Chitosan acetate based in-situ solid dispersions of carbamazepine

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Abstract

Chitosan is a polysaccharide comprising of co-polymers of glucosamine and N-acetyl glucosamine and is widely used in drug delivery applications such as dissolution enhancer, permeation enhancer, bioadhesive polymer, matrix former etc. Chitosan acetate based solid dispersions and physical mixtures were prepared to improve the biopharmaceutic characteristics of carbamazepine. The prepared formulations were characterized for its chemical compatibility (FTIR), thermal behavior (DSC), crystallinity (PXRD) and in vitro drug dissolution testing. The in vitro drug dissolution studies of physical mixtures and solid dispersions revealed that the dissolution enhancement of carbamazepine in chitosan acetate carrier. The maximum release of 90.81 % was achieved with solid dispersion formulation containing 70% of chitosan acetate. The drug release was independent of polymer concentration in formulation. Further studies required exploring the effect of processing technique on dissolution profile of formulations. The research finding concludes that chitosan acetate can be used as carrier for delivery of poor soluble drug such as a carbamazepine.

Key words: Carbamazepine, chitosan, chitosan acetate, solid dispersions, dissolution.

Introduction

Chitosan (CHN) is a polysaccharide comprising of co-polymers of glucosamine and N-acetyl glucosamine. CHN is now available in different molecular weights (polymers 50 000 Da; oligomers 2000Da), viscosity grades, and degrees of deacetylation (40–98%). CHN is insoluble at neutral and alkaline pH values, whereas it forms salts with inorganic and organic acids such as glutamic acid, hydrochloric acid, lactic acid and acetic acid. It is widely used in the food industry as a food additive and as a weight loss product. This polymer is now also in development as a safe excipient in drug formulations (Baldrick 2000). It has found a number of applications in several drug delivery systems, in virtue of its high biocompatibility, biodegradability and lack of toxicity associated with gel- and film-forming abilities, bioadhesiveness, dissolution and transmucosal penetration enhancer properties (Illum 1998, Paul and Sharma 2000, Porterro et al. 1998). CHN first attracted the attention of biopharmaceutical scientists as a mucoadhesive polymer that could be useful for peptide drug delivery. Furthermore, its antacid and antiulcer activities were exploited to reduce gastric irritation caused by active compounds, such as anti-inflammatory drugs (Açikgoz et al. 1995). The low cost and abundant availability of CHN offers high flexibility for pharmaceutical scientist to use CHN as an excipient of choice in drug delivery system (Sawayanagi et al. 1982).

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CHN is widely used as a dissolution enhancer for poor solubility drugs (Sawayanagi et al. 1983, Shiraishi et al. 1990, Acarturk et al. 1993, Genta et al. 1995). However, the poor solubility of CHN in pharmaceutically acceptable solvents is considered as one of the backdrop for using CHN as a potential carrier in drug delivery research. Various derivatives of CHN were synthesized and effectively used to improve the physicochemical properties of CHN that have been used for development of dosage forms (Maestrelli et al. 2004). Inorganic and organic acid salts of CHN were synthesized for their pharmaceutical applicability.

Carbamazepine (CBZ), chemically 5H - Dibenz (b, f) azepine - 5 – carboxamide, is used in the treatment of epilepsy, trigeminal neuralgia, bipolar affective disorder and acute mania (The Merck index 2001, Physician Desk Reference 2004). CBZ has poor solubility (170μg/ml at 25° C) (Moneghini et al. 2001) and high permeability (log P: 2.93 Clog P: 1.98) (Nehal et al. 2003). CBZ, a weakly basic drug with pKa of 7.0 (Nehal et al. 2003) indicates the pH dependent solubility. The low and erratic solubility of CBZ in gastrointestinal system offers poor bioavailability and variability in pharmacokinetics (Bertilsson 1978). There have been several literature reports concerning polymorphism of CBZ and its influence on solubility, dissolution and bioavailability (Stephen et al. 2006). CBZ has at least four anhydrous polymorphic forms: a triclinic (form I), a trigonal (form II), a P – monoclinic (form III), a C- monoclinic (form IV) (Meidong et al. 2002). It also exhibits a P – monoclinic dihydrate, numerous solvates and molecular adducts and co-crystals (Haiyan et al. 2006, Peddy et al. 2006). Anhydrous P – monoclinic form III is only suitable for drug formulation because of its thermodynamic stability at ambient temperature (Hassan et al. 2005). The thermodynamic instability and hygroscopic nature of CBZ form I hinder the bioavailability and stability, respectively and hence it is not used for the development of therapeutic dosage form (Young et al. 1991). Several attempts using water soluble carriers have been made to prepare different formulation of CBZ solid dosage forms with improved dissolution properties (Sarkari et al. 2002, Kobayashi et al. 2000, Haiyan et al. 2006, Sethia and Squillante 2004, Beatrice et al. 2002). The present investigation is focused on development of CHN acetate based on solid dispersion for solubility and dissolution enhancement of CBZ. In situ formation of CHN acetate in solid dispersion of CBZ and its pharmaco-technical properties were examined.

Materials and Methods

Materials

Carbamazepine crystal was gifted from Intas Pharmaceuticals Pvt. Ltd, Ahmedabad. Chitosan (minimum of 80% deacetylated) was gifted from Panacea Biotec, Punjab. Acetic acid and dichloromethane were purchased from Loba Chemie, Mumbai, India. All other chemicals used were of HPLC grade or analytical grade.

Preparation of solid dispersions and physical mixtures: Composition of physical mixtures and solid dispersions are listed in Table 1 and 2. The specified quantity of drug was dissolved in Dichloromethane (DCM). CHN was added in the drug solution. Acetic acid was added in the drug-CHN dispersion; the resulting solution was triturated until the entire DCM gets evaporated. The wet mass obtained was freeze dried for 24 h. The obtained solid dispersions were size reduced using # 100 mesh. CHN acetate was synthesized by freeze drying 1% CHN gel in 0.1 M acetic acid for 72 h. The corresponding physical mixtures were prepared by blending the previously size reduced (mesh # 100)
drug and CHN acetate in poly bags for 10 min. Solid dispersion and physical mixtures were stored in air tight container for further analysis.

**Table 1. Composition of CBZ physical mixtures.**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Batch Code</th>
<th>CBZ (gm)</th>
<th>%w/w</th>
<th>CHN (gm)</th>
<th>%w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PM 50</td>
<td>2.5</td>
<td>50</td>
<td>2.5</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>PM 60</td>
<td>3.0</td>
<td>60</td>
<td>2.0</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>PM 70</td>
<td>2.8</td>
<td>70</td>
<td>1.2</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>PM 80</td>
<td>2.4</td>
<td>80</td>
<td>0.6</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>PM 90</td>
<td>1.8</td>
<td>90</td>
<td>0.2</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 2. Composition of CBZ solid dispersions**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Batch Code</th>
<th>CBZ (gm)</th>
<th>%w/w</th>
<th>CHN (gm)</th>
<th>%w/w</th>
<th>0.1 M AA* (ml)</th>
<th>DCM (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SD 50</td>
<td>2.5</td>
<td>50</td>
<td>2.5</td>
<td>50</td>
<td>12.5</td>
<td>50</td>
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<tr>
<td>2</td>
<td>SD 60</td>
<td>3.0</td>
<td>60</td>
<td>2.0</td>
<td>40</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>SD 70</td>
<td>2.8</td>
<td>70</td>
<td>1.2</td>
<td>30</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>SD 80</td>
<td>2.4</td>
<td>80</td>
<td>0.6</td>
<td>20</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>SD 90</td>
<td>1.8</td>
<td>90</td>
<td>0.2</td>
<td>10</td>
<td>1</td>
<td>04</td>
</tr>
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</table>

**Table 3. Pharmaceutical characterization of CHN acetate based formulation**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Formulation batch</th>
<th>% Content Uniformity ± SD</th>
<th>pH ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PM 50</td>
<td>98.7 ± 1.1</td>
<td>3.6 ± 1.8</td>
</tr>
<tr>
<td>2</td>
<td>PM 60</td>
<td>97.3 ± 0.8</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td>PM 70</td>
<td>100.2 ± 0.6</td>
<td>3.9 ± 1.2</td>
</tr>
<tr>
<td>4</td>
<td>PM 80</td>
<td>98.5 ± 2.8</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>PM 90</td>
<td>98.7 ± 1.7</td>
<td>4.7 ± 0.7</td>
</tr>
<tr>
<td>6</td>
<td>SD 50</td>
<td>99.5 ± 0.7</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>7</td>
<td>SD 60</td>
<td>102.0 ± 3.4</td>
<td>4.1 ± 1.2</td>
</tr>
<tr>
<td>8</td>
<td>SD 70</td>
<td>98.4 ± 1.2</td>
<td>4.5 ± 0.8</td>
</tr>
<tr>
<td>9</td>
<td>SD 80</td>
<td>98.6 ± 0.5</td>
<td>4.5 ± 1.1</td>
</tr>
<tr>
<td>10</td>
<td>SD 90</td>
<td>96.5 ± 1.4</td>
<td>4.3 ± 0.6</td>
</tr>
</tbody>
</table>

*Pharmaceutical characterization, Measurement of pH*: 100 mg prepared solid dispersions and physical mixtures were dispersed in 10 ml of deionized water. pH of the solutions were monitored using digital pH meter. All samples were analyzed in triplicate.

*Content uniformity studies*: Solid dispersion and physical mixture equivalent to 12.5 mg was dissolved in required quantity of distilled water in a 250 ml volumetric flask and sonicated for 20-25 min and volume made up to 250 ml with distilled water to get the concentration of CBZ within linearity range. The absorbance was measured at 287.5 nm against blank reagent using a UV Visible spectrophotometer. The concentration of the drug present in the formulation was computed from the calibration curve. Each sample was assayed in triplicate.

*Differential scanning calorimetric studies*: Differential scanning calorimetric (DSC) analyses of the drug, carrier, solid dispersion formulation and corresponding physical mixtures were carried out by using
Shimadzu TA –60 differential scanning calorimeter equipped with computer analyzer (Shimadzu Corporation, Kyoto, Japan). Samples of (3-7 mg) were heated under nitrogen atmosphere on an aluminum pan at a heating rate of 10°C/min over the temperature range of 100-210°C.

**Powder X-ray diffraction studies:** Powder X-ray diffraction (PXRD) patterns were traced employing X-ray diffractometer (Philips PW 1729) for the samples using Ni filtered CuKα radiation, a voltage of 35KV, a current of 20mA and receiving slit of 0.2 inches. The samples were analyzed over 2θ range of 5-80° with scanning step size of 0.020° (2θ) and scan step time of one second.

**Fourier transform infra red spectroscopy:** FT-IR spectra of prepared formulation were recorded on Shimadzu FT IR – 8400 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Potassium bromide pellet method was employed and background spectrum was collected under identical situation. Each spectrum was derived from single average scans collected in the region 400 – 4000 cm⁻¹ at spectral resolution of 2 cm⁻² and ratioed against background interferogram. Spectra were analyzed by software supplied by Shimadzu.

**In vitro dissolution studies:** In vitro dissolution was evaluated using conventitional dissolution test. Powder dissolution studies were carried out first on pure drug and secondly on solid dispersion with the corresponding physical mixture. Each test was carried out in 900 ml dissolution medium at 37°C (n=6) with a six flasks USP type II dissolution apparatus (Lab India Disso 2000, Digital dissolution testing apparatus). The dissolution media used was distilled water. An accurately weighed quantity of each sample equivalent to 200 mg of CBZ was subjected to the test. To avoid the aggregation of powder in contact with dissolution medium, 200 mg of samples were treated with 10 gm of silica beads. Dissolution studies were conducted in 900 ml of distilled water as a medium. Samples were taken at appropriate time interval. The volume of dissolution medium was kept constant throughout the run by replacing the removed samples with an equivalent volume of fresh dissolution medium. Samples were filtered through 0.44 μ filter, suitably diluted and analyzed at 287.5 nm by using a UV Vis spectrophotometer.

**Results and Discussion**

**Pharmaceutical characterization, Measurement of pH:** pH of the formulation is very important not only for release and absorption of drug from GIT but also to influence the gastrointestinal toxicity. Acetic acid was added to convert the CHN to CHN acetate; hence the free acetic acid, which is liberated from the formulation, may affect the normal pH of GIT. In all the solid dispersion formulations, pH was found to be within the range of 3.4-4.7 (Table 3). The acidic microenvironment can be also favor for ionization of weakly basic drug like CBZ and hence improve solubility and dissolution of CBZ in GI fluids. Moreover, the pH range obtained from the studies was found to be slightly acidic that are less than that of GIT pH in both fasted state and fed state condition. The prepared formulations may not show gastric irritation and hence it was found to be suitable for oral administration.

**Content uniformity studies:** The content uniformity studies revealed that all the prepared formulations were within the range of 96.31 -102.07% (Table 3). The content uniformity of the prepared formulations was within the limits that are specified in pharmacopeia.

**Differential scanning calorimetric studies:** DSC thermogram of CHN acetate, physical mixtures and solid dispersions are illustrated in Figure 1. CHN acetate showed the endothermic peak at 102 °C, which represented the liberation of water. CHN acetate was freeze dried to obtain a solid mass. However, the freeze dried product showed some extent of water that was identified by DSC thermogram.
There were no additional peaks observed with CHN acetate, indicating the stability of the polymer up to 200 °C. This result indicated that CHN acetate was neither decomposed nor charred in the operation temperature and hence it is suitable for pharmaceutical thermal processes such as drying, granulation etc. CBZ showed a first melting endothermic peak at 175.61 °C with the fusion enthalpy of 13.7 J/g followed by a second endothermic peak at 191.70 °C with the fusion enthalpy of 105.88 J/g. These two endothermic peaks correspond to form III and I of CBZ, respectively (Lowes et al. 1987). The DSC thermogram of prepared formulations revealed that there was no endothermic peak at 100°C. In case of formulations, during the freeze drying process, entire water was removed. The suggested reason for the absence of water is reduced viscosity of the polymer by addition of drug may facilitate the removal of water. The peak intensity of endothermic peak obtained by physical mixtures and solid dispersion formulation showed reduction in crystallinity. Moreover, the reduction in crystallinity was dependent on the concentration of CHN acetate present in the formulations. More reduction in crystallinity was observed with solid dispersion than physical mixture. However, there was compete loss of crystallinity with either solid dispersion or physical mixture formulation.
**Figure 2.** PXRD of A. CBZ, B. CHN acetate, C. PM 50, D. PM 90, E. SD 50, F. SD 90.

*Powder X-ray diffraction studies:* PXRD pattern of all the samples are shown in Figure 2. CBZ anhydrous form III has diagnostic peak at 2θ of 15.24, 15.74 and 17.19°, which matched with pure CBZ form III as reported in literature (Katzhendler et al. 1998). PXRD pattern of treated drug was found to be similar that of CBZ. The process of either solvent or acetic acid didn’t change the crystallinity of the drug. Moreover, comparison of PXRD pattern of CBZ and treated drug revealed that there was no significant change in the peak intensity of the both drug as well as treated drug. CHN didn’t show any peak, which indicated the amorphous nature of the polymer. However, a low intensity peak was observed with CHN acetate, which confirmed formation of CHN acetate crystals. All prepared formulations had shown inferior peak intensity when compared with pure drug and treated drug. The peak intensity was dependent on the concentration of polymer. High polymer concentration yielded low peak intensity and hence it was confirmed that polymer concentration reduced the crystalline nature of the drug. Solid dispersion formulation had shown low peak intensity than their corresponding physical mixture formulations. There was no correlation between crystallinity and rate and extent of dissolution. The suggested reason might be physical interaction between drug and CHN retarding the drug release, even though the drug existed in low crystalline state.
**Fourier transform infrared spectroscopy:** FTIR spectra of CBZ, CHN, CHN acetate, physical mixtures and solid dispersions were recorded and illustrated in Figure 3. CBZ was identified with the peaks at 3473.19 cm\(^{-1}\) and 3339.78 cm\(^{-1}\) corresponding to symmetric and asymmetric N-H stretching in primary amide group, respectively. The C=O stretching from amide group of the drug appeared at 1689.55 cm\(^{-1}\). The peak at 1615.70 cm\(^{-1}\) is due to C=C stretching in aromatic ring. FTIR spectra revealed that CHN and its acetate have band (shoulder) appearing at 2917 cm\(^{-1}\) due to NH2 stretching vibration. However, the shoulder peak appearance was found to have significant difference between CHN and CHN acetate. The peak 3400 cm\(^{-1}\) represented the OH group of the CHN, whereas there was no characteristic peak that represents the OH group in CHN acetate. Furthermore, the high frequency shorter wavelength peak at 1743 cm\(^{-1}\) C=O stretching in CHN acetate represents the formation of CHN acetate. There was no difference in FTIR spectra of CBZ and CBZ treated with acetic acid. The results revealed that there was no known chemical degradation of CBZ in presence of acetic acid. This result was further confirmed by DSC and UV visible absorption spectra studies. All the physical mixtures and solid dispersion FTIR spectra revealed that there was no chemical interaction between the drug and polymer.

**In vitro dissolution studies:** In vitro dissolution profile of CBZ, physical mixtures and solid dispersions are illustrated in Figure 4 and 5. In vitro dissolution studies of CBZ and CBZ
treated with acetic acid-DCM revealed that there was no difference in dissolution profile of both treated and untreated drug. DE$_{30 \text{ min}}$ of CBZ was found to be 38.91%, whereas 39.44% with treated CBZ. The research finding states that there was neither effect of DCM nor acetic acid on dissolution of CBZ. In contrast to previous studies (Sawayanagi et al. 1982), high polymer ratio retards the drug release in both solid dispersion and physical mixture formulations. 50% w/w of CBZ contained solid dispersion formulation has shown minimum drug release of 52.44% in 60 min. The increasing extent of CBZ was obtained up to 70% w/w of CHN and above it the drug release was independent of polymer concentration in solid dispersion formulations. The maximum release of 90.81% was achieved with formulation containing 70% of CHN. The suggested reason for retardation of drug release in high proportion polymer formulations is gelling ability and/or complex forming nature of CHN.

**Figure 4.** Dissolution profile of CHN acetate based solid dispersion formulations

All the physical mixture formulations had shown low release profile than solid dispersion formulation except 50% w/w of CHN formulation. The maximum release of 66.69% was achieved with low CHN contained formulation (PM 90). However, there was no statistical difference in drug release was found in all physical mixture formulation. Moreover, the drug release was neither dependent on the concentration of polymer nor CBZ. The minimum release of 45.18% was achieved with PM 80, but the reason for low release was not clear and unknown. It may be concluded from the dissolution studies that CHN acetate based formulation may be a useful dosage form for biopharmaceutical optimization of BCS Class drug such as CBZ. Further research like optimization of suitable technology is required to develop this formulation.

**Figure 5.** Dissolution profile of CHN acetate based physical mixture formulation.
Conclusion

It can be concluded from the present investigation that in situ formation of CHN acetate is feasible during the granulation by addition of acetic acid. Addition of acetic acid during the formation of CHN acetate may not have any influence on either physical or chemical stability of the drug like CBZ. It was found that freeze drying process was considered as a best technique for drying the solid dispersion that results in absence of moisture liberated during formation of CHN acetate. Analysis of surface pH of the solid dispersion formulation proved that the prepared formulations are in the physiological pH range that may not cause any irritation or toxicity to gastrointestinal tract. The sharp endothermic peak in DSC thermogram revealed that the drug neither interacted with polymer nor decomposed during formation of CHN acetate based solid dispersions. PXRD studies stated that there was profound reduction in crystallinity of both physical mixtures and as well as solid dispersion formulation. However, as observed by amorphous nature of the drug was obtained by this technique. Both physical mixture and solid dispersion formulation showed better dissolution profile than that with pure drug alone and the drug treated with acetic acid. Moreover, expected dissolution enhancement was observed with both physical mixture and solid dispersion formulation but the effect of polymer concentration on drug release was unclear. Other processing techniques such as melt extrusion (because of high chemical stability of CBZ), dry granulation and spray drying should be investigated to improve dissolution and bioavailability of CBZ loaded CHN acetate formulation that may provide clue of effect of processing technique on formulation.

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