FORMULATION, OPTIMIZATION AND EVALUATION OF STOMACH SPECIFIC IN SITU GEL OF HYDROCHLOROTHIAZIDE
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ABSTRACT
The objectives of this research work were preparation, optimization and evaluation of in situ gelling system of Hydrochlorothiazide based on pectin that retains in the stomach by adherence to gastric wall providing an increased gastric residence time resulting in prolonged drug delivery in gastrointestinal tract. Pectin was used as a polymer and CaCO3 as a cross-linking agent. The in situ formulation exhibited the expectations, viscosity, drug content and sustained drug release. This study reports that oral administration of aqueous solutions containing pectin results in formation of in situ gel and such formulations are homogenous liquids when administered orally and become gel at the contact site. The results of a 32 full factorial design revealed that the concentrations of pectin and CaCO3 significantly affected the dependent variables of viscosity, drug content (%t) and Q50. These in situ gels are, thus, suitable for oral sustained release of Hydrochlorothiazide.

INTRODUCTION
In-situ gel forming drug delivery is a type of mucoadhesive drug delivery system. In-situ gel forming drug delivery systems are a revolution in oral drug delivery1,2. These hydrogels are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH3,4. These have a characteristic property of temperature dependant and cation induced gelation. This gelation involves the formation of the double helical junction zones followed by aggregation of the double helical segments to form a three dimensional network by complexion with cations and hydrogen bonding. Hydrochlorothiazide (HCTZ) is the diuretics of the benzothiadiazine group and has proved very important in the management of mild to moderate hypertension. It inhibits sodium reabsorption in the distal tubules causing increased excretion of sodium and water as well as potassium and hydrogen ions. Hydrochlorothiazide is poorly water soluble drug having plasma half life of 6-8 hours5,6. The main objectives of this research work are preparation and evaluation of In situ gelling system of HCTZ based on Pectin that retains in the stomach by adheres with gastric wall. Provide an increased gastric residence time resulting in prolonged drug delivery in gastrointestinal tract.

EXPERIMENTAL AND METHODS
Materials
HCTZ, hydroxypropyl methylcellulose K-15 M (HPMC K-15M) and pectin were received as gift samples from Lincoln Pharmaceuticals Ltd., Ahmedabad, India. All other reagents and chemicals were of analytical grade.

Pre-formulation study
Differential Scanning Calorimetry (DSC) studies
DSC scans of the powdered sample of Hydrochlorothiazide and its physical mixtures with formulation excipients were recorded to check compatibility of ingredients using DSC-Shimadzu 60 with TDA trend line software. DSC thermograms of pure drug (HCTZ), HPMC K-15M, pectin and Calcium carbonate were taken for their identical endothermic reaction. Further HCTZ and its physical mixtures with formulation excipients were also studied for DSC to check interactions. The samples (6-7 mg) were accurately weighed in crimped aluminum pans and heated from 50°C to 300°C, at a scanning rate of 10°C/min under air flow (100 mL/min). DSC thermograms were shown in [Figure 1].

Preparation of In-situ Gel of Hydrochlorothiazide (HCTZ)
Active material (HCTZ) was passed form sieve no. 60 while other inactive ingredients were passed form sieve no.40, and then aqueous solution of HPMC K-15M was prepared. HCTZ (25) was added slowly to the above solution while stirring on a magnetic stirrer so that there was proper and homogeneous dispersion of the drug. In other beaker, different concentrations of pectin solutions were prepared by adding the alginate to purified water containing sodium methyl paraben and sodium propyl paraben and heating to 60°C. After cooling to below 40°C, both solutions were mixed while stirring on magnetic stirrer. Then appropriate amount of calcium carbonate was added while stirring. The above formulation was sonicated in a bath sonicator for 15 minutes and then pH and viscosity of the solutions were determined. pH of solution was adjusted to 6.5 - 7.0 with 0.1N sodium hydroxide solution and then prepared solution was added in pH 1.2 buffers, to observe the gel formation, physical appearance and dissolution behavior.

Optimization by using 32 full factorial designs
On the basis of the preliminary trials in the present study a 32 full factorial design was employed to study the effect of independent variables, i.e. concentration of pectin (X1) and the concentration of HPMC K-15 M (X2) on dependent variables % drug release at Q1h, drug content and viscosity. A

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statistical model (see equation) incorporating interactive and
to evaluate the responses.

\[ Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_2^2 \]

Where, Y is the dependent variable, \( b_0 \) is the arithmetic mean response of the nine runs, and \( b_i \) is the estimated coefficient for the factor \( X_i \).

The main effects (\( X_1 \) and \( X_2 \)) represent the average result of changing one factor at a time from its low to high value. The interaction terms (\( X_1X_2 \)) show how the response changes when two factors are simultaneously changed. The polynomial terms (\( X_1^2 \) and \( X_2^2 \)) are included to investigate non-linearity\(^7\).

**Evaluations**

**Physical appearance and pH**

All the prepared pectin based In-situ solutions of HCTZ were checked for their clarity and the type of the solutions. After administered of the prepared solutions in pH 1.2 buffer also checked the time required for gel formation and type of gel formed. The pH was measured in each of the solution of pectin based In-situ solutions of HCTZ, using a calibrated digital pH meter at 27°C.

**Determination of viscosity**

Viscosity of the samples was determined using a Brookfield digital viscometer (Model no. LVDV 2P230) with spindle number 1. The sample temperature was controlled at 25 ± 1°C before the each measurements. The viscosity of the solutions prepared in water was determined at ambient condition using 2 ml aliquot of the sample. Increasing the concentration of a dissolved or dispersed substance generally gives rise to increasing viscosity (i.e. thickening), and also as molecular weight of a solute increases viscosity increases\(^8\).

**Drug content (%)**

Weigh accurately about 50 mg of HCTZ Reference Standard and transfer it to 25 ml volumetric flask. Add about 10 ml of methanol and sonicate to dissolve. Make up the volume with methanol. Take 10 ml of this stock solution to a 50-ml volumetric flask, dilute with methanol to volume, and mix. Pass a portion of this solution through a 0.5 mmv or finer porosity, and use the filtrate as the standard preparation\(^9\).

**In vitro drug release study**

The drug release study was carried out in USP XXVI dissolution test using basket apparatus (Electrolab, TDT-06T, Mumbai, India) at 37 ± 0.5°C and 100 rpm using 900 ml of pH 1.2 buffer as a dissolution medium (n = 3)\(^10\). In-situ gels equivalent to 150 mg of HCTZ were used for the test. 10 ml of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µ membrane filter, diluted and analyzed spectrophotometrically. Equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample.

**Kinetics modeling of drug dissolution profiles**

The dissolution profile of all the batches was fitted to Zero order, First order and Higuchi model to ascertain the kinetic modeling of the drug release\(^11-14\).

**Measurement of water uptake by the gel**

The water uptake by the gel of the selected formulation of pectin can be determined using a thermo gravimetric analyzer. But in this present study a simple method has been adopted to determine the water uptake by the gel. The In-situ gel formed in 40 ml of gastric acid buffer (pH 1.2) was used for this study. From each formulation the gel portion from the buffer was separated and the excess buffer was blotted out with a tissue paper. The initial weight of the gel taken was weighed and to this gel 10 ml of distilled water was added and after every 30 minutes of the interval water was decanted and the weight of the gel was recorded and the difference in the weight was calculated and reported\(^15\).

**In-vitro buoyancy study**

*In-vitro* buoyancy study was carried out by using 100 ml 0.1 N HCL. Prepared formulation was poured into the solution of 0.1 N HCL and time for buoyancy being noted.

**Stability study**

Prepared pectin based In-situ gel of HCTZ was stored in a glass containers (well Stoppard) for three months and the stability of the aqueous solutions of the pectin based In-situ gels of HCTZ was monitored up to 3 months at accelerated stability conditions (45°C temperature and 75 ± 5 % RH). Periodically (initial 1, 2 and 3 months interval) samples were removed and characterized by pH, viscosity and drug content\(^16-21\).

**RESULTS AND DISCUSSION**

**DSC Study**

DSC enables the quantitative detection of all processes in which energy is required or produced (i.e. endothermic or exothermic phase transformations). The thermo grams for pure Hydrochlorothiazide, HPMC K 15 M, Pectin, Calcium carbonate and physical mixture of Hydrochlorothiazide with excipients are presented in Figure 2. DSC curve of Hydrochlorothiazide displayed a sharp endotherm at 273.55°C, which was due to drug melting, characteristic of an anhydrous crystalline substance. In the physical mixing systems, peaks due to excipients are clearly observed with distinguishable the Hydrochlorothiazide endothermic peak at 274.90°C [Figure 2]. This indicates that in such systems the Hydrochlorothiazide has basically maintained its original crystallinity and hence Hydrochlorothiazide is compatible with selected formulation excipients. Thus from DSC study it can be concluded that there is no interaction between drug and other excipients. So, selected active material and other excipients are compatible with each other [Figure 1].

**Factorial equation for viscosity**

The viscosity is an important variable because it affects the gelation of the solutions, the flow of the formulation and time required for the gelation. The viscosity is dependent on the concentration of the polymer and concentration of the calcium carbonate. The viscosity of the pectin solutions varied from 170 to 325 cps which was measured at 150 rpm (Table 2) and showed good correlation coefficient as 0.989. Results of the equation indicate that the effect of \( X_1 \) (concentration of pectin) is more significant than \( X_2 \) (concentration of CaCO\(_3\)). Moreover, volume of CaCO\(_3\) had a negative effect on the viscosity, i.e. as the volume of cross-linking agent increase, the viscosity increases and has no significant effect on drug release, Figure 2. The viscosity of the pectin based In-situ formulation batches F1 to F9 are depicted in Table 3.
Hydrochlorothiazide

Figure 1: DSC spectra of HCTZ and its physical mixture with formulation excipients

HPMC K 15 M

Pectin
Calcium carbonate

Physical Mixture of HCTZ with excipients

Table 1: Results of preliminary trial batches for pectin

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Concentration of Pectin (%)</th>
<th>Viscosity (cp)</th>
<th>Drug Content (%)</th>
<th>Characteristic of in situ gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1.0</td>
<td>89</td>
<td>82.0</td>
<td>Gel is not formed properly and less drug content</td>
</tr>
<tr>
<td>T2</td>
<td>1.5</td>
<td>110</td>
<td>85.23</td>
<td>Gel formed but not good and Less drug content</td>
</tr>
<tr>
<td>T3</td>
<td>2.0</td>
<td>178</td>
<td>92.08</td>
<td>Gel formation and drug content are slightly better</td>
</tr>
<tr>
<td>T4</td>
<td>2.5</td>
<td>223</td>
<td>97.12</td>
<td>Gel formation and drug content are excellent</td>
</tr>
<tr>
<td>T5</td>
<td>3.0</td>
<td>289</td>
<td>98.09</td>
<td>Gel formation and drug content are good and stiff</td>
</tr>
</tbody>
</table>

Table 2: $3^2$ full factorial design layouts

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Variables levels in coded form</th>
<th>Viscosity (cps)</th>
<th>Drug content (%)</th>
<th>% Drug release ($Q_{10}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
<td>$X_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>-1</td>
<td>-1</td>
<td>170</td>
<td>89.45</td>
</tr>
<tr>
<td>F2</td>
<td>-1</td>
<td>0</td>
<td>176</td>
<td>92.12</td>
</tr>
<tr>
<td>F3</td>
<td>-1</td>
<td>+1</td>
<td>180</td>
<td>94.48</td>
</tr>
<tr>
<td>F4</td>
<td>0</td>
<td>-1</td>
<td>234</td>
<td>95.67</td>
</tr>
<tr>
<td>F5</td>
<td>0</td>
<td>0</td>
<td>255</td>
<td>97.36</td>
</tr>
<tr>
<td>F6</td>
<td>0</td>
<td>+1</td>
<td>279</td>
<td>98.42</td>
</tr>
<tr>
<td>F7</td>
<td>+1</td>
<td>-1</td>
<td>286</td>
<td>95.23</td>
</tr>
<tr>
<td>F8</td>
<td>+1</td>
<td>0</td>
<td>300</td>
<td>96.44</td>
</tr>
<tr>
<td>F9</td>
<td>+1</td>
<td>+1</td>
<td>325</td>
<td>93.25</td>
</tr>
</tbody>
</table>

Translation of coded levels in actual units

<table>
<thead>
<tr>
<th>Variables level</th>
<th>Low (-1)</th>
<th>Medium (0)</th>
<th>High (+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of Pectin ($X_1$)</td>
<td>2.0 %</td>
<td>2.5 %</td>
<td>3.0 %</td>
</tr>
<tr>
<td>Concentration of Calcium carbonate ($X_2$)</td>
<td>1.5 %</td>
<td>2.5 %</td>
<td>3.5 %</td>
</tr>
</tbody>
</table>
Figure 2: Effect of variable on Viscosity (HCTZ)

Figure 3: Effect of variable on Drug content (%)

Figure 4: Effect of variable on % Drug release (Q18)
Factorial equation for drug content
Data and graph of drug content for all the batches are mentioned in Table 2 and Figure 3 respectively. The drug content varied from 89.45 % to 98.42 % in batches F1 to F9 pectin based In-situ formulations of HCTZ and showed good correlation coefficient as 0.98. Results of the equation indicated that both the concentration of the X1 and X2 were responsible for the drug content of the In-situ formulations but the effect of X1 (concentration of pectin) is more significant than X2 (concentration of CaCO3), the effect of the X2 was very less so it was considered non significant compared to the concentration of X1.

Factorial equation for Q18
The amount of drug released of at 18 h is also important parameters for sustained action of the formulations. The Q18 for all the batches F1 to F9 varied from 80.56 % to 99.02 % (Table 2) and showed good correlation coefficient as 0.988. Results of the equation indicated that the effect of the concentration of pectin (X1) was more while the effect of the concentration of CaCO3 (X2) was also in minus sign but it was lower than X1 so the concentration of the X2 was very less effective as controlled release action of the gels than the concentration of the X1.[Figure 3].

Table 3: Summary of results of regression analysis for pectin based In-situ gel of CP

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>b0</th>
<th>b1</th>
<th>b2</th>
<th>b11</th>
<th>b22</th>
<th>Multiple R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity</td>
<td>245.55</td>
<td>64.16</td>
<td>15.66</td>
<td>-0.74</td>
<td>-0.46</td>
<td>0.978</td>
</tr>
<tr>
<td>Drug content (%)</td>
<td>94.12</td>
<td>1.33</td>
<td>0.45</td>
<td>-1.45</td>
<td>-1.78</td>
<td>0.846</td>
</tr>
<tr>
<td>Q18 (%)</td>
<td>67.07</td>
<td>-19.51</td>
<td>-6.18</td>
<td>2.98</td>
<td>-0.65</td>
<td>0.988</td>
</tr>
</tbody>
</table>

In-vitro release study
The result of the regression from zero order, first order, higuchi model and krosmeyer peppas model showed that all the batches of pectin based in-situ gels of Hydrochlorothiazide F1-F9 followed krosmeyer peppas model because good correlation coefficient obtained by this model and the batch F5 containing good correlation coefficient 0.988. The release data shows that drug release from the formulation was in the range of 99.02 % to 80.56 % for 18 h [Figure 5].

Figure 5: In-vitro dissolution study data of F1 to F9

Table 5: Composition of Optimized Formulation (F5)

<table>
<thead>
<tr>
<th>Composition</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCTZ</td>
<td>25 mg</td>
</tr>
<tr>
<td>Pectin</td>
<td>2.5 % (w/w)</td>
</tr>
<tr>
<td>CaCO3</td>
<td>2.5 % (w/w)</td>
</tr>
<tr>
<td>methyl paraben</td>
<td>5 mg</td>
</tr>
<tr>
<td>propyl paraben</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

Release mechanism
The result of the regression from zero order, first order and higuchi model all the batches of pectin based In-situ gels of HCTZ F5 followed zero order because good correlation coefficient obtained by this model and the selected batch F5 containing good correlation coefficient 0.998.

Table 5: Release kinetics for pectin base In-situ formulation batch F5

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero order kinetic</td>
</tr>
<tr>
<td>F5</td>
<td>0.998</td>
</tr>
</tbody>
</table>

In-vitro buoyancy time of optimized formulation
In-vitro buoyancy study of optimized formulation of batch F5 shows that after oral administration, formed in-situ gel remains buoyant for greater than 20 h in the presence of 0.1 N HCL.

Table 6: In-vitro buoyancy time

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Buoyancy Time (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>&gt; 20</td>
</tr>
</tbody>
</table>

Stability study
Short-term stability study of pectin based In-situ gel of HCTZ was carried out for 3 months at accelerated stability conditions. The results of the stability study for the selected batch of pectin based In-situ formulation is given in Table 6. Stability study revealed that no any major changes taken place throughout the stability study for three months so we can say that pectin based In-situ formulation has the good stability.
CONCLUSION
The present investigation deals with the formulation, optimization and evaluation of pectin based In-situ gel of HCTZ. Pectin used as a polymer and CaCO₃ was used as a cross-linking agent. The In-situ formulation exhibited well, viscosity, drug content and sustained drug release; this study reports that oral administration of aqueous solutions containing pectin results in formation of In-situ gel, such formulations are homogeneous liquid when administered orally and become gel at the contact site. The results of a 3² full factorial design revealed that the concentration of pectin and concentration of CaCO₃ significantly affected the dependent variables viscosity, drug content (%) and Q₂₆. These In-situ gels are, thus, suitable for oral sustained release of HCTZ.

REFERENCES

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