DESIGN OF FLOATING IN SITU GEL OF MUCOLYTIC AGENT BY CATION INDUCED GELATION OF NATURAL POLYSACCHARIDES

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ABSTRACT
The aim of this study is to formulate and evaluate of oral floating in situ gel of Ambroxol hydrochloride by using natural polymer i.e. gelrite and sodium alginate. Ambroxol hydrochloride is an oral systemically active mucolytic agent. Materials and Methods: This study involved formulation of Ambroxol hydrochloride oral solution which become gel upon direct contact with acidic environment pH 1.2 and then floated. The floating oral in situ gel under goes gelation by ion sensitive mechanism. These floating in situ gel formulations were prepared by using different concentration of gelrite (F1 to F3), sodium alginate (F4 to F6) and in combination of gelrite and sodium alginate (F7 to F8). The prepared formulations were evaluated for physicochemical parameters like pH, in vitro gelling capacity, gelling time, viscosity, floating lag time, floating duration, drug content uniformity, in vitro drug release study. The best formulation was selected and stability studies were performed as per ICH guidelines. Results And Discussion: Formulation F3 (1% w/v gelrite) and F9 (0.75% w/v gelrite and 0.25% w/v sodium alginate) were selected as best formulation regarding %CDR, in vitro gelling capacity, viscosity and other factors. During stability study, no any evidence of significant changes was observed in terms of physicochemical properties which confirm stability. Physicochemical parameters and in vitro drug release of the formulation found proportional to the concentration of polymer used in the formulation. The best formulations were found to have viscosity which favors oral administration, acceptable drug content and %CDR and acceptable stability. Conclusion: Study indicates that floating in situ gel of Ambroxol hydrochloride can be
formulated as F3 follows Korsmeyer- Peppas used for stomach specific controlled drug delivery system through 24h.

KEYWORDS: Ambroxol hydrochloride, mucolytic agent, In situ gel, Gelrite and sodium alginate.

INTRODUCTION
Drug delivery system (DDS) is becoming increasing sophisticated as pharmaceutical scientists acquire a better understanding of physiochemical and biological parameters pertinent to their performance.

The oral route is considered as the most favored, popular and practiced way of drug administration, because of its ease of administration, flexibility in designing, ease of production and low cost. For immediate release to site specific delivery, oral dosage forms have really progressed and large number of the drug available in the market is administered by oral route. Oral controlled release drug delivery have recently gained lots of interest in pharmaceutical field to achieve improved therapeutic advantages, such as ease of administration, patient compliance and flexibility in formulation.

The present investigation deals with the formulation, optimization and evaluation of gelrite and sodium alginate based floating oral In situ gel of Ambroxol hydrochloride. Oral administration is most convenient and preferred means of any drug delivery to the systemic circulation. Oral sustained release drug delivery recently have been increasing interest in pharmaceutical field to achieve improved therapeutic advantages, such as ease of dosing administration, patient compliance and flexibility in formulation. In-situ forming polymeric formulations drug delivery systems are in solution form before administration in the body, but once administered, undergoes gelation in-situ to form a gel. The formulation of gel depends upon factors like temperature modulation, pH changes, presence of ions and ultraviolet irradiation, from which drug gets released in sustained and controlled manner. The objective of this study was to develop a novel in- situ gel system for sustained drug delivery using a natural gelling agent. The system utilizes gelrite and sodium alginate that exhibit solution-to-gel phase transition due to changes in specific physicochemical parameters. In-situ gel was formed at a biological pH from a designed set of experiments, it was evident that formulation containing 0.5%,0.75%,1%w/v of sodium alginate control the release of drug for longer duration. The in-situ gel exhibited the expected, viscosity, drug content, pH, in vitro
gelling capacity, *in vitro* floating ability and sustained drug release. Ambroxol is indicated as "secretolytic therapy in bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport. It promotes mucus clearance, facilitates expectoration and eases productive cough, allowing patients to breathe freely and deeply. In the present study, an attempt was made to develop an *in situ* gelling liquid formulation using Ambroxol for local release in the stomach. Gastro-retentive *in situ* gelling liquid formulations were formulated using different grades and concentrations of sodium alginate. Sustained release forms: The goal of any drug delivery system is to provide a therapeutic amount of the drug to the proper site in the body to achieve promptly, and then maintain, the desired drug concentration. That Spatial placement relates to targeting a drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue. An approximately designed, sustained release drug delivery system can be a major advance toward sowing these two problems. Most sustained-release forms are designed so that the administration of a single dosage unit provides the immediate release of an amount of drug that promptly produces the desired therapeutic effect and gradual and continual release of additional amounts of drug to maintain this level of effect over an extended period, usually 8 to 12hrs. In general, the drugs best suited for incorporation into a sustained release product have the following characteristics. 1. They exhibit neither every slow nor very fast rates of absorption and excretion. 2. They are uniformly absorbed from the gastrointestinal tract. 3. They possess a good margin of safety.

**MATERIALS AND METHODS**

**Materials**

Ambroxol hydrochloride sample from Karnataka antibiotics Pvt Ltd, Banglore, gelrite from Yarrow chemicals Mumbai, sodium alginate, calcium chloride and methyl paraben from SD fine chemical Mumbai. sodium citrate from Thomas baker Mumbai.

**Drug - Excipients Compatibility Studies**[3-6]

Compatibility of drug with ingredients, sample of drug alone and with excipients used were kept in amber colored vials at room temperature for a period of 4 weeks. Samples were periodically evaluated and observed for its physicochemical change, i.e. appearance, color change, $\lambda_{\text{max}}$ and FTIR results compared with its initial results.
Determination of absorbance maximum (λ max) of Ambroxol Hydrochloride\(^{[7-8]}\)

Weighed 10 mg of pure Ambroxol hydrochloride added to 10 ml of methanol and diluted up to the mark with 0.1 N HCL to obtain a concentration of 100μg/ml as a stock solution. From the stock solution aliquot was withdrawn to obtain a concentration of 5μg/ml and scanned over the wavelength range of 400 NM to 200 NM using UV-spectrophotometer against same dilutions as blank. The spectrum of absorbance versus wavelength was recorded and analysed for the absorbance maximum (λ max) and its wavelength.

Fourier Transform Infrared Spectroscopy

Drug-excipients compatibility studies were carried out using FT-IR spectroscopy. The FT-IR spectrum of pure drug and drug-excipients mixture was taken in between 600-4000 cm\(^{-1}\) using the FTIR technique. The spectrum was compared for any possible incompatibilities between the drug and excipients used in the formulation.

Method of Formulation

a. Preparation of \textit{in situ} gelling solution of Gelrite (Gellan gum)\(^{[9-13]}\)

A gelrite solution of different concentration was prepared in around 30 ml deionized water containing sodium citrate Then the solution was heated to 90°C with stirring individually and cooled below 40°C. Appropriate amounts of calcium chloride were added. In another beaker Ambroxol hydrochloride was dissolved in deionized water with continuous stirring and finally poured into the above solution with continuous stirring. Preservative methyl paraben (0.02% w/v) was added to the above solution. Then final volume was adjusted to 100 ml with deionized water with constant stirring. The resulting Gelrite \textit{in situ} gelling solution stored in amber colored narrow mouth bottle until further use.

b. Preparation of \textit{in situ} gelling solution of Sodium alginate

Same as above procedure in place of gelrite sodium alginate was added.

c. Preparation of \textit{in situ} gelling solution with combination gelrite and sodium alginate

Gelrite solution was prepared by dissolving desired quantity of gelrite in around 30 ml of deionized water containing sodium citrate heated the solution to 90°C and cool below 40°C (1st solution). Similarly sodium alginate solution was prepared by dissolving desired quantity of sodium alginate in around 30 ml deionized water separately, heated the solution to 90°C and cool below 40°C and calcium chloride and Ambroxol hydrochloride were added to it with continuous stirring (2nd solution). The 1st and 2nd solution was mixed with constant
stirring then preservative methyl paraben was added to above mixture. Then final volume was adjusted to 100 ml using deionized water. The resulting in situ gelling solution containing Ambroxol hydrochloride was finally stored in amber colored narrow mouth bottle until further use. [Table1].

<table>
<thead>
<tr>
<th>Composition of floating in situ gel Ingredients in formulations</th>
</tr>
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<tbody>
<tr>
<td>Sample name (mg)</td>
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<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Ambroxol Hcl</td>
</tr>
<tr>
<td>Gelrite</td>
</tr>
<tr>
<td>Sodium alginate</td>
</tr>
<tr>
<td>Sodium citrate</td>
</tr>
<tr>
<td>Calcium chloride</td>
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<tr>
<td>Methyl paraben</td>
</tr>
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</table>

1. pH measurement\[14-25\] pH of the each prepared gelrite and sodium alginate based in situ gelling solutions of Ambroxol hydrochloride were checked using a calibrated pH meter at 25°C.

2. Gelling time

Gelling time was determined by mixing each of the formulation with 0.1 N HCl (approx. 50 ml) pH 1.2 in a beaker and the gelation was assessed by visual examination. The time required for the detection of gelation of in situ gelling system is noted down as gelling time and the integrity of gel was also observed.

3. Gel strength

50 ml of the formulation was placed in a 100 ml graduated cylinder and gelled by adding pH 1.2 buffer. Weight of 50 gm was placed onto the gel surface for measuring gel strength. Then gel strength, which is an indication of the ability of formed a gel to maintain its integrity, was measured, as time in seconds. The time taken from the weight to penetrate 5 cm down through the gel was noted down as gel strength.

4. Determination of drug content

Prepared 10 ml of in situ gel (containing equivalent to 60 mg of Ambroxol hydrochloride) from different batches were measured and transferred to 100 ml volumetric flack. To this 70 ml 0.1 N HCl was added and sonicated for 30 minutes. After that volume was adjusted to 100 ml with 0.1 N HCl. Complete dispersion of content was ensured visually and filtered using whatmann filter paper. From this solution 10 ml of sample was withdrawn and diluted to 100
ml with 0.1 N HCl. The absorbance of these solutions was measured in UV-Visible spectrophotometer at 244 nm against same dilutions without drug as a blank solution.

5. Floating behavior (Buoyancy)
Floating study of prepared in situ gel was carried out using 0.1 N HCl (pH 1.2). 10 ml of the formulation (in situ gelling solution) was added to a dissolution vessel containing 900 ml 0.1 N HCl without many disturbances. The time taken by formulation to emerge on the medium surface (floating lag time) and the duration of time the formulation constantly floated to the surface of the medium (floating time) were noted.

6. In vitro gelling capacity
The in vitro gelling capacity of the formulations was measured by placing 5 ml of the gelation solution (0.1N HCl, pH 1.2) in a 15 ml borosilicate glass test tube maintained at 37 ± 1°C temperatures. The formulation (1 ml) was added slowly by placing the pipette at surface of fluid in a test tube. As the solution comes in contact with gelation solution, it is immediately converted into a stiff gel like structure.

7. Viscosity study
The viscosity was measured using Brookfield DV-II+ viscometer using LV-2 spindle. The formulation was taken into sample holder and angular velocity increased gradually from 10 to 100 rpm, with await period of 30 sec at each speed. The torque value was found to be maximum of 30 rpm for the spindle LV-2, which has been selected for the entire study. The viscosity measurement was performed at room temperature. For all the formulation volume of the sample was adjusted with the mark given in the spindle to measure accurate viscosity.

8. In vitro dissolution study
The drug release study was carried out using USP-type II apparatus (rotating paddle type) at 37±0.2 °C and 50 rpm using 900 ml of 0.1 N HCl as a dissolution medium. In situ gelling solution equivalent to 60 mg of Ambroxol hydrochloride (10 ml) was used for study. 5 ml of sample solution was withdrawn at predetermined time intervals. After performing suitable dilution samples were analyzed spectrophotometrically at 244 nm. Equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample.

9. Drug release kinetic studies
The drug release kinetic studies were done by various mathematical models (zero order, first order, Higuchi’s square root, Hixson-Crowell cube
root law and Pappas equation). The model that best fits the release data is selected based on the correlation coefficient (R) value in various models. The model that gives high ‘R’ value is considered as the best fit of the release data. The release constant was calculated from the slope of the appropriate plots, and the regression coefficient (R2) was determined.

RESULTS AND DISCUSSION

Standard plot of Ambroxol hydrochloride in 0.1N HCL at 244nm.

Standard solution of Ambroxol hydrochloride (5μg/ml) scanned in the range of 200-400 nm showed maximum absorbance at 244 nm in 0.1N HCL. The standard calibration curve of Ambroxol hydrochloride was drawn by plotting absorbance v/s concentration. The value of regression coefficient was found to be 0.9996. The regression equation generated was y = 0.0237x+0.0058. The standard curve obeyed Beer’s law at the given concentration range of 2-20 μg/ml.

FT-IR spectrum of Ambroxol hydrochloride + Gelling agents and Excipients

1. Comparison of the IR spectra of pure drug to that of drug-excipients mixture after interpretation confirmed that there was no significant interaction between the drug excipients, as all the major peaks were identified after comparison of spectra’s which
ensured that there was no chemical interaction between them. This confirms the compatibility of drug and excipients used in formulation.

2. **pH Measurement**

pH measurement is very important for oral preparation otherwise it leads to irritation of the throat. Formulation F1 to F9 was checked for their pH using a calibrated pH meter. The pH of the formulations was found to be in the range of 6.32-6.84. The measurement of pH of each formulation was in triplicate and the average values were taken.

3. **Floating time**

Floating time of the prepared formulation was noted, all the formulations shown good floating ability, More than 8hrs.

4. **Gelling time**

Gelling time of the formulations was determined by mixing each of the formulation with 0.1 N HCl (approx. 50 ml) pH 1.2 in a beaker and the gelation was assessed by visual examination. Gelling time found to be vary according to the concentration of the polymer used proportionally.

5. **Drug content estimation**

Drug content of the developed formulations F1 to F9 was found to be in the range of 94.68±0.25% to 98.54±0.31%, which were within the limit (not < 94% and not > 106%) a Specified in U.S.P. Estimation was performed in triplicate. This study concludes that the drug was uniformly distributed in the formulation.

6. **Viscosity study**

Prepared formulation was evaluated for their rheological property using a brookfield viscometer. All the formulation exhibited pseudo-plastic rheology. Formulations having different concentrations of Gelrite alone, Sodium alginate alone and combination of both polymer Gelrite and sodium alginate found to affect its viscosity. Results of viscosity studies show that as the concentration of polymers increased in formulations, its viscosity also increases. The result obtained shows formulation consisting Gelrite exhibit more viscosity in comparison to sodium alginate.
7. Gel strength
Formulation F1, F2 and F6 to F9 shows good gel strength. F3 shows excellent gel strength. In case of formulation F4 and F5 shows poor gel strength. Gel strength found to depend on the concentration of the polymers used in the formulation. As the concentration increased the gel strength was also found to be increased proportionally. The result obtained shows formulation consisting Gelrite exhibit better gel strength in comparison to sodium alginate.

3. In vitro dissolution study
In vitro dissolution study was carried out for a period of 8 hrs for evaluating the drug release pattern from the formulations. The amount of cumulative drug release from the gel at the end of 8 hrs was found to be highest in formulation F4 and lowest in F3. This could be due to the effect of polymer concentration. The higher the concentration of polymer showed less cumulative release than that of the lower concentration of polymer. Gelrite based formulations exhibit delayed release rate in comparison to sodium alginate based formulation.

Comparative in vitro drug release profile of F1, F2, F3 formulation.

Comparative in vitro drug release profile of F4, F5, F6 formulations.
4. Release mechanism

The result of the regression analysis of zero order, first order, Higuchi model, Korsmeyer-Peppas model was shown in Table no. for all the formulations. Best formulation F3 shows highest value of $R^2$ for Korsmeyer-Peppas model (0.9877) with significant difference to that of another model. So formulation F3 follows Peppas kinetic model (with n value 0.6778) for drug release because of the good linearity obtained for the model. Since n value is greater than 0.45 and lesser than 0.89 so it indicates non-Fickian drug transport as the mechanism of release. Another best formulation F9 shows highest value of $R^2$ Higuchi model (0.9885) with significant difference to that of another model. Thus F9 follows Higuchi kinetic model for drug release.

5. Stability study

Stability studies were carried out on the selected best formulations F3 and F9 as per ICH Guidelines. The formulation was stored in sealed aluminium foil at 25±2°C, 60±5% RH for 3 months. The formulation was periodically evaluated for physicochemical parameter such as pH, viscosity, drug content, in vitro drug release and no significant changes were observed while comparing with the initial results which ensures the stability of the formulation.

CONCLUSION

In the present study, an attempt was made to formulate and evaluate floating in situ gel of Ambroxol hydrochloride by using natural polysaccharides i.e. Gelrite and sodium alginate. The main interest in such dosage form was to target the drug to its site of action (stomach) by preparing gastro retentive drug delivery system. At the outset, pre-formulation studies and estimation of drug by UV visible spectrophotometer was carried out. The possible interaction
between the drug and excipients was studied by FT-IR spectroscopy, which showed that there was no significant interaction between Ambroxol hydrochloride and other selected excipients under study.

Oral floating in situ gel of Ambroxol hydrochloride was prepared by Ambroxol chloride, polymers such as Gelrite, sodium alginate and other excipients as shown in formulation chart. In this study, various formulations were prepared using different concentration of Gelrite alone, sodium alginate alone and in combination of gelrite and sodium alginate in different ratios. Calcium chloride was used as a source of cation for cation induced cross linking of polymer. Formulation F3 (Gelrite alone) and F9 (gelrite and sodium alginate) were selected as best formulation because of good gelling capacity, optimum viscosity and acceptable % cumulative drug release aster 8 hrs shown by them. Formulation F3 followed Korsmeyer-Peppas Model kinetic (non- fickian transport) with R2 value 0.9877 and n value 0.6778 for the drug release from the formulation. Formulation F9 followed Higuchi model kinetic with R2 value 0.9885 for the drug release from the formulation. Results of the stability study confirmed the stability of selected formulations as there were no significant changes found in the parameters considered after 3 month stability study. Gelrite based formulations are shown higher viscosity and gel strength, whereas slower rate of drug release in sodium alginate formulations.

**BIBLIOGRAPHY**


