Some Variable Affecting Formulation of Tinidazole Mucoadhesive Oral Gel

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Abstract:

Tinidazole (TZ) is an antibacterial drug used for treatment of periodontitis. More benefit may be obtained by the application of a localized oral drug delivery system consist of mucoadhesive polymers which increase the contact time between the base and the oral tissue. Different gel formulations were prepared using the bioadhesive polymers carbomer 941, sodium carboxymethylcellulose (SCMC), and guar gum. The influence of polymer type and polymers blend in varying ratio on the viscosity, bioadhesive strength, swelling index and drug release were evaluated. SCMC based gel showed fastest release in comparison with carbomer and guar gum based gel. Using polymer blend of SCMC with either carbomer or guar gum resulted in a modification of both release and physical properties. The release of TZ was decreased with increasing amount of carbomer and guar gum and decreasing amount of SCMC. And increasing the viscosity of the gel formulations resulted in a retardation effect on the release of the drug. The study also showed that...
formulas containing carbomer exhibited maximum swelling values with lower release rates and best mucoadhesion.

**Keyword:** tinidazole, mucoadhesive oral gel, carbomer, sodium carboxymethylcellulose, guar gum.

**Introduction:**

Bacterial plaque is believed to be the main etiological agent of periodontal disease. The sub gingival micro flora associated with destructive periodontitis is predominantly Gram-negative and anaerobic [1]. Large doses of systemic antibiotics must be taken in order to achieve sufficient concentration in the gingival crevicular fluid of the periodontal pocket; this may raises a number of issues, like bacterial resistance to administered antibiotics and unpleasant or toxic side effects [2]. The lack of drug retention in the periodontal pocket is probably the chief reason for these mixed results. The attractiveness of treating periodontal disease using the sustained release of antimicrobial agent is based on maintaining effectively high level in the gingival crevicular fluid [3].

Local delivery in the oral cavity had particular application in the treatment of tooth ache, periodontal diseases and bacterial infection [4]. Of all topical formulations available, gel bases which is likely to stay in the mucosal surface seems to be the most suitable vehicle for drug delivery to the oral cavity tissue.

To increase the adherence between the bases and the oral tissues, polymers with bioadhesive properties are selected as gelling agent [5], [6]. Mucoadhesive polymers of natural, semisynthetic or synthetic origin are able to form hydro gel which swell in presence of water and physically entrap drug molecule for subsequent slow release by diffusion or erosion. Among bioadhesive polymer, poly (acrylic acid)-based polymers like carbopol and polycarbophil, cellulose derivatives like sodium carboxymethylcellulose, hydroxy propylmethyl cellulose and methyl cellulose and natural gum like xanthan gum and guar gum [4] Several buccal devices were formulated with tinidazole which is close analogue to metronidazole for treatment of periodentitis like tinidazole dental implant and tinidazole stilus [7,8].

In the present work mucoadhesive gels of tinidazole that adhere with gums for a prolonged period of time were prepared. The mucoadhesive gels were prepared by using hydrophilic polymers (carbopol-941, guar gum and sodium carboxymethylcellulose). The effect of type and polymer ratio on the mucoadhesivness and release of tinidazole were studied in addition to evaluating the swelling index and the rheological behavior of the prepared gels.

**Material and Method:**

Tinidazole (TZ) ( Sigma Chemical Co), Carbomer 941 (Goodrich, USA), Sodiumcarboxymethylcellulose (SCMC) (BDH chemical, Ltd, Pool, England), Guar gum, Methylparaben (MP) and Propylp-araben (PP) (Samra Drug
Industries), Triethanolamine (TEA) (Hopkins and Williams, England), Mannitol (E. Merck, Darmstadt).

**Preparation of Gel:**

1- **Preparation of single polymer gel:**

0.2% w/w MP, 0.02% w/w PP and 2% w/w mannitol were dissolved in distilled water, 4% w/w of each of carbomer, guar gum and SCMC powder were added slowly to the previous solution under continuous stirring at 50 rpm. A (0.5) ml of TEA was added to carbomer dispersion with continuous stirring till transparent clear gel was formed. The resultant gel masses were left over night at room temperature for complete swelling. Part of the prepared gel was added to 1% of the powdered drug with gentle stirring to produce a smooth layer of the gel. The rest of the gel was added gradually portion by portion with continuous gentle stirring to avoid air entrapment till a homogenous dispersion was obtained [9,10].

2- **Preparation of combination polymer gel:**

Different gel formulations were prepared with various ratios of carbomer to SCMC and guar gum to SCMC of 3:1, 1:1 and 1:3 by the same method mentioned previously. Different formulations of TZ mucoadhesive oral gel are given in (table-1).

**pH determination:**

Accurately 2.5 gram of gel was weighed and dispersed in 25 ml of water and then the pH was measured [11].

**Physical examination:**

The prepared gel formulations were inspected visually for color, homogeneity and consistency.

**Drug content analysis:**

A modified assay method was adopted to determine the drug content of the prepared gel.

1 gram gel was accurately weighed and dissolved in 25 ml methanol in tightly closed volumetric flask. The closed flask was shaken for 10 minute, then the mixture was filtered. The volume of filtrate was made up to 50 ml with methanol. One ml of the above solution was further diluted to 25 ml with methanol. The total TZ content was determine by comparing the U.V. absorbance of the resultant solution at a wave length of 310 nm to the standard curve of TZ in methanol [12].

**Rheological study:**

Gel viscosity measurement was evaluated at 25°C using rotation viscometer. The samples were sheared with spindle R7 by applying increasing value of shear rate over the range of speed setting from 1.5 to 12 rpm. The sample was allowed to settle for 5 minutes prior to taken the reading, then in a descending order [13].
Determination of the mucoadhesive force:

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from buccal mucosal tissue using modified physical balance method\textsuperscript{[14]}. A section of buccal mucosa was cut from the sheep buccal cavity and instantly fixed with the mucosal side out, on to glass vial using a rubber band. The diameter of each exposed mucosal membrane was (1.8) cm. The vial with buccal tissue were stored at 37°C for 10 minutes. Another vial with mucosal tissue was connected to the left side of two-arm balance and the stored vial was fixed on a height adjustable-pan. To the exposed tissue on this vial, a constant amount of 0.1 gram gel was applied. The height of the vial was adjusted so that the gel could adhere to the mucosal tissue of both vial. A force of 0.1 N was applied for 2 minutes to ensure intimate contact between the tissue and the sample. After removal of preload force, water was added slowly to previously weighed beaker placed on the right hand pan until vial get detach. The bioadhesive force expressed as the detachment stress in dyne/cm\textsuperscript{2}, was determined from the minimal weight that detach the tissue from the surface of each formulation using the following equation\textsuperscript{[15,16]}.

\textbf{Detachment stress (dyne/cm\textsuperscript{2})} = \frac{m \times g}{A} \ldots \text{eq (1)}

Where:
m: the weight added to the balance in gram
\(g\): acceleration due to gravity taken as 980 cm/sec\textsuperscript{2}
\(A\): area of tissue exposed

Swelling index study:

In this study, 1 gram sample was put into a stainless steel basket with 200 mesh of aperture, and weighed. The basket was then placed in 100 ml distilled water, allowing the gel to swell at 25°C for 6 hours. The basket was periodically weighed after removing the excess water on the surface with filter paper:

\[\frac{[W_t-W_o]}{W_o} \times 100\ldots \text{eq (2)}\]

Swelling \% = \frac{[W_t-W_o]}{W_o}

Where \(W_t\) is the weight of basket at time \(t\) and \(W_o\) is the initial weight of the basket\textsuperscript{[17]}.

In vitro study of drug release:

The release study was carried out with USP dissolution apparatus type I at 50 rpm and 100 ml phosphate buffer pH6.8 maintained at 37°C. The apparatus was slightly modified to overcome the small volume of the dissolution medium using a suitable glass beaker inside the dissolution flask. A basket of 2.5cm in diameter was enclosed with multifold filter paper filled with 1 gm of TZ gel, immersed to about 1 cm of its surface in the dissolution medium\textsuperscript{[18]}. Samples collected(1ml) at 15, 30, 45 minutes 1, 2, 3, 4, 5 and 6 hours and replaced immediately with the same volume of dissolution medium. The samples following suitably dilution were assayed spectrophotometrically at 320nm\textsuperscript{[12]}.
Stability study:
The selected formula was stored in well-sealed glass vials for a period of 4 months at 40°C, 50°C and at 4°C. at predetermined intervals, samples were collected and drug content was analysed to predict the expiration date. The physical properties were also evaluated.

Results and Discussion:
Physicochemical properties:
The physicochemical properties of the prepared formulation are shown in (table-1). It is clearly evident that all the gel formulations are homogenous, smooth with acceptable consistency. The physical appearance of the prepared gel was transparent or opaque in nature with pH range of 6.4-6.93 which lies in pH range of the oral mucosa which is reported to be between 6.2-7.4. Furthermore, the three buffer systems of the salivary system are able to maintain a non-harmful pH (6.0-7.5) in the oral cavity.[19]. Thus all the formulation considered to be not acidic, so it may not cause any damage to the hard and soft oral tissue.

Rheological study:
All the gel formulation demonstrated pseudoplastic flow with thixotropy. The flow curve of formula F6 is shown in (figure-1) as an example of the flow behavior of the gel formulations. Shear thinning phenomenon, an advantageous property of buccal gel, was observed for all the gel tested. In this flow the molecule at rest entangled with the association of the immobilized solvent. Under the influence of shear, the molecule tends to become disentangled and align themselves in the direction of flow. The molecules thus offer less resistance to flow and this together with the release of entrapped water account for the lower viscosity.[20].

The viscosity of different gel bases is described in (table-2). The apparent viscosity values were used as a measure of gel consistency. Although solid content were equal, these values appear to be markedly different, revealing variability in net work structure.[21]. Carbomer based gel showed higher viscosity values indicating higher consistency which may be due to its cross-linked structure and the molecular weight between crosslink, reflects this rheological behavior.[22].

Mucoadhesive force:
The ex-vivo mucoadhesive property of the gels were determined using sheep buccal mucosa. Mucoadhesive force in term of detachment stress, (table-2), indicated that the bioadhesive force for carbomer is much more than SCMC and guar gum which may be attributed to the high viscosity of carbomer based gel.[23]. Carbomer also has a very high percentage (58-68) of carboxylic group in its chemical structure that gradually undergo hydrogen bonding with sugar residue in the oligosaccharide chain of the mucous membrane resulting in the formation of strengthened network between polymer and mucus. In addition
may also adopt more favorable macromolecule confirmation with the accessibility of its functional group for hydrogen bonding, while other polymers only undergo superficial bioadhesion\textsuperscript{[24,25]}. On the other hand, the charge of the polymer tended to affect the mucoadhesive force, where nonionic polymer appear to undergo a smaller degree of adhesion compared to anionic polymer\textsuperscript{[26]}. This explains why the formula (F1), anionic polymer based gel, had mucoadhesive force higher than formula (F2), nonionic polymer based gel\textsuperscript{[27]}.

**Swelling index:**

The swelling index as a function of time is shown in (table-3). As the time increase, the swelling index increase, because weight gain by the gel increased proportionally with rate of hydration, later on the swelling index of the formulas (F2, F3, F7, F8 and F9) decrease gradually due to dissolution of outer most layer of the gel in the dissolution medium\textsuperscript{[28]}. The direct relation ship was observed between swelling index and carbomer concentration, this could be attributed to the ionization of the carboxylated moiety at the pH environment of the dissolution medium. Ionization of carbomer leads to the development of negative charges along the backbone of the polymer. Repulsion of the like charges uncoils the polymer into an extended structure. The counter ion diffusion inside the gel creates an additional osmotic pressure differences across the gel leading to a considerable swelling of the polymer\textsuperscript{[29]}. The swelling of formula F3 is relatively higher than the formula F2 since water causes ionization of carboxylic group of SCMC with subsequent relaxation and repulsion of the polymer chain that result in an increase in water penetration and hence increase in swelling index by time, while guar gum is neutral polymer\textsuperscript{[27,30]}. On the other hand the swelling index increases in the same order of increasing viscosity. These findings are in agreement with those obtained by Pakah et al, who reported that the water absorption rate increases as the viscosity of the polymer increases\textsuperscript{[31]}.

**The in vitro release of TZ:**

**Effect of polymer type:**

Gels with particular polymer were prepared to study the effect of polymer type on the release profile. (Figure-2) show the release profile of TZ from formulas (F1, F2 and F3). Being an anionic and water soluble, SCMC based gel (formula F3) released more than 94\% of TZ within 3 hours, and approximately 30-40\% of drug released within 30 minutes. This formula showed burst release due to rapid dissolution of the gelling polymer in the pH of the dissolution medium. Carbomer and guar gum gel showed integrity beyond 6 hours and did not dissolve completely even after 6 hours. More retardant effect was obtained with carbomer this may be attributed to the highest viscosity and swelling of this gel than other tested gel preparations. It was demonstrated that under the condition of the dissolution medium swelling of carbomer increases rapidly and consequently the viscosity. In contrary, guar gum gel showed relatively higher
percentage of drug release than carbomer gel, since it is non ionic polymer and so the pH has no effect on their swelling and viscosity \[9, 27\].

**Effect of polymer combination:**

To obtain adequate release of the drug, it is thought to prepare formulas containing mixture of SCMC (fast drug release polymer) and guar gum or carbomer (slow drug release polymers). (Figures-3 and 4) show the release profile TZ from gel with combination polymers. F8and and F9 showed burst release (approximately 30-40% of drug release within 30 minutes) and almost complete drug release within 5 hours. These gel preparations where unable to give prolonged action and maintain the therapeutic action for longer period of time. The drug release rate constants (table-4) appear to decrease significantly (p<0.05) with decreasing amount of SCMC and increasing amount of guar gum. The inclusion of higher percentage of guar gum (F7) provide prolonged release of drug through its property of slow eroding and as a rigid gel structure forming agent\[10\]. The release rate constants for carbomer: SCMC gels decreased significantly (p<0.05)with decreasing amount of SCMC and increasing amount of carbomer, this could be described as the corresponding reduction in the number and dimension of the channel by increasing viscosities of the formulations\[3\]. As it is illustrated in (table-3) and (figure-3) although gels containing carbomer:SCMC exhibit maximum swelling, they showed lower rate of release which could be attributed to higher hydrophillicity and water uptake of carbomer which produce water swollen gel that may substantially reduce the penetration of the dissolution medium into the gel and as the result the drug release \[33\].

**Kinetics of drug release:**

The in-vitro release of TZ generated linear relationship between the amount released and square root of time as shown in (figure-5) with good correlation of coefficient(r2) over 0.99 for all formulations (table-4), indicating that the release kinetic followed Higuchi-diffusion model \[34\].

\[ F=k\sqrt{t} \quad \text{eq}(3) \]

Where F is the fraction of drug released, k is the release constant, and t is the time.

Diffusion is related to transport of the drug from the gel matrix into the surrounding in vitro dissolution medium and it depend on drug concentration. As gradient varies, the drug is released and the distance for diffusion increase. This could explain why the drug is diffuses at a comparatively slower rate as the distance for diffusion increases \[35\]. Based on the release rate constants, formulas F6 and F7 showed long term controlled release kinetics, while formulas F4 and F5 showed slow release kinetics.

**Stability study (effect of storage time):**

The selected formula F6 showed good physical stability, as there was no discoloration, precipitation, or any physical changes after storage. (Figure-6) shows the effect of different temperatures on the percentage of TZ remaining.
The results obtained showed linear profiles, from which the degradation rate constants (k) were calculated from the slopes. They were found to be $7.43 \times 10^3$, $9.34 \times 10^{-3}$ and $3.37 \times 10^{-3}$ (month$^{-1}$) at $40^\circ$C, $50^\circ$C and $4^\circ$C respectively. The rate constant (k) at room temperature was determined by Arrhenius plot. The expiration date of formula F6 was found 1.83 years with a pH value of 6.64.

**Conclusion:**

A mucoadhesive system for the controlled release of TZ was developed by using carbomer and SCMC in appropriate ratio. The release rate of TZ from the prepared gel as well as the physical properties is affected by the type and the change in polymer mixing ratio. Lower release rate was observed by lowering the content of SCMC in carbomer: SCMC and guar gum: SCMC containing formulation. The mucoadhesive TZ oral gel containing 1% carbomer and 3%SCMC showed suitable release kinetics and adhesion property may be considered useful formula for delivery of TZ into the periodontal pocket.

<table>
<thead>
<tr>
<th>Batch codes</th>
<th>TZ</th>
<th>Carbomer (g)</th>
<th>Guar gum (g)</th>
<th>SCMC (g)</th>
<th>MP (g)</th>
<th>PP (g)</th>
<th>Mannitol (g)</th>
<th>Water (g)</th>
<th>pH</th>
<th>Physical appearance</th>
</tr>
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<tbody>
<tr>
<td>F1</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.02</td>
<td>2</td>
<td>100</td>
<td>6.51</td>
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</tr>
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<td>4</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.02</td>
<td>2</td>
<td>100</td>
<td>6.89</td>
<td>opaque</td>
</tr>
<tr>
<td>F3</td>
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<td>4</td>
<td></td>
<td></td>
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<td>0.02</td>
<td>2</td>
<td>100</td>
<td>6.47</td>
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</tr>
<tr>
<td>F4</td>
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<td>3</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.02</td>
<td>2</td>
<td>100</td>
<td>6.44</td>
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<td>F5</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.02</td>
<td>2</td>
<td>100</td>
<td>6.65</td>
<td>transparent</td>
</tr>
<tr>
<td>F6</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.02</td>
<td>2</td>
<td>100</td>
<td>6.81</td>
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</tr>
<tr>
<td>F7</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.02</td>
<td>2</td>
<td>100</td>
<td>6.71</td>
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<tr>
<td>F8</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.02</td>
<td>2</td>
<td>100</td>
<td>6.89</td>
<td>opaque</td>
</tr>
<tr>
<td>F9</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.02</td>
<td>2</td>
<td>100</td>
<td>6.93</td>
<td>opaque</td>
</tr>
</tbody>
</table>

**Table-1: Formulations of TZ mucoadhesive oral gel (% W/W)**

<table>
<thead>
<tr>
<th>TZ Gel</th>
<th>$\eta_{\text{max}}$ (poises)</th>
<th>$\eta_{\text{min}}$ (poises)</th>
<th>Mucoadhesive force (dyne/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>3076.20</td>
<td>13350.00</td>
<td>9425.6</td>
</tr>
<tr>
<td>F2</td>
<td>535.55</td>
<td>1957.40</td>
<td>6125.0</td>
</tr>
<tr>
<td>F3</td>
<td>646.10</td>
<td>2103.18</td>
<td>6329.8</td>
</tr>
<tr>
<td>F4</td>
<td>4674.54</td>
<td>10250.78</td>
<td>7690.2</td>
</tr>
<tr>
<td>F5</td>
<td>2545.04</td>
<td>4727.06</td>
<td>6805.5</td>
</tr>
<tr>
<td>F6</td>
<td>1079.60</td>
<td>4311.71</td>
<td>6465.8</td>
</tr>
<tr>
<td>F7</td>
<td>270.16</td>
<td>1994.56</td>
<td>6111.0</td>
</tr>
<tr>
<td>F8</td>
<td>396.43</td>
<td>2048.12</td>
<td>5988.4</td>
</tr>
<tr>
<td>F9</td>
<td>6599.00</td>
<td>2083.39</td>
<td>5921.8</td>
</tr>
</tbody>
</table>

**Table-2: Physical evaluation of different TZ mucoadhesive oral gels**

* $\eta$ Viscosity at high rate of shear (14.68 sec$^{-1}$)
* $\eta$ Viscosity at low rate of shear (2.24 sec$^{-1}$)
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Formulations code</th>
<th>%Swelling index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F9</td>
<td>F8</td>
</tr>
<tr>
<td>44.1</td>
<td>35</td>
<td>34.3</td>
</tr>
<tr>
<td>47.2</td>
<td>46</td>
<td>44.7</td>
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<td>51.6</td>
<td>49.5</td>
<td>49.3</td>
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<td>48.3</td>
<td>56.8</td>
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<tr>
<td>46.6</td>
<td>58.3</td>
<td>52.4</td>
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<tr>
<td>43.1</td>
<td>56.4</td>
<td>51.1</td>
</tr>
<tr>
<td>40.7</td>
<td>51.8</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Table-3: In-vitro swelling study of mucoadhesive oral gels of TZ

<table>
<thead>
<tr>
<th>Formulations code</th>
<th>K (µg. hour$^{1/2}$.ml$^{-1}$)</th>
<th>Correlation coefficient ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>4.107</td>
<td>0.998</td>
</tr>
<tr>
<td>F2</td>
<td>5.734</td>
<td>0.9960</td>
</tr>
<tr>
<td>F3</td>
<td>10.463</td>
<td>0.993</td>
</tr>
<tr>
<td>F4</td>
<td>4.910</td>
<td>0.996</td>
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<tr>
<td>F5</td>
<td>5.900</td>
<td>0.996</td>
</tr>
<tr>
<td>F6</td>
<td>7.954</td>
<td>0.995</td>
</tr>
<tr>
<td>F7</td>
<td>8.496</td>
<td>0.996</td>
</tr>
<tr>
<td>F8</td>
<td>9.251</td>
<td>0.996</td>
</tr>
<tr>
<td>F9</td>
<td>9.473</td>
<td>0.984</td>
</tr>
</tbody>
</table>

Table-4: Release rate constants for TZ mucoadhesive oral gels

* Significant at $P<0.05$
* * Highly Significant at $P<0.001$

Figure-1: Rheogram of formula F6 (1% carbomer:3% SCMC)
Figure-2: The effect of polymer type on the release of TZ from mucoadhesive oral gel at pH6.8 and 37° C.

Figure-3: The effect of polymer combination on the release of TZ from (carbomer: SCMC) mucoadhesive oral gel at pH6.8 and 37° C

Figure-4: The effect of polymer combination on the release of TZ from (guar gum: SCMC) mucoadhesive oral gel at pH6.8 and 37° C
Figure-5: In vitro release of TZ from oral gel formulation containing different mucoadhesive gelling agent.

Figure-6: Degradation of tinidazole in formula F6 at 50°C, 40°C and 4°C

Figure-7: Arrhenius plot for expiration date estimation of tinidazole mucoadesive oral gel (formula 6)
References:
4- Ravi Bhalodia; Biswajit Basu; Kuldeep Metha; Review, A.; Pharma, J. and BioSiences. (2010). Buccoadhesive drug delivery system, 1.2.


