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Significance of lipid matrix aging on in vitro release and in vivo bioavailability

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Abstract

A polyglycolised glyceride carrier, Gelucire 50/13, was incorporated with paracetamol as a model drug, filled into hard gelatin capsules and stored at three different temperatures for various lengths of time. The resultant solidified matrix within the capsule was subjected to thermal analysis using differential scanning calorimetry (DSC) to ascertain its supramolecular structure. Polymorphic transformations towards more stable gelucire forms were observed upon aging the matrices, with samples stored at a temperature near the melting range of the lower temperature gelucire melting fraction showing the most profound changes. The increase in the rate of drug release from aged samples could be correlated to the alterations to the supramolecular structure of the gelucire. Accelerated drug release from aged samples could also be seen from in vivo studies using healthy human volunteers, although the extent of absorption was not affected. Therefore, even though the sustainability of release may be compromised by aging the gelucire matrices, the bioavailability of the incorporated drug is unlikely to be affected.

Keywords: Lipid matrices; Gelucire®; Aging; In vivo; Sustained release; DSC

1. Introduction

Lipid based materials are fast becoming the carriers of choice for oral delivery of new chemical entities (NCE) and well-established active agents (Mueller et al., 1994; Constantinides, 1995; Chambin et al., 2004). NCEs that had been developed are mostly hydrophobic, thus presenting associated problems such as low and erratic bioavailability. Solubilisation into lipid carriers followed by intraluminal processing circumvent these inherent limitations (Humberstone and Charman, 1997). However, more hydrophilic drugs which are already in existence can also be reformulated into certain lipid based carriers for modification of their release profiles (Saraiya and Bolton, 1990; Esquisabel et al., 1996; Hamdani et al., 2002). These carriers include the gelucires, which are a family of polyglycolised glyceride bases, consisting of polyethylene glycol (PEG) esters of various fatty acids, tri-, di-, and monoglycerides of the fatty acids, with some of the corresponding free fatty acids and PEGs present in small quantities.

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Each type of gelucire is characterized by two numbers, the first being the nominal melting point and the second being the hydrophile–lipophile balance (HLB) number. In general, the higher melting bases with a bigger proportion of lipophilic components in them are used as coating and matrix agents for sustained release formulations, whilst those with more hydrophilic components within are suitable as bioavailability enhancers. The amphiphilicity of the base conferred by the long hydrocarbon chain and the alcohol moieties means that both hydrophilic and hydrophobic drugs can potentially be incorporated into these carriers.

However, lipid based carriers are known to be affected by stability issues, particularly those associated with the changes to the matrix structure upon aging. These changes have been attributed to polymorphic transformations within the lipid component and also to the progressive increase in the crystallinity of the matrix components (Sutananta et al., 1994; Hamdani et al., 2002), which in turn could result in a modification of the in vitro and in vivo releases of the incorporated drugs. In a system such as gelucire whereby the characterization of the glycerides is made even more complex by esterification with PEG, the poor understanding of the phase changes has made it very difficult to predict its release behaviour. Variations in the results of drug release studies upon aging is a function of the components within the particular gelucire, conditions of storage and incorporated drugs (Remuñán et al., 1992; Sutananta et al., 1995; San Vicente et al., 2000). Even though these investigations were vital in establishing the role of aging in altering drug release in vitro, studies that examine the relevance of these findings in vivo are scarce. Dennis et al. (1990) found that aged formulations of ketoprofen dispersed in G50/13:G50/02 base mixture gave an increased rate of drug release in vitro but this observation was discovered to be insignificant in vivo.

In our current study, Gelucire 50/13 (G50/13), which has a sufficiently high melting point to form sustained release matrices and a balance of components that allows both hydrophobic and hydrophilic drugs to be incorporated within was chosen as the model gelucire. G50/13 has the capacity to alter its dimensions through its gelling and swelling abilities up to the extent necessary for sustaining its controlled-release properties (Kopcha et al., 1991; Prapaitakul et al., 1991; Esquisabel et al., 1996). Most dosage forms, including lipid containing ones, are usually stored for a certain length of time before being used. The storage conditions that these forms are subjected to on leaving the manufacturing plant are not always easily controlled and it would be unduly optimistic to predict an absence of temperature fluctuations within their environs. As these factors have the potential of altering the performance of the dosage form, it is essential to attempt to elucidate any mechanism of change within the solid-state structure, especially in relation to its consequent behaviour in vivo. Therefore, our current study aimed to address the above issue by investigating the release behaviour of a relatively hydrophilic drug from a lipid matrix before and after storage at different temperatures, which represent the aging process. The association of structural modifications to the matrix after aging, as indicated by thermal analysis, to changes in drug release was investigated. More importantly, the significance of such modifications as shown in in vitro systems, to drug release in healthy human volunteers was determined.

2. Materials and methods

2.1. Materials

Gelucire 50/13 was purchased from Gattefossé (Saint-Priest, France). The model drug used was paracetamol (Wenzhou Pharmaceutical, China) and the hard gelatin capsules used were of size 0. The paracetamol was sieved to give particles of ≤300 μm in size. All other chemicals and reagents used were either of analytical reagent (AR) grade or of high performance liquid chromatography (HPLC) grade.

2.2. Preparation of liquid filled hard gelatin capsules

10% w/w of the drug was weighed accurately, added to the molten gelucire at 75 °C and mixed using a magnetic hot plate stirrer for 10 min to ensure homogeneity and removal of thermal history. The drug did not completely dissolve in the molten carrier and remained as a dispersion. This was also observed by Khan and Craig (2003) at the same drug loading. The mixture was then filled into hard gelatin capsules size 0 using preheated pasteur pipettes and left to set under ambient conditions. The capsules were stored in amber bottles containing silica gel for 24 h at room temperature for samples designated as freshly prepared, and in a refrigerator at 4 °C, at room temperature (24.0 ± 0.5 °C), or in a temperature-controlled oven at 37 °C until required for samples designated as aged. Before any analysis was performed, the aged capsules were equilibrated at room temperature for 24 h. The mean fill weight of the capsules was 682.6 ± 19.1 mg with the coefficient of variation being 2.8%. The prepared capsules fulfilled the criteria for the uniformity of dosage units as specified in US Pharmacopeia 24 (2000).

2.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) scans were performed on Perkin-Elmer Pyris 6 DSC (Beaconsfield, UK) equipped with an intracooler, at the rate of 2 °C/min. Purge gas used was helium flowing at the rate of 20 ml/min and the instrument was calibrated using indium and zinc standards. The 9.0–10.5 mg of samples obtained from the content of the capsules were put into non-hermetic aluminium pans (Perkin-Elmer, UK) and sealed. The melting point was taken to be the temperature where the rate of melting was the highest, that is at the tip of the endothermic curve. The heat or enthalpy of fusion was calculated from the area under the curve from the first inflection of the baseline to the temperature where the endotherm returned to the baseline (Khan and Craig, 2003). Replicates of four scans were performed for each sample.

2.4. In vitro dissolution studies

The dissolution of the liquid filled hard gelatin capsules was determined following the basket method of the USP 24 (2000) in a Type PTW S III apparatus (PharmaTest, Germany). The test was run under sink conditions with 900 ml distilled water maintained at 37 °C being used as the dissolution medium and the basket rotation speed set at 100 rpm. Samples were collected from the dissolution of six capsules using SDX automated fraction collector (Sadex, Malaysia) up to 14 h. Concentration of the dissolved drug was measured spectrophotometrically at 243 nm (Hitachi, Japan). Mean dissolution time (MDT) is the mean ratio of the first to zero moments of the dissolution rate–time curve and was calculated using the following equation (Brockmeier and Von Hattinberg, 1982):

$$\mathrm{MDT} = \frac{\mathrm{ACC}}{M_{\infty}} \tag{1}$$

where ACC is the area complimentary to the area under the accumulated dissolution curve and M_{∞} is the accumulated amount dissolved at maximum time. MDT has also been described as the sum of different periods of time during which fractions of the dose stay in the polymer matrix before release, divided by the total dose (Lindner and Lippold, 1995).

2.5. In vivo study protocol

Twelve healthy adult male volunteers not receiving any medication prior to and during the sampling period participated in the study. The study followed a three treatment, three periods, three sequence crossover design with a washout period of 1 week. The samples for this study were the liquid filled hard gelatin capsules prepared according to Section 2.2, which had been freshly prepared or subjected to the different storage temperatures of 4 and 37 °C. The volunteers were randomly divided into three groups of four and were administered the samples, according to the schedule shown in Table 1.

Five capsules containing a total amount of 300 mg of drug were administered after an overnight fast with 240 ml of water. Drinks were available ad libitum 2 h after dosing whilst standardised meals were given at 4 and 10 h after dosing. Blood samples of 5 ml in volume were collected at 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 14, 18 and 24 h after dosing via an in-dwelling cannula placed in the forearm, centrifuged for 15 min at 3500 rpm, and the plasma transferred into glass containers to be kept frozen until analysis.

Table 1
The schedule for the administration of samples to the volunteers at the three different study periods

Group	Period I	Period II	Period III
1	Freshly prepared	Storage at 4 °C	Storage at 37 °C
2	Storage at 37 °C	Freshly prepared	Storage at 4 °C
3	Storage at 4 °C	Storage at 37 °C	Freshly prepared

2.6. Analysis of the drug concentration in plasma

The plasma samples were analysed using a reversed-phase HPLC method described by Yuen et al. (1997).

2.7. Data analysis

The pharmacokinetic parameters, namely the extent of absorption ($\mathrm{AUC}_{0-\infty}$), maximum plasma concentration ($C_{\max}$), time to reach maximum concentration ($T_{\max}$) and mean residence time (MRT) were calculated from the individual plasma drug concentration data. $C_{\max}$ and $T_{\max}$ were obtained directly from the plasma values (Weiner, 1981). $\mathrm{AUC}_{0-\infty}$ was obtained by adding the area under the plasma drug concentration–time curve from time 0 to the last measurable concentration (AUC_{0-t}) and the area from the last measurable concentration to infinite ($\mathrm{AUC}_{t-\infty}$). The former was calculated using the trapezoidal formula whilst the latter was calculated by dividing the last measurable concentration with the apparent elimination rate constant (k_e). k_e was estimated from the terminal slope of the individual plasma drug concentration–time curves after logarithmic transformation of the plasma drug concentration values and the application of linear regression (Gibaldi and Perrier, 1982).

The Wagner–Nelson equation, which can be used for one as well as two compartments open model was employed to calculate in vivo absorption from plasma drug concentration data (Wagner and Nelson, 1963; Wagner, 1975):

$$\%\ \text{absorbed at time}\ t = \frac{C_t + k_\mathrm{e}\mathrm{AUC}_{0-t}}{k_e \mathrm{AUC}_{0-\infty}} \qquad (2)$$

where C_t is the plasma concentration at time t.

MRT was calculated from the individual plasma drug concentration–time curves using the Statistical Moment Theory, according to the equation:

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} \qquad (3)$$

where AUMC is the first moments curve and AUC is the area under the plasma drug concentration–time curve.

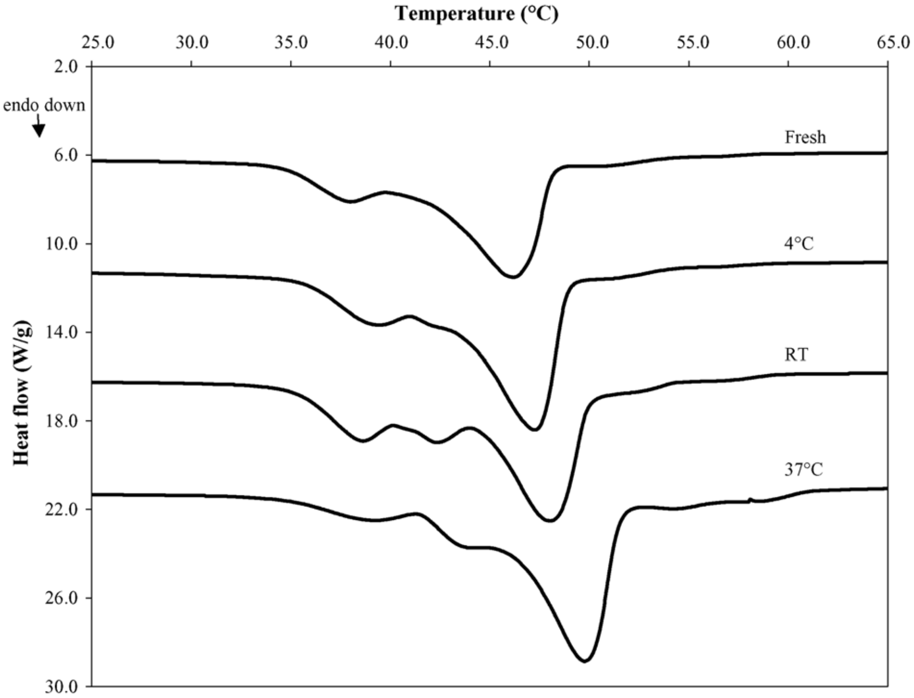

Fig. 1. DSC thermal profiles of Gelucire 50/13 samples which had been freshly prepared, or stored at 4 °C, room temperature (RT) or 37 °C for 22 weeks.

These parameters were calculated using the trapezoidal rule.

3. Results

3.1. Physical characterization of the matrices using DSC

Fig. 1 shows the thermal profiles obtained from the DSC scans of the samples containing G50/13 which had been freshly prepared, or stored at 4 °C, room temperature or 37 °C. For clarity, only the thermal profiles for the aged samples as stored for 22 weeks are shown. Several peaks could be seen within the broad melting endotherm of the gelucire and this is due to the heterogeneity of components within the carrier. The first prominent peak is designated the lower melting gelucire fraction from hereon, and the second prominent peak which commenced from about 40 °C together with the succeeding shoulder are designated the higher melting gelucire fraction. Out of the three aged samples, the ones stored at 4 °C showed the least variation to the thermal profiles of the freshly prepared samples. However, a new shoulder could be detected at the lower temperature end of the higher melting gelucire fraction, preceeding the second prominent peak, which was apparent for all the aged samples. An increase in the melting temperature of the higher melting gelucire fraction was observed for all the aged samples, with the samples stored at 37 °C showing the biggest change.

Elevations in the heat of fusion values were also observed upon aging, with 160.7 ± 6.3 J/g for the samples that were freshly prepared to 176.0 ± 8.5 J/g and 172.5 ± 6.8 J/g for the samples stored for 22 weeks at 4 °C and room temperature, respectively. A more drastic change was observed for the samples stored at 37 °C with the heat of fusion value after 22 weeks being 187.4 ± 8.2 J/g. This is a 16.6% increase from the value of freshly prepared samples, compared to 9.5% and 7.3% increases for the samples stored at 4 °C and room temperature, respectively. These differences were found to be statistically significant ($p < 0.01$).

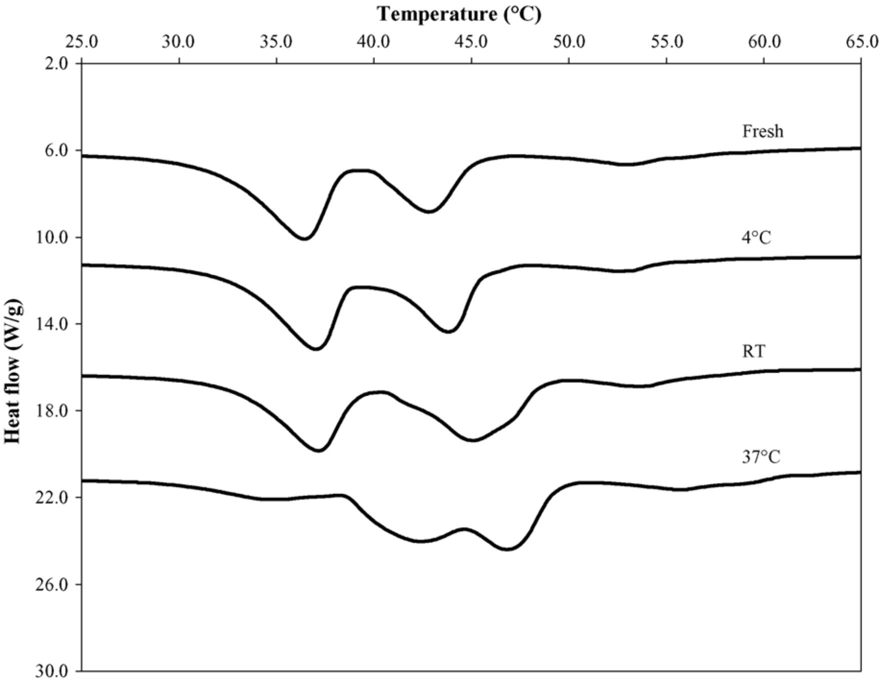

Fig. 2. DSC thermal profiles of 10% paracetamol in gelucire 50/13 dispersion samples which had been freshly prepared, or stored at 4 °C, room temperature (RT) or 37 °C for 22 weeks.

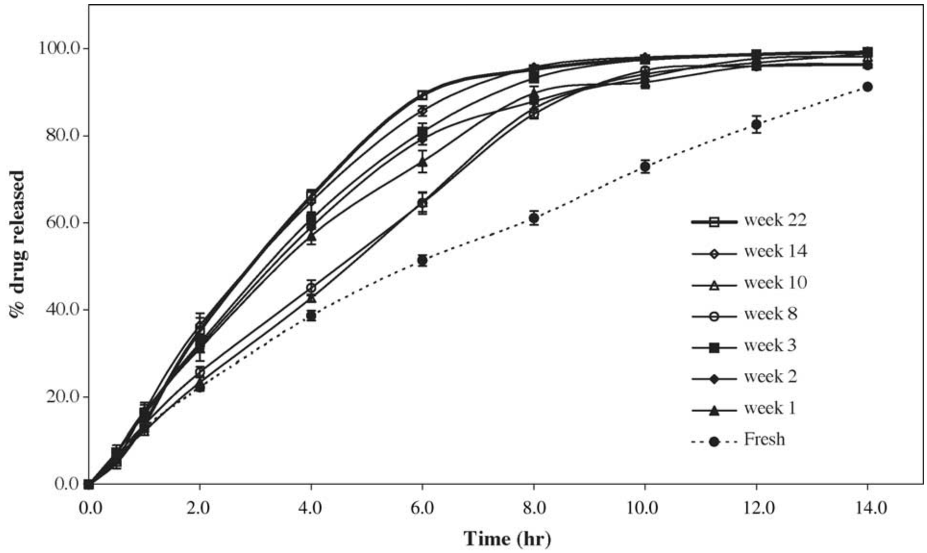

Fig. 3. In vitro dissolution profiles of paracetamol in G50/13 dispersion samples which had been freshly prepared or stored at 37 °C.

Upon the incorporation of 10% w/w paracetamol into G50/13, the lower melting gelucire fraction became more prominent. The stabilisation of this fraction by paracetamol was previously described by Khan and Craig (2003). Similar to the samples of G50/13 on its own, the total heat of fusion values for the drug dispersion samples were also elevated when stored at 4 °C, room temperature and 37 °C, from 126.0 ± 9.0 J/g when freshly prepared to the after storage values of 173.8 ± 7.7, 178.5 ± 10.5 and 196.7 ± 8.3 J/g signifying increases of 37.9%, 41.7% and 56.0%, respectively. It can be seen that there were little changes to the ther-

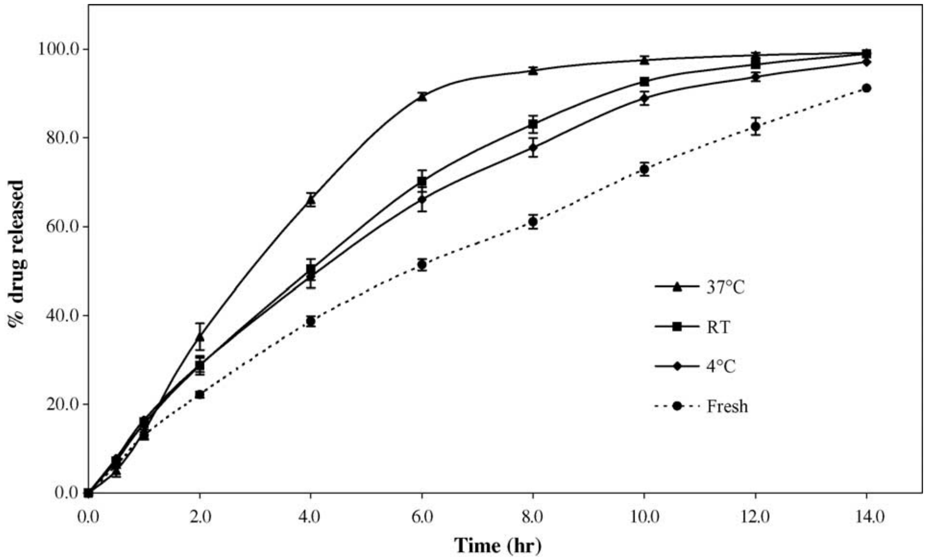

Fig. 4. In vitro dissolution profiles of paracetamol in G50/13 dispersion samples which had been freshly prepared, or stored at 4 °C, room temperature (RT) or 37 °C for 22 weeks.

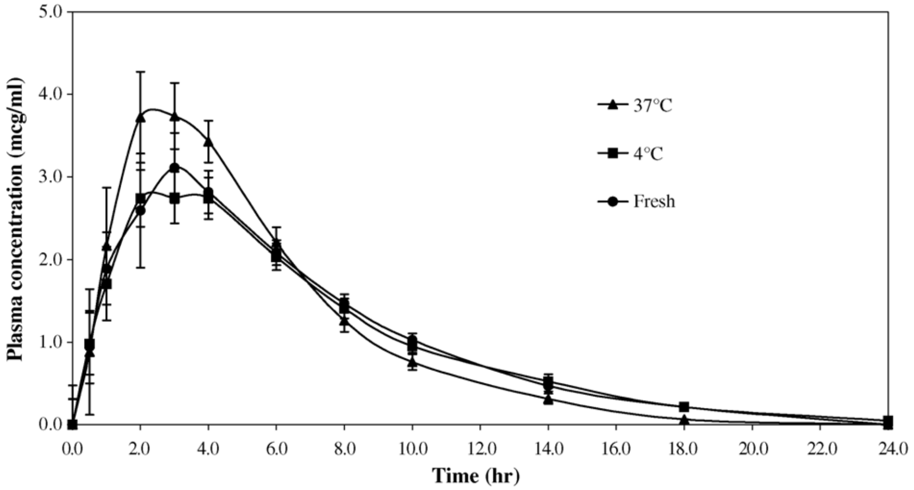

Fig. 5. Mean plasma drug concentration (μg/ml) against time (h) profiles after the oral administration of paracetamol in G50/13 dispersion samples which had been freshly prepared, or stored at 4 or 37 °C for 22 weeks.

mal profiles of the samples stored at 4 °C and room temperature (Fig. 2), but the thermal profile of the sample stored at 37 °C was vastly different from the freshly prepared sample. This modification was apparent even after only 1 week of storage at the higher temperature.

3.2. *In vitro dissolution studies*

In general, increasing the storage period under the three test conditions accelerated the release of paracetamol from G50/13 matrices, although this trend was less clearly observed in the samples which had not been subjected to prolonged storage, as exemplified in Fig. 3 by the dissolution profiles of samples stored at 37 °C. Storage at 37 °C resulted in the highest elevation in the release rates compared to storage at 4 °C and room temperature. Fig. 4 shows the comparison between the storage conditions, using the dissolution profiles of freshly prepared samples and samples stored for 22 weeks only for clarity. The mean dissolution time (MDT) values calculated reflected the changes stated above, with the MDT decreasing from 8.92 ± 0.11 h for the freshly prepared samples to 8.78 ± 0.12, 8.73 ± 0.14 and 8.47 ± 0.14 h for the samples stored for 22 weeks at 4 °C, room temperature and 37 °C, respectively. However, only the value for the sample stored at 37 °C was significantly different statistically ($p < 0.01$) from the value of the freshly prepared sample.

3.3. *In vivo study*

Fig. 5 shows the mean plasma concentration–time profiles after the oral administration of 300 mg paracetamol as the drug dispersion samples which had been freshly prepared, or stored at 4 or 37 °C for 22 weeks. The $AUC_{0\text{-}\infty}$ values of 26.2 ± 7.9, 26.2 ± 8.3 and 26.4 ± 8.9 μg/ml for the freshly prepared, stored at 4 and 37 °C samples respectively were found to be not significantly different from each other ($p > 0.9$) indicating that the extent of bioavailability of the drug was not affected by the storage conditions imposed on the matrices. Similarly, no statistically significant difference ($p > 0.05$) was observed among the $T_{\max}$ values of the samples, that were 3.2 ± 0.9, 3.0 ± 1.2 and 2.8 ± 1.1 h for the samples in the same order as above. $C_{\max}$ values for the samples were 3.3 ± 1.6, 3.1 ± 0.9 and 4.4 ± 1.5 μg/ml respectively and a statistically significant difference was observed among the logarithmically transformed $C_{\max}$ values of the three samples ($p < 0.01$).

The mean in vivo paracetamol absorption-time profiles (Fig. 6) showed that the absorption of the drug, as calculated from Eq. (2), was the fastest from the matrices stored at 37 °C. This is in accord with the in vitro dissolution profile of the same samples which showed the fastest release compared to release from freshly prepared samples or samples stored at 4 °C. However, the in vivo absorption profiles of the lat-

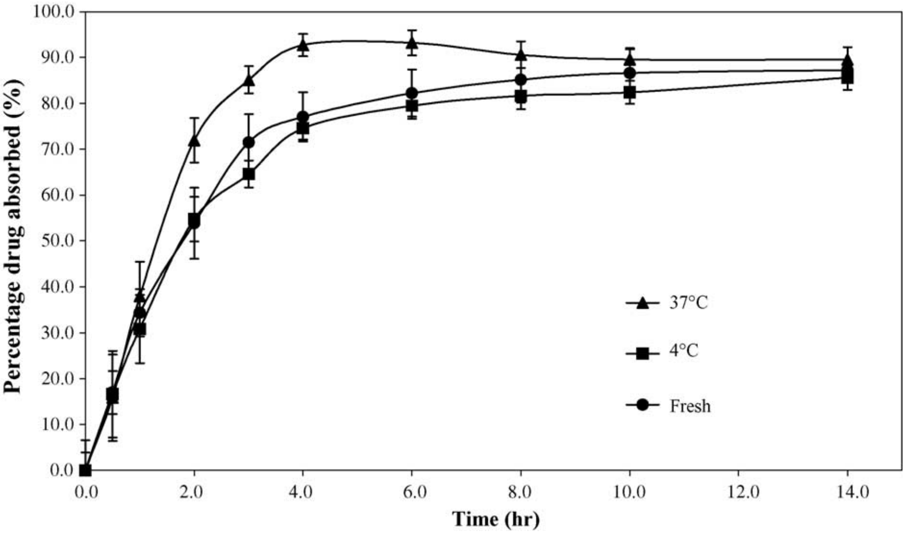

Fig. 6. Mean percentage drug absorbed (%) against time (h) profiles after the oral administration of paracetamol in G50/13 dispersion samples which had been freshly prepared, or stored at 4 or 37 °C for 22 weeks.

ter two samples were similar even though the in vitro release indicated a notable difference with the release from the samples stored at 4 °C being much faster than release from freshly prepared samples. When the mean residence time (MRT) values were calculated, it was revealed that the samples stored at 37 °C had the lowest value at 5.50 ± 0.66 h, followed by the freshly prepared samples at 6.69 ± 0.94 h and samples stored at 4 °C at 7.18 ± 0.86 h.

4. Discussion

Gelucire 50/13 is made up of many lipid components such as glycerides and free fatty acids, as well as more hydrosoluble components such as PEG esters and residual PEG 1500. These numerous components generate the endotherms observed on the thermal profiles of the samples, which in turn could be the result of several overlapping transitions. However, it may be too simplistic to suggest that the thermal profile is the simple sum of the transitions caused by individual components. The composition of the gelucire is made even more complex by the probable presence of the different polymorphic forms of the lipid components and the PEG esters of fatty acids. The different constituents may form mixed crystals with each other, leading to what had been described previously as segregations of components which melting gave rise to the endothermic peaks (Sutananta et al., 1994). Upon ageing under all three conditions, the gelucire components transformed into their more stable polymorphic forms, as evidenced by the elevated heats of fusion and the progression towards the formation of the higher melting fraction. This transformation was more pronounced in the samples stored at the highest temperature, 37 °C, which is also in the vicinity of the endotherm of the lower melting fraction. At this temperature, the solidity of the matrix is lowered due to a portion of the gelucire existing as a molten mass, which results in the more stable polymorphic form being preferred during lipid recrystallisation. This was also noted for other lipid mixtures, when the low solid content of the freshly prepared sample resulted in the crystals becoming more prone to polymorphic changes (deMan and deMan, 1994).

As gleaned from the in-vitro dissolution studies, the pattern of change in the release rates was not consistent in the earlier stages of storage (less than or at 14 weeks), which could be attributed to the rearrangement of the gelucire molecules prior to achieving a stable structure. Further storage after 14 weeks did not bring about significant alterations to the release rates, suggesting that transformations into the most stable gelucire structure possible under each condition were completed. When the MDTs were calculated from the samples that had achieved this equilibrium state, it was found that the drug release from the samples stored at 37 °C was significantly faster than from freshly prepared samples.

The in vitro dissolution profiles together with the thermal profiles obtained from DSC studies indicate an association between the supramolecular structure of the matrices and drug release. Storage at 37 °C resulted in the highest degree of transformation to a more stable form of G50/13 and also the fastest drug release, with both changes found to be significantly different statistically from the freshly prepared samples. Such an association may be explained by the formation of larger crystals in the stored samples causing a disruption to the previously compact matrix. The change in the lipid crystal structure, generally to larger sizes, is known to occur during the polymorphic transformation of a metastable to a more stable form (Hachiya et al., 1990; Heathcock, 1993). The structural alteration throughout the matrix may have increased its porosity, allowing faster and easier dissolution medium penetration into the bulk of the samples. Even though some paracetamol may have been solubilised in the molten gelucire during manufacture, the drug did not recrystallise out in its pure form during solidification, but instead induced a structural modification within the carrier matrix which was further altered during aging (Khan and Craig, 2003, 2004).

The in vivo studies demonstrated that even though the bioavailability of the drug was not altered by the aging process, $C_{\max}$ was higher for samples stored at 37 °C. Bioavailability was not affected because the chemical composition remained the same for all samples and in addition, the model drug used is not known to have a poor absorption profile. The structural changes caused by the aging process led to more drug being released in the first few hours as demonstrated by the in vitro dissolution and the higher maximum concentration achieved in vivo. These results suggest that although the extent of absorption will not be adversely affected by storage conditions, the sustainability of the release over time may be compromised especially when the gelucires are kept at temperatures near its lower melting fraction. This change in release profile may be predicted by studying the matrix structure as determined by thermal analyses.

There is no rank order correlation between MDT values obtained from the in vitro dissolution tests and MRT values obtained from the in vivo study. On closer inspection of the results, it could be seen that the samples stored at 37 °C had both the lowest MDT and MRT values, suggesting that correlation could only be seen for matrices that underwent substantial changes. As stated before, there may be disruption to the bulk of the matrix due to the formation of larger lipid crystals brought about by polymorphic transformation. The high release rates observed in vitro and in vivo for these samples may then be due to the increased preponderance of erosion as the mechanism of drug release. Shameem et al. (1995) demonstrated that the release of paracetamol was faster from one controlled release dosage form than from another, both in vitro (50–150 rpm rotating basket methods) and in vivo, due to enhanced erosion. A higher $C_{\max}$ was attributed to a “burst release effect” caused by the erosion of HPMC-Gelucire core, which was also reflected in in vitro paddle-beads dissolution tests (Mehuys et al., 2004). It has been shown previously in an in vitro study (Khan and Craig, 2003) that even though paracetamol was released from G50/13 through both erosional and diffusional mechanisms, the latter was more dominant for freshly prepared matrices. The current study suggests that even though the in vitro release for the freshly prepared samples was the slowest due to the more diffusional release, the hydrodynamic conditions in the gastrointestinal tract is sufficiently destructive to promote the faster erosional process, leading to the obscuration of any minor differences such as those seen between the freshly prepared samples and those stored at 4 °C. Hence, this led to the poor rank correlation between the MDT and MRT values of these two samples. However, this finding suggests that keeping gelucire containing preparations in a low temperature environment such as the refrigerator could ensure drug releases that are consistent with those prior to storage.

5. Conclusion

The extent of the structural supramolecular changes seen upon aging was greatly affected by the storage conditions, with storage temperature, which was close to the melting range of the unstable gelucire form causing the biggest modifications. The structure with the greatest polymorphic stability and crystallinity gave the highest rate of release. However, in practical terms, such changes as detected by physical characterization and in vitro techniques did not cause major alterations to in vivo performance. This study indicates that the extent of bioavailability can be maintained at least for hydrophilic drugs that are incorporated into polyglycolised glycerides, even when the lipid carriers have been subjected to drastic environmental changes. However, the sustainability of the drug concentration level over time in the body could be influenced by such alterations to controlled release preparations containing polyglycolised glycerides.

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