RESEARCH ARTICLE......!!!

Received: 23-07-2013; Revised; Accepted: 21-04-2014

DESIGN AND EVALUATION OF SOLID SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS (S-SNEDDS) FOR NEBIVOLOL HYDROCHLORIDE

Rahul S. Narkhede, Kishor N. Gujar, Vaishali M. Gambhire*

Department of Pharmaceutics, Sinhgad College of Pharmacy, Pune-411 041.

Keywords:
Nebivolol Hydrochloride, Freeze Drying, Solid-SNEDDS

For Correspondence:
Vaishali M. Gambhire
Department of Pharmaceutics, Sinhgad College of Pharmacy, Pune-411 041
E-mail: vaishaligambhire@gmail.com

ABSTRACT

Nebivolol hydrochloride (NEB) is third generation beta-blocker, approved by the FDA to treat a hypertension. It’s a racemic mixture of a d-Nebivolol and l-Nebivolol. Oral delivery of the NEB shows a lower bioavailability due to its poor solubility and permeability. In a present study, solid self nanoemulsifying drug delivery (S-SNEDDS) is formulated to increase the bioavailability of drug by increasing solubility and permeability through the gastro intestinal membrane. The S-SNEDDS get formulated using Optimized SNEDDS formulation with the different adsorbent D-mannitol, Trehalose, and Lactose. Further, the effect of Dilution on the globule size, globule size and PDI, differential scanning calorimetry, Fourier transformed infrared spectroscopy of S-SNEDDS was studied. In-vitro drug release study was performed using dialysis bag method. Ex-vivo drug release studies were also carried out to determine the permeability of S-SNEDDS through the stomach and intestinal membrane. The best of adsorbent determined D-mannitol with a globule size of 156.34 nm. In-vitro drug release study and ex-vivo permeation study showed significant increase in dissolution rate and permeability respectively, as compared to the drug suspension and marketed preparation (NEBISTAR™).
INTRODUCTION
Approximately 40% of new drug candidates have poor water solubility and oral delivery of such drugs is frequently associated with implications of low bioavailability, high intra- and inter subject variability, and lack of dose proportionality. To increase the solubility and bioavailability of such drug molecule various approaches used are pH adjustment, CoSolvency, Particle Size Reduction, Solid dispersion, Hydrotrophy, Micellar Solubilization, Complexation, lipid based formulations. Among these commonly used approach to improve the oral bioavailability of poorly water soluble compounds, lipid-based formulations such as incorporation of drug into oils and in these self-nanoemulsifying formulations are mostly preferred. Self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of drug, lipids and surfactants, usually with one or more hydrophilic co-solvents or co-emulsifiers that form fine oil in water Nanoemulsions upon mild agitation in an aqueous medium with a droplet sizes ranging 20–200 nm. The digestive motility of the stomach and intestine providing the agitation required for self-emulsification in vivo. In SNEDDS, surfactant enhance permeation across the intestinal membrane, reduce or eliminate food effect and enhance drug bioavailability, also reduce a hepatic clearance of the drug, in addition to increasing its solubility. For a suitable self-emulsifying vehicle in formulation, it is important to assess droplet size distribution following self-emulsification. After this it is important to design adequate dosage form for the administration of these lipid systems, because liquids produce some disadvantages; the large quantity of surfactants in the formulations can induce gastrointestinal irritation. To solve this problem the various alternatives are used like hard gelatine capsules, pellets, tablets, freeze drying. Nebivolol, a third-generation beta blocker, is approved by the FDA for the treatment of hypertension. There is a great difference in bioavailability of drug in extensive metabolizers (12%) and for poor metabolizers (96%) Nebivolol shows the lower aqueous solubility it gets responsible for the lower bioavailability. The bioavailability can be increased by increasing its solubility and reducing first pass metabolism. The result gets reported for lercanidipine, carvediol, Adefovir dipivoxil, tamoxifine citrate, ibuprofen etc. like potential to enhancement in absorption of drug, increase in the bioavailability, potential to improve the oral bioavailability and thus the therapeutic efficacy, useful for controlling the release rate of poorly water soluble drugs. In order to increase the aqueous solubility of drug which may further result in bioavailability enhancement, the present study is aimed to develop S-SNEDDS of nebivolol.
MATERIAL AND METHOD

1) Chemicals and Reagents
Nebivolol hydrochloride was obtained from Torrent Pharmaceuticals, Gujarat, India. Capmul MCM EP was obtained as gifts from Abitech Co. (USA), diethylene glycol monoethyl ether (Transcutol HP®) were kindly donated by Gattefosse Co. (Canada). polyethylene glycol (PEG)-400, D-mannitol, trehalose and lactose were purchased from Loba Chemie Pvt. Ltd., Mumbai (India). All other chemicals used were of analytical grade.

2) Preparation of Solid Self Nano-Emulsifying Drug Delivery System (S-SNEDDS)17
The liquid NEB SNEDDS having composition Capmul MCM EP 25%, Tween-60 50%, PEG-400 12.5% and Transcutol P 12.5% loaded with NEB (38.596 mg/5ml) were transformed into solid self-nanoemulsifying drug delivery system (S-SNEDDS) by freeze drying. 15 % solution of adsorbent/ cryoprotectant in distilled water was used as the aqueous phase for reconstituting the optimized drug loaded liquid SNEDDS. Formulation S-1, S-2, S-3 get formulated using D-mannitol, trehalose, lactose solution respectively as reconstituting media. Then resulting nanoemulsion was frozen to −70 °C in a deep freezer for 24 h and then lyophilized under negative displacement pressure for 24 h to obtain S-SNEDDS of NEB.

3) FORMULATION OF S-SNEDDS:-
The S-SNEDDS formulated using the different adsorbents D-mannitol, trehalose, lactose using freeze drying method. Optimization of the S-SNEDDS formulations (S1, S2 and S3) was done based on the result obtained from the following tests.

4) CHARACTERIZATION OF S-SNEDDS
Percent Transmittance17
The NEB SNEDDS were reconstituted with double distilled water and the resulting nanoemulsion was observed visually for any turbidity. Thereafter, its percent transmittance was measured at 638.2 nm using UV–Vis spectrophotometer (UV-1800 Shimadzu, Japan) against double distilled water as the blank. The studies were conducted at 50, 100, 250 and 1000 times dilution.

Measurement of Mean Globule Size and PDI20
The globule size and zeta potential of the reconstituted NEB SNEDDS were determined using Malvern Zetasizer (Nano ZS 90, UK). The samples were put in ‘folded capillary cells’ and results obtained for size, PDI were recorded.
Robustness to Dilution\textsuperscript{21}

Five formulations of NEB SNEDDS were diluted with 0.1 N HCl and phosphate buffer pH 6.8 and the % transmittance of the resultant nanoemulsion were measured at 638.2 nm by using UV-spectrophotometer.

Differential Scanning Calorimetry (DSC)\textsuperscript{22}

The possibility of any interaction between the drugs and the carriers using different approaches was assessed by carrying out thermal analysis of drug as well as the optimized formulation, using DSC. DSC analysis was performed (using Shimadzu-Thermal Analyzer DSC 60, Kyoto, Japan) on 1 to 5 mg samples. Samples were heated in an open aluminum pan at a rate of 10 °C/min conducted over a temperature range of 30 to 350 °C under a nitrogen flow of 50 ml/min.

Fourier-Transform Infrared Spectroscopy (FT-IR)\textsuperscript{22}

The infrared spectrum of NEB sample was recorded by Perkin-Elmer Spectrum BX-200 FTIR Spectrometer, equipped with a DTGS detector. The Sample was prepared by the KBr disc method (~2 mg sample in 100 mg KBr) and examined in transmission mode. Each spectrum was measured over a frequency range of 4000-400 cm\textsuperscript{-1}. The software used in the data analysis was Perkin-Elmer 3.02. The peaks obtained in the spectra were then compared with corresponding functional groups in the structure of NEB.

Drug Content Determination

Liquid-SNEDDS formulation equivalent to 5 mg was accurately weighed and dissolved in 100 ml of methanol. Further suitably diluted with methanol and NEB dissolved was quantified using RP-HPLC (LC 2010 C HT, Shimadzu. Japan) at 280 nm. Blank was prepared by dissolving blank SNEDDS in methanol with same dilution as for the samples.

Drug Release Studies

A) In-Vitro Studies\textsuperscript{23}

SNEDDS of NEB was filled in size ‘0’ hard gelatin capsules. In-vitro release profile of SNEDDS was studied using USPXXIII apparatus I at 37±0.5 °C with a rotating speed of 100 rpm in dissolution media namely, pH1.2 and 6.8 buffers so as to evaluate the effect of pH on in-vitro dissolution. During the study, 10 ml of the aliquots was removed at predetermined time intervals (10, 20, 30 and 45 min) from the dissolution medium and replaced with fresh buffer. The amount of NEB released in the dissolution medium was determined by UV spectrophotometrically at λ\textsubscript{max} 284nm.
B) In-Vitro Diffusion Profile by Dialysis Bag Method

In vitro release of NEB SNEDDS was carried on (Electrolab TDT-08 L Mumbai) by dialysis method. After NEB SNEDDS was instilled into the dialysis bag (MWCO 10000), the dialysis bag was firmly sealed and was placed in 250 ml, pH 1.2 and pH 6.8 buffer (containing 0.5% of Tween 80) as the dissolution medium at 37 °C. The revolution speed of the paddle was maintained at a rate of 100 rpm. Aliquots were withdrawn from the flask at periodic time intervals, replaced with equivalent amounts of fresh media and analyzed spectrophotometrically at λmax 284 nm.

C) Ex-Vivo Release Profile

Ex-vivo drug release was studied using, sacrificed Male Sprague-Dawley rat stomach and small intestine, were isolated and thoroughly washed with phosphate buffer saline (PBS) to remove the mucous and lumen contents. NEB SNEDDS diluted separately with 0.1 N HCl and phosphate buffer pH 6.8 were filled in the stomach and intestine respectively. An equivalent amount of plain NEB suspensions in HCl buffer and phosphate buffer respectively were used for comparison. Both the ends of the tissues were tied properly to avoid any leakage and were put into beakers containing 40 ml of phosphate-buffered pH 7.4 as the acceptor phase with the continuous aeration supply under gentle stirring at 37±2 °C. Samples were withdrawn from the acceptor phase at periodic time intervals and subjected to spectrophotometric analysis. All the experiments were performed in triplicate.

Stability Studies

The SNEDDS formulations were filled into empty hard gelatine capsules (size 0) and subjected to stability studies at 25 °C/ 60 % relative humidity (RH) and 40±2 °C/ 75±5% RH. Samples were charged in stability chambers (Thermolab, TH 200S, Mumbai) with humidity and temperature control. They were withdrawn at specified intervals for analysis over a period of 3 months. Drug content of the capsules was analyzed using a previously developed and validated stability-indicating RP-HPLC method.

RESULT AND DISCUSSION

Percent transmittance

The percentage transmittance is directly related to the droplet size of the emulsion, to study the effect of dilution on the droplet size the percent transmittance studies were conducted after 50, 100, 250 and 1000 times dilution. The result of this study is given in a Table 1.

Measurement of Mean Globule Size

The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption.
From Table 2 it can be said that, as the adsorbent shows the surfactant concentration increases, globule size decreases. The globule size distribution and polydispersity index revealed that, amongst all, the S-1 formulation shows the closer globule size distribution and also produces the finest emulsion.

**Fourier-Transform Infrared Spectroscopy (FT-IR)**

Significant vibrations detected in the spectrum of D-mannitol (Figure 1) is the OH stretching at 3495.30 cm$^{-1}$, C–H stretching at 2914.04 cm$^{-1}$, C=O stretching at 1738.50 cm$^{-1}$, and the C–O stretching at 1114.38 cm$^{-1}$. The spectrum of D-mannitol S-SNEDDS (Figure 1) appears to be the summation of those of drug and carrier. In the spectrum of D-mannitol fewer peaks of the drug were observed indicating a greater degree of trapping of NEB inside the D-mannitol matrix. In addition, relatively broad peaks were observed possibly due to the partially amorphous nature of the dispersion. An absorption band in the region 2909 cm$^{-1}$ appears in the spectrum is probably due to combination C–H stretching. The absence of any other new peaks in the solid dispersions indicates that the NEB has not undergone any polymorphic change during its preparation and that no well-defined chemical interactions have occurred.

Important vibrations detected in the spectrum of trehalose (Figure 1) are the C–N stretching at 1376.24 cm$^{-1}$, C–H stretching at 2961.83 cm$^{-1}$ and the CH$_2$ stretching at 2883.40 cm$^{-1}$. The spectra of trehalose (Figure 1) can be simply regarded as the superposition of NEB. Slight difference was seen in the position of the absorption bands of trehalose whereas the minor peaks due to NEB were absent indicating NEB was dissolved inside the trehalose matrix. However, the major characteristic peaks for NEB were still present. Moreover, the spectrum for NEB and trehalose S-SNEDDS showed no peaks other than those assigned to NEB and trehalose which indicates the absence of any well-defined chemical interactions.

Important vibrations detected in the spectrum of lactose (Figure 1) are the OH stretching at 3423.67 cm$^{-1}$, C–H stretching at 2883.83 cm$^{-1}$ and the C–O–C stretching (ether) at 1108.40 cm$^{-1}$. The spectra of lactose (Figure 1) S-SNEDDS can be simply regarded as the superposition of the NEB. Slight difference was seen in the position of the absorption bands of lactose whereas the minor peaks due to NEB were absent indicating trapping of NEB inside the lactose matrix. However, the major characteristic peaks for NEB were still present. Moreover, the spectrum for lactose S-SNEDDS showed no peaks other than those assigned to NEB and lactose which indicates the absence of any well-defined chemical interactions.
**Differential Scanning Calorimetry (DSC)**

The DSC curve of NEB profiles a sharp endothermic peak at 233.3°C corresponding to its melting, and indicating its crystalline nature. The thermograms of neat lactose and trehalose exhibited a sharp endothermic peaks corresponding to their melting points at 233.3 °C and 97.4 °C respectively.

A complete disappearance of the NEB melting peak was observed in D-mannitol S-SNEDDS (Figure 2) which is attributable to the strong interaction of drug with the melted carrier. No considerable broadening of peaks was observed in trehalose S-SNEDDS but, a substantial decrease in peak intensities of the drug was evident. The less intense peaks observed in the S-SNEDDS is a result of the decrease in the heat of fusion of these dispersions as compared to the carrier and the drug alone. This suggests that a physical interaction between the drug and carrier is taking place. This decrease in the heat of fusion indicates a reduction in crystallinity of the drug and carrier which also means that the prepared S-SNEDDS had achieved at least some degree of amorphosity.

A complete disappearance of the drug melting peak was observed in lactose S-SNEDDS (Figure 2) which is attributable to the dissolution of drug in the melted carrier before reaching its fusion temperature whereas one endothermic peak at temperatures slightly lower than that of the lactose fusion was observed which may be attributed to the fusion of an eutectic mixture between NEB and lactose.

The thermogram of lactose exhibited sharp endothermal peak corresponding to its melting points at 223.4 °C. No considerable peaks were observed in lactose S-SNEDDS (Figure 2) but, a substantial decrease in peak intensities of the drug as well as that of the respective carrier was evident. This suggests that an interaction between the drug and carrier is taking place which is not a chemical but physical interaction. The less intense peaks observed in the solid-snedds is a result of the decrease in the heat of fusion of these S-SNEDDS as compared to the carrier and the drug alone. Apart from this, no changes were observed in any of the optimized formulations. The results from all the above-depicted thermograms were in accordance with those obtained from FT-IR studies.

**Drug content determination of S-SNEDDS**

The drug content determination of S-SNEDDS formulations carried out by HPLC was within the acceptable limits for all formulations as shown in Table 2.
Drug release studies
A) In-Vitro Studies drug release studies: the dissolution profile of NEB from S-SNEDDS was slower than that from L-SNEDDS and was affected by the pH of the dissolution medium (Figure 3). This may be because the drug remained solubilized in the S-SNEDDS but was in a solid state in the S-SNEDDS. Complete release was achieved within 120 min in 0.1 N HCl, while only 85% drug was released in phosphate buffer pH 6.8 in 240 min. This slower rate of dissolution in pH 6.8 may be because of the lower solubility of the NEB at higher pH. Although pH dependency was observed in drug release from S-SNEDDS, it may not affect its in-vivo dissolution as complete release of the drug was found within 2 h in 0.1 N HCl medium, which is the average gastric residence time.

B) In-Vitro Diffusion Profile by Dialysis Bag Method: In vitro dissolution profile of NEB S-SNEDDS in 0.1 N HCl and phosphate buffer pH 6.8 is shown in Figure 4. More than 55% drug was released from S-SNEDDS within 120 min. It was also evident that the release profile of NEB from S-SNEDDS was unaffected by the pH of dissolution medium, indicating the elimination of pH effect. The S-SNEDDS formulation using D-mannitol as adsorbent shows the highest drug release among the all S-SNEDDS formulation, this effect due to the cryoprotectant effect of D-mannitol.

C) Ex-vivo drug release study: The cumulative percent release drug from rat stomach and intestine (Figure 5) of different S-SNEDDS formulation, marketed preparation and pure drug. It was observed that the release of the drug was enhanced from the reconstituted S-SNEDDS formulation as D-mannitol adsorbent, as 95.26 % drug was released within 120 min for S-SNEDDS in comparison to 44% drug release in 120 min from plain drug suspension and 50% drug release in 120 min form marketed preparation. It can be noted that absorption of the drug from the intestine can be enhanced with S-SNEDDS using mannitol as adsorbent compare to other S-SNEDDS formulation, fulfilling our objective of increasing intestinal absorption for enhancing the bioavailability of NEB.

Stability Studies
The developed formulations were found to be physically and chemically stable for 3 months at 30±2 °C/ 65±5 %RH and 40±2 °C/ 75±5 % RH. No change in the physical appearance of S-SNEDDS. The Z-average size was similar at both the storage conditions for NEB S-SNEDDS. Also, no significant decrease in the NEB content was observed indicating that NEB remained chemically stable in the S-SNEDDS. Thus, it can be concluded that the NEB SNEEDDS would remain physicochemically stable at long term stability conditions (2-8 °C), (30±2 °C/ 65±5 %RH) and (40±2 °C/75±5% RH) for 3 months.
CONCLUSION
The present study has clearly showed the potential utilization of S-SNEDDS for formulating NEB with improved aqueous solubility, stability and in-vitro drug release. The S-SNEDDS with relatively high cryoprotectant effect was prepared which self-emulsified easily with mean emulsion droplet size of 156.34 nm. Stability study and cloud point study confirmed that the S-SNEDDS had no dilution effect and was stable at 0.1 N HCl and phosphate buffer 6.8 without any change in emulsion droplet size.

ACKNOWLEDGEMENT
The authors would like to thank M/s Torrent Pharmaceutical, Gujarat, India for providing gift sample of nebivolol hydrochloride. M/s Abitec Corporation USA and M/s Gattefosse Canada are acknowledged for providing the gift samples of surfactants and oils used in the study.

REFERENCES


**FIGURE NO. 1**

![Figure 1: FT-IR Spectra of Pure NEB and Three Different Adsorbents (D-Mannitol, Trehalose, Lactose), Freeze Dried S-SNEDDS Formulation by Using an Adsorbent D-Mannitol, Trehalose, Lactose.](image-url)
FIGURE NO.: -2

Figure 2: DSC Thermogram of NEB S-SNEDDS and different adsorbents. (A: NEB, B: D-mannitol, C: S-1, D: Trehalose, E: S-2, E: Lactose, F: S-3)

FIGURE NO.: -3

Figure 3: In-vitro drug release of different S-SNEDDS formulation using a) 0.1 N HCl as a dissolution media b) phosphate buffer pH 6.8 as a dissolution media. (PD: Pure Drug, MT: Marketed Formulation)
Figure 4: In-Vitro Diffusion Profile by Dialysis Bag Method of different S-SNEDDS formulation using a) 0.1 N HCl as a dissolution media b) phosphate buffer pH 6.8 as a dilution media. (PD: Pure Drug, MT: Marketed Formulation)

Figure 5: Ex-vivo drug release studies (a) from rat stomach of reconstituted S-SNEDDS, (b) from rat intestine of reconstituted S-SNEDDS (PD: Pure drug, MT: Marketed Preparation).
Table 1: Effect of dilution on different S-SNEDDS formulations using double distilled water as a dilution medium

<table>
<thead>
<tr>
<th>Dilution</th>
<th>% Transmittance at 638.2 nm*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-1</td>
</tr>
<tr>
<td>50 Times</td>
<td>78.25±0.0325</td>
</tr>
<tr>
<td>100 Times</td>
<td>88.68±0.0215</td>
</tr>
<tr>
<td>250 Times</td>
<td>94.80±0.0145</td>
</tr>
<tr>
<td>1000 Times</td>
<td>97.87±0.0596</td>
</tr>
</tbody>
</table>

* = expressed as a mean ±SD (n=3)

Table 2: Globule Size Distribution and PDI of S-SNEDDS

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>S-SNEDDS</th>
<th>Z-average diameter (nm)*</th>
<th>PDI*</th>
<th>% Drug Content*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S-1</td>
<td>156.34±0.175</td>
<td>0.117±0.0125</td>
<td>99.73±0.116</td>
</tr>
<tr>
<td>2</td>
<td>S-2</td>
<td>173.64±0.158</td>
<td>0.229±0.0119</td>
<td>99.14±0.214</td>
</tr>
<tr>
<td>3</td>
<td>S-3</td>
<td>167.43±0.127</td>
<td>0.224±0.0201</td>
<td>99.25±0.124</td>
</tr>
</tbody>
</table>

* = expressed as a mean ±SD (n=3)

Table 3: Stability Studies of S-SNEDDS

<table>
<thead>
<tr>
<th>Days</th>
<th>Temperature condition</th>
<th>Globule size*</th>
<th>PDI*</th>
<th>% Transmittance*</th>
<th>Drug content*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2-8 °C</td>
<td>156.54±0.197</td>
<td>0.125±0.0137</td>
<td>99.88±0.11</td>
<td>99.73±0.025</td>
</tr>
<tr>
<td></td>
<td>30±2 °C/ 65±5 % RH</td>
<td>156.54±0.197</td>
<td>0.125±0.0137</td>
<td>99.88±0.11</td>
<td>99.73±0.025</td>
</tr>
<tr>
<td></td>
<td>40±2 °C/ 75±5 % RH</td>
<td>156.54±0.197</td>
<td>0.125±0.0137</td>
<td>99.88±0.11</td>
<td>99.73±0.025</td>
</tr>
<tr>
<td>30</td>
<td>2-8 °C</td>
<td>156.08±0.041</td>
<td>0.141±0.0176</td>
<td>99.81±0.14</td>
<td>99.54±0.014</td>
</tr>
<tr>
<td></td>
<td>30±2 °C/ 65±5 % RH</td>
<td>155.87±0.154</td>
<td>0.164±0.0137</td>
<td>99.79±0.08</td>
<td>99.27±0.007</td>
</tr>
<tr>
<td></td>
<td>40±2 °C/ 75±5 % RH</td>
<td>156.57±0.208</td>
<td>0.178±0.0112</td>
<td>99.76±0.24</td>
<td>99.14±0.011</td>
</tr>
<tr>
<td>60</td>
<td>2-8 °C</td>
<td>156.12±0.107</td>
<td>0.149±0.0184</td>
<td>99.82±0.17</td>
<td>99.38±0.009</td>
</tr>
<tr>
<td></td>
<td>30±2 °C/ 65±5 % RH</td>
<td>155.94±0.039</td>
<td>0.186±0.0125</td>
<td>99.88±0.09</td>
<td>99.06±0.017</td>
</tr>
<tr>
<td></td>
<td>40±2 °C/ 75±5 % RH</td>
<td>156.74±0.315</td>
<td>0.198±0.0125</td>
<td>99.71±0.12</td>
<td>98.92±0.019</td>
</tr>
<tr>
<td>90</td>
<td>2-8 °C</td>
<td>156.22±0.133</td>
<td>0.151±0.0201</td>
<td>99.79±0.13</td>
<td>99.31±0.012</td>
</tr>
<tr>
<td></td>
<td>30±2 °C/ 65±5 % RH</td>
<td>156.41±0.011</td>
<td>0.208±0.0095</td>
<td>99.84±0.11</td>
<td>98.88±0.013</td>
</tr>
<tr>
<td></td>
<td>40±2 °C/ 75±5 % RH</td>
<td>156.94±0.177</td>
<td>0.217±0.0175</td>
<td>99.68±0.11</td>
<td>98.77±0.004</td>
</tr>
</tbody>
</table>

* = expressed as a mean ±SD (n=3)