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# Design & Fabrication of Tramadol HCl loaded Multiparticulate Colon Targeted Drug Delivery System

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# ABSTRACT

The aim of the present study is to develop a multiparticulate system containing pectin microspheres for the colon targeted delivery of Tramdol HCl (TMD) for the treatment of irritable bowel syndrome. This work combines pH-dependent solubility of shellac polymers and microbial degradability of pectin polymers. Pectin microspheres containing TMD were prepared by emulsion cross linking method using different ratios of TMD and pectin (1:2 to 1:5), stirring speeds (500-2000 rpm) and emulsifier concentrations (1.0 % - 2.0% wt/vol). The yield of preparation and the encapsulation efficiencies were high for all pectin microspheres. Microspheres prepared by using drug: polymer ratio 1:3, stirring speed 1000 rpm, and 1.25% wt/vol concentration of emulsifying agent were selected as an optimized formulation. Shellaccoating of pectin microspheres was performed by oil-in-oil solvent evaporation method using coat: core ratio (5:1). Microspheres were evaluated for surface morphology, particle size and size distribution, swellability, percentage drug entrapment, and in vitro drug release in simulated gastrointestinal fluids (SGF). The release profile of TMD from Shellac-coated pectin microspheres was pH dependent. In acidic medium, the release rate was much slower; however, the drug was released quickly at pH 7.4. It is concluded from the present investigation that Shellac-coated pectin microspheres are promising controlled release carriers for colon-targeted delivery of TMD.

**Keywords:** Tramdol HCl; pectin, microspheres; shellac coating; colon targeting; multiparticulate system.

#### INTRODUCTION

Colon-specific drug delivery is intended to improve the efficacy and reduce side effects by exerting high drug concentrations topically at the disease site. Because of the distal location of the colon in the gastrointestinal (GI) tract, an ideal colon-specific drug delivery system should prevent drug release in the stomach and small intestine, and affect an abrupt onset of drug release upon entry into the colon. This requires a triggering mechanism built in the delivery system responsive to the physiological changes particular to the colon. However, the physiological similarity between distal small intestine and the proximal colon presents very limited options in selecting an appropriate drug release triggering mechanism. Commonly used pharmaceutical strategies to achieve a colon-specific drug delivery include timed-release approximating the GI transit time, pH-sensitive polymer coating, prodrug, and colonic microflora activated delivery system [1].

Single unit colon targeted drug delivery system may suffer from the disadvantage of unintentional disintegration of the formulation due to manufacturing deficiency or unusual gastric physiology that may lead to drastically compromised systemic drug bioavailability or loss of local therapeutic action in the colon. Recently, much emphasis is being laid on the development of multiparticulate dosage forms in comparison to single unit systems because of their potential benefits like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying. The purpose of designing multiparticulate dosage form is to develop a reliable formulation that has all the advantages of a single unit formulations and yet devoid of the danger of alteration in drug release profile and formulation behaviour due to unit to unit variation, change in gastro-luminal pH and enzyme population [2].

Tramadol HCl is a centrally-acting analgesic, used in treating moderate to moderately severe pain, used in the manufacture of a pharmaceutical preparation for the treatment of functional gastrointestinal disorders such as irritable bowel syndrome. The most common functional GI disorders are irritable bowel syndrome and non-cardiac chest pain. It is estimated 15-20% of the general population are affected by IBS at some time. It has been reported that the most-commonly occurring adverse side effects during treatment of pain with tramadol preparations are gastrointestinal upsets. Between one third and a half of patients suffer nausea and vomiting initially when started on tramadol pain therapy. Patients with IBS exhibit increased gut sensitivity, suggesting that at least part of the problem may be because the nerves that carry information from the gut to the brain, the afferent neurons, produce a response greater than that expected to be produced by the stimuli they have received, which results in non painful stimuli being perceived as painful (visceral hyperalgesia) [3, 4].

Targeting of TMD to the colon may provide adequate treatment for IBS and allow a reduction in dosage and possible systemic side effects.

Successful targeted delivery of drugs to the colon via the gastrointestinal tract requires the protection of a drug from degradation, release and/or absorption in stomach and small intestine and then ensures abrupt or controlled release in the proximal colon. This might be achieved by the use of specially designed drug delivery system (DDS) that can protect the drug during its

transfer to colon [5, 6]. In this work, a multiparticulate system of TMD for the treatment of irritable bowel syndrome was developed by utilizing the pH-dependent solubility of Shellac polymers and microbial degradability of Pectin polymers. TMD-loaded Pectin microspheres were prepared, which is then microencapsulated with Shellac polymer. This polymer shows the solubility at or above pH 7.

# **EXPERIMENTAL SECTION**

#### Chemicals

The drug, Tramadol HCl (TMD) was purchased from Mundi Pharma, Merrut, India. Pectin and shellac was obtained from HiMedia Laboratories Ltd, Mumbai, India. Acetone, n-Hexane, and light liquid paraffin were purchased from Qualigens Fine Chemicals, Mumbai. Span 80 was obtained from S. D. Fine Chemicals, Mumbai. Pectinase was procured from HiMedia Laboratories Ltd, Mumbai, India. All other chemicals used were of analytical reagent grade and were used as received.

**Fabrication of Tramadol HCl loaded pectin microspheres:** Pectin microspheres were prepared by emulsion cross-linking method [5, 6]. Pectin dissolved in 20 ml of distilled water and uniform solution was prepared. Dispersion of Tramadol HCl (TMD) was added to the uniform polymeric solution with stirring. To produce an emulsion aqueous polymeric solution containing drug molecules was dispersed in 40 ml of light liquid paraffin containing span 80 (1.25% w/v) and stirred at 1000 rpm continuously to obtain stable w/o emulsion. The solution was rapidly cooled to 5° c by placing the beaker in an ice bath. After 20 min of stirring 10 ml of 1.3% w/v cacl<sub>2</sub> was added gradually to the system and stirred for 1 hr (allows the time for cross-linking). Resultant microspheres was filtered and washed with n- hexane and then dried. Pectin microspheres were prepared by using different ratios of TMD: Pectin (ie, 1:2, 1:3, 1:4, and 1:5), by varying surfactant concentration (ie .75%, 1%, 1.25% and 2%) and by varying stirring speed (ie. 500, 1000, 1500 and 2000).

# Microencapsulation of Pectin microspheres:

The TMD loaded Pectin microspheres were microencapsulated by emulsion–solvent evaporation technique [7]. The Pectin microspheres (100 mg) were suspended in 20 ml of coating solution prepared by dissolution of shellac (500 mg) in ethanol –acetone mixture and then emulsified into 40 ml of light liquid paraffin containing span 80. The emulsification process was carried out for 2 h at 1000 rpm with mechanical stirrer. The Shellac coated microspheres were collected and rinsed with n-hexane and dried.

Prepared microspheres were evaluated for following parameters:

#### **1.** Particle size analysis:

Particle size distribution of the microspheres was determined by optical microscopy using calibrated ocular eyepiece. Product dispersed in light liquid paraffin and a smear of the dispersion was observed under compound microscope.

The size of 100 microspheres was measured in each case against a calibrated eyepiece in micrometer [8, 9].

#### 2. Determination of shape and spherecity:

Morphological appearance and surface characteristics of the microspheres were studied by dispersing the microspheres in liquid paraffin and observed under light microscope [8, 9].

# 3. Scanning Electron Microscopy

The shape and surface morphology of pectin microspheres and shellac-coated pectin microspheres were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Jeol JSM-1600, Tokyo, Japan) [10].

#### **Percentage Yield**

Percentage practical yield is calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of microspheres recovered from each batch in relation to the sum of starting material. The percentage yield of prepared microspheres was determined by using the formula [8].

% yield = <u>Total wt of microparticle</u> X 100 Total wt of drug and polymer

# **Determination of Drug Content**

Microspheres were accurately weighed (50 mg), triturated and digested in 10 ml pectinase solution (4% wt/v) and kept overnight for extraction of drug for the determination of entrapment efficiency. The digested homogenate was centrifuged and supernatant was collected. After appropriate dilution of supernatant with pH 7.4 phosphate buffer, aliqutes were assayed by UV spectrophotometry at suitable wavelength using a shimadzu UV visible spectrophotometer (SHIMADZU, Spectrascan-2200, Japan). Corresponding drug concentrations in the sample were calculated from the calibration curve [7, 9].

# **Determination of % Drug Entrapment**

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula:

Theoretical drug content was determined by calculation assuming that the entire drug present in the pectin solution used gets entrapped in microspheres and no loss occurs at any stage of preparation of microspheres [8].

## Swellability / Degree of Swelling

The swelling ability of the microspheres on physiological media was determined by suspending them in the PBS buffer (pH 7.4). Accurately weighed amount (100 mg) of various tramadol-

loaded pectin microspheres and shellac-coated pectin microspheres were placed in enzyme-free simulated intestinal fluid (pH 7.4 Phosphate buffer) in vials and allowed to swell for the required period of time. The microspheres were periodically removed and blotted with filter paper; then their change in weight (after correcting for drug loss) was measured until attainment of equilibrium. Degree of swelling was then calculated using the following formula [7]:

Degree of swelling =  $(W_g - W_i) / W_g \ge 100$ 

Where W<sub>i</sub>, initial weight of microspheres; and W<sub>g</sub>, final weight of microspheres.

# In Vitro Drug Release Study

Microspheres were evaluated for the in vitro drug release in simulated GI fluids (SGF). The drug dissolution test of microspheres was carried out using USP rotating basket method. Microspheres (100 mg) were weighed accurately and placed in the dissolution medium. The content was rotated at 50 rpm at  $37^{\circ}C \pm 0.5^{\circ}C$ . Perfect sink conditions prevailed during the drug dissolution study period. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 hours using 0.1 N HCl. Then KH<sub>2</sub>PO<sub>4</sub> (1.7 g) and Na<sub>2</sub>HPO<sub>4</sub>.2H<sub>2</sub>O (2.2 g) were added to the dissolution medium, adjusting the pH to 6.8 with 1.0 M NaOH, and the release rate study was continued for an additional 5 hours. After 5 hours, the pH of the dissolution medium was adjusted to 7.4 with 0.1 N NaOH and Simulated to colonic fluid by addition of 4 % w/v pectinase enzyme and maintained this condition up to 24 hours. The samples were withdrawn from the dissolution medium at various time intervals using a pipette fitted with a microfilter (0.45-µm). The rate of drug release was analyzed at 272 nm using UV Spectrophotometer. The receptor volume was maintained constant by replacing equivalent amount of SGF. The concentration of drug in the samples was calculated based on average calibration curves (n = 3). All dissolution studies were performed in triplicate [5, 7].

#### **Release Kinetics**

In order to investigate the mode of drug release from microspheres the release data were analyzed with the following mathematical models: zero-order kinetic (Eq. (1)); first-order kinetic (Eq. (2)); square root of time equation (Higuchi equation, Eq. (3)) and Korsmeyer equation Eq. (4)).

Q = k0t ......(1)

 $\ln(100 - Q) = \ln Q_0 - k_1 t \quad \dots \dots \quad (2)$ 

$$Q = k_{\rm H} t^{1/2}$$
 .....(3)

 $M_t M_{\infty} = \mathrm{kt}^n \quad .... \quad (4)$ 

In equations Q is the percent of drug released at time t and  $k_0$ ,  $k_1$  and  $k_H$  are the coefficients of the equations,  $M_t/M_{\infty}$  is the fraction of drug release at time t, k is the release rate constant and n is the release exponent indicative of the mechanism of release. When n approximates to 0.5, a

Fickian/diffusion-controlled release is implied, where 0.5 < n < 1.0 non-Fickian transport and n = 1 for zero order (case II transport). When the value of *n* approaches 1.0, phenomenologically one may conclude that the release is approaching zero order [10, 11].

#### **Stability Studies**

The stability studies were performed as per ICH guidelines at temperature of  $40^{\circ}$  C / 75% RH (Long term stability study) for 3 months. The optimized formulation was analyzed for drug content and % drug release [13]

#### **RESULTS AND DISCUSSION**

Pectin microspheres of TMD were successfully prepared by emulsion cross linking method. Uniform, surface cross-linked and almost spherical microspheres were obtained, as shown in SEM photomicrographs (Fig. 1A). The pectin microspheres were coated with shellac by emulsion solvent evaporation method, using coat: core ratio 5:1. The coated microspheres were found to be of spherical shape as observed in SEM photomicrographs (Fig. 1B). The method was optimized using different drug polymer ratio, stirring rate and emulsifier concentration to produce microspheres of small size and narrow size distribution, high drug loading efficiency, and controlled drug release at the colonic pH.



Figure 1: scanning electron photomicrographs of (A) pectin microsphere (B) shellac coated pectin microsphere

# **Effect of Drug-Polymer Ratio**

The mean particle size, % yield and % entrapment efficiency of microspheres containing various amounts of polymer were determined. The amount of drug was kept constant and concentration of pectin was varied (1:2, 1:3, 1:4 and 1:5). The mean diameter of microspheres varied from 30  $\mu$ m to 34  $\mu$ m with varying pectin concentration from 2% wt/vol to 5% wt/vol. The percentage drug entrapment varied from 82% to73% with varying drug polymer ratio from 2% to 5%. The highest drug loading efficiency was found with 2% pectin. A higher concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres [12]. Fig 2 shows the effect of drug - polymer ratio on particle size, % drug entrapment and % yield.



Figure 2: Effect of drug - polymer ratio on particle size, % drug entrapment and % yield



Figure 3: Effect of surfactant concentation on particle size, % drug entrapment and % yield

#### **Effect of Surfactant Concentration**

The effect of surfactant concentration on mean particle size ( $\mu$ m), % yield and % entrapment efficiency of microspheres were determined. The amount of surfactant was varied (1%, 1.25, 1.50% and 2%). The mean diameter of microspheres was found to vary from 35  $\mu$ m to 28  $\mu$ m on varying emulsifier concentration (Span 80) from 1 % wt/vol to 2% wt/vol. Increased surfactant concentration led to the formation of particles with a lower mean geometric diameter. Increasing Span 80 concentration from 1% to 2 % wt/vol led to stabilization of the emulsion droplets avoiding their coalescence, resulting in smaller microspheres. The drug loading efficiency varied from 77% to 81% with varying emulsifier concentration from 1% to 2% during preparation of

pectin microspheres [7]. Fig 3 shows the effect of surfactant concentration on particle size, % drug entrapment and % yield.

# **Effect of Stirring Speed**

Effect of stirring speed on mean particle size ( $\mu$ m), % yield and % entrapment efficiency of microspheres were determined. The speed of stirrer was varied (500 rpm, 1000 rpm, 1500 rpm and 2000). The mean diameter of microspheres decreased from 36  $\mu$ m to 30  $\mu$ m with increasing agitation speed of the mechanical stirrer from 500 rpm to 2000 rpm. This result was expected because high stirring rates provide the shearing force needed to separate the oil phase into smaller globules. The stirring speed of 1000 rpm was found to be optimum for pectin microspheres, as the drug loading efficiency was 78%. High stirring speed produced an irregular shape of microspheres but a slightly increased entrapment efficacy was found [6]. Fig 4 shows the effect of stirring speed on particle size, % drug entrapment and % yield.



Figure 4 : Effect of stirring speed on particle size, % drug entrapment and % yield

# **Degree of Swelling:**

Swellability of different microspheres was determined. No significant swelling was observed with shellac coated pectin microspheres as compared with pectin microspheres, thus ensuring better resistance of shellac-coated microspheres in the upper GI tract to swelling and preventing subsequent drug release at the nontarget site. Fig 5 shows the swelling ratio of pectin microspheres (TMD) and shellac coated pectin microspheres (S-TMD).



Figure 5: Degree of Swelling of Pectin Microspheres (TMD) and shellac-coated Pectin Microspheres (S-TMD)

# In - Vitro Drug Release Studies in Simulated Gastrointestinal Fluids:

In vitro drug release study of Pectin microspheres and Shellac-coated pectin microspheres was performed in pH progression medium at  $37^{\circ}C \pm 0.5^{\circ}C$ . The plot of cumulative drug release vs. time in simulated gastrointestinal conditions is shown in Figure 6 and 7, respectively.

The drug release patterns shown in Figure 6 indicate that the rate of release of TMD from pectin microspheres was mainly influenced by polymer concentration. When drug to polymer ratio increases from 1:2 to 1:5 a decreased in release rate was observed. TMD release from pectin microspheres in SGF (Simulated Gastric Fluid) followed the order P1 > P2 > P3 > P4 (Figure 6). The in vitro drug release studies of P1-P4 formulations in simulated gastro intestinal fluids showed a burst release pattern in the initial hour. A high burst release of drug was observed from the formulation P1, which contain 1:2 drug polymer ratio; whereas a less burst release of drug was observed from P4. These results indicated that formulation with lesser drug-polymer ratio shows higher drug release. This type of high drug release in stomach and small intestine is not satisfactory for a formulation, which is supposed to release its contents in the colon. The burst release may be due to solubility of pectin in the acidic pH. In order to prevent the drug release in stomach and small intestine these pectin microspheres were encapsulated with shellac, which shows solubility at a pH  $\geq$ 7. The cumulative percentage drug release from Shellac-coated pectin microspheres showed the desired rate, as there was no measurable drug release observed up to 2 hours in SGF (pH 1.2), while in SIF (Simulated Intestinal Fluid) (pH 6.8), the drug release was quite insignificant (>1%) up to 5 hours. But in colonic fluid maximum drug release was observed due to dissolution of the shellac coat at pH 7.4 and the pectin microspheres were degraded on exposure to the colonic fluid and results in higher percentage of drug release. Significant release TMD release from Shellac- coated pectin microspheres in SGF followed the order S-P1 > S-P2 > S-P3> S-P4 (Figure 7).

The release of drug from microspheres decreased as the polymer concentration increased,

suggesting that drug release could be controlled by varying the polymer concentration. This could be attributed to an increase in the density of the polymer matrix and the diffusional path length that the drug has to traverse.



Figure 6 Percentage cumulative in vitro TMD release from pectin microspheres containing different drug: pectin ratios (1:2 to 1:5) in simulated gastrointestinal fluids of different pH. Values are average of 3 readings ± standard deviation.



Figure 7 Percentage cumulative in vitro TMD release from different Shellac-coated pectin microspheres in simulated gastrointestinal fluids of different pH. Values are average of 3 readings ± standard deviation.

#### **Release Kinetics**

Data obtained from in-vitro release studies was utilized for release kinetics. The values of invitro release were attempted to fit into various mathematical models i.e. zero order, first order,

higuchi matrix, Korsmeyer-peppas and hixson crowell. These values were compared with each other for model fitting equation. Based on highest regression value (r), formulations gave good fit to the Zero order and Korsmeyer- Peppas model. Since the diffusion exponent (n) value was greater than 1, the drug release follows super case II transport.

#### **Stability study**

In view of the potential utility of optimized formulation for targeting of TMD to colon, stability studies were carried out at  $40^{\circ}$ C / 75% RH for 3 months to assess their long term stability. There is no appreciable change in drug content (Table 1) and dissolution profile of optimized formulation after storage at  $40^{\circ}$ C / 75% RH for 3 months as shown in Fig 8.





Figure 8 Percentage drug release of optimized formulation subjected for stability testing

#### CONCLUSION

The designed site-specific delivery of Tramadol hydrochloride from the system may reduce the side effects of the drug caused by its absorption from the upper part of the GI tract when given in conventional dosage forms such as tablets and capsules. The approach described appears promising for the colonic delivery of drugs.

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