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FORMULATION AND *IN-VITRO* EVALUATION OF METOCLOPRAMIDE MICROSPHERES

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ABSTRACT
Microspheres are sub-micron size polymeric drug carrier systems in which therapeutic agents are loaded micrometer. These particles are made up of a covering substance and the medication as the core component. The regulated and targeted drug delivery technology known as microspheres is thought to be very promising. The physicochemical, pharmacokinetic, and pharmacological characteristics of medicines are the foundation for the formulation and therapeutic use of microspheres. Metoclopramide is a 5-HT_3 receptor antagonist referred to as prokinetic agent. It increases contraction of muscles of gastrointestinal tract. It works by antagonizing central and peripheral dopamine two receptors in the areas that trigger nausea and vomiting. It is used to treat GERD (gastroesophageal reflux disease), diabetic gastroparesis, nausea and vomiting.

KEYWORDS: Microspheres, metoclopramide hydrochloride, hydroxyl propyl methyl cellulose and ethyl cellulose.

INTRODUCTION
Oral route nowadays represent predominant and most preferable route for drug delivery. It allows ease of administration by the patients. It is divided into immediate and modified release system. Immediate release DDS (Drug delivery system) are intended to disintegrate rapidly and exhibit instant release of drug.[1]

Modified release systems have been developed to improve the pharmacokinetic profiles of active pharmaceutical ingredients (API) and patient compliances, as well as reduce side effects.[2]

Oral modified release delivery system is most commonly used for

1) Delayed release (e.g. By using enteric coating)
2) Extended release (e.g. zero-order, first-order etc.)
3) Programmed release (e.g. pulsatile, triggered etc.)
4) Site specific or timed release (e.g. for colonic delivery or gastric retention).

The term oral controlled release implies a system that provides continuous delivery of drug for a predetermined period in a predictable and reproducible manner which increases the bioavailability.

Oral drug delivery system
Most limiting biological factor in development of once daily oral controlled release is the transit time of dosage form through the G.I. tract.

Anatomy and physiology of stomach[3,4]
STOMACH
Stomach is located in the upper left – hand portion of the abdomen just below the diaphragm. The main function of the stomach is to store food temporarily, grind and then release it slowly into the duodenum.
Stomach has four regions mainly [figure 1]

1. Cardia
2. Fundus
3. Body
4. Pylorus.

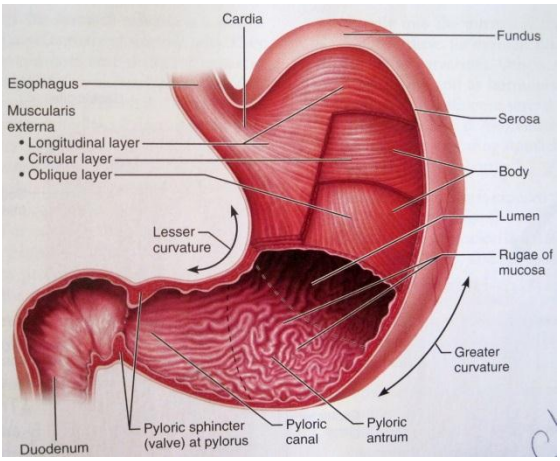

Figure 1: Anatomy of stomach.

Anatomy of Stomach

Stomach is composed of fundus and body regions. Stomach lining is devoid of villi and it consists of considerable number of gastric pits that contributes to storage capacity of stomach. Antrum region is responsible for mixing and grinding of gastric content. There are two main secretions, mucus by goblet cells and gastric acid by parietal cells.

Physiology of stomach

Gastric absorption of most of the drugs is insignificant, because of limited surface area, (0.1-0.2) m^2 thick layer of mucous coating, absence of villi on mucosal surface and short residence time.

pH range of GIT
Stomach - 1-3
Small intestine - 5-7.5
Large intestine - 7.9-8
Rectum - 7.5-8.

Gastro-retentive drug delivery system (GRDDS)

GRDDS is developed in order to prolong contact time of drug with the GI mucosa leading to higher bioavailability, higher therapeutic efficacy, reduced time intervals for drug administration and thus improved patient compliance.

Physiological factors affecting gastric retention

Gastric emptying process[3,5]

The passage from stomach to small intestine is referred as gastric emptying. Delayed gastric emptying is useful in particular where

- Drugs are absorbed from proximal part of small intestine e.g. vitamin B_2 and vitamin C.
- Drug dissolves slowly e.g. griseofulvin.
- Food promotes drug dissolution and absorption e.g. griseofulvin.[6]

Process of gastric emptying occurs in both fasting and fed state; however, the pattern of motility differs like in fasted state, it is characterized by an inter-digestive series of electrical events in a cyclic manner both through stomach and small intestine every 2-3 hours. This activity is referred to as migrating myoelectric complex (MMC), which is divided into 4 consecutive phases as described below

- **Phase-1** = Quickest period lasting from 30 to 60 minutes with no contraction.
- **Phase-2** = Consists of intermittent contraction that gradually increases in intensity as phase progresses and it last about 20 to 40 minutes.
- **Phase-3** = Short period of intense distal and proximal gastric contraction (4-5 contraction per minute) lasting about 10-20 minutes. These contractions are known as "house-keeper wave" i.e. sweep gastric contents down the small intestine.
- **Phase-4** = Short transitory period of about 0-5 minutes and contractions dissipate between the last part ofphase 3 and consequences of phase 1.[7]

Factors affecting gastric emptying and hence gastric retention time of an oral dosage form include[8,9]

Volume of meal: Larger the bulk of meal, longer will be the gastric emptying time.

Composition of meal: Fat promotes the secretion of bile, which has an inhibitory effect on gastric emptying time.

Physical state of foodand dosage form: Viscous material empty slowly than less viscous material.

Exercise: Retards gastric emptying time.

Age: Increase in age decreases gastric motility there by increasing the gastric emptying time.

Drug therapy: It also plays an important role in gastric emptying e.g. prokinetic drugs like cisapride and mosapride increases gastric emptying time whereas imipramine and atropine retard it.

Posture: Gastric emptying is favored by standing and by lying on the right side since normal curvature of a

stomach provides a downhill path whereas lying on left side or in supine position retards it.

Drug candidates for gastric retention

1. Locally acting on the stomach, such as antibiotics and antacids.
2. Absorbed partially as a result of the comparatively small G.I.T absorption window for drugs like cyclosporine, ciprofloxacin, and riboflavin.
3. Unstable in the environment of the colon or intestine, such as captopril. 4. Show limited solubility at high pH values, such as diazepam and verapamil.

Advantages of GRDDS[10]

1. Enhanced bioavailability
2. reduced frequency of dosing
3. Patient compliance
4. Improved therapeutic efficacy
5. Targeted therapy for local ailments in the upper GIT.

Disadvantages of GRDDS[11,12]

1. Requirement of high level of fluid in the stomach for the delivery system to float and work efficiently.
2. Requires the presence of food to delay gastric emptying.
3. Drug having solubility or stability problems in the highly acidic gastric environment cannot be formulated as GRDDS.

Approaches to GRDDS

Various approaches have been pursued to prolong the residence time of oral dosage forms in the stomach, these methods includes.

Bio/Muco-adhesive system[13,14]

Bio/muco-adhesive systems are those which bind to the gastric epithelial cell surface or mucin and serve as a potential means of extending the gastro retention of drug delivery system (DDS) in the stomach by increasing the intimacy and duration of contact of drug with the biological membrane.

Raft forming systems[15]

Raft forming systems have received much attention for the delivery of antacids and drug delivery for gastrointestinal infections and disorders. The mechanism involved in the raft formation includes the formation of viscous cohesive gel in contact with gastric fluids, wherein each portion of the liquid swells forming a continuous layer called a raft. This raft floats on gastric fluid because of low bulk density created by the formation of CO2 Usually, the system contains a gel forming agent and alkaline bicarbonates or carbonates responsible for the formation of CO to make the system less dense and float on the gastric fluids.

Low density systems[16]

Gas-generation systems inevitably have a lag time before floating on the stomach contents, during which the dosage form may undergo premature evacuation through the pyloric sphincter. Low-density systems (< 1 g/cm³) with immediate buoyancy have therefore been developed. They are made of low-density materials, entrapping oil or air. Most are multiple unit systems and are also called "microballoons" because of the low-density core. Generally, technique used to prepare hollow microspheres involves simple solvent evaporation or solvent diffusion methods. Polycarbonate, Eudragit S, cellulose acetate, calcium alginate, agar and low methoxylated pectin are commonly used as polymers. Buoyancy and drug release are dependent on quantity of polymer, the plasticizer-polymer ratio and solvent used.

Swelling and expanding systems[17]

Swelling type dosage forms are such that after swallowing, these products swell to an extent that prevents their exit from the stomach through the pylorus and as a result the dosage form is retained in the stomach for a longer period of time. These systems may be referred to as plug type systems.

High-density system[18]

Gastric contents have a density close to water (1.004g/cm³). When high-density pellets are given to the patient, it will sink to the bottom of the stomach and are entrapped in the folds of the antrum and withstand the peristaltic waves of the stomach wall. Sedimentation has been employed as a retention mechanism for high-density systems. A density -3g/cm' seems necessary for significant prolongation of gastric residence time. Barium sulphate, zinc oxide, iron powder, titanium dioxide may be used to formulate such high-density systems due to their high density.

Floating system[19,20]

Floating system or hydrodynamically controlled systems are low density system that has sufficient buoyancy to float over the gastric contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. This results in an increased GRT and a better control of the fluctuations in plasma drug concentration. Many buoyant systems have been developed based on granules, powders, capsules, tablets, laminated films and hollow microspheres.

Microspheres

Small, spherical particles with a diameter in the micrometer range—typically between 1um and 1000um (1 mm)—are referred to as microspheres. Microparticles are anothername formicrospheres. They are made of polymer, wax, or another type of protective material, such as a natural product or a synthetic polymer that degrades quickly.

The two types of microparticulate drug delivery systems are matrix type and reservoir type. Drug molecules that

are scattered in the matrix type of substance can be found in crystalline or solution form. The reservoir system consists of spherical particles in which drug is concentrated at the middle core.

Advantages of microsphere technology[21]
- Taste and odour can be masked.
- Conversion of oil and other liquids into pseudo solids for ease of handling.
- Delay in volatilization.
- Separation of incompatible material.
- Improvement in flow properties of powder.

Applications of microspheres[22]
- Separation of incompatible entities.
- Conversion of liquid to free flowing powder.
- Improvement in the dissolution rate and bioavailability of drug.
- Disguising unfavorable odor and taste.
- Sustained and prolonged release of drug.

Preparation techniques for microspheres[23,24,25]
The microspheres can be prepared by using the several techniques.

Single emulsion technique: The microparticulate carrier of natural polymers, i.e. those of protein and carbohydrate are prepared by single emulsion technique. The natural polymers are dispersed in aqueous medium followed by dispersion in non-aqueous medium e.g. oil. Then cross linking of dispersed globules are carried out. The cross linking can be achieved either by means of heating or by using the chemical cross linker, e.g. glutaraldehyde, formaldehyde etc.

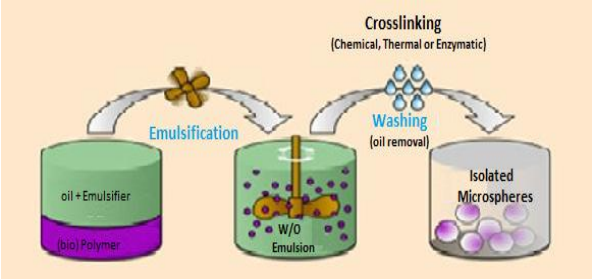

Figure 2: Single emulsion method for microsphere preparation.

Double emulsion technique: This method of microsphere preparation involves the formation of multiple emulsions or the double emulsion of type w/o/w and it is best suited to the water soluble drugs, peptides, proteins and the vaccines. The aqueous protein solution is dispersed in a hypophilic organic continuous phase. This protein phase may contain active ingredients. The continuous phase is generally consisted of polymer solution that eventually encapsulates the protein contained in dispersed aqueous phase. The primary emulsion is then subjected to the homogenization or the sonication before addition to the aqueous solution of poly vinyl alcohol (PVA). This results in the formation of the double emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction process.

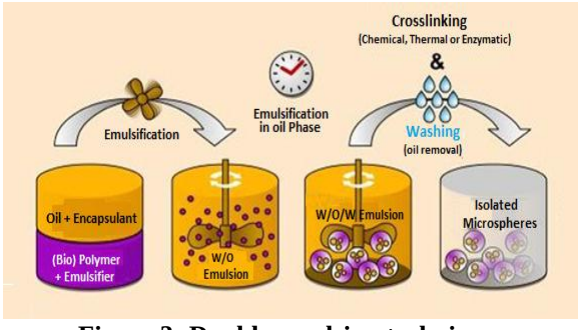

Figure 3: Double emulsion technique.

Polymerization technique
Normal polymerization: The normal polymerization is carried out in a liquid phase and carried out by using different techniques as bulk, suspension, precipitation, emulsion and miceller polymerization processes. In bulk, a monomer or a mixture of monomer along with initiates polymerization. Polymer so obtained may be moulded or fragmented as microspheres. Drug loading may be done during the process of polymerization.Suspension polymerization also referred as bead or pearl polymerization. It is carried out by heating the monomer or its mixture as droplet dispersion in a continuous aqueous phase. Emulsion polymerization is different from suspension polymerization as due to the presence of initiator in the aqueous phase. which later on diffuses to the surface of micelles or the emulsion globules. The bulk polymerization has an advantage of formation of the pure polymer, but it also suffers a disadvantage as it is

very difficult to dissipate the heat of reaction, which can adversely affect the thermolabile active ingredients.

Interfacial polymerization: It involves the reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase.

Phase separation and coacervation technique: Specially designed for preparing the reservoir types of the system, i.e., to encapsulate the water soluble drugs e.g. peptides, proteins, matrix type, particularly, when the drug is hydrophobic in nature e.g., steroids. In matrix type devices, the drug or the protein is soluble in the polymer phase. The process is based on the principle of decreasing the solubility of polymer in the organic phase to affect the formation of the polymer rich phase called coacervates. The coacervation can be brought about by addition of the third component to the system which results in the formation of the two phases, one i.e. supernatant, depleted of the polymer. In this technique, the polymer is first dissolved in a suitable solvent and then drug is dispersed by making its aqueous solution, if hydrophilic or dissolved in theformulation and in vitro evaluation of metoclopramide microspheres polymer solution itself, if hydrophobic. Phase separation is then accomplished by changing the solution condition.

Spray drying technique: These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leadingthe formation of the microspheres in the sizerange 1-100 um. Microparticles are separated from the hot air.

MATERIALS AND METHODS

Materials

Chemicals and Reagents

Materials used in the formulation are Metoclopramide hydrochloride, ethyl cellulose, hydroxyl propyl methyl cellulose (HPMC), dichloromethane, ethanol, potassium dihydrogen orthophosphate, sodium hydroxide, fused calcium chloride and HCl.

Equipment and Instruments

Following equipments were used: UV-visible spectrophotometer, hot air oven, electronic weighing balance, flash shaker, melting point apparatus, dissolution apparatus, hot plate, scanning electron microscope, projection microscope and propeller type agitator.

METHODOLOGY

Characteristics of pure drug

Physical description of drug

It is characterized by the nature of drug and organoleptic properties which include appearance, color, odour and taste of the drug.

Pre-formulation studies

Pre-formulation studies involving melting point, drug-excipients compatibility and FT-IR studies were performed for the drug.

Determination of melting point

Melting point of drug was determined by taking small amount of drug in a capillary tube closed at one end. The capillary tube was placed in a melting point apparatus and the temperature at which drug melts was recorded. This was performed thrice and average value was taken.

Drug excipients compatibility study

100 mg of each polymer/excipients was weighed accurately. To each of them 100 mg of drug was mixed in a pestle mortar. Four set of prepared physical mixtures were placed in a glass vials which were then tightly sealed. Vials were kept at 25°C/60 % RH and 40°C/75 % RH for 4 weeks. The incompatibility in the form caking, liquification and odour or gas formation was observed.

FT-IR spectroscopy: In the preparation of microspheres, drug and polymers may interact as they are in close contact with each other, which could lead to instability of drug. Pre-formulation studies regarding the drug polymer interaction are therefore very critical in selecting appropriate polymers. FT-IR spectroscopy was employed to ascertain the compatibility between metoclopramide and selected polymers. The pure drug and drug excipients were scanned separately by FT-IR spectrophotometer. All the powder samples were dried prior to obtaining any spectra in order to remove the influence of residual moisture.

Analytical methodology

Scanning of metoclopramide in 0.1N Hydrochloric acid

Accurately weighed 50 mg of metoclopramide was dissolved in 100 ml 0.1N HCI solution. From this solution 1 ml was pipette out in the 50 ml volumetric flask and volume was made up to 50 ml with 0.1N HCI (pH1:2) (concentration 10 μg/ml). The solution containing 10 μg/ml of metoclopramide in 0.1N HCI (pH-1.2) was scanned over the range of 200-400 nm against 0.IN HCl as blank using double beam spectrophotometer. The maximum obtained in graph was considered λ_{max} for pure drug.

Standard calibration curve in 0.IN HCI

Accurately weighed 10 mg of drug was dissolved in 100 ml of 0.1 N HCI to get a solution containing 100p/ml. This stock solution was used to prepare further dilutions of standard solutionAliquots (0.5-5 ml) of stock solution

were transferred into a series of 10 mi volumetric flasks. The volume was made up to mark with water to produce the concentration ranging from 5-50µg/ml. Theabsorbance of each prepared solution was measured at lambda and 272nm using double beam UV spectrophotometer against 0.1 N HCL.

Scanning of metoclopramide in distilled water

Accurately weighed 50 mg of metoclopramide was dissolved in 100 ml distilled water. From this solution 1 ml was pipette out in the 50 ml volumetric flask and volume was made up to 50 ml with distilled water (concentration 10µg/ml). The solution containing 10 µg/ml of metoclopramide in distilled water was scanned over the range of 200-400 nm against distilled as blank using double beam spectrophotometer. The maximum obtained in graph was considered beta max for pure drug.

Standard calibration curve in distilled water

Accurately weighed 10 mg of drug was dissolved in 100 ml of distilled water to get a solution containing 100µg/ml. This stock solution was used to prepare further dilutions of standard solution. Aliquots (0.5-5 ml) of stock solution were transferred into a series of 10 ml volumetric flasks. The volume was made up to mark with water to produce the concentration ranging from 5-50 µg/ml. The absorbance of each prepared solution was measured at lambda max 273 nm using double beam UV spectrophotometer against distilled water.

Scanning of metoclopramide in phosphate buffer (pH 6.8)

Accurately weighed 50 mg of metoclopramide was dissolved in 100 mi phosphate buffer pH 6.8. From this solution 1 ml was pipette out in the 50 ml volumetric flask and volume was made up to 50 ml with phosphate buffer (6.8 pH) (conc10 µg/ml). The solution containing 10 µg /ml of metoclopramide in phosphate buffer (pH 6.8) was scanned over the range of 200-400 nm against phosphate buffer (6.8 pH) as blank using double beam spectrophotometer. The maximum obtained in graph was considered lambda max for pure drug.

Standard calibration curve in phosphate buffer (pH 6.8)

Accurately weighed 10 mg of drug was dissolved in 100 ml of phosphate buffer (pH 6.8) to get a solution containing 100 µg /ml. This stock solution was used to prepare further dilutions of standard solution Aliquots (0.5-5 ml) of stock solution were transferred into a series of 10 ml volumetric flasks. The volume was made up to mark with water to produce the concentration ranging from 5-50µg/ml. The absorbance of each prepared solution was measured at lambda max 273 nm using double beam UV spectrophotometer against phosphate buffer (pH 6.8).

Scanning of metoclopramide in phosphate buffer (pH 7.4)

Accurately weighed 50 mg of metoclopramide was dissolved in 100 ml phosphate buffer pH 7.4. From this solution 1 ml was pipette out in the 50 ml volumetric flask and volume was made up to 50 ml with phosphate buffer (7.4pH) (conc. 10 µg/ml). The solution containing 10 µg /ml of metoclopramide in phosphate buffer (pH 7.4) was scanned over the range of 200-400 nm against phosphate buffer (7.4pH) as blank using double beam spectrophotometer. The maximum obtained in graph was considered lambda Buax for pure drug.

Standard calibration curve in phosphate buffer (pH 7.4)

Accurately weighed 10 mg of drug was dissolved in 100 ml of phosphate buffer (pH 7.4) to get a solution containing 100µg/ml. This stock solution was used to prepare further dilutions of standard solution. Aliquots (0.5 - 5ml) of stock solution were transferred into a series of 10 ml volumetric flasks. The volume was made up to mark with water to produce the concentration.

PREPARATION OF MICROSPHERES

Preliminary trials of microspheres

Preliminary trials were done in order to select the experimental domain to fabricate microspheres. The experimental field should not be too large, leading to non-realistic experiments and not too small, far from optional region.

Preliminary trials for independent variable

EC (x)) and stirring speed [RPM (x) were tried as independent variables for preparation of microspheres.

Design of experiment for preparation of microspheres

A 2-factor, 3-level design was used for exploring quadratic response surfaces and constructing polynomial models with Design Expert version 8.0.7.1. The two independent variables such as EC (A) and stirring speed (B) were selected on the basis of the preliminary studies carried out before the experimental design being implemented. The experimental design was applied to investigate the effect of different independent variables such as A and B. The interaction term (AB) shows how the response changes when two factors are changed simultaneously. The polynomial term (A'B') is included to investigate non linearity.

Table 1: Independent variables in 3^2 factorial design.

Correlation of actual and coded values			
Factor	**Coded value**	**Actual value**	
		EC	**Stirring**
LOW	-1	60	900
MEDIUM	0	120	1200
HIGH	+1	180	1500

Preparation of microspheres

The microspheres were prepared using solvent evaporation technique with polymers such as ethyl cellulose and HPMC. The organic solvent system used was solution of dichloromethane and ethanol (1:1). Tween 80 was used in 0.01% concentration.

The polymers were dissolved in the organic solvent with continuous stirring using propeller type agitator.

Stages in preparation of microspheres

Drug was weighed accurately

↓

Polymers in different ratio were weighed accurately

↓

Polymers added organic solvent

↓

Distilled water with 0.01% tween 80 heated upto 40°C separately

↓

Addition of organic phase to aqueous phase at 900 rpm

↓

Heated (40°C) and stirred the mixture for about half an hour to evaporate volatile solvent

↓

Microspheres were filtered and dried.

Characterization of formulation

Percent yield analysis[26]

The prepared floating microspheres were collected and weighed. The measured weight was divided by total amount of all nonvolatile components, which were used for the preparation of microspheres. The % yield was calculated using following formula

% Yield= (Actual weight of product/Total weight of non-volatile excipients and drug) X 100

Particle size analysis[27]

Measurements of the particle size distribution of microspheres were carried out with a projection microscope. Stage micrometer was used to calculate calibration factor. The particle size was calculated by multiplying the number of divisions of the ocular discoccupied by the particle with calibration factor. Fifty randomly chosen spheres were taken to measure their individual size.

Flow properties[28]

- **Angle of repose**

For the measurement of angle of repose, a glass funnel was secured with its tip at given height (h) above a piece of graph paper placed on a horizontal surface. Microspheres were poured through the funnel until the apex of the conical pile touched the tip of the funnel. The angle of repose was calculated with the formula

$\tan \theta = h/r$

$\theta = \tan^{-1} (h/r)$ (Where. = angle of repose, H=height of the heap, r=radius of the heap)

Angle of repose is affected by particle size. Larger the particle size, freely will be the flow.

- **Bulk density**

For determination of bulk density, weighed quantities of microspheres were introduced into graduated measuring cylinder and were tapped mechanically of either manually till constant volume was obtained. The bulk density of microspheres depends on particle size distribution and particle shape. Bulk density is calculated by the mass of microspheres divided by the volume of the microspheres.

Bulk Density= Mass of microspheres/Bulk volume of microspheres.

- **Tapped density:** The cylinder containing known amount of microspheres was given 100 taps on tap density apparatus. It is calculated by formula

Tapped density= (Mass of microspheres/Volume of microspheres after tapping) X100.

- **Carr's compressibility index**: It is an important property in maintaining uniform weight. It is calculated using following formulae

%Compressibility index= (Tapped density-bulk density / Tapped density) X 100.

- **Hausner's ratio:** A similar index like percentage compressibility index has been defined by Hausner. Hausner value less than 1.25 indicates good flow, where as greater than 1.25 indicates poor flow. It is calculated by the following formulae

Hausner's ratio = Tapped density / Bulk density.

Drug entrapment efficiency[29]

50 mg of metoclopramide loaded microspheres were accurately weighed. They were dissolved in minimal amount of dichloromethane and the drug was extracted into 0.1N HCI by evaporating dichloromethane. The volume was adjusted to 100 ml with HCI. The resulting solution was then suitably diluted and analyzed for drug content spectrophotometrically at 273 nm using HCl pH (1.2) as blank. Encapsulation efficiency was calculated using formula given below

%Drug entrapment = (Calculated drug concentration / Theoretical drug concentration) X 100

Buoyancy percentage[30]

Microspheres were spread over the surface of dissolution apparatus (Type II) filled with 900 ml of 0.1N HCl containing 0.02% tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12 h. The floating and the settled portion of microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of microspheres that remained floating and the total mass of the microspheres.

Buoyancy (%) = (Q_F/ Q_F+Qs) X 100 [where **Q_F** and **Qs** is weight of floating and the settled microspheres respectively].

***In-vitro* drug release**[31]

The *in vitro* release of drug from floating microspheres was carried out using paddle type Electro lab dissolution apparatus USP XXIII. Drug loaded microspheres equivalent to 30 mg of drug was introduced into 900 ml of the dissolution medium (0.1 N HCI) maintained at 37 ± 0.5°C with paddle rotating at 100 rpm. Aliquots were withdrawn at regular intervals and analyzed spectrophotometrically using UV spectrophotometer. The dissolution studies were carried out in 0.IN HCI for 24 hours. The 5 ml sample was withdrawn at predetermined intervals and filtered. Equal volume of dissolution mediumwas replaced after each withdrawl to maintain sink condition. The collected samples were suitably diluted with 0.1 N HCI and analyzed spectrophotometrically at nm to determine the concentration of drug in the dissolution medium.

Drug release kinetics

In the present study, raw data obtained from in vitro release studies were analyzed. Wherein data were fitted to different equations and kinetic models to calculate the percent drug release and release kinetics of metoclopramide microspheres. Table-shows the cumulative percentage drug release, log of cumulative percent drug release and remaining at various time points. The results of in vitro release profile of optimized batch were fitted into four models of data treatment as follows

- Cumulative percent drug released versus time (zero-order kinetic model) Log cumulative percent drug remaining versus time (first order kinetics)
- Cumulative percent drug released versus square root of time (Higuchi's model) Log cumulative percent drug remaining versus log time (Korsmeyer's-Peppas model)

Zero order kinetics

A zero order release would be predicted by the following equation: $A_t = A_o - K_o t$

Where, A_t = release at time t

A_o= initial drug concentration

K_o= zero order rate constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero order release kinetics, with a slope equal to K_o.

First-order kinetics

First order release would be predicted by the following equation: $\boldsymbol{log\ C = log\ C_o - K_t / 2.303}$ Where:c = amount of drug remained at time t C_o = initial amount of drug K= first-order rate constant hr^{-1}.

Higuchi's model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation

$$Q = \frac{D}{\lambda\tau}(2A - \epsilon Cs)]\ Cst^{1/2}$$

Q = amount of drug released at time t

D = diffusion coefficient of the drug in the matrix A = total amount of drug in unit volume of matrix C_s = solubility of the drug in the diffusion mediumϵ= porosity of the matrix τ= tortuosity t = time at which Q amount of drug is released.

Korsmeyer-peppas model

The release rate from controlled release polymeric matrices can be described by the equation

$$Q = Kt^n$$

Q = percentage of drug released at time t

K = solubility of the drug in the diffusion medium.

RESULTS AND DISCUSSION

Characterization of drug

Physical description of metoclopramide

Physical characterization

Nature- Soft powder

Color- White

Odour- Odorless

Taste- Bitter.

Pre-formulation studies
Pre-formulation studies involving melting point, drug-excipients compatibility and FT-IR studies were performed for the drug.

Determination of melting point Melting point of metoclopramide hydrochloride was found to be 145°C as specified in monograph which confirms the purity of drug as per IP.

Drug-excipients compatibility study Drug was subjected to the compatibility study with different excipients/polymers at 25°C / 60% RH and 40°C/75% RH for 4 weeks and was observed for physical changes (color changes, liquification and gas or odour formation). After 2 to 4 weeks no physical change was observed in the vial containing drug and excipients/polymers which shows that drug is compatible with selected excipients/polymers.

Result: No physical incompatibility was found with any of the excipients/polymers.

FT-IR spectral studies
Chemical interaction between drug and polymeric material was studied by using FT-IR. From the infrared spectra, it is clearly evident that there were no interactions of drug with the polymer as functional groups responsible for antiemetic action of drug was unaltered. The main peaks in the spectrum of the drug metoclopramide do not show any substantial difference. The LR spectrum shows a peak at 3392.40cm^{-1}, which signifies presence of N-H stretching. Peak at 2623.3cm^{-1}shows a presence of N-H stretch, peak at 1594.1 cm^{-1}shows presence of C=O functional group. Peak at 1532.86cm^{-1} signifies the presence of c-o-c. Simultaneously, peak at 678.82 cm^{-1} signifies presence of C-C stretching. All peaks were observed in the finger print region of FT-IR spectra. This proves that there is no drug-excipients interaction.

Analytical method development

Determination of absorption maxima(λ_{max}) of metoclopramide inhydrochloric acid pH 1.2

The solution containing 20μg/ml was scanned between 200-400 nm. The $\lambda_{max}$was found to be 273 nm which indicates purity of sample drug metroclopramide hydrochloride.

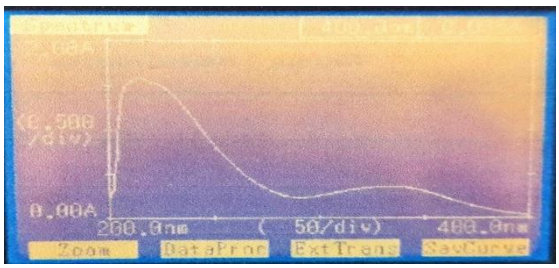

Figure 4: $\lambda_{max}$of metoclopramide in 0.1N HCI.

The absorbance of the drug solution was estimated at λ_{max}273 nm in Shimadzu UV-1700 spectrophotometer against 0.1N HCI (1.2 pH) as blank.

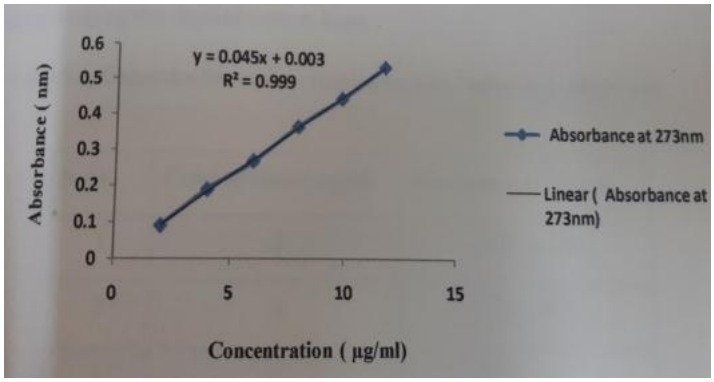

Figure 5: Standard plot of metoclopramide in 0.1N HCl.

Distilled water: UV scan of metoclopramide was done in distilled water and λ_{max} at 273nm was observed.

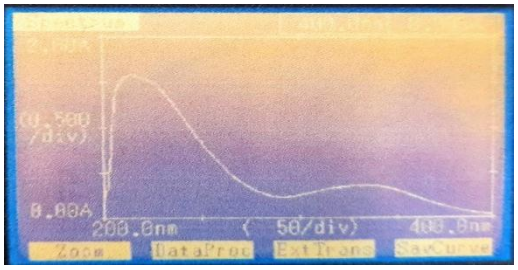

Figure 6: λ_{max} of metoclopramide in distilled water.

The absorbance of each dilution was measured at 273nm in Shimadzu UV-1700 spectrophotometer against distilled water as blank.

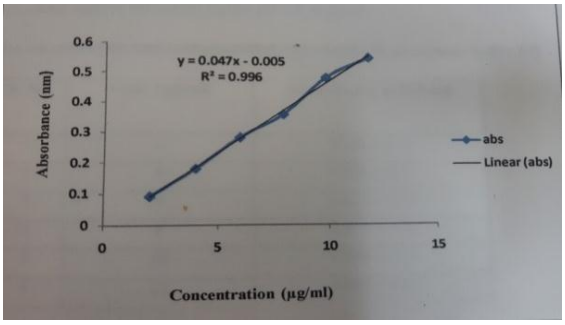

Figure 7: Standard plot of metoclopramide in distilled water.

The graph obeyed beer Lambert's law in this selected concentration range. The calibration equation for straight line was observed to be y = 0.0476x - 0.0054 with correlation coefficient as 0.9964, which was further used for determination of concentration of unknown sample.

With phosphate buffer pH 6.8

The absorbance of the drug solution was estimated at lambda max 273 nm.

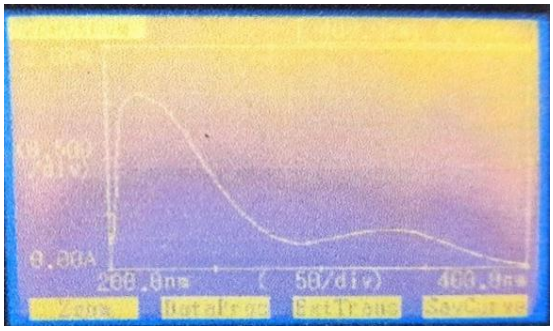

Figure 8: $\lambda_{max}$of metoclopramide in pH 6.8 phosphate buffer.

The absorbance of each dilution was measured at 273 nm in Shimadzu UV-1700 spectrophotometer against phosphate buffer pH 6.8 as blank.

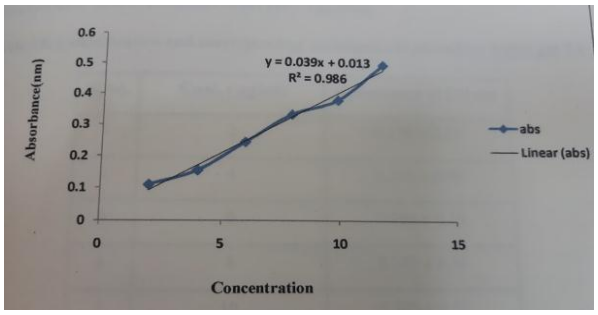

Figure 9: Standard plot of metoclopramide in phosphate buffer 6.8.

The graph obeyed beer Lambert's law in this selected concentration range. The calibration equation for straight line was observed to be y = 0.0582x-0.0243 with correlation coefficient as 0.998, which was further used for determination of concentration of unknown sample.

With phosphate buffer 7.4

The absorbance of the drug solution was estimated at λ_{max} 273 nm.

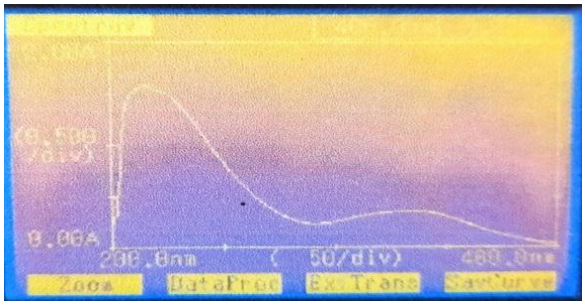

Figure 10: λ_{max} of metoclopramide in pH 7.4 phosphate buffer.

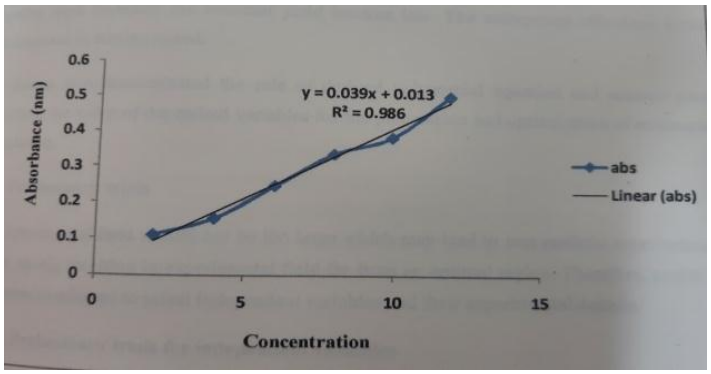

Figure 11: Standard plot of metoclopramide in phosphate buffer pH 7.4.

Characterization of microspheres

The microspheres of metoclopramide y were prepared by using solvent evaporation method. Total 9 formulations was prepared (using 3 factorial designs) and subjected to various physical and in pre studies. The result of these characterization are given below

Percentage yield

% yield= (Actual weight of microspheres / Total weight of nonvolatile components) X 100.

The percentage yield was in the range 73.7 to 96.2 %. It was observed that as the stirring speed increases, the yield of the microspheres increases. It was also found that at low speed polymer aggregated around the propeller shaft resulting in low yield.

Particle size analysis

The particle size of the microspheres was measured by projection microscope and the average particle size of 100 microspheres was taken. The particle size increases with increase in polymer ratio, but size decreases with increase in stirring speed. This is because smaller emulsion droplets are produced through stronger shear forces and increased turbulence. Particle size of all 9 formulations was in the range 104 to 190 μm.

Flow properties

- **Angle of repose**

Angle of repose is defined as maximum angle possible between the surface and the horizontal plane. Angle of repose for all the nine formulations were in the range 25.40° to 38.77 which shows that flow property of all the nine formulation were in the acceptable range. Angle of repose depends on the particle size, larger the particle size, more will it flow freely.

- **Bulk density**

Bulk density of all nine formulations was in the range of 0.153 to 0.592 g/cm^3 which was less than that of gastric fluid 1.004 g/cm^3.

- **Tap density**

Tap density of all nine formulations was in the range of 0.170 to 0.622 g/cm^3 which was less than of gastric fluid 1.004 g/cm^3.

- **Carr's compressibility index**

The Carr's index for all the nine formulations ranged from 9.54 to 18.31 which is well acceptable within the limits.

- **Hausner's index**

Hausner's index for all the nine formulations ranged from 1.04 to 1.15 which is well acceptable within the limits.

Drug entrapment study

As the concentration of ethyl cellulose increased more than 120 mg the drug entrapment decreased due to agglomeration of polymer but increase in stirring speed from 900 to 1500 rpm increased the entrapment due to more amount of polymer being available to entrap the drug. The drug entrapment of different batches was in the range 75.25 to 96.55% w/w.

Buoyancy time

The floating ability of all the 9 formulations was found to be different and varied according to concentration of ethyl cellulose. Increase in EC concentration showed good floating property which could be due to insolubility of EC in gastric fluid. The buoyancy time was calculated using USP dissolution apparatus containing 0.1 N HCl (900 ml).

***In vitro* drug release**

Release studies are required for predicting the reproducibility of rate and duration of drug release. The importance of polymer dissolution on drug release from the matrices has been known for ensuring the sustained release performance. For in vitro release studies, we employed paddle type dissolution apparatus. The result indicates that increase in the concentration of the ethyl cellulose slows the drug release due to its hydrophobic nature As the RPM increases, particle size decreases which fasten the drug release due to increase in surface area. The formulation F7 showed best results in 24 hrs. The release kinetics was evaluated by subjecting the data to zero order, first order, Higuchi's equation and Korsameyer's equation.

Table 2: *In vitro* drug release of F1 to F9 formulations.

% Cumulative Drug Release														
S. No	Formulation Code	Time (hours)												
		1	2	3	4	5	6	7	8	9	10	11	12	24
1	F1	14.94	22.89	29.31	36.62	42.45	48.31	56.89	65.41	73.22	82.73	85.33	90.45	-
2	F2	9.99	11.34	15.62	19.29	26.55	32.09	47.51	53.77	68.71	77.01	84.00	91.12	-
3	F3	15.01	21.11	29.65	34.19	42.71	49.55	54.18	59.32	65.28	78.32	89.31	94.89	-
4	F4	8.39	12.65	17.20	22.45	29.30	34.28	39.31	45.44	51.36	55.39	59.50	65.64	94.11
5	F5	9.54	13.33	19.64	23.30	29.34	33.21	39.12	42.03	48.09	53.46	60.00	64.19	95.40
6	F6	6.32	11.25	21.03	29.64	33.44	39.28	44.81	48.92	53.60	57.34	60.53	64.85	95.33
7	F7	5.65	10.59	17.60	21.11	28.09	37.31	42.93	49.00	52.07	56.30	58.03	60.00	97.52
8	F8	4.25	9.65	15.88	20.09	27.78	32.26	38.77	43.10	48.50	54.49	57.24	62.61	95.00
9	F9	6.36	11.01	18.10	23.20	29.19	33.76	40.28	47.32	51.23	56.17	60.59	64.57	96.29

CONCLUSION

The objective of this research work was to formulate and characterize microspheres of metoclopramide hydrochloride. It may be concluded that floating microspheres of metoclopramide can be successfully prepared by solvent evaporation method using EC and HPMC as polymers. The prepared floating microspheres of metoclopramide may prove to be potential candidate for safe and effective sustained drug delivery over an extended period of time which can reduce dosing frequency.

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