Improving the Bioavailability of Diltiazem Hydrochloride by Forming Complexes with β-Cyclodextrin

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1 Introduction
Therapeutic effectiveness of a drug depends upon the bioavailability and eventually upon the solubility of drug substances. Solubility is prerequisite to achieve desired concentration of drug in systemic circulation, drug absorption and pharmacological response. Oral route of drug administration is the uncomplicated and easiest approach of administration of drugs as it offers good patient compliance, convenience, accurate dosing, easy production, and greater stability. Poor hydrophilic drugs encompass dissolution is rate restrictive step in the process of drug absorption¹⁻³. Imminent bioavailability exertions are prevailing with extremely hydrophobic drugs due to inconsistent or partial absorption from gastrointestinal tract (GIT). Bioavailability is the most important property of a dosage form. It is the ability of the dosage form to deliver the active ingredient to its site of action in an amount sufficient to elicit the desired pharmacological response. Bioavailability is defined more precisely as the rate and extent of absorption of a drug from its dosage form in to the systemic circulation. It is affected by a number of factors related to the drug, dosage form and patient⁴⁻⁶.

Dosage form related factors which can produce profound differences in the drug bioavailability include formulation and manufacturing variables such as particle size, the chemical form, solubility of the drug, the type and quantity of excipients used, the compaction pressure etc⁶. It is well known that the drug bioavailability and efficacy are severely limited by its poor aqueous solubility and dissolution rate. The drug in a solid dosage form (tablet) must undergo dissolution before it is available for absorption in the gastrointestinal tract⁷. Dissolution forms the rate limiting step in the absorption of drug from solid dosage forms especially when the drug is poorly soluble¹⁻⁷.

Techniques such as, solubilization, solid dispersions, micronization, complexation, salt formation with polymers, transform in physical form, drug derivatization, use of prodrugs, addition of surfactants, alteration in pH etc. have been engaged in order to get better dissolution and bioavailability of poorly soluble drugs⁸. Although salt
formation, solubilization and particle size reduction have commonly been used to increase dissolution rate and thereby oral absorption and bioavailability of poorly water soluble drugs, there are practical limitations of these techniques. Among the various approaches, the complexation technique has proved to be the most successful, simple and economic in improving the dissolution and bioavailability of poorly soluble drug\(^9\). Cyclodextrin complexation is one of the widely used techniques in enhancing the solubility and dissolution rate. The ability of cyclodextrins to form inclusion compounds through molecular encapsulation has been known for many years. Several pharmaceutical products on the market use this formulation technology with different cyclodextrin derivatives\(^{10}\).

Diltiazem hydrochloride (DLT) is a calcium channel blocker used in the treatment of hypertension, angina pectoris and cardiac arrhythmias. Its biological half life is 3 - 4.5 h, which is relatively short and patients are advised to take DLT in divided daily doses, once in every 6 to 8 h. Such frequent drug administration may lead to fluctuations of blood levels, reduce patient compliance and lower the therapeutic efficacy. The maintenance of a constant plasma level of a cardiovascular drug is important in ensuring the desired therapeutic effect.

Several approaches could be investigated to improve their oral bioavailability, among them; complexation with cyclodextrins (CD) is drawing considerable commercial attention these times, because of their low toxicity, low cost, biocompatibility, biodegradability and abundant availability. CDs and their derivatives have received considerable attention in the pharmaceutical field for the past few years and an increased number of reviews have been dedicated to their industrial and pharmaceutical applications.

Drug-CD complexation and improvement in solubility and dissolution rate is influenced by both nature of the cyclodextrin (native or chemically modified, crystalline or amorphous) and the method of complexation, viz co-grinding, kneading, solid dispersion, solvent evaporation, co-precipitation, spray drying, or freeze drying. The effectiveness of a method depends on nature of the drug and CD. In many cases, spray drying and freeze drying were found to be more effective for drug complexation.

Therefore, the interest of the present investigation is to prepare inclusion complex systems of Diltiazem hydrochloride with crystalline native β-cyclodextrin and formulate mouth dissolving tablets with fast disintegration time.

2 Materials and Methods

2.1 Materials

Diltiazem HCl gifted from Macleods Pharmaceutical Limited Mumbai, India, was off white, odourless powder.

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2.2 Methods

2.2.1 Phase solubility analysis of Diltiazem HCl

Phase solubility studies were performed for Diltiazem HCl in pH 6.8 phosphate buffer. Solubility studies were carried out by adding excess amounts of both drugs separately in quantities exceeding its aqueous solubility to 50 mL of aqueous solutions of buffers, containing increasing concentrations of cyclodextrins (2-12 mM). The resulting suspensions were shaken at room temperature for a period of 72 hrs, until equilibrium was established. The samples were filtered through a 0.45 μm membrane filter (Millipore) and suitably diluted with corresponding buffer before analysis\(^{11-13}\).

2.2.2 Preparation of taste masked complex

In the present work, inclusion complexes of the drug and β-cyclodextrin in ratio of 1:1 w/w were prepared by different methods mentioned below.

2.2.2.1 Physical mixture method

The drugs and cyclodextrins at equimolar ratio (1:1) were weighed and mixed in a mortar by geometric dilution method for sufficient time (~5 min) to obtain a homogenous powder blend, passed through sieve no. 80 and stored in a sealed glass vials and kept in desiccator over fused calcium chloride until further use.

2.2.2.2 Kneading method

Cyclodextrins were wetted in a mortar with minimum volume of water-ethanol mixture (1:1) until a paste was obtained. The required amount of drug was slowly added and the slurry was kneaded for about 45 minutes. During this process, a suitable quantity of solvent was added to maintain optimum consistency. Further the products were dried at 40 °C to constant weight, passed through sieve no. 80 and stored in a desiccator until further evaluation.

2.2.2.3 Solvent evaporation method

Required quantities of drug and cyclodextrin were dissolved in sufficient quantity of water-ethanol solvent mixture (1:1) and evaporated on a water bath at 50°C with stirring. Each solid product was sieved through #80 and stored in desiccator\(^{14-18}\).

2.2.2.4 Spray drying

In spray drying, characteristic properties of powders like particle size and shape obtained are influenced by the nozzle, the viscosity of the feeding solution, and the outlet temperature (Tout), the later being dependent on the two spray drying process variables: inlet temperature (Tin) and solution flow\(^{19,20}\). To select best optimizing conditions, SQV with βCD combination was used and the same process conditions were applied with other CDs used in complex
formation. For optimization, influence of solution flow rate and Tin were studied and the solutions were atomized at three different flow rates (2, 5 and 10 mL/min) using fixed values for compressed air (500 L/h) and aspirator (40 m3/h). Two Tin values were used: 55 °C and 70 °C. Tout was kept constant at a temperature of 50 °C14,18.

2.2.3 Evaluation of complexes

2.2.3.1 Bulk density

Bulk density was determined by placing the powders blend in a measuring cylinder and the total volume is noted. The weight of powder bed was determined by using digital weighing balance. Bulk density was calculated using the following formula:

\[ \text{Bulk Density} = \frac{\text{Weight of the powder}}{\text{Volume of the powder}} \]

2.2.3.2 Tapped density

Tapped density was determined by taking the dried powders in a measuring cylinder and measuring the volume of powders after 100 tapping’s and take weight of the total powders.

\[ \text{Tapped Density} = \frac{\text{Weight of the powder}}{\text{Tapped Volume of the powder}} \]

2.2.3.3 Angle of repose

Angle of repose was determined by measuring the height and radius of the heap of the powder bed. A cylindrical two side open tube of 6 cm length is place on graph paper. Powders are placed in the tube and slowly removed the tube vertically. With the help of scale the height and radius of the heap were measure and note.

\[ \theta = \tan^{-1} \frac{h}{r} \]

Where, h = height of heap of granular bed, r = radius of heap of granular bed.

2.2.3.4 Compressibility index

The compressibility was calculated using the equation:

Percent compressibility or carr’s index = \[ \frac{\text{[tap - bulk]/tap}}{\text{100}} \]

Where bulk is the bulk density (g/mL) and tap is the tap density (g/mL)21-23

2.2.3.5 Drug content

The drug-CD complex equivalent to 50 mg of the drug was accurately weighed and transferred to 50 mL volumetric flask. 5 mL methanol was added and continuously shaken for 10 min and final volume was made up to the mark with pH 6.8 phosphate buffer for Diltiazem HCl. Suitable dilutions were made with respective drugs for both drugs. The solution was filtered through 0.45 μm Millipore nylon filter disc and the absorbance was measured at their respective wavelength using respective buffer for both the drugs as blank.

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2.2.3.6 Dissolution characteristics

Dissolution experiments were carried out in triplicate (n = 3) with an USP XXIII paddle apparatus in 900 mL of pH 6.8 phosphate buffer for Diltiazem HCl at 37°C using a rotation speed of 50 rpm. In each study drug-CD complex equivalent to 100 mg of drug was used. 5 mL sample was withdrawn at intervals of 5, 15, 30, 45, 60 min using a syringe fitted with prefilter (0.45 μm). An equal amount of fresh dissolution medium maintained at the same temperature was replaced immediately after withdrawal of the test sample. Test samples were suitably diluted wherever necessary and the absorbance was measured as per the analytical procedures described. The mean percent of drug dissolved and the standard deviations were calculated.

2.2.4 Preparation of tablets containing complex of drug: β-CD

Tablet containing 50 mg of Diltiazem HCl was prepared by direct compression method. Drug β-cyclodextrin complex equivalent to 50 mg and all the excipients except the lubricant were passed through a #20 mesh screen. The drug blend was prepared by mixing them manually in a polyethylene bag for 10–12 min. The lubricant was added to this blend and mixed properly again for 2 min. All formulations were prepared according to the experimental design; varying concentration of disintegration-promoting agent (1, 3 and 5%), as shown in table 1. Powdered lubricated blend was compressed into tablet by compression machine.

2.2.5 Formulation design of tablets

For formulation design of tablets, the concentration of the superdisintegrants in its low concentration to high concentration range was used. Formulation code is optimized on the basis of superdisintegrants concentration variation. The data analysis was done on the basis of three parameters viz, disintegration time, drug content and drug release study. On the basis of these sequential analyses one optimized batch was selected for further evaluation24,25.

2.2.6 Evaluation of mouth dissolving tablets

2.2.6.1 Disintegration time test

The disintegration time is defined as the time necessary for the mouth dissolving tablets (MDT) to completely disintegrate until no solid residue remains. The time required for disintegration of six MDTs, placed in each tube of disintegration test apparatus, was measured at 37 ± 2 °C using distill water. A total of six tablets were tested for each concentration, and the values reported are mean ± standard deviation26,27.

2.2.6.2 Drug content uniformity test

Tablets were powdered and the blend equivalent to 50 mg of drug was weighed and dissolved in phosphate buffer pH 6.8. The solution
was then filtered and diluted suitably. The drug content was then analyzed spectrophotometrically at respective wavelength.

**Table 1 Composition of mouth dissolving tablets**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (mg)</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex equivalent to 50 mg</td>
<td>122</td>
<td>122</td>
<td>122</td>
<td>122</td>
<td>122</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Sodium starch glycolate</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1%) (3%) (5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crosscarmellose</td>
<td>-</td>
<td>3</td>
<td>9</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1%) (3%) (5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Aspartame</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aerosil</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Talc</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Mannitol</td>
<td>43</td>
<td>37</td>
<td>31</td>
<td>25</td>
<td>43</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

2.2.6.3 *In-vitro* drug release

*In-vitro* dissolution study was performed in 900 ml phosphate buffer pH 6.8 using USP type II (paddle) apparatus at 50 rpm for 10 minutes (37 ± 0.5°C). Aliquots of the dissolution medium (1 ml) were withdrawn at specific time intervals (2, 4, 6, 8 and 10 min.) and replaced immediately with equal volume of fresh medium. The samples were filtered and diluted with suitable amount of phosphate buffer pH 6.8 and analyzed for drug content by measuring the absorbance at respective wavelength. Drug concentration was calculated from the standard calibration curve and expressed as cumulative percent drug dissolved.

3 Results and Discussion

3.1 Phase solubility study

Phase solubility study was performed to determine stiochiometric proportion of Diltiazem HCl and complexing agent β-cyclodextrin.

Phase solubility analysis plot for Diltiazem HCl and β-cyclodextrin are given in figure 1.

The phase solubility diagram of Diltiazem HCl and Cilostazol with β-cyclodextrin can be observed respectively in figure 1. As can be seen this diagram is an $A_c$ type curve with a linear relationship

$$K_{1:1} = \frac{\text{Slope}}{\text{intercept} \times (1 - \text{Slope})}$$

The apparent solubility constant ($K_{1:1}$) for the Diltiazem HCl:β-cyclodextrin and Cilostazol:β-cyclodextrin complexes was calculated from the solubility data and found to be 819.13 M$^{-1}$.

![Phase solubility analysis Diltiazem HCl](image1.png)

**Figure 1 Phase solubility analysis plot for Diltiazem HCl inclusion complexes**

3.2 Physical properties of complexes

Bulk density and tapped density of different formulations were calculated. The result of bulk density range from 0.45 to 0.51 and tapped density from 0.52 to 0.64. Hausner’s ratio was found to be in between 1.14 to 1.25; and Compressibility index from 12.28 to 20.12. Angle of repose showed good to excellent flow properties of the powdered blend (Table 2).

3.3 Drug content

Taste masked complex prepared by various method were subjected for evaluation of drug content in the drug:β-cyclodextrin (1:1) complex and the data obtained is shown in table 3. It was observed that the practical concentration obtained was 9.4 to 9.9 mg which was almost 94 to 99% of theoretical concentration that is 10 mg. The maximum percentage drug content was found to be 99.54% in the freeze dried complex.

3.4 Tablets containing complex of drug: β-CD

Complex prepared by various method were subjected for evaluation of drug content in the drug:β-cyclodextrin (1:1) complex and the data obtained is shown in table 4. It was observed that the practical yield was 77 to 83%. The maximum practical yield was found to be 93.26% in the cogrounding complex.

3.5 Evaluation of taste of drug: β CD complex

Tiwari et al. Improving the Bioavailability of Diltiazem Hydrochloride between solubilized Diltiazem HCl and β-cyclodextrin. The initial linear ascending part of solubility diagram in figure is generally ascribed to the formation of 1:1 complex for both the drugs. The apparent solubility constant ($K_{1:1}$) calculated using formula

$$K_{1:1} = \frac{\text{Slope}}{\text{intercept} \times (1 - \text{Slope})}$$

The apparent solubility constant ($K_{1:1}$) for the Diltiazem HCl:β-cyclodextrin and Cilostazol:β-cyclodextrin complexes was calculated from the solubility data and found to be 819.13 M$^{-1}$.

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The bitter taste value of formulations was ranged from 2 to 5. It is clearly seen from table 5 that there is masking of bitter taste of drug by the β cyclodextrin in drug:β cyclodextrin (1:1) complex by Freeze drying method.

Table 2 Physical characterization of complexes using Diltiazem HCl

<table>
<thead>
<tr>
<th>Drug:β-CD complex</th>
<th>Bulk density (gm/cm³)</th>
<th>Tapped density (gm/cm³)</th>
<th>Carr's index %</th>
<th>Hausnears ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kneading</td>
<td>0.50±0.25</td>
<td>0.57±1.08</td>
<td>12.28±0.21</td>
<td>1.14</td>
</tr>
<tr>
<td>Coevaporation</td>
<td>0.45±1.02</td>
<td>0.52±0.93</td>
<td>14.44±0.72</td>
<td>1.16</td>
</tr>
<tr>
<td>Co-grounding</td>
<td>0.50±0.56</td>
<td>0.58±0.14</td>
<td>14.96±0.61</td>
<td>1.17</td>
</tr>
<tr>
<td>Freeze-drying</td>
<td>0.45±1.05</td>
<td>0.52±0.25</td>
<td>14.44±0.35</td>
<td>1.16</td>
</tr>
<tr>
<td>Melting</td>
<td>0.51±0.86</td>
<td>0.64±0.49</td>
<td>20.09±0.19</td>
<td>1.25</td>
</tr>
<tr>
<td>Physical mixture</td>
<td>0.51±0.34</td>
<td>0.64±1.05</td>
<td>20.12±0.54</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Value shown in tables is mean of three determinations

3.6 In vitro dissolution studies

The Diltiazem HCl complexes were subjected for evaluation of the in vitro disintegration time (Figure 2). After evaluation it was observed that the time for all the formulations varied from 50.70 to 80.85 min. It was observed that when freeze drying method was applied for the formation complexes, it disintegrated rapidly within a short time when compared with other formulations prepared using other methods.

3.7 Characterization of tablets containing complex of drug: β-CD complexes

Figure 2 Average percent drug release from Diltiazem HCl
The results of pre-compression and post-compression indicate that product complied with the physical parameters. The formulation M3 exhibited good flow properties, less disintegration time (42.49 sec) and optimum dissolution rate compared to other formulations.

In order to have improved product performance several experiments were performed varying few of the functional excipients. Batch M3 complied with all the physical parameters such as hardness, friability and disintegration time.

**Table 6 Precompression parameters of powder blend**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Bulk density (gm/cm³)</th>
<th>Tapped density (gm/cm³)</th>
<th>Carr’s index %</th>
<th>Hausners ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.51±0.15</td>
<td>0.62±0.87</td>
<td>17.76±0.14</td>
<td>1.21</td>
</tr>
<tr>
<td>M2</td>
<td>0.51±1.15</td>
<td>0.64±1.06</td>
<td>20.86±1.36</td>
<td>1.26</td>
</tr>
<tr>
<td>M3</td>
<td>0.51±0.63</td>
<td>0.62±0.65</td>
<td>18.08±0.82</td>
<td>1.22</td>
</tr>
<tr>
<td>M4</td>
<td>0.51±0.83</td>
<td>0.64±0.43</td>
<td>19.69±0.47</td>
<td>1.24</td>
</tr>
<tr>
<td>M5</td>
<td>0.51±1.02</td>
<td>0.62±1.25</td>
<td>17.44±0.62</td>
<td>1.21</td>
</tr>
<tr>
<td>M6</td>
<td>0.51±0.38</td>
<td>0.64±1.16</td>
<td>19.87±1.05</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Value shown in tables is mean of three determinations.

**Table 7 Post compression parameters of directly compressible diluents tablets**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness (Kg/cm²)</th>
<th>Friability (%)</th>
<th>Thickness (mm)</th>
<th>Average weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>2.9±1.31</td>
<td>0.76±1.25</td>
<td>3.3±0.02</td>
<td>202.70±0.60</td>
</tr>
<tr>
<td>M2</td>
<td>3.2±1.06</td>
<td>0.63±1.05</td>
<td>3.4±0.04</td>
<td>201.6±0.52</td>
</tr>
<tr>
<td>M3</td>
<td>3.4±0.89</td>
<td>0.72±0.43</td>
<td>3.1±0.02</td>
<td>200.2±1.05</td>
</tr>
<tr>
<td>M4</td>
<td>3.1±0.19</td>
<td>0.89±1.58</td>
<td>3.2±0.03</td>
<td>198.83±0.59</td>
</tr>
<tr>
<td>M5</td>
<td>2.56±0.03</td>
<td>0.69±0.64</td>
<td>3.4±0.02</td>
<td>201.93±0.90</td>
</tr>
<tr>
<td>M6</td>
<td>2.50±0.10</td>
<td>0.62±1.27</td>
<td>3.5±0.03</td>
<td>203.63±1.00</td>
</tr>
</tbody>
</table>

Value shown in tables is mean of three determinations.

3.8 In-vitro drug release

The Diltiazem HCl complexes were subjected for evaluation of the in vitro disintegration time (Table 9). After evaluation it was observed that the time for all the formulations varied from 73.47 to 87.41 min. It was observed that M3 formulation release maximum drug at 60 min compare to other formulations.

4 Conclusions

Mouth dissolving tablets were formulated employing Diltiazem hydrochloride and their βCD complexes with an objective of evaluating the feasibility of employing drug-βCD complexes in the

Tiwari et al. Improving the Bioavailability of Diltiazem Hydrochloride design of immediate release tablet formulations for obtaining flow and complete drug release in 01 h. MDT were prepared by dry granulation method with suitable quantity of superdisintegrants. All the tablets prepared by optimizing the formula and were evaluated for drug content, drug release kinetics and mechanisms. The overall results indicate that formulation M3 was better and that it satisfies all the criteria as a fast dissolving tablet.

5 References

Table 8 Drug content of mouth dissolving tablets

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>Drug content (%)</td>
<td>96.78±0.97</td>
</tr>
<tr>
<td>Disintegration time (second)</td>
<td>61.30±3.02</td>
</tr>
</tbody>
</table>

Value shown in tables is mean of three determinations.

Table 9 In-vitro drug release of different formulations

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>67.36±1.00</td>
<td>68.87±1.30</td>
<td>73.47±2.48</td>
<td>68.30±1.37</td>
<td>70.12±1.88</td>
<td>67.30±1.37</td>
</tr>
<tr>
<td>10</td>
<td>68.92±1.24</td>
<td>70.25±1.00</td>
<td>77.95±1.16</td>
<td>69.87±1.72</td>
<td>73.25±0.98</td>
<td>70.37±1.22</td>
</tr>
<tr>
<td>15</td>
<td>70.25±1.47</td>
<td>71.89±0.92</td>
<td>80.82±2.52</td>
<td>71.67±1.09</td>
<td>76.50±2.18</td>
<td>72.67±1.09</td>
</tr>
<tr>
<td>30</td>
<td>71.86±1.50</td>
<td>75.01±1.34</td>
<td>83.68±1.52</td>
<td>72.98±0.85</td>
<td>76.87±1.55</td>
<td>75.28±0.85</td>
</tr>
<tr>
<td>60</td>
<td>73.47±2.02</td>
<td>76.39±2.38</td>
<td>87.41±2.29</td>
<td>75.99±2.08</td>
<td>80.62±1.09</td>
<td>77.39±1.48</td>
</tr>
</tbody>
</table>

Value shown in tables is mean of three determinations.


