CHAPTER III

DEVELOPMENT OF NOVEL CHITOSAN MICROPARTICLES AND FORMULATION OF TABLETED MICROPARTICLES FOR THE CONTROLLED RELEASE OF CLOZAPINE

This chapter presents the experimental results on the development of novel chitosan microparticles (MPs) loaded with clozapine and their formulation as tableted microparticles for the controlled release (CR) of clozapine. The first part (III.A) of this chapter presents the production and characterization of chitosan MPs prepared by a novel sieving method. The second part (III.B) reports on the formulation of these chitosan MPs as tableted dosage forms for the evaluation of CR of clozapine.
III.A. Development and Evaluation of Clozapine Loaded Chitosan Microparticles Prepared by a Novel Method

Abstract

A simple and commercially viable method of preparation of chitosan microparticles (MPs) was adopted for the entrapment of clozapine, which can be easily scaled-up as the CR dosage form. This method is devoid of tedious processes like emulsification in oil phase, spray-drying, etc. MPs have been prepared by changing the experimental variables such as extent of crosslinking and amount of clozapine loading in order to optimize the process variables on the final drug entrapment efficiency, size of MPs and release rates. Absence of chemical interactions between drug, polymer and crosslinking agent after the production of MPs was confirmed by Fourier transform infrared spectroscopy (FTIR). Differential scanning calorimetry (DSC) and x-ray diffraction (x-RD) spectra were obtained for the clozapine-loaded chitosan MPs to understand the crystalline nature of the drug after entrapment. The results indicated a molecular level dispersion of clozapine in the polymer matrix.

Effect of crosslinking and drug loading on the thermal decomposition of chitosan was studied by thermogravimetry (TGA) and these data indicated that pure chitosan is stable as compared to clozapine-loaded chitosan. MPs produced were irregular in shape, with the average particle sizes ranging from 543 to 698 µm, as measured by laser light scattering technique. Clozapine entrapment up to 98.85 % was obtained as determined by the high performance liquid chromatography (HPLC). In-vitro release studies were performed in the phosphate buffer pH 7.4 solution and the release of clozapine was achieved up to 12 h. Swelling studies were conducted in water and diffusion coefficients (D) and diffusional exponents (n) for water transport were determined using the empirical equation. In-vivo absorption kinetics of clozapine and clozapine-loaded MPs were investigated in albino rats. These results indicated that absorption of clozapine from the MPs was delayed since the area under the curve was higher as compared to the neat clozapine.

III.A.1. Introduction

In recent years, biodegradable and biocompatible polymeric micro/nanoparticles have attracted a considerable attention as potential carriers for the controlled and site-specific delivery of drugs [1-4]. Chitosan, a deacetylated derivative of chitin, is a naturally occurring polysaccharide, found abundantly in marine crustaceans, insects and fungi. Chitosan is a cationic, biocompatible, and biodegradable polymer having many biomedical applications [5,6]. However, chitosan is insoluble in neutral or basic conditions because of the presence of free amino groups and hence, it requires the acidic pH media for complete solubilization. In acidic pH, amino groups can undergo protonation and the polymer swells. Hence, chitosan-based drug delivery devices could release most of the active ingredient in the stomach [7].

Initial burst effect is a common phenomenon with chitosan-based delivery systems when loaded with water-soluble drug and hence, their utility in the CR of drugs in gastrointestinal tract (GIT) is questionable [8]. Chitosan MPs prepared by the conventional methods have shown a porous surface due to surface-adhered free drug. Chitosan MPs loaded with water-soluble drug when brought in contact with acidic media, tend to release the active agent quite fast and thereby, resulting in a burst effect [8]. To avoid such associated initial burst effect problems, attempts have been made to modify the method of preparation of MPs [7] by coating or grafting with other polymers [9,10]. The molecular weight of chitosan has shown somewhat insignificant effect on the drug release, while the MPs of both high and low molecular weight chitosans have shown the burst effect. However, the MPs of high molecular weight chitosan showed poor wetting properties, which would help to decrease the drug release rates [11]. Majority of the methods reported in the earlier literature are based on either emulsion-crosslinking or spray-drying techniques [7,11-13]. These methods involve various critical steps that are difficult to scale-up due to intrinsic disadvantages such as burst release, thereby making them commercially less viable.
In the present chapter, the novel method of producing MPs is described. The method is easy to scale-up from laboratory to production scale, and involves simple procedures. The model drug selected, clozapine, chemically known as 8-chloro-11-methyl-(4-methyl-1-piperizinyl)-5H-dibenzo (b,e) (1,4) diazepine (1,2), an anti-psychotic agent, is used in the management of schizophrenia. Its structure is given in Figure III.A.1. Clozapine (Clozaril®) is clinically available in the form of immediate release tablets of 25 mg and 100 mg strengths. Clozapine shows wide inter individual variations in the plasma half-life with the mean value of $6.0 \pm 1.5$ h [14]. Even though clozapine is well absorbed from the GIT, it undergoes extensive first pass metabolism and only 27-50 % of the dose reaches systemic circulation unchanged. Hence, its formulation in CR form is very important. Moreover, the use of extended-release products offers potential advantages like sustained blood levels, attenuation of adverse effects and improved patient compliance. Particularly, in case of psychosis, patient is unable to take medication frequently; hence, the development of CR formulations is necessary for patient compliance. Thus, formulating clozapine in an extended release form would increase the therapeutic efficacy and patient compliance. In this chapter, we have prepared the MPs containing clozapine and studied their in-vitro/in-vivo drug release characteristics.

III.A.2. Results and Discussion

III.A.2.1. Preparation and Characterization of MPs

The novel and inexpensive method was developed for the production of chitosan MPs. Many studies have been reported in the earlier literature to evaluate the safety of glutaraldehyde, which has been proven to be noncarcinogenic and safe [15, 16]. Crosslinking of chitosan was done using GA along with clozapine. A simple and commercially viable method was developed for the production of MPs. However, if the scale-up procedure is to be adapted from the laboratory scale to a pilot plant level or even production scale (i.e., factory level), the fully automated sieving techniques are
recommended. Scrapping the particles after passing through the sieve at regular intervals of time can alleviate the agglomeration of MPs during their production. By this method, % entrapment efficiency was found to be in the range between 91.14 and 98.97.

Figure III.A.1. Chemical structure of clozapine.

FTIR was used to confirm the chemical stability of clozapine in chitosan MPs. FTIR spectra of pure chitosan (a), placebo MPs (b), clozapine-loaded MPs (c) and pure clozapine (d) are compared in Figure III.A.2. In case of pure chitosan, the characteristic band due to N-H bending vibration is observed at 1653 cm\(^{-1}\) However, after crosslinking chitosan with GA, the band due to N-H bending vibration has split into two bands at 1653 and 1560 cm\(^{-1}\) due to the formation of imine group [17]. Clozapine has shown the characteristic bands at 2968 and 2931 cm\(^{-1}\) due to the aliphatic C-H stretching. The bands at 1590 and 1551 cm\(^{-1}\) are due to C=N stretching vibrations, while those at 1462 and 1431 cm\(^{-1}\) are due to the aromatic C=C stretching vibrations. Clozapine has also shown a characteristic band due to C-Cl stretching around 820 cm\(^{-1}\). These data are in conformity with the earlier report [18].
Figure III.A.2. FTIR spectra of pure chitosan (a), placebo MPs (b), clozapine-loaded MPs, (c) and pure clozapine (d).
When the drug is incorporated into the crosslinked chitosan MPs, in addition to the characteristic bands of the crosslinked chitosan, extra bands have appeared due to the presence of clozapine in the matrix. However, some bands of clozapine are not prominent in the drug-loaded MPs due to identical stretching observed at frequencies 2863 and 1553 cm\(^{-1}\) of the placebo chitosan MPs as well as that of the drug-loaded MPs at the same wavenumber. The characteristic bands of clozapine observed at 1431 and 1462 cm\(^{-1}\) due to the aromatic C=C stretching and the band due to the C-Cl stretching at 820 cm\(^{-1}\) have also appeared in the drug-loaded matrix without any change, which further indicates that clozapine has not undergone any chemical change during the production of MPs.

TGA experiments were carried out on pure chitosan (a), drug-loaded MPs with 5.0, 7.5 and 10.0 \(\times\) \(10^{-4}\) mL of GA (b, c and d, respectively), while the curve for pure clozapine (e) are presented in Figure III.A.3. Chitosan starts to decompose after 100°C, but the weight loss remains constant up to 310°C. A sudden weight loss is observed after 310°C and the weight loss around 400°C is 47.4 %. Whereas, the crosslinked clozapine-loaded chitosan starts to decompose at 260°C, due to the decomposition of clozapine above its melting point. The drug-loaded crosslinked MPs have shown more weight loss when compared to the neat chitosan due to higher clozapine content in the matrix (50 % of dry weight of chitosan). However, significant weight loss is observed for the clozapine-loaded MPs than the pure chitosan. Melting point of clozapine is 182°C as presented by curve (e) and around 300°C, only 0.5 % of the char residue remains. Higher weight loss is due to the decomposition of the drug above 200°C. These results indicate that the drug-loaded MPs follow a very systematic decomposition pattern when compared to either the pure drug or the polymer. Since the melting point of the drug is not altered by encapsulation, which suggests the absence of its chemical interactions with the polymer, as supported by FTIR.
Figure III.A.3. TGA thermograms of pure chitosan (a), drug-loaded MPs with 5.0, 7.5 and $10^4$ mL of glutaraldehyde ((b), (c), and (d), respectively) and pure clozapine (e).
DSC thermograms of pure clozapine (a), pure chitosan (b), placebo MPs (c) and clozapine-loaded MPs crosslinked with 5.0, 7.5 and 10.0 x 10^{-4} mL of GA (d, e and f, respectively) are presented in Figure III.A.4. The polymorphism of clozapine and glass transition temperature (T_g) of the polymer was determined before and after crosslinking as well as before and after drug loading. Pure chitosan has shown an endothermic peak at 53°C, which corresponds to its T_g. After crosslinking with 7.5 x 10^{-4} of mL GA, the endothermic peak for the placebo (c) MPs has shifted to 83°C, indicating the T_g value of the placebo. The endothermic peaks for the drug-loaded MPs viz., curves (d), (e), and (f) have appeared systematically at higher temperatures with increasing crosslinking, thus making the polymer matrix more rigid and thereby shifting the endothermic peak to higher temperature. The T_g obtained from (d), (e), and (f) curves are respectively, 51°C, 54°C and 64°C. However, the changes in T_g in all the drug-loaded crosslinked MPs have shown higher values than the pure chitosan. Such a significant change in T_g may be due to physical and morphological changes of MPs after drug loading.

The x-ray diffraction spectra recorded for the placebo MPs (a), clozapine-loaded MPs (b) and pure clozapine (c) are presented in Figure II.A.5. These studies are useful to investigate the crystalline nature of the drug in the crosslinked MPs. Clozapine has shown the characteristic intense peaks between 2θ of 9 and 11° due to the presence of clozapine crystals. However, these peaks were not observed in the clozapine-loaded MPs, but instead only peaks observed in placebo were seen. The x-ray diffraction peak always depends upon the crystal size, but in the present study, for all the drug-loaded concentrations, characteristic peaks of clozapine overlap with the noise of the coated polymer itself. Further, the loaded drug is amorphous, which is very difficult to measure at the detection limit of the crystal size in the present research. This indicates that drug is dispersed at the molecular level in the polymer matrix and hence, no crystals were found in the drug-loaded matrices.

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Figure III.A.4. DSC thermograms of pure clozapine (a), pure chitosan (b), placebo MPs (c) and clozapine-loaded MPs crosslinked with 5.0, 7.5 and 10.0 x 10^{-4} mL of glutaraldehyde ((d), (e) and (f), respectively).
Figure III.A.5. X-RD diffractograms of placebo MPs (a), clozapine-loaded MPs (b) and pure clozapine (c).
MPs produced were subjected to SEM analysis as shown in Figure III.A.6. Particles are irregular in shape with rough surfaces. SEM micrographs reveal the presence of surface adhered drug. MPs produced were also subjected to the laser light diffraction technique (Mastersizer-2000, Malvern, UK) to know their size and size distribution. The volume-mean particle size of the MPs produced for these matrices with three different extents of crosslinking as well as three different % drug loadings are presented in Table III.A.1. These results indicate that as the crosslinking increases, volume-mean particle size decreases. Even though all the particles prepared were passed through the 250-micron size sieve, particles with different volume-mean particle size were formed. This is due to the formation of the rigid matrix with a reduction in particle size during drying process at higher crosslinking [19]. As the amount of clozapine increases in chitosan MPs, the volume-mean particle size also increased because clozapine might have occupied the interstitial spaces between the polymer segments.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>% Entrapment efficiency</th>
<th>Volume mean particle size (µm)</th>
<th>n</th>
<th>$D \times 10^6$ (cm$^2$/s)</th>
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<td>8.92</td>
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<tr>
<td>F2</td>
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<td>0.219</td>
<td>8.63</td>
</tr>
<tr>
<td>F3</td>
<td>96.77</td>
<td>543</td>
<td>0.188</td>
<td>8.16</td>
</tr>
<tr>
<td>F4</td>
<td>93.14</td>
<td>674</td>
<td>0.249</td>
<td>8.45</td>
</tr>
<tr>
<td>F5</td>
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<td>599</td>
<td>0.226</td>
<td>7.75</td>
</tr>
<tr>
<td>F6</td>
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<td>560</td>
<td>0.160</td>
<td>7.08</td>
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<tr>
<td>F7</td>
<td>98.97</td>
<td>698</td>
<td>0.220</td>
<td>9.95</td>
</tr>
<tr>
<td>F8</td>
<td>97.21</td>
<td>639</td>
<td>0.217</td>
<td>8.28</td>
</tr>
<tr>
<td>F9</td>
<td>97.74</td>
<td>583</td>
<td>0.202</td>
<td>8.04</td>
</tr>
</tbody>
</table>
Figure III.A.6. SEM micrographs of group of MPs (a) and single MP (b).
III.A.2.2. Swelling Studies

MPs produced with different extent of crosslinking were subjected to dynamic swelling studies in water. Swelling data were further analyzed by an empirical equation [20] to predict the drug release by using % water uptake data of the MPs crosslinked with (5.0, 7.5 and 10.0) x 10^{-4} mL of GA loaded with 50 % of clozapine. The data shown in Figure III.A.7 suggest that as the matrix crosslinking increases, swelling capacity of the MPs decreases. MPs having the least extent of crosslinking have shown the maximum swelling up to 180 % per dry weight of the polymer. All the MPs have shown more than 50 % swelling instantly within the first minute and later, swelling becomes slow. This indicates that only water might have transported within the porous polymer matrix. The initial 60 % of the total water uptake data have been fitted to Eq. (III.A.1) to investigate the mechanism of water transport through the MPs [20].

\[ \frac{M_t}{M_\infty} = k t^n \]  

(III.A.1)

In the above equation, the least-squares method was used to estimate the values of \( k \) and \( n \) at 95 % confidence limit. The results of \( n \) presented in Table III.A.1 are in the range of 0.160 to 0.249, indicating that the drug release deviates from the Fickian trend. The calculated values of \( n \) are < 0.5 due to irregular shaped particles. The values of \( n \) decrease systematically with increasing extent of crosslinking.

Diffusion coefficient of water within the MPs was calculated [19] by using Eq. (III.A.2),

\[ D = \left( \frac{r \theta}{6 M_w} \right)^2 \pi \]  

(III.A.2)
where $\theta$ is slope of the linear portion of the plot of $M_t/M_\infty$ vs. $t^{1/2}$, $r$ is radius of the particles and $M_\infty$ is the maximum sorption value. It may be noted that diffusion coefficients have been estimated based on the Fickian diffusion model. Diffusion coefficients calculated are in the range of $(7.0$ to $9.9) \times 10^{-6}$ cm$^2$/s and, these values are found to depend on the extent of crosslinking. For instance, $D$ values show a systematic decrease with increasing crosslinking of the matrix in all the formulations. This is obvious because of the increased rigidity of the chain due to increased crosslinking, thereby prohibiting the transport of more of water molecules.

**III.A.2.3. In-vitro Drug Release**

*In-vitro* drug release was performed in pH 7.4 phosphate buffer solution for 12 h. Results of % drug release vs. time for pure clozapine, and clozapine-loaded MPs crosslinked with $(5.0$, $7.5$ and $10.0) \times 10^{-4}$ mL of GA with 50 % of...
clozapine loading are presented in Figure III.A.8, whereas MPs loaded with 25,
50 and 75 % of clozapine crosslinked with 10.0 x 10^-4 mL of GA are presented
in Figure III.A.9. Dissolution of pure clozapine occurred very fast i.e., it is
completed within the first two hours (see Figure III.A.8). On the other hand,
the drug-loaded formulations released clozapine slowly over a period of 12 h. It
may be noted that the release rates increased with decreasing crosslinking
because the loosely crosslinked matrices allow a faster diffusion of the drug
into the dissolution media. It may be noted that the initial release studies were
carried out in 0.1 N HCl in the first hour, wherein we could observe the burst
release. The initial release was 30-40 % of the total drug loading. Since the
conversion of a glassy to a rubbery polymer could take place very fast in the
acidic media, the initial drug release may be governed by the polymer water
uptake capacity, which is related to the polymer chain relaxation. The drug
release was almost identical in all the formulations prepared. However, the
burst release was observed in all formulations, but the release was extended up
to 12 h. Such an instant burst release could help to maintain the patient
therapeutic regime, while the sustained release will maintain the plasma
concentration level.

In an effort to study the effect of drug loading on the release rates, we
have taken the formulations F3, F6 and F9 with the respective weight % of the
drug loading (25, 50 and 75 %) and compared their release rates with the pure
clozapine drug. Here also, we found that the release rates vary depending upon
the amount of drug present in the matrices, i.e., the release was slower for those
formulations having lower amount of the drug. However, the burst release is
again prevalent for these systems, suggesting the rigidity of the polymer
matrix. Release of the drug from chitosan MPs involves three different
mechanisms: (a) release from the surface of the particles, (b) diffusion through
the swollen rubbery matrix and (c) release due to polymer erosion. These
mechanisms are schematically presented in Figure III.A.10. Initially, higher
release rates are observed due to the dissolution of the surface-adhered drug. At
Figure III.A.8. Effect of different crosslink densities on in-vitro release profiles.

Figure III.A.9. Effect of different percent drug loading on in-vitro release profiles.
longer time, the drug release is due to the diffusion process, which is much slower when compared to the initial release. None of the formulations have shown the 100% release, but complete release of the drug from the matrix occurs only after the complete erosion or degradation of the chitosan matrix.

![Diagram of drug release mechanism](image)

**Figure III.A.10.** Mechanism of drug release from particulate systems.

**III.A.2.4. In-vivo Drug Absorption**

Absorption patterns of pure clozapine as well as clozapine-loaded MPs have been studied in albino rats given through the peroral route. The amount of clozapine reaching the blood at different times was analyzed by HPLC. The HPLC curves for the plasma of control group animals (a), plasma of test group animals (b), and pure clozapine dissolved in mobile phase (c) are presented in Figure III.A.11. The retention time (RT) for pure clozapine as well as clozapine extracted from the plasma were observed between 3.0 and 3.1 min. The administration of clozapine and clozapine MPs was done along with 2% acacia
gum to avoid the settling of MPs before the administration. The amount of unchanged clozapine reaching the plasma at different time intervals in test (a) and standard (b) group animals are presented in Figure III.A.12. These studies were performed in triplicate for each of the formulations and the results are described as the mean ± SD in Figure III.A.12. Absorption of pure clozapine reached a maximum peak (t_{max}) at 3 h, which is comparable with the literature data [14]. Afterwards, its concentration decreased showing the maximum drug concentration (C_{max}) of 7.5 ± 0.6 µg/mL in the plasma. However, the absorption of clozapine from the MPs has shown different trends. The highest clozapine concentration (C_{max}) in the plasma was 6.8 ± 0.5 µg/mL at 3 h from MPs.

It may be noted that even though, clozapine is well absorbed from GIT, it undergoes extensive first pass metabolism. The increase in area under the curve (AUC as calculated by the trapezoidal method) can be achieved by delaying its absorption from the GIT. AUC_{0-10} for the test group was found to be 57.0 µg.h/mL, while for the standard, it was 45.5 µg.h/mL. The statistical evaluation of differences between AUC_{0-10} of the test group and the standard group was performed by Student’s t-test. The results indicate that the calculated value of AUC_{0-10} of the test group is statistically significant when compared to that of the standard group (P < 0.05). These results suggest that there is a significant increase in AUC_{0-10} and a decrease in C_{max} of the test group when compared to the standard group. The decrease in plasma concentration at 8 h is less in test group than that of the standard group. These results suggest that the amount of clozapine in the plasma is increased when the clozapine-loaded MPs were administered due to delayed absorption of clozapine. This clearly indicates that the absorption of clozapine from the MPs is delayed. Improvement in the plasma concentration was observed when clozapine was administered in controlled dosage form. This may be because of the reduction in the first pass metabolism, which in turn, increases the amount of drug reaching the systemic circulation unchanged.
Figure III.A.11. HPLC curves of plasma of control group animals (a), plasma of test group animals (b) and pure clozapine dissolved in the mobile phase (c).
Figure III.A.12. *In-vivo* absorption of clozapine from clozapine solution and clozapine-loaded MPs in albino rats.

### III.A.3. Conclusions

Chitosan-based MPs prepared by the novel sieving technique were irregular in shape with a narrow size distribution. Clozapine was stable in the chitosan matrices without undergoing any chemical changes during the particle production. The *in-vitro* release studies indicated that the MPs produced could be used as the effective CR devices for the release of clozapine. The release of clozapine was found to depend on the extent of matrix crosslinking as well as drug loading. *In-vivo* studies indicated that the absorption pattern of clozapine from the MPs followed in a controlled manner.
III.A.4. Literature Cited


III.B. Formulation and Evaluation of Novel Tableted Chitosan Microparticles for the Controlled Release of Clozapine

Abstract

This section presents results on the formulation of clozapine-loaded chitosan microparticles as tableted dosage and characterization of the tablets. Controlled release formulations of clozapine microparticulate tablets were prepared by using chitosan. Microparticles were characterized for particle size and size distribution. Microparticles were compressed into tablets using the directly compressible excipients. SEM photographs of the fractured part of the tablet revealed the presence of discrete particles in the tablets, suggesting that the system chosen is ideal for tableting. Drug release from the tableted microparticles exhibited an initial burst effect, but the release decreased with increasing extent of crosslinking. Tablets were coated with chitosan or cellulose acetate, which significantly lowered the initial burst effect when compared to the uncoated tablets. Drug release from the chitosan-coated tablets was slightly higher than the tablets coated with cellulose acetate. Tablets prepared were effective in delivering clozapine over a period of 12 h.

Results of this section have appeared in: J. Microencapsulation, 21 (2004) 709-718.
III.B.1. Introduction

Oral controlled release (CR) multiple unit dosage forms such as microparticles, beads and pellets are gaining considerable importance and in recent years in view of their added advantages over the conventional single unit formulations. Once the tablet or capsule containing multiple units disintegrate, particles spread uniformly throughout the GIT. This will avoid the release of the drug at one particular site thus, avoiding the risk of toxicity caused by the locally restrained tablet within the GIT. Uniform distribution of multiple units in GIT results in more reproducible absorption and will reduce the risk of local irritations when compared to the single unit systems [1]. Multiple units can be filled into hard gelatin capsules or they can be compressed into tablets. However, the formulation of multiple units into tablets has the advantage of preventing tampering as in case of capsules, but the merger of these multiple systems during compression can produce variations in the CR of the drug. Among the many biodegradable polymers, chitosan has been used extensively in pharmaceutical research due to its biodegradability and non-toxic nature [2,3]. Chitosan is a naturally occurring polysaccharide, found abundantly in marine crustaceans, insects and fungi. Since chitosan has numerous favorable physico-chemical and biological properties, it has gained widespread applications in pharmaceutical and medical fields [4]. Hejazi and Amiji [5] have summarized the recent advances in chitosan-based gastrointestinal delivery systems.

Drugs can be released in a controlled manner by entrapping or encapsulating them into particulate systems. Recently, Aminabhavi et al., [6-8] have demonstrated that CR of drugs can be achieved by encapsulating them into the particulate systems. Tableting of microparticles has been reported to reduce the release of drugs, which results as CR formulation [9,10]. After tableting of microparticles, the particles may remain intact within the tablet without undergoing merging or rupturing and hence, the drug release will take place from the individual microparticles; if not the microparticles may merge.
or rupture to become bigger compacts. In such cases, the release will occur from compacts in the tablet formulation. Ideally, the drug release should occur from the individual particles, which should not be affected from the compression process. However, the excipients used in tableting should provide sufficient cushioning effect to withstand the compression force and thereby, prevent the merging or rupturing of the microparticles.

In our continuing research on developing the particulate drug delivery systems, we have recently developed a simple and commercially viable method of producing chitosan microparticles for the CR of clozapine [11]. In continuation of this study, we now prepare the microparticles encapsulated into tablets to develop the novel CR dosage forms. Clozapine, an anti-psychotic agent, used in the management of schizophrenia, was chosen as a model drug. Even though clozapine is well absorbed from GIT, it undergoes extensive first pass metabolism and about 27-50 % of the dose reaches systemic circulation unchanged. However, by using the extended-release dosage forms, potential advantages like sustained plasma levels, attenuation of adverse effects, improved therapeutic efficacy and patient compliance can be achieved. The formulations developed in this research have been characterized by scanning electron microscopy, particles size analyzer and in-vitro drug release characteristics.

III.B.2. Results and Discussion

III.B.2.1. Physical Characteristics of Tablets

Clozapine-loaded microparticles were compressed into tablets by using directly compressible excipients. Compositions of the tablets are given in Table III.B.1. Tablets were characterized for drug content, hardness, thickness and disintegration time. These data are presented in Table III.B.2. During preliminary studies, tablets were produced without poly(vinyl pyrrolidone) K-30. However, tablets with sufficient hardness to withstand the conditions of friability could not be produced. On the other hand, tablets with good mechanical strength properties were obtained after adding poly(vinyl
pyrrolidone) K-30 with the hardness ranging from 5.4 ± 0.7 to 6.9 ± 0.6 kg/cm². Hardness was found to depend upon the quantity of microparticles in the tablet. Tablets produced with a higher amount of microparticles showed lower hardness. Drug content, friability and thickness of the tablets were well within the acceptable limits.

The drug content was analyzed on five tablets of each formulation individually, but only the mean values with the standard deviation i.e., ± SD are presented in Table III.B.2. The drug content was found to vary between 92.21 ± 1.03 and 97.21 ± 0.97. Since none of the formulations contained any disintegrating agent, the disintegration time was long, which varied between 26 and 34 min. The goal here was to prolong the disintegration time to prevent the burst release effect. During the disintegration of tablets, it was noticed that individual microparticles were separated without any agglomeration of the microparticles. This is indeed advantageous in the CR studies.

III.B.2.2. Particle Size, Size Distribution and Morphology

Particle size and size distributions were analyzed by the laser light diffraction technique (Mastersizer-2000, Malvern, UK). Particles produced by this method were irregular [11]. The volume-mean particle sizes of the microparticles produced by three different cross-linking extents are also included in Table III.B.2. Results indicated that as the extent of crosslinking increases, the volume-mean particle size decreased. On a population basis, particle size distribution was found to be unimodal. The microparticles used for formulations T1, T2 and T3 have shown the mean sizes of 698, 639 and 583 μm, respectively. Results are graphically presented in Figure III.B.1. Even though a wide range of size distribution of microparticles was observed ranging from 200 to 1200 μm, the majority of particles were in between 500 and 800 μm size. SEM photograph of the cross section of the tableted microparticles is given in Figure III.B.2, from which it can be seen that within the tablets, microparticles are present as individual particles without compacting during compression. Since this is an ideal requirement for producing the tableted microparticles, the procedure used in this research is suitable for tableting.
Table III.B.1
Composition of Different Formulations (in mg)

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<th>Tablet content</th>
<th>Drug-loaded microparticles</th>
<th>Pure drug</th>
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<td></td>
<td>T1</td>
<td>T2</td>
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<tr>
<td>Drug-loaded microparticles or pure drug</td>
<td>163.0</td>
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<td>Microcrystalline cellulose</td>
<td>227.5</td>
<td>219.0</td>
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<td>Lactose</td>
<td>227.0</td>
<td>218.5</td>
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<tr>
<td>Poly(vinyl pyrrolidone)</td>
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</tr>
<tr>
<td>Magnesium stearate</td>
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</tbody>
</table>

Table III.B.2
Results of Drug Content, Volume Mean Particle Size and other Properties of the Tablets

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug content (%)</th>
<th>Volume mean particle size (μm)</th>
<th>Hardness (kg/cm²)</th>
<th>Thickness (mm)</th>
<th>Disintegration time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>95.68</td>
<td>698</td>
<td>5.7 ± 0.5</td>
<td>3.78 ± 0.06</td>
<td>30</td>
</tr>
<tr>
<td>T2</td>
<td>94.20</td>
<td>639</td>
<td>5.6 ± 0.4</td>
<td>3.79 ± 0.07</td>
<td>29</td>
</tr>
<tr>
<td>T3</td>
<td>92.21</td>
<td>583</td>
<td>5.4 ± 0.7</td>
<td>3.84 ± 0.07</td>
<td>26</td>
</tr>
<tr>
<td>T10</td>
<td>97.21</td>
<td>-a</td>
<td>6.9 ± 0.6</td>
<td>3.66 ± 0.07</td>
<td>34</td>
</tr>
</tbody>
</table>

-a Not applicable
Figure III.B.1. Particle size distribution of microparticles used in formulating tablets.

Figure III.B.2. SEM photomicrograph of the cross section of tablet containing microparticles.
In-vitro drug release was performed in 0.1 N HCl followed by phosphate buffer pH 7.4 solution to simulate the GIT conditions and the release was continued up 12 h. Formulation of pure clozapine was done using the same excipients and the release was also studied to compare the release rates with that from the tableted microparticles. In the previous study [11], the release of clozapine from microparticles was investigated and these data (F7, F8 and F9) are also utilized here to compare the release from microparticles after tableting. Results of % drug release vs. time from the tableted microparticles and neat microparticles are presented in Figure III.B.3. Release rates of clozapine from the tableted microparticles are smaller than the neat microparticles. Initial high release rates were observed for microparticles due to the immediate dissolution of the surface-adhered clozapine, whereas after tableting, the initial burst release was slightly reduced. This may be due to the smaller contact surface area of the tableted microparticles and coating of the microparticles by the hydrophobic excipients like magnesium stearate during the preparation of tablets.

As observed previously [11], the release rates were found to depend on the extent of crosslinking of the matrix. Release rates decreased with an increase in crosslinking. Among the tableted microparticles studied, T3 showed the least drug release due to higher crosslinking. Comparison of drug release rates from microparticles before and after tableting was statistically evaluated by analysis of variance (ANOVA). The $F$ value was found to be 0.973 (df = 41, $P < 0.05$), which indicates that there is no significant difference in the release rates before and after tableting. In an effort to restrict the entry of water into the tablet and to reduce the burst effect, tablets were coated with either chitosan or cellulose acetate. Results of drug release vs. time for the pure clozapine tablet, uncoated tablet and tablets coated with either chitosan or cellulose acetate are compared in Figure III.B.4. It was noticed that when compared to the uncoated tablets, the coated tablets released clozapine much more slowly and the burst
Figure III.B.3. Comparison of drug release rates of microparticles before and after tableting vs. time for different formulations.

Figure III.B.4. Effect of coating of tableted microparticles on drug release rates vs. time for different formulations.
effect was drastically reduced. Cellulose acetate has been widely used for coating of tablets to reduce the burst effect. In the present study, we have used cellulose acetate for coating and also, we have evaluated chitosan for coating to control the burst release. After coating with chitosan, it was crosslinked in the gaseous phase to control the burst release.

The present results suggest that both cellulose acetate and chitosan coating could control the initial burst release of clozapine. However, due to the hydrophobic nature of cellulose acetate and reduction in swelling capacity of chitosan after crosslinking, it was possible to restrict the entry of water molecules into the tablets. Drug release from the chitosan-coated tablets was slightly higher than that of cellulose acetate-coated tablets. Again here, the drug release rates were statistically compared by ANOVA test to know the effect of coating on the release rates. The $F$ value was found to be $6.628$ (df = 62, $P < 0.05$), which indicates that there is a significant difference in the release rates before and after coating of the tablets. ANOVA was further extended by the least significant difference (LSD) procedure. The results indicate that the release rates of chitosan or cellulose acetate coated tablets are significantly different from those of the uncoated tablets, and there is no significant difference in the release rates between chitosan and cellulose acetate coated tablets.

III.B.3. Conclusions

Microparticles are the popular and advantageous systems for the CR of drugs. In this investigation, tableted microparticles containing clozapine drug having good mechanical strength properties have been prepared by using the directly compressible excipients along with a dry binder. Hardness of the tablets was inversely proportional to the amount of microparticles present in the tablets. Formulation of the tableted microparticles is useful in reducing the initial drug release. Coating of the tablets with chitosan or cellulose acetate could help to minimize the initial burst release. The in-vitro release studies indicated that the tableted microparticles developed in this research could be used successfully as CR devices for the release of clozapine.
III.B.4. Literature Cited