Hypertension is the most dominating disease of today’s fast life. The style of living full of tension and high cholesterol diets increases the risk of hypertension and cardiac disorders like myocardial infarction. Atenolol is a $\beta_1$-blocker which can lower the blood pressure by increasing the cardiac output and simvastatin is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor that can, on the one side, lower the peripheral resistance and, on the other side, save patient from cardiac risks. Its GIT adverse effects are abdominal cramps, constipation, diarrhea, flatulence, heartburn, altered taste, dyspepsia and nausea. Atenolol blocks stimulation of $\beta_1$ (myocardial)-adrenergic receptors and does not usually affect $\beta_2$ (pulmonary, vascular, uterine) receptor sites. It decreases blood pressure, heart rate and frequency of attacks of angina pectoris (1).

Granulation is a technique that is used to entrap drugs for multiple reasons that include the instability of drug in acid medium, GIT disturbances etc. However, stability and biological activity of the drug should not be affected during granulation. Product yield and drug encapsulation efficiency should be high and drug release profile should be reproducible in specific limits (2). Ethylcellulose (EC) is a biodegradable polymer that can be used for the entrapment of many harmless drugs for oral use (3). EC is preferably used as it can easily bear the transport shocks.

Literature studies revealed that there is no single dosage form contained both the drugs for treatment of hypertension. These two drugs are mostly prescribed in combination so the purpose of this novel study was to formulate a combined dosage form having both drugs with extended release behavior. Non-solvent addition technique of granulation was used. The prepared granules were characterized in different ways. Release behavior was evaluated using different kinetic models. Moreover, solvent used, i.e., toluene, is a class II solvent so its complete removal from drug delivery system is necessary. A further study for its complete removal and evaluation using high performance liquid chromatography and gas chromatography is in progress in our lab.
MATERIALS AND METHODS

Materials
Simvastatin was gifted by ZAFA Pharmaceuticals (Pvt.) Ltd., Karachi, Pakistan. Atenolol was a donation of ICI, Karachi, Pakistan. Toluene and EC (22cps) were purchased from Merck, Germany. Polyisobutylene (PIB, m.w. 2,800) was purchased from Acros Organics, USA. Petroleum ether (40–60°C) was from BDH, England. Distilled water was obtained from Industrial Laboratory, Islamia University, Bahawalpur.

Preparation of granules
Weighed EC was added into toluene solution with 6% w/w polyisobutylene in a beaker and stirred at 500 rpm for 6 h using magnetic stirrer (Velp, Europe). Atenolol and simvastatin were dispersed in it. The solution was stirred for 15 min and then petroleum benzine (non-solvent) was added to induce phase separation. The granules formed were transferred to ice bath for solidification. These granules were treated with chilled petroleum benzine five times for complete granulation with continuous stirring. Granules were then washed with n-hexane and dried in an oven (Memmert, Germany) at 50°C for 3 h to ensure complete removal of all organic solvents (4). Drug polymer ratio was varied as 1:1 (M1), 1:2 (M2) and 1:3 (M3).

Micromeritic test
The granules were characterized to check flow properties of combined granules.
Bulk density and tapping density was determined as:

\[
D_b = \frac{\text{Sample weight}}{\text{Sample volume}} \tag{1}
\]

Tapped density \(D_t\) = weight of granules/volume of granules after 100 tapings \(\tag{2}\)

Compressibility index was determined by the following formula:

\[
C_i = \left[\frac{(D_b - D_t)}{D_b}\right] \times 100 \tag{3}
\]

Hausner ratio of granules was also determined by using the following equation:

\[
\text{Hausner ratio} = D_b/D_t \tag{4}
\]

The frictional forces in a loose powder can be measured by the angle of repose, \(\Phi\). This is the maximum angle possible between the surface of a pile of powder and horizontal plane. Mathematically:

\[
\Phi = \tan^{-1} \frac{h}{r} \tag{5}
\]

where, \(h\) = height of heap, \(r\) = radius of heap (5). The size of the granules was determined using light microscope attached with a graduated stage (4).

Encapsulation efficiency and production yield
Encapsulation efficacy of granules was determined to know the amount of drugs entrapped within the granules. The known amount of granules was crushed and dissolved in distilled water with 0.5% sodium dodecyl sulfate. It was stirred continuously up to 2 h. The solution was filtered and checked by the UV spectroscopy at 238 nm for simvastatin and at 275 nm for atenolol to check the amount of encapsulated drug after extraction from granules (6). Loading efficacy was determined as:

\[
\text{Drug loading} = \frac{\text{Amount of drug in microparticles}}{\text{Amount of microparticles}} \times 100 \tag{6}
\]

Drug encapsulation efficiency = \(\frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100 \tag{7}\)

The production yield of the method was calculated by formulas (7) and (8).

production yield \(\% \) = \(\frac{M}{M_t} \times 100 \tag{8}\)

where, \(M\) and \(M_t\) are the weights of granules and starting materials (\(M_t = \) total weight of DS and EC), respectively.

Dissolution test
Combined granules were subjected to the dissolution using USP apparatus I (Pharma test, Germany). Dissolution medium was distilled water with 0.5% sodium dodecyl sulfate as a surfactant. The surfactant was used to dissolve the drugs well in the medium. Dissolution medium (900 mL) was maintained at 37 ± 0.05°C. Weighed quantities of granules were placed in the dissolution medium. Samples were collected at time 0, 30, 60, 90, and 120 min, and so on up to 12 h at different intervals using automatic sample collector (Pharma Test, Germany). The samples were studied at wavelength of 273 nm for atenolol and 238 nm for simvastatin.

Kinetic analysis
Data were studied after fitting them into different kinetic models. These models were (4):

Zero order kinetic model, \(D_t = D_0 + K_1t \tag{9}\)

First order kinetics model, \(\ln D_t = \ln D_0 + K_1t \tag{10}\)

Higuchi kinetic model, \(D_t = D_0 + K_2t^{\frac{1}{2}} \tag{11}\)

Hixson Crowell kinetic model, \(D_t^3 - D_0^3 = K_3t \tag{12}\)

Korsmeyer–Peppas kinetic model, \(D_t/D_0 = K_4t^n \tag{13}\)

where, \(M_t\) is the cumulative amount of drug which is released at any specified time point and \(M_0\) is the initial amount of drug in the formulation. \(K_0, K_1, K_2, K_3\), and \(K_4\) are rate constants for zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models, respectively. In equation (5), \(M_t/M_0\) is the fraction of the drug release at time t and n is
the release exponent that characterizes different release mechanisms. The n-value is calculated from the slope of Korsmeyer-Peppas plot.

**FTIR analysis**

FTIR spectroscope (MIDAC M2000, USA) was used to done the FTIR measurements. Samples were prepared by KBr disc methods and 2 mg of granules were contained in 200 mg of KBr disc. Scanning was done in the range of 450–5000 cm⁻¹.

**XRD analysis**

D8 Discover (Bruker, Germany) apparatus was used for x-rays’ diffraction to confirm any drug-drug or drug-polymer interaction. Sample was scanned in the range of 8 to 70° with Cu-Kα radiation at 1.5406 Å (source). Scanning speed was 4°/min. Scintillation detector was used for detection with primary slit of 1 mm and secondary slit of 0.6 mm.

**Thermal analysis**

Diffraction scanning calorimetry is the technique to determine the energy consumed to create the zero temperature difference between sample and standard inert reference. Both the specimens were retained at controlled temperature whether heated or cooled. Thermograms were recorded on a scanning calorimeter TA instrument (USA). Instrument was calibrated by the use of indium as standard. The samples were heated on the aluminum pan at 250°C, scanning rate of 20 °C/min under the flow of nitrogen. An empty aluminum pan was used as reference.
RESULTS AND DISCUSSION

Preparation of granules

The polymer and both drugs were encapsulated by non solvent addition technique. Toluene was used as solvent for EC to dissolve it well. Petroleum benzin was added as non solvent to induce granulation. After granulation, particles were dried in air and in oven for complete removal of toluene and benzin from granules.

Micromeritic test, sizing and morphology of granules

Sieve analysis was done to determine size of granules. Rheological property was also analyzed. It was seen that the compressibility index of granules was below 15%, which indicated excellent flow property of granules. Hausner’s ratio of granules was below 1.29, which was indicative of free flowing property. The angle of repose was approximately 20°. It was also seen that the flow property of drugs in combined formulation was far better than that of pure drugs. So it can be concluded that granulation improved flow property of drugs (Table 1). The granules were rough, whitish and irregular in shape. Granules were of non porous surface due to the slow evaporation (Fig. 1).

Encapsulation efficiency and production yield

Encapsulation efficacy of simvastatin was determined as 68.71, 70.28 and 71.32%, whereas that of atenolol was 89.67, 90.92 and 90.01 for 1:1, 1:2 and 1:3 drug-polymer ratio, respectively. It can be suggested that EC entrapped hydrophilic drug more than hydrophobic drug. The production yield
was approximately 89% for atenolol and 72% for simvastatin for all batches.

**FTIR Analysis**

Both pure drugs, ethylcellulose and granules were analyzed by FTIR for the study of drug-drug and drug-polymer compatibility. The spectra of pure drugs show specific peaks, which are also present in the spectra of granules (Fig. 2). This result confirms the absence of any chemical drug-drug and drug-polymer interaction.

**XRD analysis**

Both pure drugs, ethylcellulose and granules were analyzed by XRD for the study of their physical states. The diffractograms of pure drugs contained many characteristic diffraction peaks revealing that both drugs are crystalline in nature (Fig. 3), whereas the diffraction phenomenon observed from ethylcellulose showed a single minor characteristic peak which confirms its amorphous or semi-crystalline structure. The drugs encapsulated in granules also exhibited identical bands as those of their pure samples. It indicates the absence of any chemical drug-drug and drug-polymer interaction.

**Thermal analysis**

Both pure drugs, ethylcellulose and granules were analyzed by DSC for the study of their physical states. The DSC results of pure simvastatin and atenolol showed endothermic peaks at 130°C and 150°C, respectively, which are the melting points of these two drugs (Fig. 4). These both endothermic peaks were also observed in the results of granules but with lower intensity, showing some minor reduction in crystalline nature during granulation.

**Dissolution test**

The dissolution study of both atenolol and simvastatin from ethylcellulose granules was conducted in distilled water (Fig. 5). Sodium dodecyl sulfate was added to the dissolution medium to enhance the solubility of drugs in distilled water. Two distinctive dissolution profiles were observed in dissolution test, indicating the release of two different drugs from the granules. The release of atenolol was better controlled than simvastatin from all granule formulations. An initial burst release of both drugs from granules was observed followed by slow release phase till 12th hour but this burst effect was more severe for simvastatin than for atenolol (Fig. 5). The release of both drugs was better controlled from granules prepared by 1:3 drug polymer ratio (Fig. 5). The t_{80%} for atenolol and simvastatin was 8.16 h (release was slower) and 6.23 h (release was quicker), respectively.

The initial burst release of both drugs can be due to the following reasons: (i) drug particles embedded on the external surface of granules due to imperfect encapsulation and/or (ii) difference in their solubilities (solubility of atenolol and simvastatin was approximately 89% for atenolol and 72% for simvastatin for all batches.

### Table 1. Micromeritic values of granules, pure atenolol and simvastatin

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Average diameter (µm)</th>
<th>Bulk density (g/mL)</th>
<th>Taped density (g/mL)</th>
<th>Compressibility index (%)</th>
<th>Hausner’s ratio</th>
<th>Angle of repose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>98 ± 5.48</td>
<td>0.14</td>
<td>0.15</td>
<td>17.1</td>
<td>2.35</td>
<td>43.92°</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>79 ± 7.20</td>
<td>0.14</td>
<td>0.16</td>
<td>16.8</td>
<td>2.28</td>
<td>48.54°</td>
</tr>
<tr>
<td>Combined 1:1</td>
<td>280 ± 8.12</td>
<td>0.22</td>
<td>0.25</td>
<td>10.8</td>
<td>1.10</td>
<td>20.41°</td>
</tr>
<tr>
<td>Combined 1:2</td>
<td>323 ± 1.08</td>
<td>0.24</td>
<td>0.28</td>
<td>11.1</td>
<td>1.54</td>
<td>21.91°</td>
</tr>
<tr>
<td>Combined 1:3</td>
<td>619 ± 6.11</td>
<td>0.27</td>
<td>0.30</td>
<td>09.3</td>
<td>1.23</td>
<td>19.28°</td>
</tr>
</tbody>
</table>
tatin in dissolution medium was 86 mg/mL and 100 mg/mL, respectively) (4). Higher solubility of simvastatin was also a reason of its quicker release from granules through water-filled channels present in hydrophobic ethylcellulose granules that were created during the process of phase separation (9). These channels facilitate the quicker diffusion of medium into the granules and thus release of drugs in a burst manner without degrading ethylcellulose granules (4, 10, 11). This burst effect was more intense from 1:1 ($t_{80\%} = 6.12$ and 2.28 h for atenolol and simvastatin, respectively) and 1:2 ($t_{80\%} = 6.67$ and 3.88 h for atenolol and simvastatin, respectively) drug-polymer ratio granules than that of 1:3 ($t_{80\%} = 8.16$ h and 6.23 h for atenolol and simvastatin, respectively, Fig. 5). It could be due to the higher amount of ethylcellulose aggregation around 1:3 ratio granules resulting in thicker polymer coating. It produced a shield against the diffusion of dissolution medium into the core of granules (4). In addition, more porous structure of 1:1 and 1:2 ratio granules is evident from Figure 1, which is responsible for quicker release of drugs from these granules, while the structure of 1:3 ratio granule seems less porous. This is the reason of slow release of drug from these granules and also reduced burst effect.

Regression coefficient ($R^2$) was determined for both drugs by curve fitting method in different kinetic models. The highest value of $R^2$ was seen in Higuchi model for atenolol and simvastatin that indicated the release of drugs from granules depending on square root of time. The value of diffusion coefficient ($n$) indicated that the release of drug from granules was by diffusion.

CONCLUSION

It can be concluded from the results that simultaneous granulation of atenolol and simvastatin is possible by non solvent addition technique using ethylcellulose without disturbing the physico-chemical nature of drugs and polymer used.

DECLARATION OF INTEREST

The authors declare no conflict of interest regarding the contents of this article.

REFERENCES


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