Formulation and Characterization of Microspheres of Simvastatin for the Management of Hypercholesterolemia

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ABSTRACT

The aim of the present study was to prepare and evaluate the microspheres of Simvastatin. Simvastatin microspheres were prepared by ion gelation using polymers such as HPMC (K 15 M), and sodium alginate. Totally 6 different formulations of Simvastatin were prepared by using the above polymers. The microspheres were characterized for drug content, entrapment efficiency, in-vitro drug release etc. The formulation F1 was selected as an ideal formulation based on the in vitro release profile which shows an extended drug release of 98.8 ± 3.13 % upto 24 hours in phosphate buffer of pH 7.0. FT-IR studies indicated the lack of drug-polymer interactions in the ideal formulation. The in vitro release data of all microsphere formulations were plotted in various kinetic equations to understand the mechanisms and kinetics of drug release. The optimized formulation followed zero order kinetics. From the stability study it was observed the formulation was found to be stable.

Keywords: Simvastatin, HPMC (K 15 M), ion gelation method, Sodium Alginate, zero order kinetics.

INTRODUCTION

A well-developed controlled drug delivery system (CDDS) can overcome some of the problems of conventional dosage forms and improve therapeutic effect of a given drug. To get maximum therapeutic effect, it is essential to deliver the drug to the target tissue in the optimal amount in the right period of time there by causing little toxic effect and minimum side effects. There are different approaches to deliver a drug to the target site in a controlled release pattern. One such approach is using microspheres as carriers for drugs. To overcome the relativity short gastro intestinal time and improve localization for oral controlled or sustained release drug delivery systems. The polymers are incorporated to modify release profiles of dosage forms.

Simvastatin is antihyperlipidemic used to control hypercholesterolemia. It is belonging to statin class of pharmaceuticals, and a synthetic derivative of a fermentation product of Aspergillus Terreus. It is structural analog of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme). It inhibits the enzyme hydroxymethylglutaryl-CoA (HMG-CoA) reductase. Simvastatin is inactive lactone prodrug and hydrolyzed in the gastrointestinal tract to the active β-hydroxy derivative. It decreases total cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, and apolipoprotein B, while increasing high density lipoprotein (HDL).

In the present study simvastatin microspheres by ion gelation technique using polymers such as sodium alginate, and HPMC (K 15 M). The prepared microspheres were evaluated for micromeritic properties, drug content, entrapment efficiency, in vitro drug release studies and in vitro drug release kinetic studies.

MATERIALS AND METHODS

Materials

Simvastatin was obtained from Research-lab fine chem. Industries India. HPMCK15M, Sodium alginite were purchased from S.D. Fine Chemicals Ltd, Mumbai, India. All reagents were of A.R. grade. Double distilled water was used throughout the experiment.

Methods

Compatibility study

Fourier Transform Infrared Spectroscopy (FTIR) study

In this method individual samples as well as the mixture of drug and excipients were ground mixed thoroughly with potassium bromide (1:100) for 3-5 minutes in a mortar and compressed into disc by applying pressure of 10 kg/cm² to form a transparent pellet in hydraulic press. The pellet was kept in the sample holder and scanned from 4000 to 400 cm⁻¹ in FTIR spectrophotometer. Then the characteristics peaks were obtained of all sample as well as mixtures.
Preparation of microspheres

Microspheres were prepared by ion gelation method. A solution was prepared by dissolving 40 mg equivalent of Simvastatin in 5 ml distilled water. The solution was dispersed in 10 ml of polymeric solution which contained required amount of sodium alginate and HPMC K15M (1%, or 2% of alginate solution was prepared by taking 1 g, or 2 g of sodium alginate in 100 ml of water respectively and measured amount of HPMC K15M was added in 100 ml of water i.e. 0.2 g, 0.6 g or 1 g of HPMC K15M to prepare 0.2%, 0.6% or 1% of HPMC K15M solution respectively) as shown in Table 1. The resulting solution was dropped through a 22G syringe needle into 5% (w/v) CaCl₂ solution. The solution containing suspended particles was kept for specific time to improve the mechanical strength. The fully formed microspheres were collected washed with distilled water subsequently air dried and stored in desiccator. The different formulations F1 to F6 have different levels of process variables which are given below.

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Conc. of Drug (mg)</th>
<th>Conc. of Sodium alginate (%)</th>
<th>Conc. of HPMC K15M (%)</th>
<th>Amount of Sodium alginate (mg)</th>
<th>Amount of HPMC K15M (mg)</th>
<th>Hardening time (h)</th>
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</thead>
<tbody>
<tr>
<td>F1</td>
<td>40</td>
<td>1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.02</td>
<td>3</td>
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<tr>
<td>F2</td>
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<td>0.6</td>
<td>0.1</td>
<td>0.06</td>
<td>5</td>
</tr>
<tr>
<td>F3</td>
<td>40</td>
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<td>1</td>
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<tr>
<td>F5</td>
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</tr>
<tr>
<td>F6</td>
<td>40</td>
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<td>0.2</td>
<td>0.1</td>
<td>5</td>
</tr>
</tbody>
</table>

Characterization of Microspheres

**Micromeritic characterization**

**Angle of repose:**

Fixed funnel method determines angle of repose. The required quantity of microspheres was taken in funnel where funnel tip touched the microspheres heap. The granules were permitted to fall through the funnel. Powder cone radius was determined and followed by determination of angle of repose using given equation

\[
\tan \theta = \frac{h}{r} \quad \text{(1)}
\]

\[
\theta = \tan^{-1} \left( \frac{h}{r} \right) \quad \text{(2)}
\]

Where \( \theta \) is the angle of repose, \( h \) is the height of cone in cm and \( r \) is the radius of the cone base in cm.

**Bulk density (\( e_b \)):**

Bulk density apparatus (Sisco, India) was used to determine bulk density of microspheres. First granules mass (\( m \)) and bulk volume (\( V_b \)) was noted. The given equation was used to calculate bulk density.

\[
e_b = \frac{m}{V_b} \quad \text{(3)}
\]

**Tapped density (\( e_t \)):**

Bulk density apparatus (Sisco, India) was used to determine tapped density of microspheres using standard procedure. First granules mass (\( m \)) and tapped volume (\( V_t \)) was noted. From the given equation 4 tapped density was calculated

\[
e_t = \frac{m}{V_t} \quad \text{(4)}
\]

**Compressibility index (Carr’s index):**

Carr’s index is used to estimate powder flow characteristics. The Carr’s index can be calculated by the following equation 5.

\[
\% \text{Carr’s index (C.I.)} = \frac{e_t - e_b}{e_t} \times 100 \quad \text{(5)}
\]

Where \( e_t \) is the tapped density of granules and \( e_b \) is bulk density of granules

**Hausner’s ratio:**

Hausner’s ratio is another parameter to estimate powder flow characteristics. It is the ratio of tapped density to bulk density. It is shown in equation 6.

\[
\text{Hausner’s ratio (H.R.)} = \frac{e_t}{e_b} \quad \text{(6)}
\]

**Physical characterization**

**Particle Size of microspheres:**

The size distribution in terms of average diameter of microspheres were determined by optical microscope (Unilab RH-62-B, India). The optical microscope was fitted with a calibrated ocular micrometer and a stage micrometer by which the size of the microspheres could be determined. 10 particles of each formulation were studied and the mean of the particles were taken into account.

**Drug content and Entrapment Efficiency (E.E.) (%):**

Microspheres equivalent to 40 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres by the help of mortar and pestle and extracting with aliquots with pH 7 phosphate buffer and repeatedly. The extract was
transferred to a 50 ml volumetric flask and the volume was made up using pH 7 phosphate buffer. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV/Visible spectrophotometer Double Beam, Shimadzu, 1800 Japan) at 247 nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula:

\[
\text{Entrapment Efficiency (E.E.) } (\%) = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100 \ldots \ldots \ldots (7)
\]

**Percentage yield:**

The prepared microspheres were collected and weighed. The measured weight was divided by total amount of all nonvolatile components which were used in the preparation of microspheres.

\[
\% \text{ Yield} = \frac{\text{Total weight of microspheres}}{\text{Total weight of the drug and excipients}} \times 100 \ldots \ldots (8)
\]

**Swelling Index Studies (S.I)**

Swelling index of the all formulations were carried out using BP method. The dynamic swelling property of microspheres depicted in table was determined in the phosphate buffer pH 7. Microspheres of known weight (20 mg) from different batches were added in dissolution medium (phosphate buffer pH 7) for 6 h and the swollen microspheres were collected by a centrifuge and the wet weight of the swollen microspheres was determined by first blotting the particles with filter paper to remove absorbed water on surface and then weighing immediately on an electronic balance. The percentage of swelling of microspheres in the dissolution media was then calculated by using equation given below.

\[
\text{S.I } (\%) = \frac{W_t - W_0}{W_0} \times 100 \ldots \ldots (9)
\]

Where, S.I (\%) = Percentage of swelling of microspheres,

\[W_t = \text{weight of the microspheres at time } t,\]

\[W_0 = \text{initial weight of the microspheres}\]

**In-Vitro drug release study:**

The in-vitro release study of the formulations was carried out using USP II paddle type dissolution test apparatus (Electrolab 8 station). A weighed quantity of the formulation was introduced into the dissolution chamber which was filled with 900 mL of phosphate buffer of pH 7 prepared according to USP specification and the whole system was stirred at 50 rpm and maintained at constant temperature (37 ± 0.5°C). At specific time intervals, 5 ml of the aliquot was withdrawn and replaced by an equal volume of fresh pre-warmed dissolution medium. After suitable dilution, the samples were analyzed at 247 nm using UV-Visible spectrophotometer (Shimadzu 1800 Japan). Dissolution studies were performed three times and the mean values were taken.

**MDT Calculation:**

Mean dissolution time (MDT) value is used to characterize drug release rate from a dosage form and indicates the drug release retarding efficiency of polymer. Mean dissolution time (MDT) values for the release curves of all the formulations were calculated. A mean dissolution time (MDT) for release of drug were calculated using the following expression

\[
\text{MDT}_{\text{in vitro}} = \frac{\sum_{i=1}^{n} T_{\text{mid}} \Delta M}{\sum_{i=1}^{n} \Delta M}
\]

\[ \ldots \ldots (10) \]

Here, \(i\) is the dissolution sample number, \(n\) is the number of dissolution sampling times, \(T_{\text{mid}}\) is the midpoint between times \(T_i\) and \(T_{i-1}\), and \(\Delta M\) is the amount of CA dissolved between times \(T_i\) and \(T_{i-1}\).

**Drug release mechanism and kinetics:**

Different kinetic models (zero-order, first-order, Higuchi’s and Korsmeyer-Peppas) were applied to interpret the release profile (the order and mechanism of drug release) from microsphere system. Based on in-vitro release studies, all data were fitted to various kinetic equations to find out the mechanism of drug release from the drug loaded microspheres.

**Accelerated stability study**

The stability study of the optimized formulation was carried out according to the ICH guideline. The stability studies of the optimized formulation were performed at 25°C/60%RH, room temperature and 45°C/75% RH for 3 month in airtight sealed vials. The studies were performed in the stability chamber for 3 month. At the end of the storage period, the formulation was observed for physical appearance, size, shape, surface morphology, in-vitro drug release to find out whether any significant difference was there or not.

**RESULTS AND DISCUSSION**

**Fourier Transform Infrared Spectroscopy (FTIR) study**

From the FTIR study it was found that there was no interaction between the drug, and polymer Hence drug-excipient mixture revealed that here was no incompatibility in the developed microspheres.

**Micromeritic characterization of Microspheres**

The micromeritic studies lend a hand to estimate the flowability of microsphere after a successful drug entrapment. All the microspheres were evaluated for micrometric properties such as angle of repose bulk density, tapped density, Carr’s index and Hausner’s ratio. All were found to be acceptable limits reported in Table-2. The angle of repose was found in the ranges from 14.03 ±0.63 to 19.65 ±0.54 degrees, bulk density of microspheres

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was found to be in the range of 4.01 ±0.43 to 5.81 ±0.52 gm/ml, tapped density in the range of 4.71 ±0.47 to 6.68 ±0.28 gm/ml, the Carr’s index values were in the range of 4.21 ±0.36 to 15.1 ±0.57 %, and the Hausner’s ratio was in the range between 1.04 ±0.02 to 1.17 ±0.08.

Characterization of microspheres:

All the formulated batches of microspheres were evaluated to find out Particle size, Sphericity, Swelling index, T50% and MDT (80%). It is shown in Table 3a and Table 3b. The drug content was found in the range of 96.5±0.46 to 99.5±0.52, % Yield in the range of 70.3±2.16 to 87.5±1.32, Entrapment Efficiency (%) in the range of 80±1.64 to 100±2.16 (Table 3a), Particle size (µm) range in 375±1.03 to 595±1.85, sphericity in the range 0.78±0.54 to 1.02±0.63, Swelling index (%) in range 112.4±3.47 to 135.2±3.17, T50% in the range 9.4 to 12.7 and MDT in the range 5.65 to 7.84 h (Table 3b).

In vitro dissolution study

The in vitro drug release was carried out in phosphate buffer of pH 7 to found out cumulative drug release (CDR). The % CDR from formulations F1, F2, F3, F4, F5 and F6 at the end of 24 h were found to be 98.8 ± 3.13, 95.2 ± 3.08, 96.1±2.25, 97.4±2.47, 95.8±2.53 and 93.2±2.62% respectively. The in vitro drug release data is given in Table 4 and its graph is shown in Figure 1. Out of all formulations F1 batch showed highest amount of drug release at the end of 24h.

In Vitro drug release kinetic study

From the kinetic it is observed that all the formulations follow non-Fickian transport mechanism and following zero order kinetics. It is shown in table 5. The optimized batch F1 followed zero order kinetics.

Table 2: Micromeritic properties of the microspheres

<table>
<thead>
<tr>
<th>Formulation codes</th>
<th>Angle of repose (degree)* ± SD</th>
<th>Bulk density (gm/ml)* ± SD</th>
<th>Tapped density (gm/ml)* ±SD</th>
<th>Carr’s index(%)*± SD</th>
<th>Hausner’s ratio* ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>14.03 ±0.63</td>
<td>4.70 ±0.35</td>
<td>5.33 ±0.34</td>
<td>11.7 ±1.07</td>
<td>1.13 ±0.04</td>
</tr>
<tr>
<td>F2</td>
<td>18.43 ±0.43</td>
<td>5.01 ±0.15</td>
<td>5.71 ±0.53</td>
<td>12.5 ±1.13</td>
<td>1.14 ±0.01</td>
</tr>
<tr>
<td>F3</td>
<td>15.25 ±0.46</td>
<td>4.01 ±0.43</td>
<td>4.71 ±0.47</td>
<td>15.1 ±0.57</td>
<td>1.17 ±0.08</td>
</tr>
<tr>
<td>F4</td>
<td>17.12 ±0.73</td>
<td>5.81 ±0.52</td>
<td>6.66 ±0.24</td>
<td>11.7 ±0.35</td>
<td>1.13 ±0.06</td>
</tr>
<tr>
<td>F5</td>
<td>19.65 ±0.54</td>
<td>5.71 ±0.45</td>
<td>6.68 ±0.28</td>
<td>14.2 ±0.54</td>
<td>1.17 ±0.03</td>
</tr>
<tr>
<td>F6</td>
<td>18.43 ±0.83</td>
<td>5.71 ±0.23</td>
<td>5.93 ±0.32</td>
<td>4.21 ±0.36</td>
<td>1.04 ±0.02</td>
</tr>
</tbody>
</table>

N.B. * Mean ± SD, n=3

Table 3a: Characterization of microspheres:

<table>
<thead>
<tr>
<th>Formulation codes</th>
<th>Drug content (%)* ± SD</th>
<th>Yield (%)* ± SD</th>
<th>Entrapment Efficiency (%)* ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>99.5±0.52</td>
<td>87.5±1.32</td>
<td>87±1.62</td>
</tr>
<tr>
<td>F2</td>
<td>97.8±0.65</td>
<td>82.5±1.45</td>
<td>83.5±0.97</td>
</tr>
<tr>
<td>F3</td>
<td>97.7±0.73</td>
<td>85.4±2.13</td>
<td>80±1.64</td>
</tr>
<tr>
<td>F4</td>
<td>98.4±1.02</td>
<td>79.1±2.34</td>
<td>81.6±1.29</td>
</tr>
<tr>
<td>F5</td>
<td>96.5±0.46</td>
<td>70.3±2.16</td>
<td>85.7±0.91</td>
</tr>
<tr>
<td>F6</td>
<td>96.7±0.41</td>
<td>82.1±1.98</td>
<td>83.5±1.27</td>
</tr>
</tbody>
</table>

* Mean ± SD, n=3

Table 3b: Characterization of microspheres

<table>
<thead>
<tr>
<th>Formulation codes</th>
<th>Particle size (µm)# ± SD</th>
<th>Sphericity* ± SD</th>
<th>Swelling index (%)*± SD</th>
<th>T50% (h)</th>
<th>MDT (80%) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>595±1.85</td>
<td>0.78±0.54</td>
<td>112.4±3.47</td>
<td>9.4</td>
<td>5.65</td>
</tr>
<tr>
<td>F2</td>
<td>477±0.97</td>
<td>0.82±0.26</td>
<td>125.2±3.17</td>
<td>10.2</td>
<td>5.94</td>
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<tr>
<td>F3</td>
<td>410±1.08</td>
<td>0.88±0.72</td>
<td>132.6±2.74</td>
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<td>6.28</td>
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<tr>
<td>F4</td>
<td>462±1.54</td>
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<td>119.4±2.79</td>
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<td>6.72</td>
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<td>F5</td>
<td>375±1.03</td>
<td>1.02±0.63</td>
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<td>12.6</td>
<td>7.37</td>
</tr>
<tr>
<td>F6</td>
<td>538±2.86</td>
<td>0.97±0.78</td>
<td>135.2±3.17</td>
<td>12.7</td>
<td>7.84</td>
</tr>
</tbody>
</table>

N.B. *Mean ± SD, n=3, # Mean ± SD, n=10
Table 4: % CDR of Simvastatin of different batches

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>F1 ± S.D</th>
<th>F2 ± S.D</th>
<th>F3 ± S.D</th>
<th>F4 ± S.D</th>
<th>F5 ± S.D</th>
<th>F6 ± S.D</th>
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</thead>
<tbody>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>1</td>
<td>7.6±0.09</td>
<td>7.2±0.13</td>
<td>6.4±0.11</td>
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<td>4.3±0.02</td>
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<td>2</td>
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<td>12.1±0.56</td>
<td>10.7±0.64</td>
<td>9.5±0.57</td>
<td>8.3±0.42</td>
<td>7.8±0.53</td>
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<td>22.6±1.14</td>
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<td>19.1±1.52</td>
<td>17.2±1.64</td>
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<td>12</td>
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<td>48.2±1.33</td>
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<td>18</td>
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<td>80.1±1.84</td>
<td>79.7±1.84</td>
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<td>95.2±3.08</td>
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<td>97.4±2.47</td>
<td>95.8±2.53</td>
<td>93.2±2.62</td>
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</table>

N.B. Mean ± SD, n=3

Figure 1: %CDR profile of batches F1-F6 of Simvastatin microspheres

Table 5: The drug released kinetic parameters of all formulations

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Correlation Coefficients (R²)</th>
<th>Kores Meyer- Peppas</th>
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<tr>
<td></td>
<td>Zero-order</td>
<td>First-order</td>
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<td>F1</td>
<td>0.9933</td>
<td>0.9351</td>
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<td>0.9951</td>
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<td>0.9336</td>
</tr>
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<td>F5</td>
<td>0.9998</td>
<td>0.9413</td>
</tr>
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<td>F6</td>
<td>0.9995</td>
<td>0.9581</td>
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</tbody>
</table>
Stability studies

From short term stability studies of optimized formulation F1, showed that there was no significance change in, drug content and % CDR. Hence the formulation was found to stable in storage condition.

CONCLUSION

Ionic orifice gelation method was found to be one of the good methods for the preparation of microspheres. Sodium alginate and HPMC K15M were found to be good polymer for sustaining drug from microspheres. All characteristic parameters of the optimized formulation were found to be in good acceptable range. Stability study of the optimized product for 3 months indicated that the product is stable.

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