) subjects. The study produced following results: half life ($t_{1/2}$), time of maximum concentration ($T_{max}$), maximum concentration ($C_{max}$), area under curve ($AUC_{0-\infty}$) and Cl/F for olanzapine were found as 32 hours, 3 hours, 7.6 ng/mL & 272 ng.h/mL and 18.4 L/h respectively (Gossen et al, 2002). In another pharmacokinetics study of olanzapine, following 10
mg olanzapine (Zyprexa®) tablets administered to 24 healthy adult male human subjects, the following results were achieved: half life ($t_{1/2}$), time of maximum concentration ($T_{\text{max}}$), level of maximum concentration ($C_{\text{max}}$), area under curve ($\text{AUC}_{0-\infty}$) and $K_e$ for olanzapine were found as 32 hours, 6 hours, 11 ng/mL, 367 ng.h/mL and 0.02 Litres/hour respectively (Gossen et al, 2002).

Olanzapine is metabolized primarily by glucuronidation and cytochrome P450-mediated oxidation into pharmacologically inactive metabolites, 10-$N$-glucuronide olanzapine and 2-hydroxymethylolanzapine, 4$'$-$N$-desmethyl olanzapine and 4$'$-$N$-oxide olanzapine (Kassahun et al, 1997; Ring et al, 1996; Keck and McElroy, 2002)

**Prochlorperazine maleate**

Prochlorperazine maleate, a phenothiazine derivative (molecular formula: $C_{20}H_{24}ClN_3S.2C_4H_4O_4$; molecular weight: 606.09) has been designated as first generation antipsychotic (FGA). It was used in the treatment of psychosis and the manic phase of bipolar disorder but now-a-days it is seldom used for this purpose because other safe drugs are available. It is widely used for the short term treatment of nausea and vomiting caused by chemotherapy (Olver et al, 1992; Crucitt et al, 1996), radiotherapy (Tramer et al, 1998) and surgery (Patterson et al, 1993). Prochlorperazine is more effective than octreotide (Miller et al, 2009), metoclopramide (Coppola et al, 1995, Jones, 1996), ketorolac and droperidol (Brousseau et al, 2004) in the clinical management of migraine symptoms (Pain and vomiting). Prochlorperazine is insoluble in water and alcohol. It is slightly soluble in dichloromethane, but soluble in methanol, diethyleneether, ter-butylether and chloroform.

Samples of blood collected from 18 healthy Chinese human male subjects receiving single dose of 15 mg prochlorperazine maleate tablets orally produced the following results: half life ($t_{1/2}$), time of maximum concentration ($T_{\text{max}}$), maximum concentration ($C_{\text{max}}$) and area under curve ($\text{AUC}_{0-\infty}$) were found as 8.2 ± 2.2 hours, 3.2 ± 0.9 hours, 3.6 ± 0.8 ng/mL and 29.5 ± 4.5 ng.h/mL respectively (Yan et al, 2009).
1.6: MOTIVATION BEHIND THE CURRENT WORK

The use of antipsychotics has been associated with frequently happening poor compliance causing a raise in personal burden and economic cost (Fenton et al 1997). The prochlorperazine is commonly prescribed to be taken three times a day for prevention and treatment of nausea and vomiting and thus, has more chances for non-compliance. On the other hand, frequent occurrence of side effects has been reported with the use of SGAs such as risperidone and olanzapine which are concentration-dependent. With the development of the extended release formulations for risperidone and olanzapine, it is expected to optimize the drug concentration in blood which may lead to reduction of their side effects. Thus, the above mentioned drugs were considered good candidates for presenting as extended release tablets.

The new available fine particle ethylcellulose with average particle size range of 6 to 50 µM are chemically equivalent to the existing standard ethoxyl grade of coarse ethylcellulose materials (Pollock and Sheskey, 1996). The effect of particle size of an excipient on the characteristics of the final product is well documented (Bodmeier and Chen, 1989; Bolhuis and Chowhan, 1996). Thus, these smaller ethylcellulose particle sizes may offer the new options for modulating the drug release besides addressing problems in the areas of tablet physical properties, active release profiles, and manufacturing limitations. The combined use of the HPMC and EC has not been studied thoroughly so far. Thus, the combination of these polymers was employed to design extended release tablet formulations of the model drugs.

1.7: OBJECTIVES, HYPOTHESIS AND AIMS

1.7.1 Objectives

The objectives of this dissertation was to develop extended release matrix (ERM) tablets of the three commonly used antipsychotic drugs, risperidone, olanzapine and prochlorperazine maleate with pH independent zero order release mechanism.
1.7.2 **Hypothesis:**

Hydroxypropylmethylcellulose, HPMC-2208 (Methocel® K100 LV-CR; a hydrophilic polymer) in combination with fine particle ethylcellulose-FPEC (Ethocel® Standard 7FP Premium; a hydrophobic polymer) may produce the desired 24 hour extended release matrix tablets of risperidone, olanzapine and prochlorperazine maleate with pH independent zero order mechanism of release.

1.7.3 **Aims**

1. To Study the effects of the following variables on the release rate and release mechanisms of respective drugs from the test tablet formulations:
   a. Different ratios of hydroxypropylmethylcellulose (Methocel® K100 LV-CR) and Fine Particle Ethylcellulose (Ethocel® Standard 7FP Premium).
   b. Compressional hardness
   c. Dissolution Medium such as 0.1 N HCl, pH 1.5 adjusted with 1 M NaOH and Phosphate Buffer, pH 6.8 adjusted with 0.1 N HCl or 1 M NaOH

2. To compare the *in-vitro* release profiles of the optimized extended release matrix tablets of the model drugs.

3. To evaluate the stability of the selected/optimised extended release tablets of the risperidone, olanzapine and prochlorperazine maleate matrix tablets formulations

4. To evaluate the bioavailability (BA) of the selected/optimised extended release matrix tablets and compare their BA with the commercially available conventional tablets (used as control)

5. To determine the level of *In-vitro/In-vivo* correlation between results of dissolution tests in vitro and bioavailability tests In-vivo for the formulations under study.
2.1: ANIMALS, MATERIALS AND EQUIPMENTS

2.1.1 Animals

<table>
<thead>
<tr>
<th>Animals</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbits</td>
<td>Local breed rabbits of either sex (Himalayan angora, male and female); purchased from Board Bazaar, Peshawar, Pakistan</td>
</tr>
</tbody>
</table>

2.1.2 Materials

2.1.2(a) Chemicals and reagents

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Source/Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetinitrile HPLC grade</td>
<td>Merck, Germany</td>
</tr>
<tr>
<td>Acetonitrile, HPLC-ECD grade</td>
<td>Fisher, UK</td>
</tr>
<tr>
<td>Colloidal silicon dioxide-Aerosil®</td>
<td>Maximum, Karachi, Pakistan, received through Bryon Pharmaceuticals, Peshawar; Pakistan</td>
</tr>
<tr>
<td>Ammonium Acetate, HiperSolve for HPLC</td>
<td>BDH, England</td>
</tr>
<tr>
<td>Dichloromethane stabilized with 20ppm amylase, PAI 361254</td>
<td>Panreac, France</td>
</tr>
<tr>
<td>Ethocel® Standard 7 FP Premium (fine particle ethyl cellulose-Ethocel®)</td>
<td>Dow Chemical Company, As a gift Provided by Colorcon Asia Pvt Ltd Lot No.119013T10, India; received through Bryon Pharmaceuticals, Peshawar; Pakistan</td>
</tr>
<tr>
<td>Glacial Acetic acid</td>
<td>Scharlau, France</td>
</tr>
<tr>
<td>Hydrochloric acid 37%, PA 131020 1612</td>
<td>Panreac, France</td>
</tr>
<tr>
<td>Lactose</td>
<td>Meggle, Germany; received through Bryon Pharmaceuticals, Peshawar; Pakistan</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>Solmom Enterprises, Karachi, Pakistan, received through Bryon Pharmaceuticals, Peshawar; Pakistan</td>
</tr>
<tr>
<td>Methanol HPLC grade</td>
<td>Merck, Germany</td>
</tr>
<tr>
<td>Methanol HPLC-ECD grade</td>
<td>Fisher, UK</td>
</tr>
<tr>
<td>Methocel® K 100 LV-CR (hydroxypropylmethylcellulose-HPMC)</td>
<td>Dow Chemical Company, as a gift sample; Provided by Colorcon Asia Pvt. Ltd. Lot No.SK050112N21, India; received through Bryon Pharmaceuticals, Peshawar; Pakistan</td>
</tr>
<tr>
<td>n-Pentane, PRS 142006.1612</td>
<td>Panreac, France</td>
</tr>
<tr>
<td>Sodium dihydrogen orthophosphate (NaH₂PO₄)</td>
<td>Fisher, UK</td>
</tr>
<tr>
<td>Sodium hydroxide (NaOH), Extra Pure; 50 wt% solution</td>
<td>Acros Organics, USA</td>
</tr>
<tr>
<td>Drug</td>
<td>Source</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sodium octylsulfonate</td>
<td>Fisher, UK</td>
</tr>
<tr>
<td><strong>2.1.2(b) Drugs</strong></td>
<td></td>
</tr>
<tr>
<td>Risperidone</td>
<td>Jubilant Organosis, India, received through Bryon Pharmaceuticals, Peshawar; Pakistan</td>
</tr>
<tr>
<td>9-Hydroxyrisperidone</td>
<td>Purchased from TLC PhamaChem, Canada</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>RPG Life Sciences, India, received through Danas Pharmaceuticals, Islamabad; Pakistan</td>
</tr>
<tr>
<td>Prochlorperazine maleate</td>
<td>Mehta API, India, received through Drug testing laboratory NWFP; Pakistan</td>
</tr>
<tr>
<td>Risperidone-Risperdal® tablets (4mg) by Johnson &amp; Johnson</td>
<td>Purchased from local market</td>
</tr>
<tr>
<td>Prochlorperazine maleate-Stemetil® tablets (5mg) by Sanofi Aventis</td>
<td>Purchased from local market</td>
</tr>
<tr>
<td>Olanzapine-Zyprexa® tablets (10mg) by Eli Lilly</td>
<td>Purchased from local market</td>
</tr>
</tbody>
</table>
### 2.1.3 Equipments

<table>
<thead>
<tr>
<th>Equipment/Model</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Balance AX 200</td>
<td>Shimadzu, Japan</td>
</tr>
<tr>
<td>Centrifuge (Table Top Centrifuge), Model-DSC 200A-2</td>
<td>Digisystem Laboratory Instruments Inc.</td>
</tr>
<tr>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>Column, RP C18 Shim Pack, CLC-ODS 150× 6 × 5 µM</td>
<td>Shimadzu, Japan</td>
</tr>
<tr>
<td>Column, PurospharR Star RP.C18e, HibarR RT 250-4.6 (5 µM)</td>
<td>Merck, Germany</td>
</tr>
<tr>
<td>Dissolution Tester DT6, 6Pot</td>
<td>Erweka, Germany</td>
</tr>
<tr>
<td>Water Bath</td>
<td>WisBath, Pakistan</td>
</tr>
<tr>
<td>Filter paper, Sartorius with diameter of 47 mm and pore size of 0.45 µM</td>
<td>Sartorius AG, Germany</td>
</tr>
<tr>
<td>Friability Tester FB-994</td>
<td>Curio, Pakistan</td>
</tr>
<tr>
<td>Hand held Sieve (Stainless steel), Local</td>
<td>Peshawar, Pakistan</td>
</tr>
<tr>
<td>Hardness, diameter and thickness Tester, CHT-901</td>
<td>Curio, Pakistan</td>
</tr>
<tr>
<td>High performance liquid chromatography (HPLC) system, including</td>
<td>Shimadzu Japan</td>
</tr>
<tr>
<td>A) CBM (communication boss module)-20A</td>
<td></td>
</tr>
<tr>
<td>B) Double Pumps-LC-20AT</td>
<td></td>
</tr>
<tr>
<td>C) Injection port, 7725i (Rheodyne, USA) coupled with</td>
<td></td>
</tr>
<tr>
<td>*ECD detector, Coulchem 111, model -5300, (ESA USA) along with dual analytical cells, model 5011 (Shimadzu, Japan) and *UV detector, SPD-20A (Shimadzu, Japan)</td>
<td>Shimadzu Japan</td>
</tr>
<tr>
<td>Micropipette (10-200 µL)</td>
<td>TreffLab, France</td>
</tr>
<tr>
<td>pH meter, 420A</td>
<td>Orion USA</td>
</tr>
<tr>
<td>Rotary Tablet Press ZP-17</td>
<td>Schangai Tiafeng Pharm. Machine Manufac. Com, China</td>
</tr>
<tr>
<td>Sonicator, SD-120H</td>
<td>Mujigae, Korea</td>
</tr>
<tr>
<td>Spectrophotometer UV-Visible, UV-1700</td>
<td>Shimadzu, Japan</td>
</tr>
<tr>
<td>Stability chamber, Ti-Sc-THH-07-0400</td>
<td>Faisalabad; Pakistan</td>
</tr>
<tr>
<td>Vacuum Filtration Assembly</td>
<td>Boeco, Germany</td>
</tr>
<tr>
<td>Vacuum Pump</td>
<td>Roeker 300, Taiwan</td>
</tr>
<tr>
<td>Viscometer LVDV-11+PRD</td>
<td>Brookfield USA</td>
</tr>
<tr>
<td>Vortex mixer, Gyromixer</td>
<td>Pakland scientific, Pakistan</td>
</tr>
</tbody>
</table>
2.2 GENERAL METHODS

2.2.1 Tablets manufacture

2.2.1(a) Preparation of powders-mix

Blends of Methocel® K100LV-CR (Hydroxypropylmethylcellulose, HPMC-2208) and Ethocel® Standard 7FP Premium (Fine particle ethylcellulose) were used as recommended by Tatavarti and Hoag (2006). Based upon trials and errors during the present work, three constrained mixtures of Methocel® K100-LV CR and Ethocel® Standard 7FP Premium were employed to manufacture the extended release matrix tablets using formulations F1, F2 and F3 of the three antipsychotic drugs (risperidone, olanzapine and prochlorperazine maleate).

For tablet of 200 mg, the compositions of relevant formulations are shown in Table 2.1. The amounts of lactose (filler); colloidal silicon dioxide- Aerosil® (glidant) and magnesium stearate (lubricant) in the tablets were kept fixed in all formulation (Table 2.1) in order to determine precisely the effect of the type and concentrations of the polymers added into the blends. The powders-mix for 600 tablets was prepared separately for each batch by geometric dilution method using polythene bags.

Table 2.1: Composition of designed extended release tablets of risperidone, olanzapine and prochlorperazine maleate

<table>
<thead>
<tr>
<th>Drug</th>
<th>Formulation</th>
<th>Methocel (%)</th>
<th>Ethocel (%)</th>
<th>Lactose (%)</th>
<th>Magnessium Stearate (%)</th>
<th>Colloidal silicon dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risperidone (2%)</td>
<td>F1</td>
<td>60</td>
<td>30</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>45</td>
<td>45</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>30</td>
<td>60</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Olanzapine (5%)</td>
<td>F1</td>
<td>60</td>
<td>30</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>45</td>
<td>45</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>30</td>
<td>60</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prochlorperazine maleate (12%)</td>
<td>F1</td>
<td>58</td>
<td>28</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>43</td>
<td>43</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>28</td>
<td>58</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: Weight of each tablet was 200 mg
2.2.1(b) Granulation and tableting

Slugs were prepared using Tablet Press, ZP-17 Shangai, China; equipped with a flat faced tooling, 17 mm in diameter. The slugs were crushed manually in pestle and mortar and then passed through an oscillating granulator equipped with 20 mesh screen to obtain granules of size less than 1 mm. Weighed amount of magnesium stearate (lubricant) was screened through sieve # 40 and mixed geometrically with the granules. The mixed granules of every formulation of each drug were divided into three batches to be compressed into tablets of 9 kg, 12 kg and 15 kg hardness.

The tablet formulations were manufactured by double compression (slugging) method using blends of release retarding polymers, Methocel K100 LV-CR (hydrophilic) and Ethocel Standard 7 FP Premium (hydrophobic). The total amount of the polymers blend in each formulation was kept fixed at 90% level in case of designed tablets of risperidone and olanzapine and at 86% level in case of designed tablets of prochlorperazine maleate.

Before compression, the corresponding amounts of Active Pharmaceutical Ingredients (APIs) such as risperidone, olanzapine and prochlorperazine maleate; Methocel® K100LV-CR and Ethocel® Standard 7 FP Premium, lactose, colloidal silicon dioxide-Aerocel® and magnesium stearate were weighed and screened through sieve # 40. The screened powders were mixed thoroughly in polythene bags for 10 minutes, using geometric dilution method. The following steps were followed for the preparation of tablets.

**Slugging**

The powder mix was compressed into slugs (rough tablets), each one weighing 700 – 800 mg using multiple punch tablet press (ZP 17; Shangai, China) equipped with flat faced tooling, 17 mm in diameter. The prepared slugs were stored in polythene bags till further processing.
Chapter-2: Methodology

Milling

The slugs were initially crushed in a pestle with the help of a round bottom mortar to obtain granules of larger particle size, which were further processed through an Oscillating granulator fitted with a 20 mesh screen for getting granules of size less than 1000 μM. The two step granulation process led to production of the desired size granules with a suitable proportion of fines.

Lubrication

The lubricant magnesium stearate and Aerosil® were added in two steps. In the first step, 50% of the pre-screened magnesium stearate and Aerosil® were added to the powders-mix before slugging and the remaining 50% of them were added after crushing of the slugs into granules. The whole mixing process was carried out manually in polythene bag, using geometric dilution method.

Compression

The prepared granules were divided into three batches for being compressed into 9 kg, 12 kg and 15 kg hard tablets. Each batch was compressed into 200 mg tetragonal tablets, using a Rotary Tablet Press (ZP-17, Shangai China) equipped with flat faced tooling with 8×3.5 mm dimensions.

Storage of manufactured tablets

The prepared tablets were stored in airtight, amber coloured glass bottles till further evaluation

2.2.2 In-vitro evaluation

2.2.2(a) Physicochemical evaluation of powders-mix and granules

Angle of repose

Angle of repose (AR) of the powders-mix was tested by the fixed funnel method (USP 30, 2007). The accurately weighed powders were taken in a funnel with orifice 8 mm in diameter. The powders were allowed to flow through the funnel orifice freely on a powder paper to form a cone like heap. The height of the heap of powder was adjusted in such a way that the tip of the funnel just touched the apex of
the heap of the powders. The diameter (base) and height of the powder cone were measured with the help of a ruler and the angle of repose was calculated using the following equation:

\[ \text{AR} = \tan \theta = \frac{h}{r} \]

Where \( h \) = height of the cone and \( r \) = radius or half of diameter (Base) of the cone formed by falling of the powder or granules on a smooth surface respectively from a funnel.

Granules were prepared by crushing the slugs in an oscillating granulator equipped with 20 mesh screen and AR of the granules were measured as mentioned for powders.

**Compressibility index and Hausner ratio**

To measure the compressibility index (CI) and the Hausner ratio (HR), a quantity of 100g material (powders or granules) from each Test formulation was gently poured into a 250 mL measuring cylinder. After noting the initial volume \( V_0 \), the tapping was employed on the cylinder by allowing it to fall under its own weight onto a hard surface from the height of 3 cm at few seconds intervals. The tapping was continued until no further change in volume was observed. The volume measured after tapping was taken as the final tapped volume, \( V_f \). The compressibility index and the Hausner ratio were calculated according to the standard USP30 method with the following equations.

\[ \text{CI} = \left( \frac{V_0 - V_f}{V_0} \right) \times 100 \]

\[ \text{HR} = \frac{V_0}{V_f} \]

Where \( V_0 \) is the initial volume before being tapped and \( V_f \) is the final volume after tapping of the powder or granules respectively in a cylinder (USP30, 2007).

**Drug content**

Accurately weighed 200 mg of the prepared granules were taken in a 250 mL volumetric flask, to which was added 100 mL of methanol and then shaked thoroughly for half an hour. The extract was suitably diluted before filtration through
0.45 mL filter membrane and thus content of drug in granules was analysed. Risperidone, olanzapine and prochlorperazine were analysed at their wavelengths of maximum absorbance, $\lambda_{\text{max}}$ 280 nm $\lambda_{\text{max}}$ 270 nm and $\lambda_{\text{max}}$ 254 nm respectively, using UV-Visible Spectrophotometer (Schimadzu, Model UV-1700, Japan). The experiment was repeated three times to find out average drug content and standard deviation (SD).

2.2.2 (b) Physicochemical evaluation of Tablets

**Weight Variation**

Twenty tablets from each batch of test formulations were accurately weighed individually in milligrams (mg) using an analytical balance (AX-200, Schimadzu; Japan). Average weight and standard deviation were calculated, using computer based excel programme and the results were recorded accordingly.

**Tablet hardness and dimensions**

The hardness and dimensions of the 10 pre-weighed tablets were accurately measured individually from each batch of the prepared tablet, using a dual function hardness and thickness tester (Curio H901; Pakistan). The average dimensions and standard deviation were calculated and noted accordingly.

**Friability**

Pre-weighed twenty tablets ($W_1$) from every batch of each formulation were rotated at 25 rpm for 4 minutes in the chamber of friability testing apparatus (Curio FB-994, Pakistan). Then the tablets were de-dusted well with the help of a blower and re-weighed the same twenty tablets ($W_2$) to determine their loss in weight. Percent Friability (F %) was thus calculated according to the following formula:

$$ F(\%) = 1 - \frac{W_2}{W_1} \times 100 $$

**Drug content**

10 tablets, each one weighing 200 mg; were weighed individually and then crushed to a fine powder. Accurately weighed 200 mg of this powdered material was transferred to a 250 mL volumetric flask and then extracted the drug with 100 mL
methanol and analysed as given above. The experiment was repeated ten times to find out average drug content and standard deviation (SD) in order to find out content uniformity of dosage units.

2.2.2(c) Drug dissolution

In-vitro drug release test were performed for the prepared tablets in accordance with the monograph “Dissolution Procedure” <711>, USP30 (2007) over a 24 hour period, using an Apparatus 2, Paddle Dissolution System (DT6, Erweka, Germany). The paddles were run at 50 rpm in 900 mL dissolution medium, thermostatically maintained at 37°C. Six tablets from each batch of every formulation were tested. The release of drug was measured at 1, 2, 4, 6, 8, 10, 12 and 24 hours. The release and dissolution of the risperidone, olanzapine and prochlorperazine maleate from their respective extended release tablets was analysed, using UV-Visible Spectrophotometer, UV-1700; Shimadzu Japan. The drug release was tested in two different dissolution media, including 0.1 N HCl, pH 1.5 and phosphate buffer, pH 6.8.

Drug Release Kinetics

To study the release kinetics of the drug from the test formulations, data obtained from dissolution studies in both dissolution media, 0.1N HCl, pH-1.5 and phosphate buffer, pH-6.8 were fitted in various kinetic models such as zero-order, first-order, Higuchi model and Hixson-Crowells cube root law and power law. The value of K (release rate constant) was calculated from the slope of the dissolution profiles. Kinetic models of evaluations are summarized in Table-2.2
Table 2.2: Kinetic models used for drug release analysis

<table>
<thead>
<tr>
<th>Kinetic Model</th>
<th>Equation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order (Hajiianou et al, 1993)</td>
<td>( W_t = K_0 t )</td>
<td>Whereas the ( W_t ) and ( W_0 ) represent percent drug release at time ( t ) and percent drug present in the Tablets initially (i.e. 100 percent) respectively and the ( K_0 ), ( K_1 ), ( K_H ) and ( K_{HC} ) are release rate constants of Zero order, First order Higuchi’s and Hixon-Crowel’s kinetic models respectively. ( M_t / M_\infty ) indicates fractional drug release from matrix into the dissolution media, ( k_{KP} ) is a release rate constant incorporating structural and geometric characteristics of the matrix and “n” is a diffusion exponent indicative of drug release mechanism.</td>
</tr>
<tr>
<td>First order (Singh et al, 1967)</td>
<td>( \log W_t = \log W_0 - \frac{K_1 \times t}{2.303} )</td>
<td></td>
</tr>
<tr>
<td>Higuchi square root time equation (Higuchi, 1963)</td>
<td>( W = K_H t^{1/2} )</td>
<td></td>
</tr>
<tr>
<td>Hixon and Crowel’s Cube root law equation (Hixon and Crowel, 1931)</td>
<td>( W_0^{1/3} - W_t^{1/3} = K_{HC} t )</td>
<td></td>
</tr>
<tr>
<td>Korsemeyer-Peppas (Power law) equation, (Korsemeyer et al 1983)</td>
<td>( M_t / M_\infty = k_{KP} t^n )</td>
<td></td>
</tr>
</tbody>
</table>

The criterion for selecting the most appropriate model was chosen on the basis of values of “n” and \( R^2 \) of best fit. To elucidate the mechanism of drug release from six tablets, dissolution data for the first 60% of drug release were fitted in Power law (Korsmeyer et al, 1983), as log cumulative percent of drug released versus log time, calculating the exponent “n” through the slope of the straight line.

Fickian diffusion refers to diffusion of drug through pores of the matrix, zero order demonstrates release of drug with erosion of the polymeric chains and anomalous transport refers to release of drug by a combined process of diffusion and erosion (Peppas, 1985). The criterion for selecting the most appropriate model (among the mathematical models mentioned above) was chosen on the basis of a “n” values or goodness of fit test (i.e. coefficient of determination \( R^2 \) value falling near to 1.0, which shows linearity of regression line) where necessary.
Table 2.3: Interpretation of release exponent “n” in Power law for release mechanism of different geometries (Ritger and Peppas, 1987a; Ritger and Peppas, 1987b)

<table>
<thead>
<tr>
<th>Exponent “n” for</th>
<th>Thin film</th>
<th>Cylinder</th>
<th>Sphere</th>
<th>Drug release mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.5</td>
<td>&lt; 0.45</td>
<td>&lt; 0.43</td>
<td></td>
<td>Fickian diffusion</td>
</tr>
<tr>
<td>0.5 &lt; n &lt; 1.0</td>
<td>0.45 &lt; n &lt; 0.89</td>
<td>0.45 &lt; n &lt; 0.85</td>
<td>Anomalous</td>
<td></td>
</tr>
<tr>
<td>&gt; 1.0</td>
<td>&gt; 0.89</td>
<td>&gt; 0.85</td>
<td></td>
<td>Zero order</td>
</tr>
</tbody>
</table>

Note: The word cylinder refers to tablets

Testing dissolution equivalency

Model independent method was used to test dissolution equivalency as suggested by Shah (1998) and Fassihi and Pillay (1996) and recommended for use by the US FDA (1997). Model independent method is a simple approach that uses a difference factor $f_1$ and a similarity factor $f_2$ to compare dissolution profiles. The difference factor $f_1$ calculates the percent difference between the two dissolution profiles at each time point and is a measure of the relative error between the two curves,

$$f_1 = \frac{\sum_{i=1}^{n} |R_i - T_i|}{\sum R_i} \times 100$$

Where $n$ is the number of pull points, $R_i$ is the dissolution value of the Reference tablet at time $t$ and $T_i$ is the dissolution value of the Test tablet at time $t$.

The similarity factor $f_2$ is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the curves,

$$f_2 = 50\log\left\{\left[1 + \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2\right]^{-0.5}\right\} \times 100$$

Where $n$ is the number of data points collected, $R_i$ and $T_i$ are the percent drug dissolved at each time point for Reference and Test tablets respectively.
Two release profiles are considered similar if the values of difference factor $f_1$ is close to 0 and similarity factor $f_2$ is close to 100. Generally, $f_1 \leq 15$ and $f_2 \geq 50$, indicates an average difference of not more than 10% at the sample time points (Gohel and Panchal, 2002; Shah, 1998; US FDA, 1997).

### 2.2.2(d) Selection of tablet formulation

For each drug, three tablet formulations, F1, F2 and F3 with different ratios of polymer and co-polymer were prepared. Each formulation was compressed at different hardness levels of 9 kg, 12 kg and 15 kg and thus, by this way, 9 batches in case of each drug were prepared. Out of the nine batches, one formulation F3 with 12 kg hardness was selected for further testing based upon the 24 hours release period and optimum level of physicochemical characteristics, release rate and release mechanism.

### 2.2.2(e) Reproducibility and accelerated stability study

The selected optimised tablet formulations in case of each drug were stored in high density polyethylene (HDPE) Jars and tested for stability under the short term accelerated storage conditions. Short term stability study was conducted under the International Commission for Harmonisation (ICH) guidelines for accelerated storage conditions i.e. at $40 \pm 2\^\circ C/75 \pm 5\%$ Relative humidity (RH) conditions. At predetermined time intervals of 0, 1, 2, 3, 4, 5 and 6 months the tablets were evaluated for their different physicochemical parameters like the appearance, hardness, friability, weight variation and content uniformity.

### 2.2.3: In-vivo evaluation

#### 2.2.3(a): Study protocol and design

The study protocol was approved by Research and Ethical Committee (REC) of the HMC Hospital, Peshawar, Pakistan in its 3rd meeting. The procedure was carried out in accordance with the Animal Scientific Procedure Act, 1986. The pharmacokinetic parameters of the optimised Test tablet and the respective Reference (conventional) tablets for comparison were studied in two animal groups, each comprising of 6 rabbits in a parallel study design. The relative bioavailability and In-vitro In-vivo correlation were determined with the help of suitable formulas. The details of the study design has been given in Table 2.4.
Table 2.4: Study design for pharmacokinetics and bioavailability of risperidone, olanzapine and prochlorperazine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>conventional olanzapine tablets (10 mg) <strong>Zyprexa®</strong></td>
<td>1 tablet</td>
</tr>
<tr>
<td>2</td>
<td>Test extended release tablet of olanzapine (10 mg)</td>
<td>1 tablet</td>
</tr>
<tr>
<td>3</td>
<td>conventional risperidone tablets (4 mg) <strong>Risperdal®</strong></td>
<td>1 tablet</td>
</tr>
<tr>
<td>4</td>
<td>Test extended release tablet of risperidone (4 mg)</td>
<td>1 tablet</td>
</tr>
<tr>
<td>5</td>
<td>conventional prochlorperazine maleate tablets <strong>Stemetil®</strong> (8 mg equivalent 5 mg base)</td>
<td>1 tablet × 3</td>
</tr>
<tr>
<td>6</td>
<td>Test extended release tablet of prochlorperazine maleate (24 mg equivalent 15 mg base)</td>
<td>1 tablet</td>
</tr>
</tbody>
</table>

**Note:** Treatment 1, 2, 3, 4 and 6 with dose of 1 tablet means that one tablet of the drug was administered to each rabbit of the group (n = 6), while treatment 5 with dose of 1 tablet × 3 means that one tablet 8 hourly (3 tablets per day) was administered to each rabbit of the group (n = 6)

**Animals**

The local breed rabbits of either sex, weighing 2.5 ± 0.3 kg were used for In-vivo studies as were used by Jing et al (2006); Ishikawa et al (2000) and Maeda et al (1979). For assessment of the relative pharmacokinetics of the test formulations F3 with 12 kg hardness and the commercial formulation, rabbits were divided into two groups, each one six in number. The rabbits were kept fasting for 24 hours before administration of tablets and until 24 hours post dosing. However, during this period water was allowed at libitum.

**Animal housing and maintenance**

A standard food for the rabbits was prepared in accordance with the already published recipe composed of 40% Bran, 20% Grass meal, 18% Middlings, 12% Ground oats and 10% white fish meat (Kelley et al, 1992). The standard food was given to the rabbits for at least three days prior to drug administration. The rabbits were weighed and the food withdrawn for 24 hour. Water was allowed ad libitum during the fasting period.
2.2.3(b) Administration of dose

The rabbits were shifted to placement restraints (wooden holder) and the tablets administered orally, using a 3 mL syringe with its barrel smoothly cut at the needle end (Care was taken to smoothen the edges of the cut end of the syringe for avoiding damage to the oral mucosa of rabbit). Once tablet swallowing was confirmed, the rabbit received 10 mL tape water with the help of a 10 mL syringe fitted with oral tube in order to mimic human dosing.

Figure 1: 3 mL syringe with cut end through which tablet was administered.

2.2.3(c) Withdrawal of blood Samples

The blood samples (0.7 mL each time) were collected from the marginal ear vein of the rabbits at the time points of 0, 1, 2, 4, 6, 8, 12, 24 and 48 hours for the Test and Reference tablets of olanzapine and risperidone. Blood samples were withdrawn at 1, 2, 4, 6, 8, 9, 10, 12, 14, 16, 17, 18, 20, 22, 24 and 28 hours in case of prochlorperazine maleate Reference tablets (which were administered three times a day at 8 hours intervals), and at 1, 2, 4, 6, 8, 12, 24 and 28 hours in case of the Test tablet of prochlorperazine maleate.

2.2.3(d) Extraction of drug from serum

The blood was allowed to clot for 30 minutes. The resulting clot was rimmed with a sterile wooden stick and placed it still in the original collection tube in the refrigerator for at least one hour. The processed blood samples were centrifuged at 2800 rpm for 10 minutes and collected 200 µL serum off the clot with the help of a pasture tube and centrifuged again at 2800 rpm for 10 minutes. 100 µL of clear serum so obtained was transferred to a clean glass made test tube and placed in a refrigerator until further processing.
Extraction of risperidone and 9-hydroxyrisperidone from the Serum

Risperidone and 9-hydroxyrisperidone from the serum samples were extracted by a simple one step liquid liquid extraction with slight modification in the method reported by Aravagiri et al (1998). Briefly, added 6 mL of the extraction solvent mixture, consisting of pentane and dichloromethane (85:15) to 100 µL serum sample collected after its basification with 100 µL of 1M NaHCO₃. After Vortex mixing for a few seconds, the mixture was centrifuged at 2800 rpm for 10 minutes. The supernatant liquid was collected and filtered through a 0.45μM disposable syringe filter and was then placed in a 10 mL disposable borosilicate glass made test tube. The extraction solvent was evaporated from the processed sample at 50°C in a water bath (WiseBath) under nitrogenous atmosphere. The resulting residue was dissolved in 100 µL acetonitrile and refrigerated at –20°C until analysis by HPLC.

Extraction of olanzapine from the serum

Olanzapine from the serum samples was extracted by a simple one step liquid liquid extraction in accordance with the method reported by Aravagiri et al (1997). The sample was further treated following the procedures used for extraction of risperidone and 9-hydroxyrisperidone, except, that serum sample was basified with 100 µL of 1M NaOH

Extraction of prochlorperazine maleate from the serum

Prochlorperazine from the serum samples was extracted by a simple one step liquid liquid extraction in accordance with the method reported by Fowler et al (1986). Briefly, 6 mL of the extraction solvent, chloroform was added to the collected 100 µL serum sample after its basification with 100 µL of 1M NaOH. The sample was further treated using the same procedure as given for risperidone and 9-hydroxyrisperidone except that mobile phase, consisting of ion pairing solution, methanol and acetonitrile (45:15:40) was used for dissolution of the prochlorperazine.
2.2.3(e) Determination of drug concentration in serum samples

Analysis of risperidone and 9-hydroxyrisperidone

The risperidone and 9-hydroxyrisperidone in serum were determined according to the method described by Cho and Lee (2006), using HPLC coupled with UV detector. Briefly, the HPLC system consisted of Shimadzu solvent delivery pump model LC-20 AT, fitted with a 20μl sample loop in Rheodyne injection port. The eluted chromatographic peaks were detected at $\lambda_{max}$ 280 nm by UV detector (SPD-20A), using the analytical column RP C18 HibarR RT 250 $\times$ 4.6 $\times$ 5. The mobile phase consisted of 130 mM ammonium acetate, methanol and acetonitrile (40:20:40). The pH of the mobile phase was adjusted to 6.5 by addition of a few drops of 0.1N HCl. The mobile phase was degassed by filtering through the 0.45 μM nylon filter and sonicator before use. The compounds were eluted isocratically with mobile phase flow rate of 0.9 mL (pump pressure being 120 kgf).

Analysis of olanzapine

The chromatographic separations of olanzapine were carried out with an HPLC system consisting of a Shimadzu solvent delivery pump model LC-20 AT, fitted with a 20μl sample loop in Rheodyne injection port. The eluted chromatographic peaks were detected by an ESA Coulchem 111 model 5300 electrochemical detector fitted with a dual electrode high sensitivity analytical cell (model 5011A ESA) and a Guard cell ESA (Model 5020). The analytical column was a RP C18 ShimPak 150 $\times$ 6 $\times$ 5.

The analytical cell was utilized in an oxidation (+200mv)/reduction (–200mv) mode. The Guard cell was kept at slightly higher potential (300mv). Aliquots of the serum extracts (20μl) were injected into the HPLC system, utilizing a filtered and well sonicated/degassed mobile phase consisting of 75 mM sodium phosphate (pH 7) methanol and acetonitrile (48:26:26). The chromatographic determination was carried out in the light of a method reported by Catlow et al (1995) with slight modification.
Analysis of prochlorperazine

The chromatographic separations of prochlorperazine were carried out using the same HPLC-UV system as used for the risperidone and 9-hydroxyrisperidone except that the UV detector used was set at $\lambda_{\text{max}}$ 254 nm. The USP 30 (2007) recommended mobile phase, consisting of ion pairing solution, methanol and acetonitrile (45:15:40) was used for elution of prochlorperazine. The mobile phase was deaerated by filtering through the 0.45 μM nylon filter and then sonicated before use. The compound was eluted isocratically with the mobile phase with a flow rate of 1.0 mL (pump pressure being 185 kgf). The ion pairing solution was prepared by transferring 4.33g of sodium 1-octanesulfonate to 1 litre volumetric flask and then dissolving it in 1000 mL of purified water.

Standard curves and methods validation

Separate stock solutions of risperidone & 9-Hydroxyrisperidone, olanzapine and prochlorperazine maleate were prepared by dissolving suitable amounts of them in methanol to obtain their final concentration of 0.1 mg/mL. The stock solutions were stored at –20°C till further processing.

Standard curve consisting of eight spiked samples with concentrations ranging from 2 ng/mL to 100 ng/mL were prepared in pooled serum samples collected from drug free rabbits. These samples were also used for determination of the extraction efficiency, limit of quantification and linearity of the standard curve. Precision and accuracy of the data were determined using the prepared samples three times a day on each of the three consecutive days.

2.2.3(f): Instrument and chromatographic conditions

Schimadzu HPLC System was used for determination of drug concentration in serum (blood), a detailed account of which is given in Sections 3.2.2.3(f), 4.2.2.3(f) and 5.2.2.3(f)

2.2.3(g) Pharmacokinetic analysis

The plasma concentration-time data of the risperidone and olanzapine for each animal was analysed by interactive, pharmacokinetic computer software, WinNolin® Ver 5.2.1 (Pharsight Corporation, Mountain View, CA, USA). The multiple
dose-based pharmacokinetic data for prochlorperazine maleate was analyzed using software, PK-Fit; using the non-compartmental approach, based on statistical moment theory implemented in the software. The program yielded kinetic parameters of absorption, distribution and elimination for individual data set. The peak concentration \( C_{\text{max}} \) and time of its occurrence \( T_{\text{max}} \) were read directly from each concentration-time data. Area under the plasma level time curve (AUC and moment plasma level time curves (AUMC) were calculated by trapezoidal method. The ratio of the above areas was used to estimate the mean residence time (MRT) of the drug. For the computation of the terminal rate constant (Lz), the program used a minimum of three data points. Where the computation of Lz was not possible for all the animals, best fit implemented in software was used. The \( t_{1/2} \), clearance \( (C_l) \) and volume of distribution \( (V_d) \) were estimated with the help of following equations,

\[
t_{1/2} = \frac{\ln(2)}{Lz};\ C_l = \frac{\text{Dose}}{\text{AUC}_{0-\infty}} \text{ and } V_d = \frac{C_l}{Lz}\ 
\]

2.2.3(h) Relative bioavailability

Relative bioavailability was determined according to the following formula.

\[
\text{Relative bioavailability} = \frac{AUC_{0-t}^{(\text{Test})}}{AUC_{0-t}^{(\text{Reference})}} \times 100
\]

2.2.4 In-vitro and In-vivo correlation

The In-vitro and In-vivo correlation (IVIVC) of each optimized tablet was determined graphically by finding coefficient of determination \( (R^2) \) when percent drug absorbed \( (F_a) \) was plotted against percent drug released \( (F_r) \). The values of the percent drug dissolved were obtained from In-vitro release data and percent drug absorbed was calculated, using the Wagner and Nelson equation (Wagner and Nelson, 1964).

\[
F_a = \frac{C_t + K_{el} \times AUC_{0-t}}{K_{el} \times AUC_{0-\infty}} \times 100
\]

Where \( F_a \) is the fraction of drug absorbed, \( C_t \) is the serum drug concentration at time \( t \), \( K_{el} \) is the total elimination rate constant, \( AUC_{0-t} \) and \( AUC_{0-\infty} \) are areas under the curves between time zero and time \( t \) and between time zero and time infinity respectively.
Statistical analysis

The parametric data generated for individual rabbit was further processed statistically for getting mean and standard deviation. Using personal computer based PrismGraphPad, the student t test was applied to the data for determination of significance of difference ($P \leq 0.05$) between means of treatments.
3.1: INTRODUCTION

Risperidone, a second generation antipsychotic (SGA) drug, is widely used for the clinical management of psychotic illnesses including schizophrenia, bi-polar disorders and irritability in autistic disorders (Potter and Hollister, 2004; Tandon and Jibson, 2003). Risperidone is rapidly and almost completely absorbed following oral administration (Heykants et al, 1994) but approximately one third of risperidone is metabolised via first pass effect in the liver before reaching systemic circulation (Byerly and De Vane, 1996).

The primary metabolite, 9-hydroxyrisperidone is nearly equipotent with risperidone as a 5-HT₂ and D₂ receptors antagonist (Heykants et al, 1994), so the serum concentration of the active moiety is the sum of the serum concentrations of risperidone and 9-hydroxyrisperidone (De Leon et al, 2007; Hendset al, 2006; Byerly and De Vane, 1996; Huang et al, 1993). The absolute bioavailability of risperidone and 9-hydroxyrisperidone when combined is almost 100% after oral administration (Ereshefsky and Lacombe, 1993).

Time to peak plasma concentration for risperidone ranges from 1.44 to 1.67 hours (Cho and Lee, 2006). The combined Cmax of risperidone and its 9-hydroxyrisperidone metabolite has been reported to be 30 ng/mL for 4 mg tablets (Bondolfi et al, 2002) and 50 ng/mL for 6 mg tablets (Aravagiri et al, 1998) while their combined elimination half life is approximately 20 hours in both poor and extensive metabolisers (Heykants et al, 1994). Risperidone has a volume of distribution of 1.1 litres/kg (Huang et al, 1993).

Hiemke et al (2004) suggested that 20 to 60ng/mL are optimal concentrations for therapeutic activity, while Mauri et al (2007) suggested that the upper limit for extrapyramidal side effects (EPS) is 74ng/mL.

Clinical studies suggest that plasma levels of risperidone correlate with adverse drug effects (Spina et al, 2001), which in turn are associated with poor compliance (Weiden et al, 2004; Gerlach, 2002; Robinson, 2002). Current users of First generation antipsychotics (FGAs) and Second generation antipsychotic (SGAs) had a similar, dose-related increased risk of sudden cardiac death (Wayne et al, 2009),
obesity (Weiden et al, 2004; Allison et al, 1999), diabetes mellitus and dyslipidemia (Meyer and Koro, 2004), extrapyramidal side effects-EPS (De Leon et al, 2007; Lee et al, 2005). Risperidone has been worst out of SGAs for EPS in the dose range of 8-16 mg/day (Lemmens et al, 1999), which limits the use of this drug. Risperidone may share some of the adverse effects seen with the FGAs e.g dry mouth, constipation, difficulty in micturation and agitation; though the incidence and severity of such effects may vary (Sweetman, 2008).

Nonadherence with antipsychotic medication remains the highest predictor of patient relapse and rehospitalisation (Lieberman et al, 2005; Harris et al, 2002; Svarstads et al, 2001; Olfson et al, 2000). Although some studies show a significant advantage in adherence rates with use of SGAs versus FGAs (Jones, 2006; Rosenheck, 2006), others show no advantage (Gianfrancesco et al, 2006; Weiden, 2004) or mixed results (Dolder et al, 2002).

Up to 50% of individuals with schizophrenia have significant difficulties in adhering to treatment (Ascher-Svanum et al, 2006; Weiden et al, 2004; Corriss et al,. 1999) and there are reports that only 11.6% of treated patients achieved 1 year of uninterrupted antipsychotic medication therapy (McCombs et al, 1999). Consequently, such non-adherent patients are twice as likely to undergo rehospitalization from relapse (Svarstad et al., 2001), resulting in a poor quality of life and increased economic burden at health care cost (Menzin et al, 2003).

Oral extended release formulations allow reduction in dosing frequency as well as side effects as compared to formulation presented as a conventional dosage form (Qiu and Zhang, 2000). New formulations of antipsychotic drugs have shown advantages over older formulations in terms of convenience, side effects and efficacy (Keith, 2006). Improved efficacy and tolerance were observed in patients with schizophrenia taking quetiapine XR (extended release) tablets as compared to conventional tablets (Ganesan et al, 2008; Moller et al, 2008; Kahn et al, 2007; Peuskens et al, 2007). Similarly the paliperidone (Invega®) extended release tablets have shown improved treatment and tolerability profiles (Kane et al, 2007; Marder et al, 2005).
As per the available literature, no study has been reported so far regarding preparation of risperidone extended release tablets. Based upon frequent occurrence of serious side effects, the present study was aimed at the development of extended release tablet of risperidone to achieve an optimum and stable concentration of it in blood for minimizing its side effects. Such an achievement usually helps in avoiding side effects and getting improvement in compliance.

The widely recommended polymers, Methocel® K100 LV-CR and Ethocel® Standard 7FP Premium (Li et al, 2005; Tiwari and Rajabi-Siahboomi, 2008) were used in combination as polymer and copolymer to exploit their good characteristics for getting extended drug release with zero order kinetics.

3.2: MATERIALS AND METHODS

3.2.1: Materials

Risperidone (Jubilant organosys science active Ltd India) was provided by Bryon Pharma, NWFP, Pakistan as a gift and 9-hydroxyrisperidone was purchased from TLC PharmaChem, Canada. Methocel® K100LV-CR and Ethocel® Standard 7FP Premium were obtained as gift from the Colorcon Asia Ltd, India. Risperdal tablets-conventional (batch No.6914 dated May, 2007; Johnson and Johnson (Pvt) Ltd. Pakistan) containing 4 mg risperidone, were purchased from local market and used as Reference during in vivo studies. Acetonitrile and Methanol, both of HPLC grade were purchased from authorized local supplier of Merck, Germany. All the other chemicals used were of analytical or pharmaceutical grade.

3.2.2: Methods

3.2.2.1: Tablet manufacture

3.2.2.1(a): Preparation of powders-mix

The powders-mix of risperidone, Methocel®, Ethocel®, lactose, colloidal silicon dioxide-Aerosil® and magnesium stearate was prepared for six hundred tablets, using the method given in chapter-2, Section 2.2.1(a).
3.2.2.1(b) Granulation and tableting

Matrix tablets were prepared by dry granulation (slugging) method, using the ingredients mentioned in the Table-2.1. The granules of each formulation were divided into three batches for compression into tablets of 9 kg, 12 kg and 15 kg hardness as mentioned in Chapter-2, Section 2.2.1(b). Rotary tablet machine (Tablet Press, ZP-17 Shangai, China); with flat faced tooling of 8.00 × 3.5 mm diamensions was used in the manufacture of the tablets.

3.2.2.2: In-Vitro evaluation

3.2.2.2(a): Physicochemical evaluation of powders-mix and granules

Physical characteristics, such as the angle of repose (AR), compressibility index (CI), and Hausner ratio (HR) of the powders-mix and granules (prepared by crushing of compacted slugs) were determined as outlined in Chapter-2, Section 2.2.2 (a). Risperidone content in a sample of 200 mg prepared granules was analysed at wavelength of maximum absorbance, $\lambda_{\text{max}}$ 280 nm; using UV-Visible Spectrophotometer (Schimadzu, Model UV-1700, Japan), as outlined in Chapter-2, Section 2.2.2(a).

3.2.2.2(b): Physicochemical evaluation of tablets

The prepared tablets of risperidone were evaluated for various physicochemical characteristics, such as friability, hardness, weight variation and drug content. Risperidone content of the tablets was determined at $\lambda_{\text{max}}$ 280 nm with UV-Visible Spectrophotometer as described in Chapter-2, Section 2.2.2(b)

3.2.2.2(c): Drug dissolution

The drug release profiles of six tablets from each batch was measured as outlined in Chapter-2, Section 2.2.2(c), using UV-Visible Spectrophotometer at $\lambda_{\text{max}}$ 280 nm. The drug release data were fitted to various kinetic models including zero order, first order, Higuchi’s square root of time equation, Hixon and Crowel’s cube root equation and Power law equation, a detailed account of which is given in Chapter-2, Section 2.2.2(c)
Dissolution profiles of 9 kg, 12 kg and 15 kg hard tablets of the selected formulation F3 were determined at pH 6.8 and then compared with the dissolution profiles of these tablets determined at pH 1.5 to find out dissolution equivalency, difference factor $f_1$ and similarity factor $f_2$; as described in Chapter-2, Section 2.2.2 (c).

3.2.2.2(d): Selection of tablet formulation for stability and In-vivo studies

Optimised risperidone tablet formulation was selected based on better flowability and compressibility of granules and drug release profiles (zero order profiles with $R^2$ values approaching 1) of the prepared tablets.

3.2.2.2(e): Reproducibility and accelerated stability study:

Reproducibility of the manufacturing process was determined by preparing three repeated batches of the selected optimized formulation (F3 with 12 kg hardness) on three different occasions. The optimised tablets were stored in high density polyethylene (HDPE) jars and kept under accelerated storage conditions of $40 \pm 2 \degree C / 75 \pm 5\% \text{ relative humidity (RH)}$ in a stability chamber (Ti-Sc-THH-07-0400, Faisalabad, Pakistan) in accordance with International Commission for Harmonization (ICH) guidelines for a period of 6 months. The samples were tested for appearance, percent drug content, percent friability and hardness at predetermined time intervals of 0 time (prestorage) and after storage at 1, 2, 4 and 6 months respectively.

3.2.2.3: In-vivo evaluation

3.2.2.3(a): Study protocol and design

For in vivo experiments, the rabbits were divided into two groups, each having six animals. The first group received the Risperdal® (Johnson and Johnson) as Reference tablet and the second group received the extended release risperidone Test tablets, a detailed account of which is given in Chapter-2, Section 2.2.3(a)

3.2.2.3(b): Administration of dose

The dose of the drug was administered according to the procedure stated in Chapter-2, Section 2.2.3(b)
3.2.2.3(c): Withdrawal of blood samples

Blood samples (0.7 mL each time) were collected from the marginal ear vein at 0, 1, 2, 4, 6, 8, 12, 24 and 48 hours in test tubes and allowed to clot. A 200 µL serum was withdrawn, centrifuged at 2800rpm for 10 minutes and 100 µL of clear serum so obtained was transferred to 10 mL test tubes and stored at -20°C until the time of analysis.

3.2.2.3(d): Extraction of risperidone and 9-hydroxyrisperidone from serum

Serum samples were prepared by liquid-liquid extraction as described by Aravagiri et al (1998). Briefly, to the 100 µL spiked or sample serum, added 100 µL of 1M sodium carbonate, and 6 mL of pentane & dichloromethane (85:15) mixture and vortex mixed. The mixture was centrifuged at 2800rpm and the supernatant (organic) layer transferred into test tubes for drying under nitrogenous atmosphere. The residue obtained after drying was dissolved in 100 µL of acetonitrile by vortex mixing. 20 µL of the prepared sample was injected into the HPLC System for analysis.

3.2.2.3(e): Determination of risperidone and 9-hydroxyrisperidone concentrations in serum samples

Blood serum level of risperidone and its metabolite, 9-hydroxyrisperidone was determined by HPLC coupled with UV detector {for details see Chapter-2, Section 2.2.3(e)}

3.2.2.3(f): Instrument and chromatographic conditions

The risperidone and 9-hydroxyrisperidone in serum were determined according to the method described by Cho and Lee (2006), using an analytical column (Purosphar® Star RP.C18e Hibar® RT 250 mm×4.6×mm×5µM, Merck, Japan) fitted into the Schimadzu liquid chromatographic system-equipped with communication boss module (model 20A), two independently working pumps (model LC-20AT) and a variable wavelength UV-Visible detector (model SPD-20A) set at 280 nm. Mobile phase, consisting of 130 mM ammonium acetate aqueous solution: methanol: acetonitrile (40:20:40, pH 6.5), was used at the flow rate of 0.9 mL/min for elution of analytes.
3.2.2.3(g): Pharmacokinetic analysis

Pharmacokinetic analysis was performed by standard non-compartmental method (Gibaldi and Perrier, 1982) using interactive, pharmacokinetic computer base software, WinNolin® Ver 5.2.1 (Pharsight Corporation, Mountain View, CA, USA). The peak concentration (\(C_{\text{max}}\)) and time of its occurrence (\(T_{\text{max}}\)) were read directly from each concentration-time data. Area under the serum level-time curve (AUC) and area under moment serum level-time curves (AUMC) were calculated by trapezoidal method. The ratio of the areas was used to estimate the mean residence time (MRT) of the drug. For the computation of the terminal elimination rate constant (\(K_{\text{el}}\)), the program used a minimum of three last data points. Where the computation of elimination rate constant (\(K_{\text{el}}\)) was not possible for all the rabbits, best fit implemented in software was used. The half life (\(t_{1/2}\)), total clearance (\(C_{\text{total}}\)) and volume of distribution (\(V_{d}\)) were estimated by the formulas, \(t_{1/2} = \ln(2) / K_{\text{el}}\); \(C_{\text{total}} = \frac{\text{Dose}}{\text{AUC}_{0-\infty}}\) and \(V_{d} = \frac{C_{\text{total}}}{K_{\text{el}}}\), respectively. The parametric data generated for individual rabbit was further processed statistically for getting mean and standard deviation. Student t test was applied to the data for determination of significance of difference (\(P \leq 0.05\)) in means between different treatments.

3.2.2.3(h): Relative bioavailability

Relative bioavailability of the Test and Reference tablets were determined as outlined in Chapter-2, Section 2.2.3(h).

3.2.2.4: In vitro and in vivo correlation

The In-vitro and In-vivo correlation (IVIVC) of the optimized batch of risperidone tablets was tested according to the procedure given in Chapter-2, Section 2.2.4.

3.3: RESULTS

3.3.1.1: Physicochemical evaluation of powders-mix and granules

As shown in Table-3.1; the angle of repose (AR) for powders-mix varied from 47 ± 2° to 55 ± 4°, indicating poor flowability as compared to granules for which the angle of repose ranged from 31 ± 2° to 33 ± 2°, indicating good flowability. The compressibility index of powders-mix varied from 26 ± 3% to 31 ± 2%, indicating
poor compressibility as compared to granules, for which it ranged from 11 ± 2% to 13 ± 2% indicating good compressibility. Hausner ratio (HR) followed the same trend (1.36 ± 0.16 to 1.45 ± 0.18 for powders and 1.13 ± 0.12 to 1.18 ± 0.12 for granules) as was noted for AR and CI of powders-mix and granules. Drug content of granules were 103 ± 4%, 102 ± 3% and 104 ± 3% for formulations F1, F2 and F3 respectively.

Table 3.1: Physicochemical characteristics of powders-mix and granules prepared for manufacture of extended release tablets of risperidone (Mean ± SD, n = 3)

<table>
<thead>
<tr>
<th>Material</th>
<th>Angle of Repose (degrees)</th>
<th>Compressibility Index (%)</th>
<th>Hausner Ratio</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powders mix</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>47 ± 2</td>
<td>26 ± 3</td>
<td>1.36 ± 0.16</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>49 ± 3</td>
<td>28 ± 4</td>
<td>1.42 ± 0.13</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>55 ± 4</td>
<td>31 ± 2</td>
<td>1.45 ± 0.18</td>
<td>-</td>
</tr>
<tr>
<td>Granules</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>31 ± 2</td>
<td>11 ± 2</td>
<td>1.13 ± 0.12</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>F2</td>
<td>33 ± 2</td>
<td>13 ± 2</td>
<td>1.16 ± 0.15</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>F3</td>
<td>32 ± 2</td>
<td>12 ± 1</td>
<td>1.18 ± 0.12</td>
<td>104 ± 3</td>
</tr>
</tbody>
</table>

Note: F1 contains 60% Methocel® and 30% Ethocel®, F2 contains 45% Methocel® and 45% Ethocel®, while F3 contains 30% Methocel® and 60% Ethocel®

3.3.1.2: Physicochemical evaluation of tablets

The tablets from each batch were found uniform with respect to dimensions (length × width, 8.0×3.5mm to 8.1×3.6mm), the percent weight variation (2 ± 0.4 to 4 ± 0.5), percent friability (0.36 ± 0.05 to 0.39 ± 0.08) and percent drug content (100 ± 3 to 103 ± 2) represented by the results of the selected formulation F3 (Table 3.2); fulfilling the dosage uniformity requirements of USP.
Table 3.2: Physicochemical characteristics of extended release tablets of risperidone for its selected formulation F3 (Mean ± SD, n = 10)

<table>
<thead>
<tr>
<th>Hardness of tablets</th>
<th>Friability (%)</th>
<th>Weight Variation (%)</th>
<th>Drug Content (%)</th>
<th>Dimensions (Length × Width) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 kg</td>
<td>0.39 ± 0.08</td>
<td>4 ± 0.5</td>
<td>100 ± 3</td>
<td>8.1 ± 0.1×3.6 ± 0.1</td>
</tr>
<tr>
<td>12 kg</td>
<td>0.36 ± 0.05</td>
<td>3 ± 0.3</td>
<td>103 ± 2</td>
<td>8.0 ± 0.1×3.5 ± 0.1</td>
</tr>
<tr>
<td>15 kg</td>
<td>0.40 ± 0.04</td>
<td>2 ± 0.4</td>
<td>101 ± 3</td>
<td>8.1 ± 0.1×3.5 ± 0.1</td>
</tr>
</tbody>
</table>

Note: F3 contains 30% Methocel® and 60% Ethocel®

3.3.1.3: Drug dissolution

3.3.1.3(a): Effect of formulation on drug release

Based on the data given in Table 3.3, the Figures 3.1-3.6 demonstrate the maximum of 8 hours, 12 hours and 24 hours release periods from the designed formulations of F1, F2 and F3 respectively. The drug release rates (%/hour) were 12.09, 8.13, 4.11 for F1, F2 and F3 with 9 kg hardness; 12.29, 8.26, 4.17 for F1, F2 and F3 with 12 kg hardness and 11.91, 8.13, 4.10 for F1, F2 and F3 with 15 kg hardness in pH-1.5. Nearly same trend and levels of release rates were observed for the above mentioned tablets in pH-6.8 (Table 3.3).
Table 3.3: Effect of formulation (F1, F2 and F3), dissolution media (pH-1.5 and pH-6.8) and tablet hardness (9 kg, 12 kg and 15 kg) on release kinetics of risperidone from its extended release tablets

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness</th>
<th>pH of the dissolution medium = 1.2</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Zero order</td>
<td>Higuchi's</td>
<td>First order</td>
<td>Hixon-Crowel</td>
<td>Korsemeyer</td>
<td>Results</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td></td>
<td></td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>n</td>
<td>R²</td>
<td>Mechanism of drug release</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 kg</td>
<td>12.09</td>
<td>0.998</td>
<td>45.94</td>
<td>0.971</td>
<td>-0.213</td>
<td>0.816</td>
<td>-0.311</td>
<td>0.982</td>
<td>0.88</td>
<td>0.994</td>
<td>Anomalous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 kg</td>
<td>12.29</td>
<td>0.996</td>
<td>46.74</td>
<td>0.969</td>
<td>-0.247</td>
<td>0.778</td>
<td>-0.0317</td>
<td>0.977</td>
<td>0.94</td>
<td>0.994</td>
<td>Zero order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 kg</td>
<td>11.91</td>
<td>0.998</td>
<td>45.30</td>
<td>0.973</td>
<td>-0.212</td>
<td>0.821</td>
<td>-0.302</td>
<td>0.979</td>
<td>0.86</td>
<td>0.997</td>
<td>Anomalous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td></td>
<td></td>
<td>8.13</td>
<td>0.999</td>
<td>36.22</td>
<td>0.974</td>
<td>-0.124</td>
<td>0.768</td>
<td>-0.231</td>
<td>0.952</td>
<td>1.05</td>
<td>0.993</td>
<td>Zero order</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 kg</td>
<td>8.26</td>
<td>0.997</td>
<td>36.87</td>
<td>0.976</td>
<td>-0.126</td>
<td>0.783</td>
<td>-0.235</td>
<td>0.952</td>
<td>1.07</td>
<td>0.995</td>
<td>Zero order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 kg</td>
<td>8.13</td>
<td>0.996</td>
<td>36.29</td>
<td>0.976</td>
<td>-0.113</td>
<td>0.794</td>
<td>-0.229</td>
<td>0.962</td>
<td>1.14</td>
<td>0.994</td>
<td>Zero order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td></td>
<td></td>
<td>4.11</td>
<td>0.997</td>
<td>23.75</td>
<td>0.954</td>
<td>-0.059</td>
<td>0.839</td>
<td>-0.128</td>
<td>0.906</td>
<td>1.07</td>
<td>0.987</td>
<td>Zero order</td>
<td></td>
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</tr>
<tr>
<td>9 kg</td>
<td>4.17</td>
<td>0.998</td>
<td>24.06</td>
<td>0.949</td>
<td>-0.075</td>
<td>0.782</td>
<td>-0.127</td>
<td>0.928</td>
<td>1.01</td>
<td>0.998</td>
<td>Zero order</td>
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<tr>
<td>15 kg</td>
<td>4.10</td>
<td>0.998</td>
<td>23.45</td>
<td>0.962</td>
<td>-0.065</td>
<td>0.819</td>
<td>-0.125</td>
<td>0.917</td>
<td>0.99</td>
<td>0.997</td>
<td>Zero order</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>pH of the dissolution medium = 6.8</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td></td>
<td></td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>n</td>
<td>R²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 kg</td>
<td>11.45</td>
<td>0.998</td>
<td>43.57</td>
<td>0.975</td>
<td>-0.189</td>
<td>0.833</td>
<td>-0.287</td>
<td>0.978</td>
<td>0.81</td>
<td>0.998</td>
<td>Anomalous</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>12 kg</td>
<td>11.85</td>
<td>0.993</td>
<td>45.18</td>
<td>0.973</td>
<td>-0.246</td>
<td>0.780</td>
<td>-0.297</td>
<td>0.861</td>
<td>0.88</td>
<td>0.980</td>
<td>Anomalous</td>
<td></td>
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<tr>
<td>15 kg</td>
<td>11.55</td>
<td>0.994</td>
<td>44.23</td>
<td>0.982</td>
<td>-0.212</td>
<td>0.840</td>
<td>-0.285</td>
<td>0.958</td>
<td>0.83</td>
<td>0.985</td>
<td>Anomalous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td></td>
<td></td>
<td>8.40</td>
<td>0.997</td>
<td>37.14</td>
<td>0.976</td>
<td>-0.143</td>
<td>0.728</td>
<td>-0.242</td>
<td>0.951</td>
<td>1.11</td>
<td>0.993</td>
<td>Zero order</td>
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</tr>
<tr>
<td>9 kg</td>
<td>8.19</td>
<td>0.998</td>
<td>36.69</td>
<td>0.982</td>
<td>-0.127</td>
<td>0.799</td>
<td>-0.229</td>
<td>0.948</td>
<td>1.02</td>
<td>0.998</td>
<td>Zero order</td>
<td></td>
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</tr>
<tr>
<td>15 kg</td>
<td>8.18</td>
<td>0.994</td>
<td>36.21</td>
<td>0.956</td>
<td>-0.115</td>
<td>0.799</td>
<td>-0.234</td>
<td>0.965</td>
<td>1.01</td>
<td>0.978</td>
<td>Zero order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td></td>
<td></td>
<td>4.17</td>
<td>0.997</td>
<td>23.85</td>
<td>0.954</td>
<td>-0.076</td>
<td>0.789</td>
<td>-0.125</td>
<td>0.926</td>
<td>0.98</td>
<td>0.992</td>
<td>Zero order</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 kg</td>
<td>4.18</td>
<td>0.996</td>
<td>23.98</td>
<td>0.939</td>
<td>-0.065</td>
<td>0.811</td>
<td>-0.131</td>
<td>0.927</td>
<td>1.08</td>
<td>0.994</td>
<td>Zero order</td>
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</tr>
<tr>
<td>15 kg</td>
<td>4.20</td>
<td>0.995</td>
<td>24.25</td>
<td>0.947</td>
<td>-0.074</td>
<td>0.761</td>
<td>-0.126</td>
<td>0.924</td>
<td>0.99</td>
<td>0.992</td>
<td>Zero order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: K, R² and “n” represent release rate constant, coefficient of determination and release exponent respectively, whereas F1 contains 60% Methocel® and 30% Ethocel®; F2 contains 45% Methocel® and 45% Ethocel®, while F3 contains 30% Methocel® and 60% Ethocel®.
Figure 3.1: Comparative release profiles of risperidone from 9 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.2. (Mean ± SD, n = 6).

Figure 3.2: Comparative release profiles of risperidone from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.2. (Mean ± SD, n = 6).
Figure 3.3: Comparative release profiles of risperidone from 15 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.2. (Mean ± SD, n = 6).

Figure 3.4: Comparative release profiles of risperidone from 9 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6).
Figure 3.5: Comparative release profiles of risperidone from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6).

Figure 3.6: Comparative release profiles of risperidone from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6).
3.3.1.3(b): Effect of hardness on drug release

As shown in Figure-3.7 based on the data given in Table-3.3; the 9 kg, 12 kg and 15 kg hardness of tablets had no significant effect on drug release rate (%/hour) from all the three formulations; being 12.09, 12.29 and 11.91 respectively for F1; being 8.13, 8.26 and 8.13 respectively for F2 and being 4.11, 4.17 and 4.10 respectively for F3 in pH 1.2. Nearly same levels of release rates were observed for the above mentioned tablets in pH-6.8. Similarly there was no significant effect of hardness on drug release mechanism as all the formulations F1, F2 and F3 showed zero order release mechanism based either on their “n” values being ≥ 0.89 or goodness of fit test (R^2 approaching 1).

3.3.1.3(c): Effect of pHs of dissolution media on drug release

As shown in the Figure-3.7 and Table 3.3, drug release rates (%/hour) in pH 1.2 and pH 6.8 were noted as 12.09 vs 11.45 for 9 kg hard tablets, 12.29 vs 11.85 for 12 kg hard tablets and 11.91 vs 11.55 for 15 kg hard tablets in case of F1. For F2, it was 8.13 vs 8.40 for 9 kg hard tablets, 8.26 vs 8.19 for 12 kg hard tablets and 8.13 vs 8.18 for 15 kg hard tablets. The release rates (%/hour) were observed as 4.11 vs 4.17 for 9 kg hard tablets, 4.17 vs 4.18 for 12 kg hard tablets and 4.10 vs 4.20 for 15 kg hard tablets in case of F3. Thus there was no significant effect of pH on the drug release rates of each of the formulation tested. Similarly there was no significant effect of hardness on drug release mechanism as all of the formulations F1, F2 and F3 showed zero order release mechanism based either on their “n” values being ≥ 0.89 or goodness of fit test (R^2 approaching 1).
Figure 3.7: Effect of formulation (F1, F2 and F3); hardness (9 kg, 12 kg and 15 kg) and dissolution media (pH 1.2 and pH 6.8) on the drug release rates (K values) from risperidone extended release tablets (Mean ± SD, n = 6).
As shown in Table 3.4, the values of difference factor $f_1$ and similarity factor $f_2$ were less than 5 and greater than 80 respectively, indicating a high level of equivalence of dissolution profiles determined at pH 6.8 vs pH 1.2

Table 3.4: Difference factor $f_1$ and similarity factor $f_2$ calculated for 9 kg, 12 kg and 15 kg hard extended release tablets of risperidone, while comparing their dissolution profiles in pH-6.8 with dissolution profiles in pH-1.2

<table>
<thead>
<tr>
<th>Hardness of extended release tablets (F3)</th>
<th>Value of $f_1$ for pH-1.2 vs pH-6.8</th>
<th>Value of $f_2$ for pH-1.2 vs pH-6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 kg</td>
<td>4.48</td>
<td>80.96</td>
</tr>
<tr>
<td>12 kg</td>
<td>3.25</td>
<td>82.53</td>
</tr>
<tr>
<td>15 kg</td>
<td>2.89</td>
<td>84.95</td>
</tr>
</tbody>
</table>

3.3.1.4: Selection of the optimized Test tablets

The Test tablets of risperidone containing 30%Methocel® and 60%Ethocel® (F3) with 12 kg hardness was selected as the optimized formulation based on its better flowability (AR = 32 ± 2), compressibility index (CI = 12 ± 1) and release profiles, being 24 hours with zero order release in both dissolution media of pH 1.2 and pH 6.8. For this formulation, an optimum compression force (hardness being 12 kg) was applied for compaction.

3.3.1.6: Reproducibility and accelerated stability study:

The data on the stability parameters, such as drug content, weight variation, friability, hardness and appearance for the optimised risperidone tablet formulation F3 with 12 kg hardness is given in Table-3.5 Among the three batches of F3 produced at different occasions, there was no significant difference in drug contents (102 ± 3, 103 ± 2 and 100 ± 3). The friability of optimized formulation was noted to be 0.4 ± 0.05, 0.36 ± 0.07 and 0.5±0.03 for the three batches produced at three different occasions. In addition, there was no significant effect of ICH recommended accelerated storage conditions (40°C/75 %RH) on the percent drug content (103 ± 3, 104 ± 2, 103 ± 3, 101 ± 3 and 100 ± 2); percent weight variation (4 ± 0.3, 4 ± 0.4, 3 ± 0.4, 3 ± 0.3, 4 ± 0.4 ); percent friability (0.45 ± 0.2, 0.41 ± 0.3, 0.51 ± 0.3, 0.43 ± 0.2, 0.68 ± 0.2); hardness (12.0 ± 0.2, 12.0 ± 0.3, 12.0 ± 0.3, 12.2 ± 0.4 and 12.4 ± 0.4) and
appearance (whitish) tested at 0 time (pre-storage) and after storage at 1, 2, 4 and 6 months respectively.

Table 3.5: Stability indicating parameters (drug content, weight variation, friability, hardness and appearance) for the optimized extended release tablets of risperidone. (Mean ± SD, n = 10)

<table>
<thead>
<tr>
<th>Testing time</th>
<th>Drug content (%)</th>
<th>Weight variation (%)</th>
<th>Friability (%)</th>
<th>Hardness (kgs)</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 0 time (pre-storage)</td>
<td>103 ± 3</td>
<td>4 ± 0.3</td>
<td>0.45 ± 0.02</td>
<td>12.0 ± 0.3</td>
<td>Whitish</td>
</tr>
<tr>
<td>After 1 month</td>
<td>104 ± 2</td>
<td>4 ± 0.4</td>
<td>0.41 ± 0.03</td>
<td>12.0 ± 0.3</td>
<td>Whitish</td>
</tr>
<tr>
<td>After 2 months</td>
<td>103 ± 3</td>
<td>3 ± 0.4</td>
<td>0.51 ± 0.03</td>
<td>12.0 ± 0.3</td>
<td>Whitish</td>
</tr>
<tr>
<td>After 4 months</td>
<td>101 ± 3</td>
<td>3 ± 0.3</td>
<td>0.43 ± 0.02</td>
<td>12.2 ± 0.4</td>
<td>Whitish</td>
</tr>
<tr>
<td>After 6 months</td>
<td>100 ± 2</td>
<td>4 ± 0.4</td>
<td>0.68 ± 0.02</td>
<td>12.4 ± 0.4</td>
<td>Whitish</td>
</tr>
</tbody>
</table>

3.3.2: In vivo evaluation

Figures 3.8, 3.9, 3.10 and 3.11 are typical chromatograms of standard solution containing 50 ng/mL risperidone and 50 ng/mL 9-hydroxyrisperidone, of the blank serum, of the rabbit serum collected from its blood withdrawn 6 hours after administration of Test tablet; rabbit serum spiked with 30 ng/mL risperidone and 30 ng/mL 9-hydroxyrisperidone respectively. The retention times for 9-OH-risperidone and risperidone were noted as 4.02 minutes and 4.62 minutes respectively. The mean absolute recovery of risperidone and 9-hydroxyrisperidone determined from five aliquot samples were 91.5% ± 3 and 90% ± 2 respectively.
Comparative serum concentrations of active moieties (risperidone + 9-hydroxyrisperidone) from Reference (conventional) and Test (extended release) tablets respectively are shown in Figure 3.12, 3.13 and 3.14. The pharmacokinetic parameters based on the serum level time curve have been recorded in Table-3.6. The two tailed t-test using PrismgraphPad was carried out to test for the treatment effect i.e Test tablet vs Reference tablet. Significantly low values of C_{max} (51.50 vs 72.83 ng/mL, p<0.0001), higher values of T_{max} (11.00 vs 4.67 hours, p<0.05) and mean residence time-MRT (19.40 vs 17.24 hours, p<0.05) were observed for Test tablets, indicating that the active moiety remained in the body for longer times. Elimination rate constant, volume of distribution, total clearance and particularly area under curve for active moiety from Test tablets were not significantly different from Reference tablets, indicating bioequivalence of the Test and Reference tablets. A trend of increase in half life (18.14 vs 15.27) of active moiety from Test tablets was observed, although it was not significantly different from the half life of active moiety for Reference tablet.

For risperidone alone, three times increase in half life-t_{1/2} (15.32 vs 5.07 hours, p < 0.0001), significantly longer mean residence time-MRT (15.01 vs 7.29 hours, p < 0.0001), larger volume of distribution-Vd (0.146 vs 0.071 L/kg, p < 0.001), greater area under curves; AUC_{0-48hrs} (589.0 vs 499.8, p < 0.05) and AUC_{0-∞} (835.5 vs 518.8, p < 0.05), lower level of maximum serum concentration-C_{max} (26.00 vs 56.00 ng/mL, p < 0.0001), lower elimination rate constant-K_{el} (0.053 vs 0.140, p < 0.0001), lesser rate of total clearance-C_{l_total} (0.0051 vs 0.0078, p < 0.001) were observed from Test tablet as compared to Reference tablets, indicating its longer stay with wide open distribution in the body.
For 9-hydroxyrisperidone alone, four fold increase in half life-$t_{1/2}$ (74.72 vs 17.50 hours, $p<0.001$), significantly longer mean residence time-MRT (24.08 vs 21.05 hours, $p < 0.001$), larger volume of distribution, $V_d$ (0.184 vs 0.087 L/kg, $p < 0.001$), lesser area under curve, $AUC_{0-48}$ (745.2 vs 1109.0 ng.hr/mL, $p < 0.0001$), lower level of maximum serum concentration-$C_{max}$ (21.00 vs 33.83 ng/mL, $p < 0.0001$), lower elimination rate constant-$K_{el}$ (0.0125 vs 0.0419, $p < 0.0001$) were observed from Test tablet as compared to Reference tablets, indicating an optimum level of serum concentration, longer persistence time and larger distribution volume of 9-hydroxyrisperidone in the body.
Table 3.6: Pharmacokinetic parameters for active moiety, risperidone and 9-hydroxyrisperidone, following oral administration of once a day 4 mg Reference or once a day 4 mg Test tablets of risperidone to rabbits (Mean ± SEM, n = 6)

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter calculated for</th>
<th>Reference Tablets</th>
<th>Test Tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a) Active moiety (risperidone plus 9-hydroxy risperidone)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elimination rate constant - $K_{el}$ (hour$^{-1}$)</td>
<td>0.049 ± 0.0070</td>
<td>0.044 ± 0.0062</td>
</tr>
<tr>
<td>Half-life - $t_{1/2}$ (hours)</td>
<td>15.27 ± 2.092</td>
<td>18.14 ± 3.45</td>
</tr>
<tr>
<td>Time of maximum plasma concentration - $T_{max}$ (hours)</td>
<td>4.67 ± 0.42</td>
<td>11.00 ± 2.72*</td>
</tr>
<tr>
<td>Maximum plasma concentration - $C_{max}$ (ng/mL)</td>
<td>72.83 ± 2.12</td>
<td>51.50 ± 1.80***</td>
</tr>
<tr>
<td>Area under curve - $AUC_{0-48hrs}$ (ng.hour/mL)</td>
<td>1609.00 ± 70.72</td>
<td>1702.00 ± 54.20</td>
</tr>
<tr>
<td>Area under curve - $AUC_{0-inf}$ (ng.hour/mL)</td>
<td>1853.00 ± 146.80</td>
<td>2061.00 ± 184.80</td>
</tr>
<tr>
<td>Mean residence time - MRT$0-48hrs$ (hour)</td>
<td>17.24 ± 0.64</td>
<td>19.40 ± 0.55*</td>
</tr>
<tr>
<td>Volume of distribution - $Vd$ (litre)</td>
<td>0.047 ± 0.0029</td>
<td>0.049 ± 0.0044</td>
</tr>
<tr>
<td>Total clearance - $C_{L_{total}}$ (litre/hour)</td>
<td>0.0022 ± 0.00017</td>
<td>0.0020 ± 0.00015</td>
</tr>
<tr>
<td><strong>b) Risperidone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elimination rate constant - $K_{el}$ (hour$^{-1}$)</td>
<td>0.14 ± 0.009</td>
<td>0.053 ± 0.004***</td>
</tr>
<tr>
<td>Half-life - $T_{1/2}$ (ng.hour/mL)</td>
<td>5.07 ± 0.34</td>
<td>15.32 ± 2.08***</td>
</tr>
<tr>
<td>Time of maximum plasma concentration - $T_{max}$ (hour)</td>
<td>4.17 ± 0.17</td>
<td>3.67 ± 0.33</td>
</tr>
<tr>
<td>Maximum plasma concentration - $C_{max}$ (ng/mL)</td>
<td>56.00 ± 2.66</td>
<td>26.00 ± 1.27***</td>
</tr>
<tr>
<td>Area under curve - $AUC_{0-48hrs}$ (ng.hour/mL)</td>
<td>499.8 ± 26.72</td>
<td>589.0 ± 19.60*</td>
</tr>
<tr>
<td>Area under curve - $AUC_{0-inf}$ (ng.hour/mL)</td>
<td>518.8 ± 21.64</td>
<td>835.5 ± 107.4*</td>
</tr>
<tr>
<td>Mean residence time - MRT$all$ (hour)</td>
<td>7.289 ± 0.57</td>
<td>15.01 ± 1.09***</td>
</tr>
<tr>
<td>Volume of distribution - $Vd$ (litre)</td>
<td>0.071 ± 0.012</td>
<td>0.15 ± 0.016**</td>
</tr>
<tr>
<td>Total clearance - $C_{L_{total}}$ (litre/hour)</td>
<td>0.0079 ± 0.0003</td>
<td>0.0051 ± 0.00056**</td>
</tr>
<tr>
<td><strong>c) 9-OH-Risperidone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elimination rate constant - $K_{el}$ (hour$^{-1}$)</td>
<td>0.042 ± 0.0053</td>
<td>0.012 ± 0.0034***</td>
</tr>
<tr>
<td>Half-life - $T_{1/2}$ (ng.hour/mL)</td>
<td>17.50 ± 2.11</td>
<td>74.72 ± 16.06***</td>
</tr>
<tr>
<td>Time of maximum concentration - $T_{max}$ (hour)</td>
<td>14.67 ± 3.04</td>
<td>14.67 ± 3.04</td>
</tr>
<tr>
<td>Maximum plasma concentration - $C_{max}$ (ng/mL)</td>
<td>33.83 ± 1.276</td>
<td>21.00 ± 1.789***</td>
</tr>
<tr>
<td>Area under curve - $AUC_{0All}$ (ng×hour/mL)</td>
<td>1109 ± 54.99</td>
<td>745.2 ± 50.23***</td>
</tr>
<tr>
<td>Area under curve - $AUC_{inf}$ (ng.hour/mL)</td>
<td>1594 ± 291.0</td>
<td>2280 ± 384.5</td>
</tr>
<tr>
<td>Mean residence time - MRT$0-48hrs$ (hour)</td>
<td>21.05 ± 0.7215</td>
<td>24.08 ± 0.4496**</td>
</tr>
<tr>
<td>Volume of distribution - $Vd$ (litre)</td>
<td>0.087 ± 0.011</td>
<td>0.184 ± 0.019**</td>
</tr>
<tr>
<td>Total clearance - $C_{L_{total}}$ (litre/hour)</td>
<td>0.0029 ± 0.0004</td>
<td>0.0021 ± 0.00038</td>
</tr>
</tbody>
</table>

**Note:** Values are significantly different (* P < 0.05, ** P < 0.001, *** P < 0.0001) between means of Reference and Test tablets of risperidone (Mean ± SEM, N = 6)
**Figure 3.8:** A representative chromatogram of 9-hydroxyrisperidone (labeled as 9-OH) and risperidone (labeled as Rsp) using a standard solution consisting of a mixture of 50 ng/mL 9-hydroxyrisperidone and 50 ng/mL risperidone.

**Figure 3.9:** A representative chromatogram of extracted blank serum
Figure 3.10: A representative chromatogram of 9-hydroxyrisperidone (labeled as 9-OH) and risperidone (labeled as Rsp) extracted from a sample of rabbit serum withdrawn 6 hours after administration of Test tablet.

Figure 3.11: A representative chromatogram of 9-hydroxyrisperidone (labeled as 9-OH) and risperidone (labeled as Rsp) extracted from a sample of rabbit serum spiked with 30 ng/mL of 9-hydroxyrisperidone and 30 ng/mL of risperidone.
Figure 3.12: Comparative serum concentration-time profiles of active moiety-1 (Reference tablet) and active moiety-2 (Test tablet), following oral administration to rabbits (Mean ± SD, n = 6). Note: Active moiety represents the combined concentrations of risperidone and 9-hydroxyrisperidone.
Figure 3.13: Comparative serum concentration-time profiles of risperidone and 9-OH-risperidone following oral administration of Reference tablets to rabbits. (Mean ± SD, n = 6)

Figure 3.14: Comparative serum concentration-time profiles of risperidone and 9-OH-risperidone following oral administration of Test tablets to rabbits. (Mean ± SD, n = 6)
3.3.3: *In vitro in vivo correlation*

Percent drug absorbed (Fa, at Y axis) when plotted against percent drug released (Fr at X axis) as given in Figure 3.15, yielded linear curve with $R^2$ value equal to 0.7293, indicating a relatively good correlation of absorption with the amount of drug release up to almost 85%. These points indicate that release and absorption of risperidone occurs throughout the GIT, which further support the idea of administering risperidone in extended release tablet form.

**Figure 3.15:** *In-vitro* and In-vivo correlation of risperidone Test tablet. Percent of drug absorbed is plotted against percent of drug released at times 1, 2, 4, 6, 8, 12 and 24 hours
3.4: DISCUSSION

The concept of regulating drug delivery to the body has been in existence for a few decades because of many benefits in the industry, including decreased side effects and increased patient compliance. In the past few decades, pharmaceutical researchers have developed novel techniques of oral controlled release delivery including the hydrophilic matrix system (i.e. tablets), which is acknowledged as the simplest, most economical and widely applicable approach (Li et al, 2005; Durig and Fassihi, 2002; Sako et al, 2002; Williams et al, 2002).

The widely recommended release retarding hydrophilic polymer, Methocel® K100 LV-CR (Tiwari and Rajabi-Siahboomi, 2008; Rajabi-Siahboomi and Jordan, 2000) was initially used alone. During the pilot study Methocel® was tried from 10 to 60% level of the tablet weight, which could hardly extended the drug release period upto 8 hours. Further increase in the level of the Methocel® (i.e. 60 to 90%) could not significantly extend the release period beyond 8 hours. Therefore, Methocel® was partially substituted by a hydrophobic copolymer, Ethocel® standard 7FP premium (see Table-2.1), to complement the performance of the Methocel® as recommended by Tatavarti and Hoag (2006).

As given in Table-2.1, Chapter-2; three formulations of ER tablets of risperidone were prepared employing the dry granulation-slugging method. The formulations F1, contained 60% Methocel® and 30% Ethocel®. The F2 was prepared with 45% Methocel® and 45% Ethocel® while F3 contained 30% Methocel® and 60% Ethocel®. The other excipients such as lactose, colloidal silicion dioxide-Aerocel® and magnesium stearate were kept fixed at 6%, 1% and 1% levels respectively in order to determine clearly the effect of the polymers.

The determined higher values of angle of repose (AR), compressibility index (CI) and Hausner ratio (HR) of the powders-mix for formulations; F1, F2 and F3 indicate poor flowability and poor compressibility characteristics. All this may be attributed to the higher proportions (≥ 30%) of Ethocel® standard 7FP premium (Particle size ≤ 9.7 µM). The compression in the form of slugs and then crushing of the same for granulation resulted in a good flow and compressibility as shown by the lower values of AR, CI and HR (Table 3.1).
The granulation process resulted into an optimum sized granules containing a suitable proportion of fines. The prepared granules demonstrated better flow, desired levels of compressibility and little segregation during processing. These characteristics of granules, in turn helped in production of tablets without defects related to weight loss (friability) and content uniformity as shown in Table-3.2. As reported by Chowhan (1980) both Methocel® and Ethocel® have sufficient binding characteristics, so tablets with lowest level of friability and desired hardness at each level of compressional force were produced. Thus, the above findings were suggestive of using the two polymers Methocel® and Ethocel® in combination and the appropriateness of dry granulation method for the manufacture of extended release tablets of risperidone, fulfilling the USP requirements.

Drug release at a zero order rate provides a consistent concentration of the drug for absorption and maintains plasma concentration within a therapeutic window leading to minimizing side effects and/or reducing the frequency of dosage administration. Many researchers have sought to formulate matrices for zero order release patterns but few have been successful (Lee, 1984b; De Haan, 1986a; De Haan, 1986b).

To elucidate the mechanism of drug release from risperidone extended release tablets, dissolution data for the first 60% of drug release were fitted to the Power law equation as suggested by Bettini et al (2001). The release exponent “n” was calculated through the slope of the straight line of the above model fitting (Korsemeyer et al, 1983; Peppas, 1985). In case of cylinders (i.e. tablets), the value of “n” ≤ 0.45 shows Fickian release; 0.45 < “n” < 0.89 shows anomalous transport, while the value of “n” ≥ 0.89 shows zero order release (Ritger and Peppas, 1987a; Ritger and Peppas, 1987b).

Fickian diffusion refers to diffusion of drug through pores of the matrix, zero order demonstrates release of drug with erosion of the polymeric chains and anomalous transport refers to release of drug by a combined process of diffusion and erosion (Peppas, 1985). The criterion for selecting the most appropriate model (among the mathematical models mentioned above) was chosen on the basis of a “n” values and/or goodness of fit test (i.e. coefficient of determination R² value falling near to 1.0, which shows linearity of regression line) where necessary.
Interestingly, in total 90% of the polymeric blend, 60% Methocel® and 30% Ethocel® (F1) could hardly maintain the release period for 8 hours. However, the value of release exponent “n” was increased from 0.45 (indicating drug release by diffusion/Higuchian) to 0.79, 0.90 and 0.90 (showing anomalous drug transport and/or zero order mechanism) of 9 kg, 12 kg and 15 kg hard tablets respectively in dissolution media of pH 1.5. Nearly similar “n” values (“n” > 0.5 to 0.91) of the above mentioned tablets were observed in pH 6.8 (Table 4.3). One major difficulty faced after inclusion of 30% Ethocel® in formulation F1 was dictation of using dry granulation (slugging) method for tablet manufacture (instead of direct compression method) because of poor flow ability and poor compressibility characteristics of the powders-mix. Thereafter further substitution of 15% Methocel® by Ethocel® (F2; 45%;45%) extended the release period up to 12 hours with “n” values ≥ 0.89, indicating zero order kinetics. In case of formulation F3, 15% Methocel® was further substitution by Ethocel® (30% Methocel® and 60% Ethocel®), consequently extension in the release period up to 24 hours was achieved.

The regular but not very proportional reduction in release rates with increase in the concentration of Ethocel® may be due to slow hydration of the matrix, based upon the hydrophobic character of Ethocel®. The insoluble particles of Ethocel® may be acting as barrier to drug release in the gel layer of the Methocel®. This idea is in line with the findings of Howard and Timinis (1998), where they used water insoluble sodium alginate for reducing the release rates of basic drugs from the Methocel® based hydrophilic matrices.

A major objectionable thing in the formulations, F1, F2 and F3, seems to use a higher percentage of the polymer blend i.e 90%. It was compromised for achieving the 24 hours release period with zero order kinetics. However, the above findings are in line with the work of Agrawal (2003) who used 89% of fine particle ethylcellulose to formulate his extended release tablets. Requirement of this much higher level of the polymers blend may be due to their lowest viscosities, being 100cps for the Methocel® and 7cps for the Ethocel® as their trade name indicates (Dow Chemicals, 2002) and clearly mentioned in the Handbook of Pharmaceutical excipients (Rowe, 2006), leading to rapid disentanglement and erosion of the matrices.
Drug release mechanism based on release exponent, “n” and the highest goodness of fit test ($R^2$ approaching to 1) indicated zero order release of drug in both dissolution media of pH 1.2 and 6.8 for all the three formulations F1, F2 and F3.

Since hardness usually varies during tablet production and it has been advocated by some researchers (Agrawal et al, 2003) that hardness of tablets effects dissolution rate (K values), so the above parameter was also studied during the present work. The findings of this study demonstrated that higher compression force led to increased tablet hardness in all formulations, F1, F2 and F3 but the release rates and mechanisms remained unaffected. It can be implied that the porosity and/or tortuosity of the prepared tablets after their hydration were not influenced by increase in tablet hardness from 9 kg to 15 kg.

Rekhi et al (1999) have also reported that changes in compression force had no noteworthy effect on drug release from HPMC based matrix tablets once critical hardness was achieved. The above findings were also in line with the previous studies conducted by Ravi et al, 2008; Velasco et al, 1999; Ford et al, 1987 and Doelker, 1986).

As the tablet has to pass through different parts of the GIT with different pH environments, therefore it was considered necessary to study the effect of pH of the dissolution media (particularly the commonly encountered pH 1.2 and 6.8) on the drug release rate and mechanism. Methocel® K100 LV-CR (hydroxypropylmethcellulose), is a cellulose derivative (with methoxyl and hydroxypropyl substituents on a $\beta$-o-glucopyranosyl ring backbone) resistant to changes in pH of the dissolution medium in the range of 2 to 13, so it is relatively stable (Marcos et al, 1996). Similarly, Ethocel®, a cellulose derivative (with ethoxyl substitution on anhydroglucose ring backbone) is insoluble in water, thus its release properties are less affected by the pH changes (Atsuko et al, 2006). Therefore risperidone release from the matrices composed of these polymers was not effected by changes in pH

Two release profiles are considered similar if the values of difference factor $f_1$ is close to 0 and similarity factor $f_2$ is close to 100. Generally, $f_1 \leq 15$ and $f_2 \geq 50$, indicates an average difference of not more than 10% at the sample time points (Fassahi and Pillay, 1998; Shah et al, 1998; US FDA, 1997). In this study, the lower
values of $f_1$ (< 4) at the 9 kg, 12 kg and 15 kg hardness levels of formulation F3, show negligible difference of dissolution profiles in pH 6.8 from dissolution profiles in pH 1.2. The higher values (> 81) of similarity $f_2$ of the F3 with the hardness levels of 9 kg, 12 kg, 15 kg at the pH 6.8 and pH 1.2 was another supporting point for the conclusion that the pHs have negligible effect on the drug release from the designed matrices.

The method of HPLC-UV analysis was validated according to the FDA recommendations and International Guidelines (US FDA, 2001; Shah et al, 2000) prior to conducting HPLC analysis. The plasma levels of risperidone correlate with its adverse drug effects (Spina et al, 2001), which in turn lead to poor compliance (Weiden et al, 2004; Gerlach, 2002; Robinson et al, 2002). Thus, the extended release (ER) risperidone tablets are expected to minimize fluctuations in blood levels of risperidone and so will reduce side effects and improve compliance. The Test (ER) risperidone tablets prepared in this study maintained nearly constant and optimum therapeutic concentration i.e. $\leq 52$ ng/mL; of the active moiety (risperidone + 9-OH-risperidone) for 24 hours; as compared to the Reference tablet, which showed fluctuations (peak and trough) in drug serum concentration with time. Here it is worth to mention that the therapeutic range of active moiety is 20-60 ng/mL (Hiemke et al, 2004). Extension in half life ($t_{1/2}$) and time required for achieving maximum concentration ($T_{max}$) of Test formulation are also indicative of drug release occurring at a slower rate for extended time, eliminating the need for taking risperidone tablets in divided doses. Further nonsignificant difference in mean AUCs of Test and Reference tablets indicates that the Test tablets were bioequivalent to Reference tablets, which means that the extent of drug absorption is not reduced significantly. Relative bioavailability noted as 1.06, also support the concept of bioequivalence of the Test and the Reference tablet formulations.

In-vitro and In-vivo correlation (IVIVC) is an analytical mathematical model unfolding the relationship between an In-vitro property of a dosage form and the respective In-vivo response. Typically, the in vitro property is the rate or extent of drug release while the In-vivo response is the plasma drug concentration (US FDA, 1997).
Five correlation levels (Level A, B, C, D and E) have been defined for the *In-vivo* and *In-vitro* correlation in the FDA guidance (US FDA, 1997). The level A correlation is the highest class of correlation and represents a point-to-point relationship between *In-vitro* dissolution rate and *in vivo* absorption rate of the drug from the dosage form (USP 30, 2007). Usually, percent of drug absorbed is calculated by a model dependent Wagner-Nelson method.

To demonstrate a correlation, fraction of drug absorbed *In-vivo* is plotted against the fraction of drug released *In-vitro*. If this relationship assumes linearity with a slope of 1, then the curves are superimposable, and there is a 1:1 relationship which is termed as point-to-point or level A correlation. The correlation is considered general and could be extrapolated within a reasonable range for that formulation of the active drug moiety.

During the present work, a good *In-vitro* and *In-vivo* correlation of level A was achieved with coefficient of determination ($R^2$) being equal to 0.9273, which indicate that the formulation was successful enough for further promotion and clinical evaluation.

### 3.5. CONCLUSION

Results of the present study clearly indicate that combination of both the Methocel® K100LV-CR (a hydrophilic polymer) and the Ethocel® standard 7FP premium (a hydrophobic polymer) could be successfully employed for formulating extended release matrix tablets of Risperidone. The investigated extended release matrix tablet has shown a strong *In-vitro* and *In-vivo* correlation and nearly smooth blood levels of Risperidone throughout 24 hours. So one can expect improved therapy and reduced chances of the dose-dependent side effects, most often associated with its conventional tablets.
4.1: INTRODUCTION

Olanzapine, a second generation antipsychotic (SGA) drug, is widely used for the clinical management of psychotic illnesses including acute mania, schizophrenia and related schycotic disorders (Ciudad et al, 2005; Lieberman et al, 2005; McCormack and Wiseman, 2004). Olanzapine is almost completely absorbed following oral administration but approximately 40% is metabolised via first pass effect in the liver before reaching systemic circulation, so its oral bioavailability is 60% (Callaghan et al, 1999).

The concentration of olanzapine in serum shows a tendency to increase with the administered daily dose (Aravagiri et al, 1997). The C<sub>max</sub> for olanzapine has been reported as 5 to 50 ng/mL for 2.5 – 17.5 mg daily dose (Catlow et al, 1995) and 8 to 31 ng/mL for 10–20 mg daily dose (Aravagiri et al, 1997), while its mean time for maximum concentration (T<sub>max</sub>) and half life (t<sub>1/2</sub>) have been reported as approximately 6 hours and 33 hours respectively (Callaghan et al, 1999).

The second generation antipsychotics (SGAs) may not be remarkably different from the first generation antipsychotics (FGAs) and both are associated with an increased risk of extrapyramidal symptoms (De Leon et al, 2007; Lee et al, 2005). Olanzapine and risperidone (SGAs) probably have an intermediate extrapyramidal profile (Fernandez et al, 2003; Katz, 2004). However, olanzapine is more likely to cause weight gain (Strassning, 2009; Thase et al, 2007; Robinson et al, 2006; Newcomer, 2005), elevation of blood glucose (Guo et al, 2007; Henderson, 2002) and increased cholesterol & lipid levels (Lieberman et al, 2005; Newcomer, 2005). Patients receiving olanzapine and clozapine are at significantly increased risk for developing the metabolic syndrome as compared to other SGAs (Lamberti et al, 2006; De Hert et al, 2008).

Current users of FGAs and SGAs had a similar, dose-related increased risk of sudden cardiac death (Ray et al, 2009), obesity (Weiden et al, 2004; Allison et al., 1999) and extrapyramidal side effects-EPS (De Leon et al, 2007; Lee et al, 2005). Extended release tablets of some antipsychotic drugs, including paliperidone (Invegs<sup>®</sup>) and quetiapine XR (Seroquel XR) have shown higher efficacy and
As per the available literature, no study has been reported so far regarding preparation of olanzapine proper in extended release tablet form, although its combination with fluoxetine (Symbioxx) is available in the market. Based on its frequent and serious side effects discussed above, the present study was aimed at the development of extended release tablet of olanzapine to avoid fluctuations of drug concentrations in blood and in turn, the side effects for improvement in compliance. The widely recommended polymers, Methocel® K100 LV-CR and Ethocel® Standard 7FP Premium (Li et al, 2005; Tiwari and Rajabi-Siahboomi, 2008) were used in combination as polymer and copolymer to exploit their good characteristics for getting extended drug release with zero order kinetics.

4.2: MATERIALS AND METHODS

4.2.1: Materials

Olanzapine (RPG life sciences Ltd, India) was provided by Danis Pharma, Islamabad, Pakistan; as a gift. Methocel® K100 LV-CR (hydroxypropylmethylcellulose) and Ethocel® Standard 7FP Premium (fine particle ethylcellulose) were obtained as gift from the Colorcon Asia Ltd, India. Zyprexa® tablets-conventional (Eli Lilly Pvt Ltd, Karachi; Pakistan) containing 10 mg olanzapine, were purchased from local market and used as Reference during In-vivo studies. Acetonitrile and Methanol, both of HPLC grade were purchased from authorized local supplier of Merck, Germany. All the other chemicals were of analytical or pharmaceutical grade.

4.2.2: Methods

4.2.2.1: Tablet manufacture

4.2.2.1(a): Preparation of powders-mix

The powders-mix of olanzapine, Methocel®, Ethocel®, lactose, colloidal silicon dioxide-Aerocel® and magnesium stearate was prepared for six hundred tablets, using the method given in chapter-2, Section 2.2.1(a).
Chapter-4: Extended Release Tablets of Olanzapine

4.2.2.1(b) Granulation and tableting

Matrix tablets were prepared by dry granulation (slugging) method as given in Chapter-2, Section 2.2.1(b)

4.2.2.2: In-Vitro evaluation

4.2.2.2(a): Physicochemical evaluation of powders-mix and granules

Physicochemical characteristics, such as the angle of repose (AR), compressibility index (CI), Hausner ratio (HR) of the powders-mix and granules were determined as outlined in Chapter-2, Section 2.2.2 (a)

The olanzapine content of granules was determined using UV-Visible Spectrophotometer (Model-1700) at $\lambda_{\text{max}}$ 270 nm, as outlined in Chapter-2, Section 2.2.2 (a)

4.2.2.2(b): Physicochemical evaluation of tablets

The prepared tablets of olanzapine were evaluated for various physicochemical characteristics, such as friability, hardness, weight variation and drug content as mentioned in Chapter-2, Section 2.2.2(b).

4.2.2.2(c): Drug dissolution

The drug release profiles of six tablets from each batch was measured as outlined in Chapter-2, Section 2.2.2(c), using UV-Visible Spectrophotometer at $\lambda_{\text{max}}$ 270 nm. The drug release data were fitted to various models including zero order kinetics, first order kinetics, Higuchi’s square root of time equation, Hixon and Crowel’s cube root equation and power law equation, as given in Chapter-2, Section 2.2.2(c)

Dissolution profiles of 9 kg, 12 kg and 15 kg hard tablets of the selected formulation F3 were determined at pH 6.8 and then compared with the dissolution profiles of these tablets determined at pH 1.2 to find out dissolution equivalency (difference factor $f_1$ and similarity factor $f_2$); as described in Chapter-2, Section 2.2.2 (c)
4.2.2.2(d): Selection of tablet formulation for stability and **In-vivo** studies

Optimised olanzapine tablet formulation was selected based on better flowability and compressibility of granules and drug release profiles (zero order profiles with $R^2$ values approaching 1) of the prepared tablets.

4.2.2.2(e): **Reproducibility and accelerated stability study**

Reproducibility of the manufacturing process was determined by preparing three repeated batches of the selected optimized formulation (F3 with 12 kg hardness) on three different occasions. The optimised tablets were stored in high density polyethylene (HDPE) jars and kept under accelerated storage conditions of $40 \pm 2^\circ C / 75 \pm 5\%$ relative humidity (RH) in a stability chamber (Ti-Sc-THH-07-0400, Faisalabad, Pakistan) in accordance with International Commission for Harmonization (ICH) guidelines for a period of 6 months. The samples were tested for appearance, percent drug content, percent friability and hardness at predetermined time intervals of 0 time (prestorage) and after storage at 1, 2, 4 and 6 months respectively.

4.2.2.3: **In-vivo evaluation**

4.2.2.3(a): Study protocol and design

For in vivo experiments, the rabbits were divided into two groups, each having six animals. The first group received the Zyprexa® (Eli Lilly) as Reference tablet and the second group received the extended release Test tablets of olanzapine, a detailed account of which is given in Chapter-2, Section 2.2.3(a)

4.2.2.3(b): **Administration of dose**

The dose of the drug was administered to rabbits according to the procedure stated in Chapter-2, Section 2.2.3(b)

4.2.2.3(c): **Withdrawal of blood samples**

Blood samples (0.7 mL each time) were collected from the marginal ear vein at 0, 1, 2, 4, 6, 8, 12, 24 and 48 hours in test tubes and allowed to clot. A 200 µL serum was withdrawn, centrifuged at 2800rpm for 10 minutes and 100 µL of so obtained cleared serum was transferred to 10 mL test tubes and stored at -20°C until the time of analysis.
4.2.2.3(d): Extraction of olanzapine from serum

Serum samples were prepared by liquid-liquid extraction as described by Aravagiri et al (1997). Briefly, to 100 µL spiked or sample serum, added 100 µL of 1M sodium hydroxide, and 6 mL of pentane & dichloromethane (85:15) mixture and vortex mixed. The mixture was centrifuged at 2800rpm and the supernatant (organic) layer transferred into test tubes for drying under nitrogenous atmosphere. The residue obtained after drying was dissolved in 200 µL of acetonitrile by vortex mixing. 20 µL of the prepared sample was injected into the HPLC System for analysis.

4.2.2.3(e): Determination of olanzapine concentrations in serum samples

Blood serum level of olanzapine was determined by HPLC coupled with ECD detector, as outlined in Chapter-2, Section 2.2.3(e)

4.2.2.3(f): Instrument and chromatographic conditions

Chromatographic separation was performed according to the method described by Kasper et al (1999) using an analytical column (Shimpack, RP.C18, CLC-ODS 150mm×6mm×5µM, Shimadzu, Japan) fitted into the Schimadzu liquid chromatographic system equipped with communication boss module (CBM, model 20A), two independently working pumps (model LC-20AT) and an electrochemical detector, ESA Choulchen-111 (model 5300) equipped with a Model 5011 dual analytical cell (electrodes 1 and 2 set at +200mV and -200mV respectively) and a Model 5020 guard cell, set at -300mV. The HPLC mobile phase, consisting of 75 mM phosphate buffer, methanol, acetonitrile (48:26:26, pH 6.53), was used at the flow rate of 1.0 mL/min for elution of analytes.

4.2.2.3(g): Pharmacokinetic analysis

The concentration-time data of olanzapine from single dose 10 mg Zyprexa®, and from the single dose 10 mg extended release Test tablet, were analyzed, using the pharmacokinetic computer based software, WinNolin® Ver 5.2.1 (Pharsight Corporation, Mountain View, CA, USA). Non compartmental approach implemented in the above software was employed to calculate the pharmacokinetic parameters, as outlined in Chapter-2, Section 2.2.3 (g).
4.2.2.3(h): Relative bioavailability

Relative bioavailability of the optimized Test tablet and Reference tablet was determined as outlined in Chapter-2, section 2.2.3(h).

4.2.2.4: In vitro and in vivo correlation

The in vitro and in vivo correlation (IVIVC) of the optimized ER tablets of olanzapine was tested according to the procedure given in Chapter-2, Section 2.2.3(h).

4.3: RESULTS

4.3.1: In vitro evaluation

4.3.1.1: Physicochemical evaluation of powders-mix and granules

As shown in Table-4.1; the angle of repose (AR) for powders-mix varied from 46 ± 3° to 60 ± 3°, indicating poor flowability as compared to granules for which the angle of repose varied from 31 ± 3° to 33 ± 2°, indicating good flowability. The compressibility index of powders-mix varied from 27 ± 3% to 33 ± 3%, indicating poor compressibility as compared to granules, for which it ranged from 11 ± 2% to 12 ± 1% indicating good compressibility. Hausner ratio (HR) followed the same trend (1.32 ± 0.11 to 1.56 ± 0.13 for powders and 1.14 ± 0.12 to 1.17 ± 0.13 for granules) as was noted for AR and CI of powders-mix and granules. Drug content of granules varied from 102 ± 2% to 103 ± 3%.

Table 4.1: Physicochemical characteristics of powders-mix and granules prepared for manufacture of extended release tablets of olanzapine (Mean ± SD, n = 3)

<table>
<thead>
<tr>
<th>A. Powders mix</th>
<th>Angle of repose (degrees)</th>
<th>Compressibility index (%)</th>
<th>Hausner Ratio</th>
<th>Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>46 ± 3</td>
<td>27 ± 3</td>
<td>1.32 ± 0.11</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>51 ± 3</td>
<td>32 ± 4</td>
<td>1.43 ± 0.13</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>60 ± 3</td>
<td>33 ± 3</td>
<td>1.56 ± 0.13</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Granules</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>31 ± 3</td>
<td>11 ± 2</td>
<td>1.14 ± 0.12</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>F2</td>
<td>33 ± 2</td>
<td>13 ± 2</td>
<td>1.16 ± 0.10</td>
<td>103 ± 2</td>
</tr>
</tbody>
</table>
F3 | 33 ± 2 | 12 ± 1 | 1.17 ± 0.13 | 102 ± 2

**Note:** F1 contains 60% Methocel® and 30% Ethocel®; F2 contains 45%Methocel® and 45% Ethocel® while F3 contains 30% Methocel® and 60% Ethocel®
4.3.1.2: Physicochemical evaluation of tablets

The tablets from each batch were found uniform with respect to dimensions (length × width, 8.0×3.6mm to 8.1×3.5mm), the percent weight variation (4 ± 0.3 to 5 ± 0.5), percent friability (0.42 ± 0.04 to 0.49 ± 0.07) and percent drug content (100 ± 4 to 102 ± 3), as represented by the results of the selected formulation F3 (Table 4.2); fulfilling requirements of the USP.

Table 4.2: Physicochemical characteristics of extended release tablets of olanzapine for its selected formulation F3 (Mean ± SD, n = 10)

<table>
<thead>
<tr>
<th>Hardness of tablets</th>
<th>Friability (%)</th>
<th>Weight variation (%)</th>
<th>Drug content (%)</th>
<th>Dimensions (Length and Width in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 kg</td>
<td>0.46 ± 0.08</td>
<td>5 ± 0.5</td>
<td>100 ± 4</td>
<td>8.0 ± 0.1×3.6 ± 0.1</td>
</tr>
<tr>
<td>12 kg</td>
<td>0.49 ± 0.07</td>
<td>4 ± 0.3</td>
<td>101 ± 3</td>
<td>8.1 ± 0.1×3.5 ± 0.1</td>
</tr>
<tr>
<td>15 kg</td>
<td>0.42 ± 0.04</td>
<td>5 ± 0.4</td>
<td>102 ± 3</td>
<td>8.1 ± 0.1×3.5 ± 0.1</td>
</tr>
</tbody>
</table>

Note: F3 contains 30% Methocel® and 60% Ethocel®

4.3.1.3: Drug dissolution

4.3.1.3(a): Effect of formulation on drug release

Based on the data given in Table 4.3, the Figures 4.1 - 4.6 demonstrate the maximum of 8 hour, 12 hours and 24 hours release periods from the designed formulations of F1, F2 and F3 respectively. The drug release rates (%/hour) were 11.85, 8.19, 4.07 for F1, F2 and F3 respectively with 9 kg hardness; 11.98, 8.24, 4.13 respectively for F1, F2 and F3 with 12 kg hardness and 12.11, 8.24, 4.12 for F1, F2 and F3 respectively with 15 kg hardness in pH-1.5. Nearly same trend and levels of release rates were observed for the above mentioned tablets in pH-6.8 (Table 4.3).
### Table 4.3: Effect of formulation (F1, F2 and F3), dissolution media (pH-1.5 and pH-6.8) and tablet hardness (9 kg, 12 kg and 15 kg) on release kinetics of olanzapine from its extended release tablets

#### pH of the dissolution medium = 1.5

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness</th>
<th>Zero order</th>
<th>Higuchi</th>
<th>First order</th>
<th>Hixon-Crowel</th>
<th>Korsemeyer</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>11.85</td>
<td>0.996</td>
<td>44.87</td>
<td>0.962</td>
<td>-0.245</td>
<td>0.769</td>
<td>-0.296</td>
</tr>
<tr>
<td>12</td>
<td>11.98</td>
<td>0.998</td>
<td>45.87</td>
<td>0.986</td>
<td>-0.215</td>
<td>0.849</td>
<td>-0.301</td>
</tr>
<tr>
<td>15</td>
<td>12.11</td>
<td>0.992</td>
<td>46.64</td>
<td>0.991</td>
<td>-0.254</td>
<td>0.846</td>
<td>-0.297</td>
</tr>
<tr>
<td>F2</td>
<td>9</td>
<td>8.19</td>
<td>0.999</td>
<td>36.44</td>
<td>0.972</td>
<td>-0.145</td>
<td>0.742</td>
</tr>
<tr>
<td>12</td>
<td>8.24</td>
<td>0.999</td>
<td>36.64</td>
<td>0.971</td>
<td>-0.147</td>
<td>0.757</td>
<td>-0.222</td>
</tr>
<tr>
<td>15</td>
<td>8.24</td>
<td>0.992</td>
<td>36.89</td>
<td>0.978</td>
<td>-0.135</td>
<td>0.864</td>
<td>-0.216</td>
</tr>
<tr>
<td>F3</td>
<td>9</td>
<td>4.07</td>
<td>0.996</td>
<td>23.69</td>
<td>0.963</td>
<td>-0.065</td>
<td>0.830</td>
</tr>
<tr>
<td>12</td>
<td>4.13</td>
<td>0.990</td>
<td>24.26</td>
<td>0.978</td>
<td>-0.0757</td>
<td>0.812</td>
<td>-0.120</td>
</tr>
<tr>
<td>15</td>
<td>4.12</td>
<td>0.987</td>
<td>24.34</td>
<td>0.985</td>
<td>-0.076</td>
<td>0.826</td>
<td>-0.118</td>
</tr>
</tbody>
</table>

#### pH of the dissolution medium = 6.8

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness</th>
<th>Zero order</th>
<th>Higuchi</th>
<th>First order</th>
<th>Hixon-Crowel</th>
<th>Korsemeyer</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>K</td>
<td>R²</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>11.64</td>
<td>0.998</td>
<td>44.22</td>
<td>0.971</td>
<td>-0.209</td>
<td>0.801</td>
<td>-0.293</td>
</tr>
<tr>
<td>12</td>
<td>11.49</td>
<td>0.989</td>
<td>44.34</td>
<td>0.993</td>
<td>-0.210</td>
<td>0.844</td>
<td>-0.284</td>
</tr>
<tr>
<td>15</td>
<td>11.40</td>
<td>0.987</td>
<td>44.12</td>
<td>0.996</td>
<td>-0.245</td>
<td>0.815</td>
<td>-0.275</td>
</tr>
<tr>
<td>F2</td>
<td>9</td>
<td>8.24</td>
<td>0.998</td>
<td>36.54</td>
<td>0.965</td>
<td>-0.145</td>
<td>0.736</td>
</tr>
<tr>
<td>12</td>
<td>8.03</td>
<td>0.998</td>
<td>35.97</td>
<td>0.986</td>
<td>-0.127</td>
<td>0.810</td>
<td>-0.217</td>
</tr>
<tr>
<td>15</td>
<td>8.33</td>
<td>0.983</td>
<td>37.595</td>
<td>0.986</td>
<td>-0.150</td>
<td>0.811</td>
<td>-0.219</td>
</tr>
<tr>
<td>F3</td>
<td>9</td>
<td>4.19</td>
<td>0.986</td>
<td>24.70</td>
<td>0.979</td>
<td>-0.077</td>
<td>0.837</td>
</tr>
<tr>
<td>12</td>
<td>4.26</td>
<td>0.965</td>
<td>25.35</td>
<td>0.984</td>
<td>-0.078</td>
<td>0.858</td>
<td>-0.122</td>
</tr>
<tr>
<td>15</td>
<td>4.22</td>
<td>0.979</td>
<td>24.89</td>
<td>0.973</td>
<td>-0.067</td>
<td>0.864</td>
<td>-0.125</td>
</tr>
</tbody>
</table>

**Note:** K, R² and “n” represent release rate constant, coefficient of determination and release exponent respectively; F1 contains 60% Methocel® and 30% Ethocel®, F2 contains 45% Methocel® and 45% Ethocel®, F3 contains 30% Methocel® and 60% Ethocel®
Figure 4.1: Comparative release profiles of olanzapine from 9 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.5. (Mean ± SD, n = 6).

Figure 4.2: Comparative release profiles of olanzapine from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.5. (Mean ± SD, n = 6).
Chapter 4: Extended Release Tablets of Olanzapine

**Figure 4.3:** Comparative release profiles of olanzapine from 15 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 1.5. (Mean ± SD, n = 6).

**Figure 4.4:** Comparative release profiles of olanzapine from 9 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6).
**Figure 4.5:** Comparative release profiles of olanzapine from 12 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, N = 6).

**Figure 4.6:** Comparative release profiles of olanzapine from 15 kg hard tablets of formulations F1 (60% Methocel®, 30% Ethocel®), F2 (45% Methocel®, 45% Ethocel®) and F3 (30% Methocel®, 60% Ethocel®), in dissolution media of pH 6.8. (Mean ± SD, n = 6).
4.3.1.3(b) Effect of hardness on drug release

As shown in Figure 4.7 based on the data given in Table 4.3; the 9 kg, 12 kg and 15 kg hardness of tablets had no significant effect on drug release rate (%/hour) from all the three formulations; being 11.85, 11.98 and 12.11 respectively for F1; being 8.19, 8.24 and 8.24 respectively for F2 and being 4.07, 4.13 and 4.12 respectively for F3 in pH 1.5. Nearly same levels of release rates were observed for the above mentioned tablets in pH-6.8. Similarly there was no significant effect of hardness on drug release mechanism as the value of “n” remained > 0.89 in cases of all the formulations (F1, F2 and F3) at pH 1.5 and pH 6.8.

![Figure 4.7: Effect of formulation (F1, F2 and F3); hardness (9 kg, 12 kg and 15 kg) and dissolution media (pH 1.5 and pH 6.8) on the drug release rates (K values) from olanzapine extended release tablets (Mean ± SD, n = 6).](image-url)
4.3.1.3(c) Effect of change in pH of the dissolution media on drug release

As shown in the Figure-4.7 and Table-4.3, drug release rates (%/hour) in pH 1.5 and pH 6.8 were noted as 11.85 vs 11.64 for 9 kg hard tablets, 11.98 vs 11.49 for 12 kg hard tablets and 12.11 vs 11.40 for 15 kg hard tablets in case of F1. For F2, it was 8.19 vs 8.24 for 9 kg hard tablets, 8.24 vs 8.03 for 12 kg hard tablets and 8.24 vs 8.33 for 15 kg hard tablets. The release rate (%/hour) were noted as 4.07 vs 4.19 for 9 kg hard tablets, 4.13 vs 4.26 for 12 kg hard tablets and 4.12 vs 4.22 for 15 kg hard tablets in case of F3. Thus there was no significant effect of pH on the drug release rates of each of the formulation tested. Similarly there was no significant effect of pH on drug release mechanism as the values of “n” remained > 0.89 in cases of all the formulations F1, F2 and F3 at the 9 kg, 12 kg and 15 kg hardness levels.

As shown in Table-4.4, the values of difference factor $f_1$ and similarity factor $f_2$ were less than 9 and greater than 66 respectively, indicating a good level of equivalence of dissolution profiles determined at pH 6.8 and pH 1.5.

Table 4.4: Difference factor $f_1$ and similarity factor $f_2$ calculated for 9 kg, 12 kg and 15 kg hard extended release tablets of olanzapine, while comparing their dissolution profiles in pH-6.8 with dissolution profiles in pH 1.5

<table>
<thead>
<tr>
<th>Hardness of extended release tablets</th>
<th>Values of difference factor-$f_1$ for dissolution profiles in pH 6.8 vs pH 1.5</th>
<th>Values of similarity factor-$f_2$ for dissolution profiles in pH 6.8 vs pH 1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 kg</td>
<td>8.36</td>
<td>68.29</td>
</tr>
<tr>
<td>12 kg</td>
<td>7.12</td>
<td>66.55</td>
</tr>
<tr>
<td>15 kg</td>
<td>6.14</td>
<td>72.53</td>
</tr>
</tbody>
</table>

4.3.1.4: Selection of the optimized Test tablets

The Test olanzapine tablet containing 30% Methocel® and 60% Ethocel® (F3) with 12 kg hardness was selected as the optimized formulation based on its better flowability ($AR = 33 \pm 2$) and compressibility properties ($CI = 13 \pm 1$) and release profiles. The formulation followed the zero order release pattern in dissolution media of pH 1.5 and pH 6.8. For this formulation, an optimum compression force of 12 kg was applied for compaction.
4.3.1.5: Reproducibility and accelerated stability study:

The data on the stability parameters, such as drug content, weight variation, friability, hardness and appearance for the optimised olanzapine tablet formulation F3 with 12 kg hardness is given in Table 4.5. Among the three batches of F3 produced at different occasions, there was no significant difference in drug contents (103 ± 2, 102 ± 3 and 100 ± 4). The friability of optimized formulation was noted to be 0.3 ± 0.06, 0.41 ± 0.04 and 0.46±0.05 for the three batches produced at three different occasions. In addition, there was no significant effect of ICH recommended accelerated storage conditions (40°C/75 %RH) on the percent drug content (103±2, 102±3, 101±2, 100±3 and 100 ± 2); percent weight variation (3 ± 0.3, 4 ± 0.5, 3±0.4, 3 ± 0.5, 4 ± 0.3 ); percent friability (0.49 ± 0.2, 0.61 ± 0.3, 0.56 ± 0.3, 0.48 ± 0.3, 0.60 ± 0.3); hardness (12.0 ± 0.4, 12.0 ± 0.3, 12.0 ± 0.4, 12.2 ± 0.3, 12.4±0.3) and appearance (yellowish) tested at 0 time (pre-storage) and after storage at 1, 2, 4 and 6 months respectively.

Table 4.5: Stability indicating parameters (drug content, weight variation, friability, hardness and appearance) for the optimized extended release tablets of olanzapine. (Mean ± SD)

<table>
<thead>
<tr>
<th>Readings taken for determination of stability</th>
<th>Drug content (%)</th>
<th>Weight variation (%)</th>
<th>Friability (%)</th>
<th>Hardness (kgs)</th>
<th>Appearance (clour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 0 time (prestorage)</td>
<td>103 ± 2</td>
<td>3 ± 0.3</td>
<td>0.49 ± 0.02</td>
<td>12.0 ± 0.4</td>
<td>Yellowish</td>
</tr>
<tr>
<td>After 1 month</td>
<td>102 ± 3</td>
<td>4 ± 0.5</td>
<td>0.61 ± 0.03</td>
<td>12.0 ± 0.3</td>
<td>Yellowish</td>
</tr>
<tr>
<td>After 2 months</td>
<td>101 ± 2</td>
<td>3 ± 0.4</td>
<td>0.56 ± 0.03</td>
<td>12.0 ± 0.4</td>
<td>Yellowish</td>
</tr>
<tr>
<td>After 4 months</td>
<td>100 ± 3</td>
<td>3 ± 0.5</td>
<td>0.48 ± 0.03</td>
<td>12.2 ± 0.3</td>
<td>Yellowish</td>
</tr>
<tr>
<td>After 6 months</td>
<td>100 ± 2</td>
<td>4 ± 0.3</td>
<td>0.60 ± 0.03</td>
<td>12.4 ± 0.3</td>
<td>Yellowish</td>
</tr>
</tbody>
</table>

4.3.2: In vivo evaluation

Typical chromatograms of standard solution containing 50 ng/mL of olanzapine, of blank serum, of the rabbit serum collected 4hrs after administration of ER tablets, rabbit serum spiked with 40 ng/mL olanzapine are shown in Figure 4.8, 4.9, 4.10 and 4.11 respectively. The retention time for olanzapine was noted as 9.23 minutes. The mean absolute recovery of olanzapine determined from five aliquots of quality control samples was found to be 93%.
The comparative serum concentrations of olanzapine from Test (extended release) and Reference (conventional) tablets are shown in Figure 4.12. Based on the concentration profile data, the computed pharmacokinetic parameters are given in Table 4.6. Two tailed t-test using PrismGraphPad was carried out to test the treatment effect i.e. Test vs Reference tablets. Significantly low values of $C_{\text{max}}$ (43.17 ± 1.47 vs 82.17 ± 2.21 ng/mL, p < 0.0001) but high values of $T_{\text{max}}$ (14.00 ± 2.00 vs 8.00 ± 0.89 hours, p < 0.05); $\text{MRT}_{0-48\text{hrs}}$ (28.59 ± 0.81 vs 17.61 ± 0.36 hours, p < 0.0001); half life (17.09 ± 1.59 vs 13.13 ± 0.65 hours, p < 0.05) and volume of distribution (0.11±0.01 vs 0.081 ± 0.002, p < 0.001) were observed for Test tablets. This showed that the olanzapine remained at optimum concentration in the body for longer time with higher distribution. Elimination rate constant, total clearance and particularly areas under curves, $\text{AUC}_{0-48\text{hrs}}$ and $\text{AUC}_{0-\text{inf}}$ for olanzapine from Test and Reference tablets were not significantly different from each other, indicating nearly equal bioavailability of the Test and Reference tablets. The relative bioavailability of Test tablet was calculated as 94%.

Table 4.6: Pharmacokinetic parameters for olanzapine, following oral administration of once a day 10 mg Reference and once a day 10 mg Test tablets of olanzapine to two separate groups of rabbits (Mean ± SEM, n = 6)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters calculated for</th>
<th>Reference Tablets (olanzapine, 10 mg)</th>
<th>Test Tablets (olanzapine, 10 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elimination rate constant-$K_{\text{cl}}$ (hour$^{-1}$)</td>
<td>0.053 ± 0.0033</td>
<td>0.042 ± 0.0038</td>
</tr>
<tr>
<td>Half life-$t_{1/2}$ (hours)</td>
<td>13.13 ± 0.6494</td>
<td>17.0 ± 1.59$^*$</td>
</tr>
<tr>
<td>Time of maximum plasma concentration-$t_{\text{max}}$ (hours)</td>
<td>8.00 ± 0.89</td>
<td>14.00 ± 2.00$^*$</td>
</tr>
<tr>
<td>Maximum plasma concentration-$C_{\text{max}}$ (ng/mL)</td>
<td>82.17 ± 2.21</td>
<td>43.17 ± 1.47$^{***}$</td>
</tr>
<tr>
<td>Area under curve-$\text{AUC}_{0-48\text{hrs}}$ (ng×hour/mL)</td>
<td>2104 ± 69.69</td>
<td>1980 ± 45.06</td>
</tr>
<tr>
<td>Area under curve-$\text{AUC}_{0-\text{inf}}$ (ng×hour/mL)</td>
<td>2342 ± 107.2</td>
<td>2140 ± 79.08</td>
</tr>
<tr>
<td>Mean residence time-$\text{MRT}_{0-48\text{hrs}}$ (hours)</td>
<td>17.61 ± 0.3588</td>
<td>28.59±0.8122$^{***}$</td>
</tr>
<tr>
<td>Volume of distribution-$V_d$ (Litres)</td>
<td>0.081 ± 0.002</td>
<td>0.114 ± 0.007$^{**}$</td>
</tr>
<tr>
<td>Total clearance-$Cl_{\text{total}}$ (Litres/hour)</td>
<td>0.0043 ± 0.00019</td>
<td>0.0047 ± 0.00018</td>
</tr>
</tbody>
</table>

Note: Values are significantly different (* P < 0.05, ** P < 0.001, *** P < 0.0001) between means of Reference and Test tablets of olanzapine (Mean ± SEM, n=6)
Figure 4.8:  A representative chromatogram of olanzapine (labeled as Oln) using a standard solution of 50 ng/mL olanzapine.

Figure 4.9:  A representative chromatogram of extracted blank serum.
Figure 4.10: A representative chromatogram of olanzapine (labeled as Oln) extracted from a sample of rabbit serum withdrawn 4 hours after administration of Test tablet.

Figure 4.11: A representative chromatogram of olanzapine (labeled as Oln) extracted from a sample of rabbit serum spiked with 40 ng/mL olanzapine.
Figure 4.12: Comparative serum concentration-time profiles of olanzapine-1 and olanzapine-2, following oral administration of Reference and Test tablets respectively to rabbits (Mean ± SD, n = 6). Note: Reference tablets represents conventional Zyprexa tablets, while Test tablets represents the prepared extended release tablets
4.3.3: *In-vitro In-vivo correlation*

Percent drug absorbed (Fa at Y axis) when plotted against percent drug released (Fr at X axis) as given in Figure 4.13, yielded linear curve with $R^2$ equal to 0.9082, showing good correlation of absorption with the amount of drug release up to almost 85%.

![Figure 4.13: Percent of drug absorbed plotted against percent of drug released at times 1, 2, 4, 6, 8, 12 and 24 hours to show the *In-vitro In-vivo* correlation of olanzapine Test tablet](image)

*Figure 4.13:* Percent of drug absorbed plotted against percent of drug released at times 1, 2, 4, 6, 8, 12 and 24 hours to show the *In-vitro In-vivo* correlation of olanzapine Test tablet
4.4: DISCUSSION

Like risperidone, in a pilot study; olanzapine tablets were initially prepared with Methocel® using the direct compression method of tablet manufacture. However, up to 90% Methocel® alone could extend the release period only up to 8 hours. Then Ethocel®, in combination with the Methocel® was tried which proved successful. However the unbearable variations in the weights and hardnesses of tablets preparation by direct compression created an urge for using some granulation method, so it was considered rational to use the dry granulation-slugging method as discussed in Chapter-3, Section 3.4.

As given in Table 2.1, Chapter-2; three formulations of ER tablets of olanzapine were prepared employing the dry granulation-slugging method. The formulations F1, contained 60% Methocel® and 30% Ethocel®. The F2 was prepared with 45% Methocel® and 45% Ethocel® while F3 contained 30% Methocel® and 60% Ethocel®. The other excipients such as lactose, colloidal silicon dioxide-Aerocel® and magnesium stearate were kept fixed at 3%, 1% and 1% levels respectively. The olanzapine tablet formulation F3 with 12 kg hardness met all the compendial specifications and thus, selected as the optimized olanzapine test formulation as discussed in Chapter-3, Section 3.4.

To elucidate the mechanism of drug release from olanzapine extended release tablets, dissolution data for the first 60% of drug release were fitted to the Power law equation as discussed in Chapter-3, Section 3.4.

Interestingly, in total 90% of the polymeric blend, 60% Methocel® and 30% Ethocel® (F1) could hardly maintained the release period for 8 hours. However, the value of release exponent “n” was increased from 0.45 (indicating drug release by diffusion/Higuchiian) to 0.79, 0.90 and 0.90 (showing anomalous drug transport and/or zero order mechanism) of 9 kg, 12 kg and 15 kg hard tablets respectively in dissolution media of pH 1.5. Nearly similar “n” values (“n” > 0.5 to 0.91) of the above mentioned tablets were observed in pH 6.8 (Table 4.3). One major difficulty faced after inclusion of 30 % Ethocel® in formulation F1 was dictation of using dry granulation (slugging) method for tablet manufacture (instead of direct compression.
method) because of poor flowability and poor compressibility characteristics of the powders-mix. Thereafter further substitution of 15% Methocel® by Ethocel® (F2; 45%:45%) could extend the release period up to 12 hours with “n” values $\geq 0.89$, indicating zero order kinetics. In case of formulation F3, 15% Methocel® was further substitution by Ethocel® (30% Methocel® and 60% Ethocel®), consequently extension in the release period up to 24 hours was achieved.

The regular but not very proportional reduction in release rates with increase in the concentration of Ethocel® may be due to slow hydration of the matrix, based upon the hydrophobic character of Ethocel®. The insoluble particles of Ethocel® may be acting as barrier to drug release in the gel layer of the Methocel®. This idea is in line with the findings of Howard and Timinis (1998), where they used water insoluble sodium alginate for reducing the release rates of basic drugs from the Methocel®.

Drug release mechanism based on release exponent, “n” and the highest goodness of fit test ($R^2$ approaching to 1) indicated zero order release of olanzapine in both dissolution media of pH 1.5 and 6.8 for all the three formulations F1, F2 and F3.

The findings of this study demonstrated that higher compression force led to increased tablet hardness in all formulations, F1, F2 and F3 but the release rates and mechanisms remained unaffected. It can be implied that the porosity and /tortusity of the prepared tablets after their hydration were not influenced by increase in tablet hardness from 9 kg to 15 kg. The above findings were also in line with the previous studies conducted by Ravi et al, 2008; Rekhi et al, 1999; Velasco et al, 1999; Ford et al, 1987 and Doelker, 1986).

The olanzapine release from the matrices composed of these polymers was not effected by changes in pH. The higher values (> 66 ) of similarity $f_2$ and lower values (< 9) of difference factor of the formulation F3 with the hardness levels of 9 kg, 12 kg, 15 kg at the pH 6.8 and pH 1.5 was another supporting point for the conclusion that the pHs have negligible effect on the drug release from the designed matrices.

There was no significant difference between the results of In-vitro studies of risperidone and olanzapine because they have similar physicochemical characteristics and same concentrations of the polymers were used in fabrication of tablets.
The method of HPLC coupled with ECD analysis was validated in the light of the US FDA guidance (1997) and International Guidelines (Shah et al, 2000). As depicted by Aravagiri et al (1997), the concentration of olanzapine in serum shows a tendency to increase with the administered daily dose, which in turn cause increase in side effects and poor compliance (Weiden et al, 2004; Gerlach, 2002; Robinson et al, 2002). Thus, the extended release (ER) olanzapine tablets were developed to minimize the fluctuations in the olanzapine level of blood. The serum-concentration profile (<45 ng/mL) from the ER tablets recorded in the present study shows the ability of the Test olanzapine tablet formulation to maintain a fairly constant and optimum therapeutic level for 24 hours. A rapid rise in concentration (high peak) followed by a decline (trough) is evident in drug serum concentration time profile of the olanzapine reference formulation. Significant extension in half life-$t_{1/2}$ and time required to achieve maximum concentration ($T_{\text{max}}$) of Test olanzapine formulation is indicative of drug release at a slower rate for extended times. Thus, use of the olanzapine tablets in divided doses could be avoided. Non significant difference in mean AUCs of Test and Reference tablets indicates that the Test tablets were comparable to Reference tablets. Relative bioavailability and In-vitro In-vivo correlation were also calculated as it is the essential part of product development studies.

The relative bioavailability of 94% for Test tablets indicates nearly equal bioavailability of drug from both of the formulations. However, a stable concentration of Test tablet over 24 hours as compared to that of the Reference formulation shows the appropriateness of the developed ER formulation. An In-vitro In-vivo correlation ($R^2$) of 0.9082 of the optimized Test tablet indicated a good correlation of absorption with the amount of drug release up to almost 85%. In light of the above facts, it may be implied that the release and absorption of olanzapine occurs throughout the GIT, which is further suggestive of suitability of presenting olanzapine as the extended release tablet form.
4.5: CONCLUSION

Results of the present study clearly indicate that combination of both the Methocel® K100LV-CR (a hydrophilic polymer) and the Ethocel® standard 7FP premium (a hydrophobic polymer) could be successfully employed for formulating extended release matrix tablets of olanzapine. The investigated extended release matrix tablet has shown a strong \textit{In-vitro} and \textit{In-vivo} correlation and nearly smooth blood levels of olanzapine at therapeutic concentration throughout 24 hours. So one can expect improved therapy and reduced chances of the dose-dependent side effects from this formulation, most often associated with its conventional tablets.
5.1: INTRODUCTION

Prochlorperazine is a dopamine D2 receptor antagonist (Hamik and Paroutka (1989). Chemically, it is a phenothiazine derivative and has been placed in the first generation antipsychotics group (Potter and Hollister, 2004). Prochlorperazine and its salts are widely used for preventing nausea and vomiting, including that associated with cyclophosphamide and cisplatin-based chemotherapy (Olver et al, 1992; Crucitt et al, 1996), radiotherapy (Tramer et al, 1998) and surgery (Patterson et al, 1993; Williams and Smith, 1999). Prochlorperazine has shown a great success in the clinical management of pain and nausea associated with acute migraine headaches (Miller et al, 2009; Brousseau et al, 2004; Tanen et al, 2003; Coppola et al, 1995).

Just like chlorpromazine, prochlorperazine cause less sedation, fewer anti-muscarinic effects but more extrapyramidal side effects (Sweetman, 2008). Local irritation has been observed after the use of prochlorperazine buccal tablets. Reports of ulceration and soreness of the lip and tongue have been associated with use of prochlorperazine maleate oral tablets (Dixbury et al, 1982; Reilly and Wood, 1984). The erosive chelitis recovers after withdrawal of prochlorperazine and recurs on re-challenge.

Prochlorperazine is also effective for the short term symptomatic treatment of vertigo as occurs in miniere’s disease or labyrinths. It may be used in the clinical management of schizophrenia, mania and other psychosis (Sweetman, 2008). Prochlorperazine has been used as an adjunct therapy in the short term management of severe anxiety. Prochlorperazine base is generally administered by the rectal route and prochlorperazine maleate by the oral or buccal routes while prochlorperazine edisilate and mesilate can be given orally or parenterally.

Therapeutic level of 10 to 40 ng/mL and toxic level of 200 to 300 ng/mL for prochlorperazine have been reported by Regenthal et al (1999). Mean plasma half life ($t_{1/2}$) of 6.8 hours. $t_{max}$ of 1.5 to 5 hours and $C_{max}$ of 4 ng/mL for tablets have been reported by Taylor and Bateman (1987).

Clinical studies suggest that plasma levels of prochlorperazine correlate with its adverse drug effects (Olver et al, 1989), which, in turn are associated with poor compliance (Weiden et al, 2004; Gerlach, 2002; Robinson et al, 2002). Reports of
some investigators show that first generation antipsychotics (FGAs) and second generation antipsychotics (SGAs) had a similar dose-related increased risk of sudden cardiac death (Ray et al, 2009), obesity (Weiden et al, 2004; Allison et al., 1999), diabetes mellitus and dyslipidemia (Meyer and Koro, 2004) and extrapyramidal side effects (De Leon et al, 2007; Lee et al, 2005).

Frequently occurring noncompliance of antipsychotics (Gianfrancesco et al, 2006; Weiden, 2004) is a burning issue (Lieberman et al, 2005; Harris et al 2002) because most often such patients need rehospitalization (Svarstad et al. 2001), have poor quality of life and are increased economic burden on health care (Menzin et al. 2003). As oral extended release (ER) tablets of some antipsychotics (e.g. paliperidone (Invega®, Quetiapine XR) have shown advantages (Ganesan et al, 2008; Moller et al, 2008; Kane et al, 2007; Marder et al, 2005), so it seems rational to prepare ER tablets of prochlorperazine maleate for minimizing its side effects and optimizing its therapeutic outcomes. We have already demonstrated that the extended release tablets of risperidone and olanzapine provided optimised serum concentration profiles (see Table 3.7, Chapter-3 and Table 4.7, Chapter-4)

Currently, prochlorperazine maleate is widely used for clinical management of nausea and vomiting. Based on its commonly used 5 mg conventional tablets (given 3 to 4 times a day) for prevention of nausea and vomiting, the present study was aimed at developing once daily 15 mg extended release tablet of prochlorperazine maleate. Such tablets of prochlorperazine are expected to avoid fluctuations in blood concentration, reduce side effects and improve compliance.

The extensively recommended polymer, Methocel® K100 LV-CR and co-polymer Ethocel® Standard 7FP Premium (Li et al, 2005; Tiwari and Rajabi-Siahboomi, 2008) were used along with routinely used tablet excipients for preparing the extended drug release tablets of prochlorperazine maleate with zero order kinetics.
5.2: MATERIALS AND METHODS

5.2.1: Materials

Prochlorperazine maleate (Sanofi Aventis) was kindly provided by Drug Testing Laboratory, NWFP; Pakistan as a gift sample. Methocel® K100 LV-CR (Hydroxypropylmethylcellulose, HPMC-2208) and Ethocel® Standard 7FP Premium (Fine particle ethylcellulose) were obtained as gift from the Colorcon Asia Ltd, India. Stemetil tablets-conventional (batch No.564 bearing manufacturing date Sep, 2008; Sanofi Aventis (pvt) Ltd. Pakistan) containing 5 mg prochlorperazine base, were purchased from local market and used as Reference during in vivo studies. Acetonitrile and Methanol, both of HPLC grade were purchased from authorized local supplier of Merck, Germany. All the other chemicals used were of analytical or pharmaceutical grade.

5.2.2: Methods

5.2.2.1: Tablet manufacture

5.2.2.1(a): Preparation of powders-mix

The powders-mix of prochlorperazine maleate, Methocel®, Ethocel®, lactose, colloidal silicon dioxide-Aerocel® and magnesium stearate was prepared for six hundred tablets, as outlined in chapter-2, Section 2.2.1(a).

5.2.2.1(b) Granulation and tableting

Matrix tablets were prepared by dry granulation (slugging) method, using the ingredients mentioned in the Table-2.1. The granules of each formulation were divided into three batches for compression into tablets of 9 kg, 12 kg and 15 kg hardness as mentioned in Chapter-2, Section 2.2.1(b). Rotary tablet machine (Tablet Press, ZP-17 Shangai, China); with flat faced tooling, 17 mm in diameter was used in the manufacture of the tablets.
5.2.2.2: **In-vitro evaluation**

5.2.2.2(a): **Physicochemical evaluation of powders-mix and granules**

Physical characteristics, such as the angle of repose (AR), compressibility index (CI) and Hausner ratio (HR) of the powders-mix and granules (prepared by crushing of compacted slugs) were determined as outlined in Chapter-2, Section.

Prochlorperazine maleate content in a sample of 200 mg prepared granules was analysed at wavelength of maximum absorbance, $\lambda_{\text{max}}$ 254 nm; using UV-Visible Spectrophotometer (Shimadzu, Model UV-1700, Japan), as outlined in Chapter-2, Section 2.2.2(a).

5.2.2.2(b): **Physicochemical evaluation of tablets**

The prepared tablets of prochlorperazine maleate were evaluated for various physicochemical characteristics, such as friability, hardness weight variation and drug content. Prochlorperazine maleate content of the tablets was determined at $\lambda_{\text{max}}$ 254 nm with UV-Visible Spectrophotometer as described in Chapter-2, Section 2.2.2(b).

5.2.2.2(c): **Drug dissolution**

The drug release profiles of six tablets from each batch was measured as outlined in Chapter-2, Section 2.2.2(c), using UV-Visible Spectrophotometer at $\lambda_{\text{max}}$ 254 nm. The drug release data were fitted to various kinetic models including zero order, first order, Higuchi’s square root of time equation, Hixon and Crowel’s cube root equation and Power law equation, a detailed account of which is given in Chapter-2, Section 2.2.2(c).

Dissolution profiles of 9 kg, 12 kg and 15 kg hard tablets of the selected formulation F3 were determined at pH 6.8 and then compared with the dissolution profiles of these tablets determined at pH 1.5 to find out dissolution equivalency; as described in Chapter-2, Section 2.2.2 (c)
5.2.2.2(d): Selection of tablet formulation for stability and In-vivo studies

Optimised prochlorperazine maleate tablet formulation was selected based on better flowability and compressibility of granules and drug release profiles (zero order profiles with $R^2$ values approaching 1) of the prepared tablets.

5.2.2.2(e): Reproducibility and accelerated stability study

Reproducibility of the manufacturing process was determined by preparing three repeated batches of the selected optimized formulation (F3 with 12 kg hardness) on three different occasions. The optimised tablets were stored in high density polyethylene (HDPE) jars and kept under accelerated storage conditions of $40 \pm 2^\circ C / 75 \pm 5\%$ relative humidity (RH) in a stability chamber (Ti-Sc-THH-07-0400, Faisalabad, Pakistan) in accordance with International Commission for Harmonization (ICH) guidelines for a period of 6 months. The samples were tested for appearance, percent drug content, percent friability and hardness at predetermined time intervals of 0 time (prestorage) and after storage at 1, 2, 4 and 6 months respectively.

5.2.2.3: In-vivo evaluation

5.2.2.3(a): Study protocol and design

For in vivo experiments, the rabbits were divided into two groups, each having six animals. The first group received the Stemetil® (Aventis) as Reference tablet and the second group received the extended release prochlorperazine maleate Test tablets, a detailed account of which is given in Chapter-2, Section 2.2.3(a)

5.2.2.3(b): Administration of dose

The dose of the drug was administered according to the procedure stated in Chapter-2, Section 2.2.3(b)

5.2.2.3(c): Withdrawal of blood samples

Blood samples (0.7 mL each time) were collected from the marginal ear vein at 0, 1, 2, 4, 6, 8, 12, 24 and 28 hours in case of Test tablets and at 1, 2, 4, 6, 8, 9, 10, 12, 14, 16, 17, 18, 20, 22, 24 and 28 hours in case of Reference tablet administered three times a day, and allowed to clot. A 200 µL serum was withdrawn, centrifuged at 2800rpm for 10 minutes and 100 µL of clear serum so obtained was transferred to 10 mL test tubes and stored at -20°C until the time of analysis.
5.2.2.3(d): Extraction of prochlorperazine

Serum samples were prepared by liquid-liquid extraction as described by Fowler et al (1986). Briefly, to 100 µL spiked or sample serum, added 100 µL of 1M sodium hydroxide and 6 mL chloroform. The mixture was centrifuged at 2800rpm and the supernatant (organic) layer transferred into test tubes for drying under nitrogenous atmosphere. The residue obtained after drying was dissolved in 100 µL of the mobile phase by vortex mixing. 20 µL of the prepared sample was injected into the HPLC System for analysis.

5.2.2.3(e): Determination of prochlorperazine in serum samples

Blood serum level of prochlorperazine was determined by HPLC coupled with UV detector as described in Chapter-2, Section 2.2.3(e)

5.2.2.3(f): Instrument and chromatographic conditions

The prochlorperazine serum concentration was determined according to the method described by United States Pharmacopeia (USP 30, 2007), using an analytical column (Purosphar® Star RP.C18e Hibar® RT 250mm×4.6×mm×5µM, Merck, Japan) fitted into the Schimadzu liquid chromatographic system equipped with communication boss module (model 20A), two independently working pumps (model LC-20AT) and a variable wavelength UV-Visible detector (model SPD-20A) set at 254 nm. Mobile phase, consisting of ion pairing solution: methanol: acetonitrile (45:15:40), was used at the flow rate of 1.0 mL/min for elution of the analyte. The ion pairing solution was prepared by dissolving 4.33g of sodium 1-octanesulfonate in 500 mL water and then adding 4.0 mL glacial acid and sufficient purified water to make the final volume 1000 mL

5.2.2.3(g): Pharmacokinetic analysis

The concentration-time data of prochlorperazine from multidose Stemetil®, and from the single dose extended release Test tablet, were analyzed using the pharmacokinetic software, PK-Fit Ver 2.02. Non compartmental approach implemented in the above software was employed to calculate the pharmacokinetic parameters.
5.2.2.3(h): Relative bioavailability

Relative bioavailability of the Test and Reference tablets were tested as outlined in Chapter-2, Section 2.2.3(h).

5.2.2.4: *In-vitro* and *In-vivo* correlation

The *In-vitro* and *In-vivo* correlation (IVIVC) of the optimized batch of prochlorperazine maleate tablets was tested according to the procedure given in Chapter-2, Section 2.2.4.

5.3: RESULTS

5.3.1: *In-vitro* evaluation

5.3.1.1: Physicochemical evaluation of powders-mix and granules

Table-5.1 shows the values of angle of repose (AR) for powders mix and the granules. The AR of powder-mix was observed to be 44 ± 2° to 52 ± 4°, indicating poor flowability. The AR values of granules, 31 ± 2° to 34 ± 2° reflected good flowability. Poor compressibility indices were noted for powder (26 ± 3 to 31 ± 3) as compared to good compressibility indices for the granules (11 ± 2% to 13 ± 2%).

Hausner ratio (HR) of poweder (1.38 ± 0.17 to 1.45 ± 0.18) and that of granules (1.13 ± 0.12 to 1.17 ± 0.15) further supported the suitability of granules for tabletting. Drug contents of granules were 101 ± 4, 102 ± 3 and 102 ± 2 for F1, F2 and F3 respectively.

Table 5.1: Physicochemical characteristics of powders-mix and granules prepared for manufacture of extended release tablets of prochlorperazine maleate (Mean ± SD, n = 3)

<table>
<thead>
<tr>
<th>Material</th>
<th>Angle of Repose (degrees)</th>
<th>Compressibility Index</th>
<th>Hausner Ratio</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powders mix</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>44 ± 2</td>
<td>26 ± 3</td>
<td>1.38 ± 0.17</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>45 ± 3</td>
<td>28 ± 4</td>
<td>1.44 ± 0.17</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>52 ± 4</td>
<td>31 ± 3</td>
<td>1.45 ± 0.18</td>
<td>-</td>
</tr>
<tr>
<td>Granules</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>33 ± 3</td>
<td>12 ± 2</td>
<td>1.15 ± 0.13</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>F2</td>
<td>34 ± 2</td>
<td>13 ± 2</td>
<td>1.17 ± 0.15</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>F3</td>
<td>31 ± 2</td>
<td>11 ± 2</td>
<td>1.13 ± 0.12</td>
<td>102 ± 2</td>
</tr>
</tbody>
</table>

Note: F1 contains 58% Methocel® and 28% Ethocel®; F2 contains 43% Methocel® and 43% Ethocel®; F3 contains 28% Methocel® and 58% Ethocel®
Chapter-5: Extended Release Tablets of Prochlorperazine Maleate

5.3.1.2: Physicochemical evaluation of tablets

The prochlorperazine maleate tablets from each batch were observed to be uniform in dimensions (length × width, 8.0 ± 0.1×3.5 ± 0.1 mm to 8.1 ± 0.1×3.6 ± 0.1 mm), percent weight variation (3 ± 0.4 to 5 ± 0.4), percent friability (0.39 ± 0.04 to 0.56 ± 0.05 and percent drug content (98 ± 3 to 102 ± 2) as represented by the results of selected formulation F3 (Table 5.2). It leads us to the conclusion that all the batches of different formulations fulfills the USP requirements.

Table 5.2: Physicochemical characteristics of extended release tablets of prochlorperazine maleate for its selected formulation F3 (Mean ± SD, n = 10)

<table>
<thead>
<tr>
<th>Hardness of tablets</th>
<th>Friability (%)</th>
<th>Weight Variation (%)</th>
<th>Drug Content (%)</th>
<th>Dimensions (Length × Width in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 kg</td>
<td>0.48 ± 0.08</td>
<td>3 ± 0.5</td>
<td>98 ± 3</td>
<td>8.1 ± 0.1×3.6 ± 0.1</td>
</tr>
<tr>
<td>12 kg</td>
<td>0.56 ± 0.05</td>
<td>5± 0.4</td>
<td>102 ± 3</td>
<td>8.0 ± 0.1×3.5 ± 0.1</td>
</tr>
<tr>
<td>15 kg</td>
<td>0.39 ± 0.04</td>
<td>3 ± 0.4</td>
<td>102 ± 2</td>
<td>8.1 ± 0.1×3.5 ± 0.1</td>
</tr>
</tbody>
</table>

Note: F3 contains 28% Methocel® and 58% Ethocel®

5.3.1.3: Drug dissolution

5.3.1.3(a): Effect of formulation on drug release

The data on the effect of concentration of polymers have been given in Table 5.3, and the Figures 5.1 – 5.6. The maximum release period of 8 hours, 12 hours and 24 hours were achieved from the formulations F1, F2 and F3 respectively. The formulation F1 demonstrated significantly higher drug release rates (%/hour) as compared to that of F2 and F3, being 12.38 vs 8.29 vs 4.09 for 9 kg hard tablets; 12.24 vs 8.22 vs 4.05 for 12 kg hard tablets and 12.13 vs 8.19 vs 4.11 for 15 kg hard tablets in pH 1.2. Nearly same trend and levels of release rates were observed for the above mentioned tablets in pH-6.8 (Table 5.3).
### Table 5.3: Effect of formulation (F1, F2 and F3), dissolution media (pH-1.2, pH-6.8) and tablet hardness (9 kg, 12 kg and 15 kg) on release kinetics of prochlorperazine maleate from its extended release tablets

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness</th>
<th>pH of the dissolution medium = 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K</td>
</tr>
<tr>
<td>F1</td>
<td>9 kg</td>
<td>12.38</td>
</tr>
<tr>
<td></td>
<td>12 kg</td>
<td>12.24</td>
</tr>
<tr>
<td></td>
<td>15 kg</td>
<td>12.13</td>
</tr>
<tr>
<td>F2</td>
<td>9 kg</td>
<td>8.29</td>
</tr>
<tr>
<td></td>
<td>12 kg</td>
<td>8.22</td>
</tr>
<tr>
<td></td>
<td>15 kg</td>
<td>8.19</td>
</tr>
<tr>
<td>F3</td>
<td>9 kg</td>
<td>4.09</td>
</tr>
<tr>
<td></td>
<td>12 kg</td>
<td>4.05</td>
</tr>
<tr>
<td></td>
<td>15 kg</td>
<td>4.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness</th>
<th>pH of the dissolution medium = 6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 kg</td>
<td>12.23</td>
</tr>
<tr>
<td></td>
<td>12 kg</td>
<td>12.51</td>
</tr>
<tr>
<td></td>
<td>15 kg</td>
<td>12.35</td>
</tr>
<tr>
<td>F2</td>
<td>9 kg</td>
<td>8.13</td>
</tr>
<tr>
<td></td>
<td>12 kg</td>
<td>8.17</td>
</tr>
<tr>
<td></td>
<td>15 kg</td>
<td>8.12</td>
</tr>
<tr>
<td>F3</td>
<td>9 kg</td>
<td>4.17</td>
</tr>
<tr>
<td></td>
<td>12 kg</td>
<td>4.27</td>
</tr>
<tr>
<td></td>
<td>15 kg</td>
<td>4.21</td>
</tr>
</tbody>
</table>

**Note:** K, R² and “n” represent release rate constant, coefficient of determination and release exponent respectively, whereas F1 contains 58% Methocel® and 28% Ethocel®; F2 contains 43% Methocel® and 43% Ethocel®, while F3 contains 28% Methocel® and 58% Ethocel®.
Figure 5.1: Comparative release profiles of prochlorperazine maleate from 9 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 1.2 (Mean ± SD, n = 6).

Figure 5.2: Comparative release profiles of prochlorperazine maleate from 12 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 1.2 (Mean ± SD, n = 6).
Figure 5.3: Comparative release profiles of prochlorperazine maleate from 15 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 1.2 (Mean ± SD, n = 6).

Figure 5.4: Comparative release profiles of prochlorperazine maleate from 9 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 6.8 (Mean ± SD, n = 6).
Figure 5.5:  Comparative release profiles of prochlorperazine maleate from 12 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 6.8 (Mean ± SD, n = 6).

Figure 5.6:  Comparative release profiles of prochlorperazine maleate from 12 kg hard tablets of formulations F1 (58% Methocel®, 28% Ethocel®), F2 (43% Methocel®, 43% Ethocel®) and F3 (28% Methocel®, 58% Ethocel®), in dissolution media of pH 6.8 (Mean ± SD, n = 6).
5.3.1.3(b): Effect of hardness on release rate

Figures 5.7 and Table 5.3 show the effect of tablet hardness on the release rate of prochlorperazine maleate from its ER tablets. The tablet hardness had no significant effect on the drug release rates (%/hour) from all the three formulations, being 12.38, 12.24 and 12.13 for F1; 8.29, 8.22 and 8.19 for F2 and 4.09, 4.05 and 4.11 for F3 at pH 1.2. Nearly same levels of release rates were observed for the above mentioned tablets at pH 6.8.

![Figure 5.7: Effect of formulation (F1, F2 and F3); hardness (9 kg, 12 kg and 15 kg) and dissolution media (pH 1.2 and pH 6.8) on the drug release rates (K values) from prochlorperazine maleate extended release tablets (Mean ± SD, n = 6).](image-url)
5.3.1.3(c): Effect of pHs of dissolution media on drug release

As shown in the Figure-5.7 and Table 5.3, drug release rates (%/hour) at pH 1.2 and pH 6.8 were observed to be 12.38 vs 12.23 for 9 kg hard tablets, 12.24 vs 12.51 for 12 kg hard tablets and 12.13 vs 12.35 for 15 kg hard tablets in case of F1. For F2, it was 8.29 vs 8.13 for 9 kg hard tablets, 8.22 vs 8.17 for 12 kg hard tablets and 8.19 vs 8.12 for 15 kg hard tablets. The release rate (%/hour) at pH 1.2 and pH 6.8 were noted as 4.09 vs 4.17 for 9 kg hard tablets, 4.05 vs 4.27 for 12 kg hard tablets and 4.11 vs 4.21 for 15 kg hard tablets in case of F3. There was no significant effect of pH on the drug release rates of each of the formulation tested. Similarly there was no significant effect of pH on its release mechanism as mentioned above (Table 5.3).

For the selected tablet formulation, F3, the values of difference factor $f_1$ at pH 1.2 and pH 6.8 at hardness levels of 9 kg, 12 kg and 15 kg (Table 5.4), were found to be less than 11. The values of similarity factor, $f_2$ for the same were found to be greater than 61 as shown in Table 5.4, indicating better equivalence levels for each hardness.

Table 5.4: Difference factor $f_1$ and similarity factor $f_2$ calculated for 9 kg, 12 kg and 15 kg hard extended release optimised tablets of prochlorperazine maleate, while comparing their dissolution profiles in pH-6.8 with dissolution profiles in pH 1.2

<table>
<thead>
<tr>
<th>Hardness of extended release tablets (F3)</th>
<th>Value of $f_1$ for pH-1.2 vs pH-6.8</th>
<th>Value $f_2$ for pH-1.2 vs pH-6.8</th>
</tr>
</thead>
</table>
5.3.1.4: Selection of the optimized Test tablets

The prochlorperazine maleate tablet formulation F3 was selected as the optimised formulation. This formulation contained 28% Methocel and 58% Ethocel with 12 kg hardness. The basis of F3 selection as the optimised tablet formulation was its better flowability (AR = 31 ± 2°, compressibility properties (CI = 11 ± 2) and release rate (K = 4.05%/hour at pH-1.2 and K = 4.27%/hour at pH-6.8). The formulation followed the zero order release profile (n = 0.921, R² = 0.995 at pH-1.2 and n = 0.964, R² = 0.998 at pH-6.8).

5.3.1.5: Reproducibility and accelerated stability study

The data on drug content, weight variation, friability, hardness and appearance for the optimised prochlorperazine maleate tablet formulation is given in Table 5.5. Among the three batches of F3 at 12 kg hardness level produced at different occasions, there was no significant difference in drug contents (100 ± 3, 101 ± 3 and 103 ± 2). The friability was 0.4 ± 0.07, 0.36 ± 0.06 and 0.5 ± 0.08 for the three batches of optimised tablets produced at three different occasions. In addition there was no significant effect of ICH recommended accelerated storage conditions (40°C / 75 % RH) on the percent drug content (101 ± 3, 103 ± 4, 101 ± 4, 102 ± 2, 101 ± 3); percent weight variation (3 ± 0.4, 5 ± 0.4, 4 ± 0.3, 3 ± 0.5, 5 ± 0.3 ); percent friability (0.49 ± 0.1, 0.57 ± 0.1, 0.62 ± 0.1, 0.53 ± 0.1, 0.48 ± 0.1 ); hardness (12.0 ± 0.3, 12.0 ± 0.4, 12.0 ± 0.2 12.1 ± 0.3, 12.3 ± 0.2) and appearance (whitish) tested at 0 time (pre-storage) and after storage at 1, 2, 4 and 6 months respectively.

Table 5.5: Stability indicating parameters (drug content, weight variation, friability, hardness and appearance) for the optimized extended release tablets of prochlorperazine maleate (Mean ± SD, n = 3)
Chapter-5: Extended Release Tablets of Prochlorperazine Maleate

<table>
<thead>
<tr>
<th></th>
<th>At 0 time (pre-storage)</th>
<th>After 1 month</th>
<th>After 2 months</th>
<th>After 4 months</th>
<th>After 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>101 ± 3</td>
<td>103 ± 4</td>
<td>101 ± 4</td>
<td>102 ± 2</td>
<td>101 ± 3</td>
</tr>
<tr>
<td></td>
<td>3 ± 0.4</td>
<td>5 ± 0.4</td>
<td>4 ± 0.3</td>
<td>3 ± 0.5</td>
<td>5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.49 ± 0.01</td>
<td>0.57 ± 0.01</td>
<td>0.62 ± 0.01</td>
<td>0.53 ± 0.01</td>
<td>0.48 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>12.0±0.3</td>
<td>12.0 ± 0.4</td>
<td>12.0 ± 0.2</td>
<td>12.1 ± 0.3</td>
<td>12.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>White</td>
</tr>
</tbody>
</table>

5.3.2: *In-vivo* evaluation

Figures 5.8, 5.9, 5.10 and 5.11 are typical chromatograms of 60 ng/mL standard solution prochlorperazine maleate, of the blank serum, of the rabbit serum collected 4 hours after administration of Test tablets, of rabbit serum spiked with 60 ng/mL prochlorperazine maleate respectively. The retention time for prochlorperazine maleate was noted as 2.19 minutes. The mean relative recovery of prochlorperazine determined from five aliquots of quality control samples was found to be 87%.

The comparative serum concentrations of prochlorperazine maleate from both Reference (conventional) and Test (extended release) prochlorperazine maleate tablets are shown in Figures 5.12. Based on the concentration profile data, the computed pharmacokinetic parameters are given in Table 5.6. Two tailed t-test using prismGraphPad indicated significantly lower C$_{max}$, 43.50 ± 1.39 ng/mL (p<0.0001) for Test tablets as compared to C$_{max}$ of 80.33 ± 1.99 ng/mL for the Reference (conventional) tablets. The T$_{max}$ of Test tablets was achieved after 14.33 ± 7.16 hours as compared to significantly shorter T$_{max}$ of 4.17 ± 0.37 hours (p<0.0001) for Reference tablets. The values of MRT for Test tablets was significantly higher than that of Reference tablets (25.58 ± 4.77 hours vs 8.74 ± 1.57; p<0.0001). The half life of 16.07 ±3.97 hours of Test tablets was significantly higher (p<0.0001) than that of 5.26 ± 1.31 hrs for Reference formulation. The volume of distribution (Vss) of 244.064 ± 38.314 Litres for Test tablets was significantly higher (p<0.0001) than that of 84.327 ± 15.383 Litres for the Reference formulation. Elimination rate constant of Test tablets was significantly lower (0.046 ± 0.01 vs 0.14 ± 0.044; p<0.001) than that of the Reference formulation. There was no statistical difference between the total clearance of Test tablets (10.75 ± 1.046 Litres/hour) and that of Reference tablets (11.46 ± 1.93 Litres/hour). The area under curve, AUC$_{0-24h}$ of the Test tablets...
(1409.35 ± 145.46 ng.hour/Litre) not significantly different (p<0.05) from that of the Reference 5 mg tablets given three times a day (448.85 ± 76.66 × 3 = 1346 ± 229.98 ng.hour/Litre).
Table 5.6: Pharmacokinetic parameters of prochlorperazine following oral administration of 5 mg Reference tablets three times a day and 15 mg Test tablets administered once a day to two separate groups of rabbits (Mean ± SEM, n=6).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Reference tablets (prochlorperazine)</th>
<th>Test Tablets (prochlorperazine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elimination rate constant- $K_{el}$ (hr$^{-1}$)</td>
<td>0.142 ± 0.044</td>
<td>0.046 ± 0.01***</td>
</tr>
<tr>
<td>Half life- $t_{1/2}$ (hours)</td>
<td>5.257 ± 1.314</td>
<td>16.071 ± 3.97***</td>
</tr>
<tr>
<td>Time of maximum plasma concentration- $T_{max}$ (hours)</td>
<td>4.167 ± 0.37</td>
<td>14.33 ± 7.157***</td>
</tr>
<tr>
<td>Maximum plasma concentration- $C_{max}$ (ng/mL)</td>
<td>64.5 ± 4.031</td>
<td>45 ± 3.42**</td>
</tr>
<tr>
<td>Area under curve-AUC$_{0-}$ (ng.hour/mL)</td>
<td>448.85 ± 76.66</td>
<td>1409.35 ± 145.46***</td>
</tr>
<tr>
<td>Mean residence time-MRT$_{0-48hrs}$ (hour)</td>
<td>8.74 ± 1.57</td>
<td>25.58 ± 4.77***</td>
</tr>
<tr>
<td>Volume of distribution-Vb (L)</td>
<td>97.81 ± 10.80</td>
<td>270.49 ± 25.42***</td>
</tr>
<tr>
<td>Volume of distribution-Vss (L)</td>
<td>84.32 ± 15.38</td>
<td>244.06 ± 38.32***</td>
</tr>
<tr>
<td>Total clearance-Cl$_{total}$ (L / hour)</td>
<td>11.47 ± 1.93</td>
<td>10.75 ± 1.05</td>
</tr>
</tbody>
</table>

**Note:** Values are significantly different (* P < 0.05, ** P < 0.001, *** P < 0.0001) between means of Reference and Test tablets of prochlorperazine
**Figure 5.8:** A representative chromatogram of prochlorperazine (labeled as PCZ) from a standard solution containing 60 ng/mL of prochlorperazine maleate.

**Figure 5.9:** A representative chromatogram of extracted blank serum
**Figure 5.10:** A representative chromatogram of prochlorperazine (labeled as PCZ) extracted from a sample of rabbit serum withdrawn 4 hours after administration of Test tablet.

**Figure 5.11:** A representative chromatogram of prochlorperazine (labeled as PCZ) extracted from a sample of rabbit serum spiked with 60 ng/mL prochlorperazine maleate.
Figure 5.12: Comparative serum concentration-time profiles of prochlorperazine-1 and prochlorperazine-2, following oral administration of Reference and Test tablets respectively to rabbits (Mean ± SD, n = 6). Note: Prochlorperazine-1 represents Zyprexa (conventional) tablets while prochlorperazine-2 represents Test (extended release, prepared) tablets.
5.3.3: *In-vitro* and *In-vivo* correlation

The percent drug absorbed (Fa, Y axis) when plotted against percent drug released (Fr, X axis) produced higher linearity ($R^2 = 0.8458$) showing good correlation of absorption with the amount of drug release up to almost 85 %, as shown in Figure 5.13.

![Graph](image)

**Figure 5.13:** Percent of drug absorbed plotted against percent of drug released at times 1, 2, 4, 6, 8, 12 and 24 hours to show the *In-vitro In-vivo* correlation of prochlorperazine Test tablet
5.4: DISCUSSION

Like risperidone and olanzapine, in a pilot study prochlorperazine tablets were initially prepared with Methocel® using the direct compression method of tablet manufacture. However, upto 86%Methocel® alone could extended the release period only upto 8 hours. Then Ethocel®, in combination with the Methocel® was tried which proved successful. However the unbearable variations in the weights and hardnesses of tablets preparation by direct compression created an urge for using the granulation method, so it was considered rational to use the dry granulation-slugging method as discussed in Chapter-3, Section 3.4.

As given in Table-2.1, Chapter-2; three formulations of ER tablets of prochlorperazine were prepared employing the dry granulation-slugging method. The formulations F1, contained 58% Methocel® and 28% Ethocel®. The F2 was prepared with 43% Methocel® and 43% Ethocel® while F3 contained 28% Methocel® and 58% Ethocel®. The other excipients such as lactose, silicon dioxide (Aerocel®) and magnesium stearate were kept fixed at 3%, 1% and 1% levels respectively in order to determine clearly the effect of the polymers.

To elucidate the mechanism of drug release from prochlorperazine extended release tablets, dissolution data for the first 60% of drug release were fitted to the Power law equation as suggested by Bettini et al (2001). The release exponent “n” was calculated from the slope of the straight line of the above model fitting (Korsmeyer et al, 1983 and Peppas, 1985). In case of cylinders (i.e. tablets), the value of “n” ≤ 0.45 indicate Fickian release; 0.45 < “n” < 0.89 indicate anomalous transport, while the value of “n” ≥ 0.89 indicate zero order release.

Interestingly, in total 86% of the polymeric blend, 58% Methocel® and 28% Ethocel® (F1) could hardly maintained the release period for 8 hours. However, the value of release exponent “n” was increased from 0.45 (indicating drug release by diffusion/Higuchian) to 0.94, 0.88 and 0.86 (showing anomalous drug transport and/or zero order mechanism) of 9 kg, 12 kg and 15 kg hard tablets respectively in dissolution media of pH 1.2. Nearly similar “n” values ( “n” > 0.5 to 0.91) of the above mentioned tablets were observed in pH 6.8 (Table 5.3). One major difficulty faced after inclusion of 30 % Ethocel® in formulation F1 was dictation of using dry
granulation (slugging) method for tablet manufacture (instead of direct compression method) because of poor flowability and poor compressibility characteristics of the powders-mix. Thereafter further substitution of 15% Methocel® by Ethocel® (F2; 45%:45%) could extend the release period up to 12 hours with “n” values $\geq 0.89$, indicating zero order kinetics. In case of formulation F3, 15% Methocel® was further substituted by Ethocel® (28% Methocel® and 58% Ethocel®), consequently extension in the release period upto 24 hours was achieved.

The regular but not very proportional reduction in release rates with increase in the concentration of Ethocel® may be due to slow hydration of the matrix, based upon the hydrophobic character of Ethocel®. The insoluble particles of Ethocel® may be acting as barrier to drug release in the gel layer of the Methocel®. This idea is in line with the findings of Howard and Timinis (1998), where they used water insoluble sodium alginate for reducing the release rates of basic drugs from the Methocel®.

Drug release mechanism based on release exponent, “n” and the highest goodness of fit test ($R^2$ approaching to 1) indicated zero order release of drug in both dissolution media of pH 1.2 and 6.8 for all the three formulations F1, F2 and F3.

The results of this study demonstrated that higher compression force led to increased tablet hardness in all formulations, F1, F2 and F3 but the release rates and mechanisms remained unaffected. It can be implied that the porosity and /tortusity of the prepared tablets after their hydration were not influenced by increase in tablet hardness from 9 kg to 15 kg. Findings of the present study were in line with the previous studies conducted by Ravi et al (2008); Rekhi et al (1999); Velasco et al (1999); Ford et al (1987) and Doelker (1986). Just like risperidone and olanzapine, the prochlorperazine release from the matrices composed of these polymers was not effected by changes in pH.

There was no significant difference between the results of In-vitro studies of prochlorperazine maleate from that of risperidone, olanzapine because they have similar physicochemical characteristics and nearly same levels of the identical polymers were used in fabrication of extended release tablets of these drugs.
Chapter-5: Extended Release Tablets of Prochlorperazine Maleate

The method of HPLC-UV analysis was validated according to the FDA recommendations and International Guidelines (US FDA, 2001; Shah et al, 2000) prior to conducting HPLC analysis. The plasma levels of prochlorperazine correlate with its adverse drug effects (Olver et al, 1989), which in turn lead to poor compliance (Weiden et al, 2004; Gerlach, 2002; Robinson et al, 2002). Thus, the extended release (ER) prochlorperazine maleate tablets are expected to minimize fluctuations in blood levels of prochlorperazine and so will reduce side effects and improve compliance. The therapeutic blood concentration range of prochlorperazine is 10-40 ng/mL (Regenthal, 1999). The Test (ER) prochlorperazine maleate tablet in this study maintained nearly constant and optimum therapeutic concentration i.e. ≤ 44ng/mL; for 24 hours; as compared to the Reference tablet, which showed fluctuations (peak and trough) in drug serum concentration with time, when used in divided doses. Significant extension in half life (t₁/₂) and time required for achieving maximum concentration (Tₘₐₓ) of Test formulation are also indicative of drug release occurring at a slower rate for extended time, eliminating the need for taking prochlorperazine maleate tablets in three or four doses per day. A non-significant difference between mean AUC (1409 ± 135) of 15 mg Test tablet given once a day and AUC (449 ± 77 × 3 = 1347 ± 231) of 5 mg Reference tablets given three times a day indicates that the one Test tablets was bioequivalent to the three Reference tablets, which means that the extent of drug absorption of prochlorperazine maleate was not effected significantly by inclusion of it in extended release matrices. Relative bioavailability was noted as 1.05, which also indicate that the Test and the Reference tablet formulations were bioequivalent. The Test tablets were capable of releasing sufficient amount to maintain its therapeutic range for extended period of time. A relatively good level of *In-vitro* and *In-vivo* correlation was achieved with coefficient of determination (R²) being 0.8458, which indicate that the formulation was successful enough for further promotion and clinical evaluation.
5.5: CONCLUSION

Results of the present study clearly indicate that combination of both the Methocel® K100LV-CR (a hydrophilic polymer) and the Ethocel® Standard 7FP Premium (a hydrophobic polymer) can be successfully employed for formulating extended release matrix tablets of prochlorperazine maleate. The investigated extended release matrix tablet has shown a strong In-vitro and In-vivo correlation and nearly smooth blood levels of prochlorperazine throughout 24 hours. So one can expect better therapeutic outcomes and reduced chances of the dose-dependent side effects, most often associated with its conventional tablets.
Successful therapy depends not only on the drug efficacy but also on many other factors including optimal drug delivery and maintenance of effective drug concentration with minimum side effects. Effective drug concentration is critical for better treatment outcomes and lower side effects lead to the compliance of a drug. Side effect and compliance are the two major issues in long term treatments like psychiatric disorders. Partial or complete noncompliance has a deep association with the suboptimal treatment outcomes (Swanson, 2003; Kane, 1996; Lin et al, 1995). Compliance to antipsychotic medication plays a key role in achieving optimal long-term clinical outcomes (Aschor-Svanum, 2006)

The second generation antipsychotics (SGAs) were thought to have minimum side effects and with improved compliance (Rettenbacher et al, 2004; Nemeroff, 2003; Gray et al., 2002, Zygmunt et al, 2002; Gaebel, 1997). Advantages of SGAs over FGAs are not established uptil now (Gianfrancesco et al, 2006; Weiden, 2004; Dolder et al, 2002). Thus, noncompliance with the treatment using antipsychotics remains the major concern (Lieberman et al, 2005; Harris et al, 2002; Svarstads et al, 2001; Olfson et al, 2000).

The development of modified drug delivery systems (MDDs) have improved patient compliance, reduced side effects and optimized the dosage regimen without compromising the therapeutic efficacy (Keith, 2006). As a result of reduction in side effects of antipsychotics, some MDDS have been developed. Paliperidone (Invega®), an antipsychotic drug extended release tablets have been reported to offer improved treatment and tolerability profiles (Kane et al, 2007; Marder et al, 2005). Oral route for administration of drug is the most preferred and tablet is the most popular dosage form (Shangraw, 1989; Rudnic and Schwartz, 2005). Thus, in the present study, extended release matrix tablet formulations of the three antipsychotics, viz risperidone, olanzapine and prochlorperazine maleate were developed.

Among different technologies employed to develop ER tablet formulation, the hydrophilic matrix systems are most popular because of the simplicity of formulation, ease of manufacturing, lower cost, FDA acceptance and applicability to drugs of wide range solubility (Durig and Fassihi, 2002; Sako et al, 2002; Williams et al, 2002). In majority of cases, the hydrophilic matrices use polymers with flexible chemistry,
which offer prospect to formulate an extended release dosage form of desired characteristics.

Methocel® K100 LV-CR, the most frequently recommended version of hydroxypropylmethylcellulose-HPMC, has got great favor amongst the polymers employed in fabrication of hydrophilic matrices (Li et al, 2005; Tiwari and Rajabi-Siahboomi, 2008). However, sometimes it needs to be used in combination with another polymer (e.g Ethocel Standard 7FP Premium, an effective and popular version amongst the hydrophobic ethylcelluloses) to extend the time of drug release and minimize its abrupt burst effect. Therefore during the present work, after a long series of trials and errors in a pilot study, Methocel K100 LV-CR® and Ethocel® Standard 7FP Premium were blended together to develop the extended release matrix tablets of the model drugs under study.

The blends of the two polymers in three different ratios were able to produce different release rates ranging from 8 hours to 24 hours. The combination also reduced the burst release as desirable for smooth plasma levels. Release of drugs from polymer-based systems result from a single or combined effect of matrix hydration followed by gel formation, textural / rheological behavior, erosion, osmotic effects and dissolution and/or diffusion (Turner et al, 2004; Durig and Fassahi, 2002; Sako et al, 2002; Bettini et al, 2001; Narasimhan, 2001; Durig and Fassihi, 2000). Different factors that influence the drug release from polymeric matrices include the polymer type, physicochemical characteristics of the polymer, drug solubility, drug/polymer ratio, type and amount of tablet excipients (Levina and Rajebi-Siahboomi, 2004; Gao and Meury, 1996; Hogan, 1989). The HPMC is a frequently used polymer in hydrophilic matrix formulations due to its easy availability in a wide range of molecular weights and viscosity grades, FDA acceptance, water solubility and swelling/erosion distinctiveness (Jamzad et al, 2005; Pillay and Fassihi, 1999). Ethocel®, a hydrophobic polymer is another widely used polymer in the development of extended release matrices.

Direct compression was initially adopted for the manufacture of ER tablets of the model drugs. However, the poor flow ability and compressibility indices of all the three drugs forced the use of alternative tablet manufacturing approaches. Effective
compression of a powder into a tablet of desired properties requires a number of physical characteristics including appropriate flow ability, cohesiveness and compressibility.

Granulation methods, dry or wet are used to impart the required characteristics to the materials to be compressed. Wet granulation is performed in the presence of a liquid, such as water. This method is labor intensive, time consuming and requires special equipments and space. There is a tendency of losing material during various stages of processing, chances of incompatibility between formulation components and instability of moisture and heat sensitive drugs during wet granulation.

Stability of the model drugs used in this study has not been established yet. So dry granulation method was employed for the development of the ER tablets of the drugs because no instability problem happens while using this method. Thus, for manufacture of ER tablets of the three drugs, the dry granulation-slugging method was adopted throughout the present study.

As described in Chapter-1, Chapter-2 and Chapter-3, the heat and sensitivity bound granulation process resulted into an optimum sized (300 to 700 µM) granules containing a suitable proportion of fines for all the three drugs. The tablets resulted from granules were free of defects, regarding weight variation, weight loss (friability) and content uniformity. As reported by Chowhan (1980) both Methocel and Ethocel have sufficient binding characteristic, which was further improved by granulation step in this study, therefore, quality tablets meeting the compendia specifications were produced during this study.

When Methocel® was used alone at 10 – 90% level of the tablet weight, the desired release period could not be achieved. This may be due to the hydrophilic nature and low viscosity of the Methocel®, which led to a rapid hydration of matrix resulting in shorter release duration. Therefore, Methocel® was partially substituted by a hydrophobic copolymer, Ethocel® Standard 7FP Premium in the range of 28% to 58%. The level of colloidal silicon dioxide and magnesium stearate, each at 1% was kept fixed in all formulations of all three drugs in order to observe the effect of concentrations of the polymer and copolymer and the process variables such as tablet hardness and pH of the dissolution media on the tablet release characteristics. Lactose
was added from 0 – 6 % of tablet weight to compensate the deficiency in pre designed bulk of tablets. No lactose was added into tablet formulations of prochlorperazine maleate, 3% was incorporated into olanzapine ER tablets while 6% was added into risperidone ER tablets.

To determine the kinetics of drug release from the prepared ER tablets, the dissolution data of the respective drugs were fitted to different kinetic models. The release exponent “n” provided information on the release kinetics. The release profile was assumed to be of zero order based on the value of \( n \geq 0.89 \) and/or goodness of fit test i.e. \( R^2 \) value approaching 1 for the used kinetics equations. In case of the optimised ER tablet formulations for risperidone, olanzapine and prochlorperazine maleate, the values of the release exponent, “n” were \( \geq 0.89 \) and/or \( R^2 \) was > 0.99 which indicated zero order release in dissolution media of pH 1.2 and 6.8.

The reduction in release rates with increase in the concentration of Ethocel® may be attributed to the slow hydration of matrices, based upon the hydrophobic character of Ethocel®. The slow release may also be due to the formation of a highly tortuous matrix with reduced porosity of the matrix upon hydration of Methocel®. Up to 90 % of the polymer blend in the present study was comparable to 89% of fine particle ethylcellulose used by Agrawal (2003) to manufacture ER tablets of diphylline, theophylline and caffeine. In the present study, so much high percent of polymers content was deemed necessary to obtain the zero order release kinetics for 24 hours.

Different studies have shown contradictory effect of hardness or the crushing strengths on the drug release. Changes in compression force or the crushing strength has no major effect on drug release from HPMC based matrix tablets (Rekhi et al, 1999). Contrarily, some other studies using Fine Particle Ethylcellulose resulted in significant effects of hardness on the drug release profiles (Agrawal et al, 2003).

In this study, higher compression force led to increase in the tablet hardness in case of all formulations of each drug, but the release rates and mechanism of release remained unaffected for all the three drugs. The above mentioned findings in the present study are in line with the previous studies conducted by Ravi et al (2008) and
Velasco et al (1999). It can be implied that the porosity and tortuosity of the prepared tablets after their hydration was not influenced by increase in tablet hardness from 9 kg to 15 kg.

To mimic the effect of different pHs of gastrointestinal tract (GIT), the dissolution studies of extended release tablets of the three drugs were carried out at the most common extremes of pH 1.2 and pH 6.8. As the Methocel® is stable enough and resistant to changes in pH ranging from 2 to 13 (Marcos et al, 1996) and Ethocel® is hydrophobic/water insoluble polymer; thus, the release from the Methocel-Ethocel combination would be unaffected by the pH (Atsuko et al, 2006). All the three drugs have a relatively significant solubility at acidic pHs, thus the release profiles of the drugs from Methocel-Ethocel matrices were observed as independent of pH changes in dissolution media.

Generally, $f_1$ up to 15 and $f_2 > 50$ indicates similar dissolution profiles (Shah et al, 1998, US FDA, 1997). In this study, the values of difference factor, $f_1$ for selected ER tablets of risperidone, olanzapine and prochlorperazine maleate were less than 3.5, 7.2 and 4.7 respectively when their dissolution profiles in pH 6.8 were compared with dissolution profiles in pH 1.2. In the same way, similarity factor, $f_2$ of the selected tablets of risperidone, olanzapine and prochlorperazine were greater than 82, 66 and 78 respectively. Taking into consideration the above mentioned results, one can conclude that the pH of dissolution media does not affect the dissolution profiles of the three drugs from the designed formulations.

The drugs were analyzed by previously published HPLC methods (Aravigiri et al, 1998; Aravagiri et al, 1997; Fowler et al, 1986) after their validation for the present set up. The Test ER tablets of risperidone and olanzapine were found capable of maintaining optimum therapeutic concentrations for 24 hours as compared to the respective Reference tablets. The serum concentrations of the Reference tablet demonstrated fluctuation over time. Extension in half life and time required for achieving maximum concentrations ($T_{max}$) of Test ER formulations of risperidone and olanzapine are also indicative of the drug release at a slower rate for extended times.
Similarly, the single oral dose of 15 mg prochlorperazine produced fairly constant serum-level time curve without fluctuations up to 24 hours as compared to the multidose (3 times) Reference tablets. For most of the time, the prochlorperazine levels were noted as being in minimum effective range. Stable and constant drug absorption and a delayed $T_{max}$ value are expected to produce lesser side effects as suggested by Qiu and Zhang (2000). The prolonged MRT of the single dose (15 mg) prochlorperazine ER Test tablet than that of 3 tablets of Reference formulation was suggestive of the use of a single ER prochlorperazine tablet formulation effectively.

The relative bioavailabilities > 84% for the optimised extended release tablets in case of all three model drugs indicate bioequivalence with their respective counterpart conventional tablets. However, smooth and stable serum concentrations of these extended release tablets of risperidone, olanzapine and prochlorperazine over 24 hour shows the appropriateness of the developed formulations. The overall AUC resulting from the single dose of prochlorperazine extended release Test tablet was nearly same to that of the conventional three tablets. Nonsignificant difference in mean AUCs of Test and Reference tablet formulations of all model drugs further support the conclusion that the Test tablets of all the drugs were comparable in bioavailability to their respective Reference tablets. These findings lead us to the suggestion to use extended release tablets of the three drugs for avoiding their side effects, ultimately leading to improved compliance.

Using Wagner-Nelson method (Wagner-Nelson 1964), the In-vitro and In-vivo correlation ($R^2$) of risperidone, olanzapine and prochlorperazine maleate were found as 0.7293, 0.9082 and 0.8458 respectively, which indicate a good correlation of absorption with the amount of drug released. Furthermore, the release and absorption of risperidone, olanzapine and prochlorperazine occur throughout the GIT, which is further suggestive of the suitability of presenting risperidone, olanzapine and prochlorperazine maleate as the extended release tablet formulations.
Conclusions:

1. The extended release tablets of risperidone, olanzapine and prochlorperazine maleate were successfully developed based on the following backgrounds: a) The optimized tablets showed the most desired pH independent zero order release kinetics, b) Achievement of optimum serum concentrations for 24 hours and a good In-vitro In-vivo correlation.

2. Hardness and pH of dissolution media do not affect the drug release kinetics, when the matrix tablets are composed of the binary mixture of Methocel® K100 LV-CR and Ethocel® standard 7FP Premium.

3. Matrices composed of binary mixtures of the Methocel® K100 LV-CR and Ethocel® standard 7FP provide an appropriate environment for stability of risperidone, olanzapine and prochlorperazine maleate like basic drugs.

4. Methocel® K100 LV-CR and Ethocel® Standard 7FP Premium might produce similar constructive results in case of other low dose, water insoluble basic drugs.

For future work, it is recommended to:

1. Study further In-vivo absorption of the developed dosage form in other animal models such as dogs.

2. Conduct human studies to find out the pharmacokinetics properly and therapeutic influences of the developed formulations in clinical settings.

3. Study the affect of developed formulations on the level of neurotransmitters in the brain areas implicated in psychiatric disorders, in comparison with the conventional tablets of the same antipsychotic drugs.

4. The binary mixtures of the Methocel® K100 LV-CR and Ethocel® Standard 7FP Premium shall be further studied, using other similar drugs; for confirmation of the achievements of the present study; i.e. most desired pH independent zero order release from the binary mixture of Methocel® K100 LV-CR and Ethocel® standard 7FP.
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128
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