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FORMULATION CHARACTERIZATION AND IN VITRO EVALUATION OF BUOYANT MICROBEADS OF SELECTED ANTIMIGRAINE AND ANTIEMETIC AGENTS

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ABSTRACT

The main objective of current work is to reduce dosing frequency and improve patient compliance by designing and systematically evaluating controlled release buoyant micro beads of rizatriptan benzoate and domperidone. The limitations of conventional dosage forms can be attenuated by designing it in the form of mucoadhesive buoyant microbeads which prolong the gastric residence time at the absorption site to facilitate intimate contact with the absorption surface and thereby improve and enhance the bioavailability. The six formulations F1, F2, F3, F4C, F5C and F6C of Rizatriptan benzoate and Domperidone loaded buoyant mucoadhesive microbeads were successfully fabricated by ionotropic gelation technique using sodium alginate as the hydrophilic carrier in combination with chitosan polymer as drug release and mucoadhesive modifier in varying concentrations respectively, by adding drops of drugs loaded sodium alginate dispersion into calcium chloride solution which aid as cross-linking agent at optimum conditions. The microbeads were then evaluated for drug content, size, drug release rate, drying rate, swelling ratio, encapsulation efficiency, buoyancy duration and mucoadhesive properties using appropriate methods. Particle size distribution of drug loaded formulations were measured by an optical microscope and surface morphology of optimized microbeads was determined by SEM analysis. No significant drug-polymer interactions were observed in FT-IR studies. The *in vitro* wash-off test indicated that the sodium alginate micro beads had good mucoadhesive strength. The buoyancy and drug release of optimized batch was retained for longer duration up to 12 h. *In vitro* drug release profile of microbeads was examined and the optimized batch exhibited zero order kinetic and well fitted to higuchi model. The study demonstrated that the formulation of controlled release buoyant mucoadhesive microbeads of rizatriptan benzoate and domperidone is a promising approach in reducing the frequency of dosing. Also ionotropic gelation technique offers a flexible and easily controllable process in a cost effective manner and had better drug release for long duration of time.

INDEX TERMS: Calcium carbonate, Calcium chloride, Chitosan, Controlled release, Domperidone, Ionotropic gelation technique, Sodium alginate, Sodium bicarbonate, Rizatriptan benzoate.

I. INTRODUCTION

The most effective oral drug delivery always depends on gastric emptying process, gastro intestinal transit time of dosage form, drug release and site of absorption of drug. Gastro-retentive drug delivery system (GRDDS) has gained immense popularity in the field of oral drug delivery recently. Dosage forms that can be retained in the stomach are called as gastro retentive drug delivery systems.[1-3] The rizatriptan benzoate is a potent triptan drug which is a selective 5- hydroxytryptamine 1 receptor subtype of agonist. It is used for the treatment of migraine attack. It is used for treating acute migraine by minimising the symptoms, including pain, nausea, and photophobia or phonophobia by selectively binding to serotonin (5-HT) 1B receptors which are expressed in intracranial arteries and also to 5-HT 1D receptors located on peripheral trigeminal sensory nerve terminals in the meninges and central terminals in brain stem sensory nuclei and also relief migraine headaches by inhibition of pro-inflammatory neuropeptide release. Domperidone acts as gastrointestinal emptying adjunct and peristaltic stimulant. It has strong affinities towards the D2 and D3 dopamine receptors, which are found in the chemoreceptor trigger zone, located just outside the blood brain barrier, which among others regulates nausea and vomiting. So anti emetics are used in combination to treat migraine associated nausea and also to facilitate absorption of oral migraine treatments by improving gastric motility. So alginate microbeads of antiemetic agent, Domperidone and Rizatriptan benzoate are the best candidates for acute treatment of migraine in its combination form.

Microbeads are defined as the monolithic sphere distributed the whole matrix as a molecular dispersion of particle and molecular dispersion defined as the drug

particle are dispersed in to the continuous phase of one or more than one miscible polymers. The micro-beads were prepared by ionotropic gelation technique where the gelation of natural anionic polysaccharide sodium alginate, the natural polymer was react with oppositely charged calcium ions, acting as cross linker or counter ion, to form immediately micro-beads**.** The combined mechanisms of floating, mucoadhesive and swelling properties of microbeads will enhance the gastric retention which provide greater efficacy of dosageform.

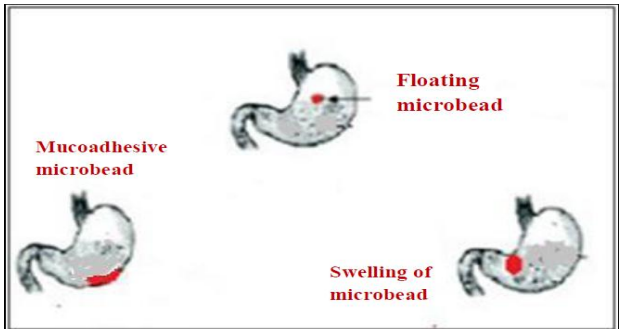

Figure 1: Various gastroretentive properties of microbeads.

MATERIALS AND METHODS

MATERIALS

The pure drugs rizatriptan benzoate and domperidone was purchased from Yarrowchem products, Mumbai, India. Also excipients sodium alginate, glacial acetic acid, calcium carbonate, sodium bicarbonate, chitosan and calcium chloride required for the study were purchased from Yarrowchem products, Mumbai, India. All the chemicals and reagents used were of analytical grades satisfying pharmacopoeias specifications.

METHODS

CHARACTERIZATION OF RIZATRIPTAN BENZOATE AND DOMPERIDONE

DETERMINATION OF λ max

In UV/VIS spectrophotometry we plot the absorbance A of a solution (A is the measure of how much light is absorbed), against the wavelength of the light reaching the solution, λ. This is called the absorption spectrum. From the spectrum we find the wavelength with the highest absorbance, the wavelength of the absorption peak, λmax. At this wavelength the spectrophotometric method is most sensitive for the analyte. Next, we determine the absorbance A at λmax for a number of standard solutions of different concentration, always starting with the lowest concentration. From these absorbance values, we plot a calibration curve for the analyte which we can then use to determine the concentration of an analyte solution of unknown concentration.[4]

PREPARING THE STOCK SOLUTION AND SIX STANDARD SOLUTIONS

Primary stock solutions were prepared by dissolving accurately weighed rizatriptan benzoate (100 mg) and domperidone (100 mg) separately in two different standard flasks using 100 ml of 0.1 N HCL to obtain a 10, 00µg/ml concentration of rizatriptan benzoate and domperidone solutions. These solutions were subjected to scanning between 200-400 nm and absorption maxima at 225 nm for rizatriptan benzoate and 284 nm for domperidone was determined.The primary stock solution was diluted serially with sufficient 0.1 N HCL to obtain the concentration range of 10-100 µg/mL for both rizatriptan benzoate and domperidone. The calibration curve for rizatriptan benzoate and domperidone is obtained by measuring the absorbance at the λ max of 225 nm and 284 nm.[5, 6]

MELTING POINT DETERMINATION

Every pure solid has a characteristics melting point therefore determination of melting point helps in identification of the compound. If the compound melts over a very narrow range, it can usually be assumed that the compound is relatively pure. Conversely, compounds that melt over a wide range are assumed to be relatively impure. Thus melting point also serves as a criterion of purity of a compound.

Small quantities of the compound whose melting point to be determined were taken on a porous plate and powder it with a spatula. Introduce the powdered compound in the capillary tube. Moisten the bulb of thermometer with conc. sulphuric acid or liquid paraffin and attach the capillary to the lower end of the thermometer. Place the thermometer with the capillary tube in the melting point apparatus. Finally noted the temperature at which the compound starts melting and completely melts. The experiment was repeated with new capillary tube and fresh quantity of the substances. The readings were taken in triplicate and average reading was compared with standard values.[7]

SOLUBILITY AND SATURATION SOLUBILITY DETERMINATION

Compounds with insufficient solubility carry a higher risk of failure during discovery and development since insufficient solubility may compromise other property assays, mask additional undesirable properties, influence both pharmacokinetic and pharmacodynamic properties of the compound, and finally may affect the ability of the compound.

The solubility of a compound depends on its structure and solution conditions. Structure determines the lipophilicity, hydrogen bonding, molecular volume, crystal energy and ionizability, which determine solubility. Solution conditions are affected by pH, co-solvents, additives, ionic strength, time and temperature. Poorly soluble compounds can dramatically reduce productivity in drug discovery and development. Drug solubility studies were performed by adding excess amounts of sample to test solvents such as deionized water, glacial acetic acid, 0.1 N HCL, calcium chloride solution, phosphate buffer of both pH 6.8 and 7.4 in test

tube and shaken vigorously.Thus both solubility and saturation solubility of APIs and excipients was obtained by determining the concentration soluble per ml of solvent.[8]

DRUG - EXCIPIENT COMPATIBILITY STUDIES

A complete characterization and understanding of physicochemical interactions of an active pharmaceutical ingredient (API) in the dosage forms is an integral part of preformulation stage of new dosage form development as it is most desirable for consistent efficacy, safety and stability of a drug product. In a dosage form, an API comes in direct contact with other components (excipients) of the formulation that facilitate the administration and release of an active component as well as protect it from the environment. Although excipients are pharmacologically inert, they can interact with drugs in the dosage form to affect drug product stability in physical aspects such as organoleptic properties, dissolution slow down or chemically by causing drug degradation.

Fourier-transform infrared spectroscopy (FTIR)

Drug –excipient compatibility was determined by FTIR analysis. It is carried out by the spectral analysis of drug and drug- excipient mixture. The changes in chemical composition of drug after mixing with excipents were determined with IR spectral analysis.IR was used because mixing of the two components in the molecular level will cause change in oscillating dipoles of the molecules. If the drug and polymer interacts, then the functional groups in FTIR spectra will show the band shift and broadening compared to that of pure compounds.

Procedure for FTIR

In this study, pelletisation of potassium bromide (KBr) was employed. Before forming the pellet of potassium bromide, it was completely dried at 100°C for one hour and after drying it was thoroughly mixed with the sample in the ratio of 1 part of sample and 100 parts of KBr. The mixture was compressed to form a disc using dies. This disc was placed in the sample chamber and a spectrum was obtained through the software program which was further subjected to interpretation. The FTIR spectrums depicted in **Figure 5** to **9** were taken by using FTIR Nicolet iS50- model where as **Figure 10** to **15** were taken by using Shimadzu FTIR 8400 spectrophotometer. The spectra so obtained were compared with standard spectra.[9]

FORMULATION

The buoyant microbeads of rizatriptan benzoate and domperidone were formulated using the natural polymers having mucoadhesive and floating property using ionotropic gelation technique. The composition of formulation was represented in the following **Table1**.

Table1: Formulation of drugs loaded microbeads.

Ingredients	Formulation code					
	F_1	F_2	F_3	F_4C	F_5C	F_6C
Rizatriptan benzoate (mg)	25	25	25	25	25	25
Domperidone (mg)	25	25	25	25	25	25
CaCO3 (mg)	-	-	-	100	150	250
NaHCO3 (mg)	100	150	250	-	-	-
Chitosan (mg)	250	350	450	250	350	450
Sodium alginate (g)	2	2.5	3	2	2.5	3
CaCl2 (g)	1.5	2.5	3	1.5	2.5	3
Glacial acetic acid (%)	1	1	1	1	1	1

METHOD OF PREPARATION

External ionotropic gelation method/Cross-linking method

Sodium alginate was dissolved in distilled water containing 1 % glacial acetic acid which was maintained at pH <6.5 and heated at 60 °C. The drug was dissolved uniformly in 50 ml of above prepared alginate solution when temperature of the solution reduced to 40°C under continuous stirring. The stirring was continued after complete addition of polymers until a uniform dispersion of drug-polymer alginate was obtained. It was kept for ultrasonication to gethomogeneous bubble free slurry dispersion. Thus the dispersion was taken into a syringe and dropped through a 21 G syringe needle into 100 ml of calcium chloride solution were represented diagrammatically in **Figure2**. The solution was kept under stirring to improve the mechanical strength of the beads and to prevent aggregation of the formed beads. Immediate formation of small alginate beads took place after 5 min of curing time. The formed beads were collected by filtration and dried at 50 ºC.[10]

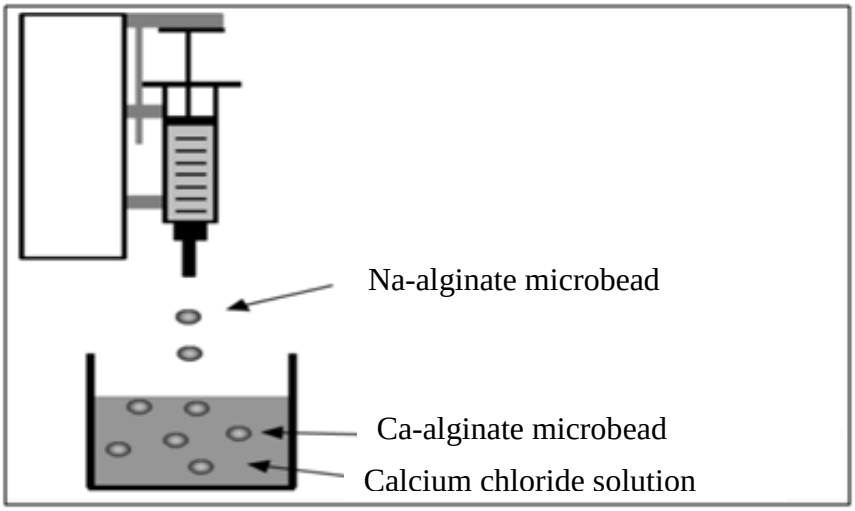

Figure2: Diagrammatic representation of external ionotropic gelation technique/Cross-linking method.

IN-VITRO EVALUATION

DETERMINATION OF VISCOSITY OF SODIUM ALGINATE SOLUTION

The viscosity of sodium alginate solutions prepared for the formulation were determined by using Brookfield viscometer, model DV-E at 100 rpm with spindle no: 62. A definite amount of sample solution with known weight concentration was placed on visometer and viscosity was expressed in centipoises.

PHYSICAL CHARACTERIZATION OF MICROBEADS

The general appearance of beads, its visual identity and overall elegance, is essential for consumer acceptance. The control of the general appearance of beads includes the measurements of a number of attributes such as a size, shape, colour, presence or absence of an odour, taste etc.

MICROMERITICS PROPERTIES

Bulk density

Bulk density is the ratio of total mass of microbeads to the bulk volume of microbead. It was measured by pouring the weighed microbeads into a measuring cylinder and the volume was noted. It is expressed in g/ml.

Bulk density = Weight of the microbeads/Bulk volume of the microbeads

Tapped density

Tapped density is the ratio of total mass of microbeads to the tapped volume of microbeads. It is determined by tapping a graduated cylinder containing known weight of microbeads manually until the microbeads bed volume has reached a minimum volume. The minimum volume so obtained is the tapped volume. It is noted and expressed in g/ml.

Tapped density = Weight of the microbeads/Tapped volume of microbeads

Angle of repose

Microbeads were allowed to fall freely through the funnel, which was fixed at 1cm above the horizontal flat surface until the apex of pile just touches the tip of the funnel. The formation of sharp cone would mean poor flow property while a good spread would indicate a superior flow property. The angle of repose (ɵ) was determined by formula, $\Theta = \tan^{-1}(h/r)$

Where, h= height of pile formed by microspheres, r= radius of circular base formed by the microbeads on the ground.

Table 2: Angle of repose and flow description.

Angle of repose(θ)	Type of flow
<25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

Carr's index

It is also known as compressibility index. The carr's index is frequently used in pharmaceutics as an indication of the flow ability of a powder. In a free-flowing powder, the bulk density and tapped density would be close in value therefore; the carr's index would be small.

On the other hand, in a poor-flowing powder where there are greater inter-particle interactions, the difference between the bulk and tapped density observed would be greater, therefore, the Carr's index would be larger. Lower compressibility index value indicates better flow.

Carr's index = [(Tapped density- Bulk density) ×100]/Tapped density

Table 3: Carr's index values and corresponding type of flow.

Carr's index	Flow description
5-15	Excellent
12-16	Good
18-21	Fair to passable
23-28	Poor
28-35	Poor
35-38	Very poor
>40	Extremely poor

Hausner's ratio

Hausner predict the flow properties of powder by using interparticle friction. This is a simple index that can be determined on small quantities of powder.

Hausner's ratio is the ratio of tapped density to bulk density.

Hausner's ratio= Tapped density/Bulk density

Table 4: Hausner's ratio values and corresponding type of flow.

Hausner's ratio	Type of flow
< 1.25	Good flow
> 1.25	Poor flow

PRODUCTION YIELD

The yields of production of prepared micro beads of all batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of micro beads and percent production yields were calculated as per the formula mentioned below.

Percentage yield = (practical yield/theoretical yield) ×100

DRUG CONTENT AND ENCAPSULATION EFFICIENCY

Accurately weighed microbeads equivalent to dose of drugs selected were suspended in 100 ml of simulated intestinal fluid of pH 1.2 ± 0.1 and kept for 24 h. Next day it was stirred for 5 min and filtered. The drug content in the filtrate was analyzed spectrophotometrically at 225 nm and 284 nm using Shimadzu UV-spectrophotometer. The obtained absorbance was plotted on the standard curve to get the exact concentration of the drug. Calculating this concentration with dilution factor we get the percentage of actual drug content and entrapment efficiency. The drug entrapment efficiency was determined using following relationship.[11]

Drug content= concentration x dilution factor x amount of stock solution

% Drug entrapment efficiency = [Actual drug content / Theoretical drug] x 100

DRYING RATE STUDY OF THE BEADS

The drying rate optimization for each formulation is important to obtain proper microbeads. Prepared beads were placed in open glass bottles and kept in an incubator maintained at 50 ºC. Initially, the beads were removed at short intervals of time (5, 10 and 15 up to obtain constant weight). These measurements were continued until attainment of constant mass and note down the temperature and time for the complete drying of the beads.

PARTICLE SIZE ANALYSIS

The sample of prepared floating microbeads was randomly selected and their size was determined using an optical microscope with the help of eye piece and stage micro meter. In all measurements at least 50 beads were examined. Each experiment was carried out in triplicate

The average particle size was determined by using the following formula,

Average particle size (D) = Σnd÷ Σn

SCANNING ELECTRON MICROSCOPY (SEM)

The shape and surface characteristics were determined by scanning electron microscopy (JSM-6390 model supplied by JEOL) using gold sputter technique. The particles were dried in vacuum, coated to 200 Aº thicknesses with gold palladium using prior to microscopy. A working distance of 20 nm, a tilt of zero-degree and accelerating voltage of 15 kv were the operating parameters. Photographs were taken within a range of 50-500 magnifications.[12, 13]

SWELLING STUDY

Swelling ratio was studied by measuring the percentage water uptake by the beads. About 250 mg of beads from all prepared placebo beads were accurately weighed and placed in 100 ml of buffer (pH 6.8, 0.1 N HCL of pH 1.2 and pH 7.4). Beads were removed from their respective swelling media after 2 h and weighed after drying the surface water using filter paper.

The water uptake was calculated as the ratio of the increase in weight of beads after swelling to the dry weight.

Swelling ratio = [(swollen weight - initial weight)/ Initial weight] x 100

BUOYANCY TEST

The floating study of all batches was carried out using USP dissolution apparatus type II. Accurately weighed 250 mg of microbeads were placed in the dissolution vessel containing 500 ml of 0.1 N HCL (simulated gastric fluid-pH 1.2) maintained at 37 ± 0.5 ºC for 10 h at 50 rpm. The buoyancy of microbeads was measured by visual observation. The time taken by beads to float on the surface of dissolution medium is noted down as floating lag time and duration of floating were recorded by weighing the floating beads. The method is performed for all the batches of formulation in the same manner. The % buoyancy for the developed beads was calculated according to the following formula,[14-17]

% Buoyancy = (Weight of floating beads /weight of taken beads) ×100

IN-VITRO MUCOADHESION TEST

The time taken for detachment of the micrbeads from stomach mucosa was measured in 0.1N hydrochloric acid (pH 1.2). This was evaluated by an in-vitro adhesion testing method, known as wash off method. A piece of goat stomach mucosa (2×2 cm) was mounted onto a glass slide with cyanoacrylate glue. About 50 microbeads were spread over the wet rinsed tissue specimen and immediately thereafter the support was hung on the arm of a USP tablet disintegrating test machine. The slides will move up and down in the test fluid at 37 ± 0.5 ºC. The adherence of beads was regularly observed. The beads that remained adhered to the mucosa were counted at regular intervals up to 12 h.[18-22]

% Mucoadhesion = [No: of adhered microbeads ÷ Total no: of applied microbeads] x 100

LOOSE SURFACE CRYSTAL STUDY (LSC)

The loose surface crystal (LSC) study was an important parameter giving an indication of the amount of drug on the surface of the microbeads without proper entrapment which shows immediate release in dissolution media. The microbeads weight equivalent to dose of drugs (10 mg) was suspended in 100 ml of 0.1 N HCL (pH 1.2). The samples were shaken vigorously for 15 min in a magnetic stirrer. The amount of drug leached out from the surface was analyzed spectrophotometrically at 225 nm and 284 nm. Percentage of drug released with respect to entrapped drug in the sample was recorded.[23, 24]

IN-VITRO DRUG RELEASE STUDIES

In-vitro release studies of prepared microbeads were carried out using 0.1 N HCL buffer (pH 1.2) using USP-basket type apparatus. Accurately weighed quantity of microbeads equivalent to dose of drugs was put into the basket rotated at a constant speed at 100 rpm and maintained temperature 37 ± 5 ºC in 900 ml of the dissolution medium (phosphate buffer pH6.8). The sample was withdrawn at 0, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h and 12 h. Each time interval 5 ml of sample was withdrawn, at the same time 5 ml of fresh dissolution media was added to maintain sink condition.

Repeated the same with buffer pH 6.8 and 7.4.The withdrawn samples were suitably diluted and measure the absorbance at 225 nm and 284 nm spectrophotometrically. The cumulative percentage drug release at regular time intervals was calculated.[25-27]

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

Organoleptic evaluation

The physical appearance of pure drugs rizatriptan benzoate and domperidone are reported in **Table5**. The colour of rizatriptan benzoate and domperidone was found to be white to off-white crystalline solid and white solid. The odour and taste of both drugs was determined as characteristic and bitter.

Table 5: Organoleptic properties of pure drugs.

Drugs	Colour	Odour	Taste
Rizatriptan benzoate	White to off-white crystalline solid	Characteristic	Bitter
Domperidone	White solid	Characteristic	Bitter

IDENTIFICATION OF DRUGS

Determination of λ max

The wavelength of maximum absorbance of rizatriptan benzoate and domperidone in 0.1N HCL was depicted in the **Table6**. The drugs rizatriptan benzoate and domperidone showed maximum absorbance at the wavelength of 225 nm and 284 nm.

Table 6: λmax values of pure drugs.

Drug	λ max
Rizatriptan benzoate	225 nm
Domperidone	284 nm

Calibration curve for rizatriptan benzoate

The graph of rizatriptan benzoate with absorbance vs. concentration was found to be linear in the concentration range of 10-100 µg/ml at 225nm were indicated in **Figure3** and **Table7** shows the calibration curve data.

Table7: Concentration and absorbance values for rizatriptan benzoate in0.1N HCL.

Sl.no	Concentration(µg/ml)	Absorbance at 225 nm
1.	10	0.1236
2.	20	0.2414
3.	40	0.4795
4.	60	0.6967
5.	80	0.9136
6.	100	1.1438

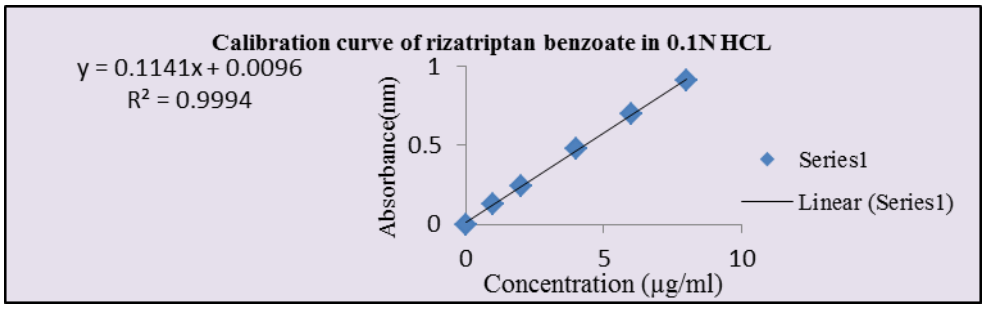

Figure3: Calibration curve of rizatriptan benzoate.

Calibration curve for domperidone

The calibration curve of domperidone showed graph of absorbance vs. concentration in a linear manner with the concentration range of 10-100 µg/ml at 284 nm were represented in below **Figure4** and the data is given in **Table8**.

Table 8: Concentration and absorbance values for domperidone in 0.1N HCL.

Sl.no	Concentration (µg/ml)	Absorbance at 284nm
1.	10	0.0287
2.	20	0.0499
3.	40	0.0989
4.	60	0.1556
5.	80	0.1996
6.	100	0.2538

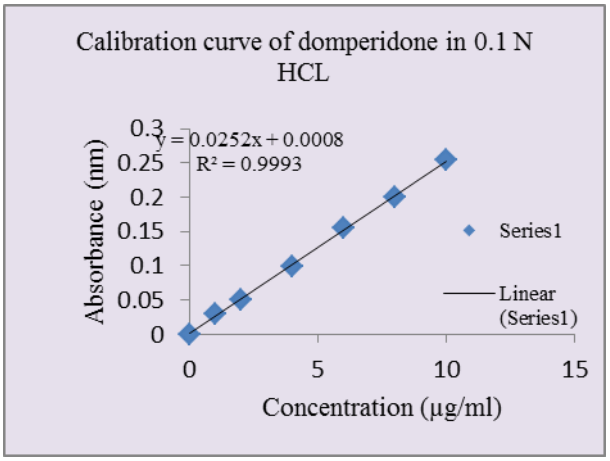

Figure4: Calibration curve of domperidone.

The R^2 of the calibration curve of both rizatriptan benzoate and domperidone was found to be 0.999, indicating that it follows the Beers Lambert law within this concentration range.

Melting point determination

It was found within the standard range 178-180° C for rizatriptan benzoate and 242.5 °C for the domperidone. The data is represented in **Table9**. Since there is not much change in the melting point it can be concluded that the supplied drugs are free of impurities.

Table 9: Data of determined melting point of pure drugs.

Drugs	Melting point determined
Rizatriptan benzoate	178 °C
Domperidone	242 °C

Solubility and saturation solubility determination

The rizatriptan benzoate and domperidone were found to be freely soluble in 0.1N HCL, glacial acetic acid, buffer with pH 1.2, 6.8 and 7.4 and calcium chloride solution. Rizatriptan was freely soluble in distilled water whereas domperidone was very slightly solubility in distilled water. The saturation solubility of rizatriptan benzoate and domperidone as observed in solvents and buffer were represented in the **Table10 & 11**.

Table 10: Saturation solubility data of rizatriptan benzoate (mg/ml).

Solvents	Solubility (mg/ml)
0.1 N HCL	46
1.2 pH buffer	50
6.8 pH buffer	49
7.4 pH buffer	50
Distilled water	45
Glacial acetic acid	52
Calcium chloride solution (1 %)	25

Table 11: Saturation solubility data of domperidone (mg/ml).

Solvents	Solubility (mg/ml)
0.1 N HCL	<1
1.2 pH buffer	<1
6.8 pH buffer	3
7.4 pH buffer	5
Distilled water	Very less soluble (1mg/L)
Glacial acetic acid	10
Calcium chloride solution (1 %)	5

Drug-excipient compatibility studies

FTIR spectroscopy

The FTIR spectrum of rizatriptan benzoate, domperidone pure drugs and excipients viz. Sodium alginate, chitosan, calcium chloride, calcium carbonate, glacial acetic acid, sodium bicarbonate in combination was taken.

Also the drug-drug interaction was checked by FTIR spectrum of both drugs in combination. The drug polymer interactions within the formulation were also studied. The spectrums so obtained are represented in the **Figure 5** to **15.**

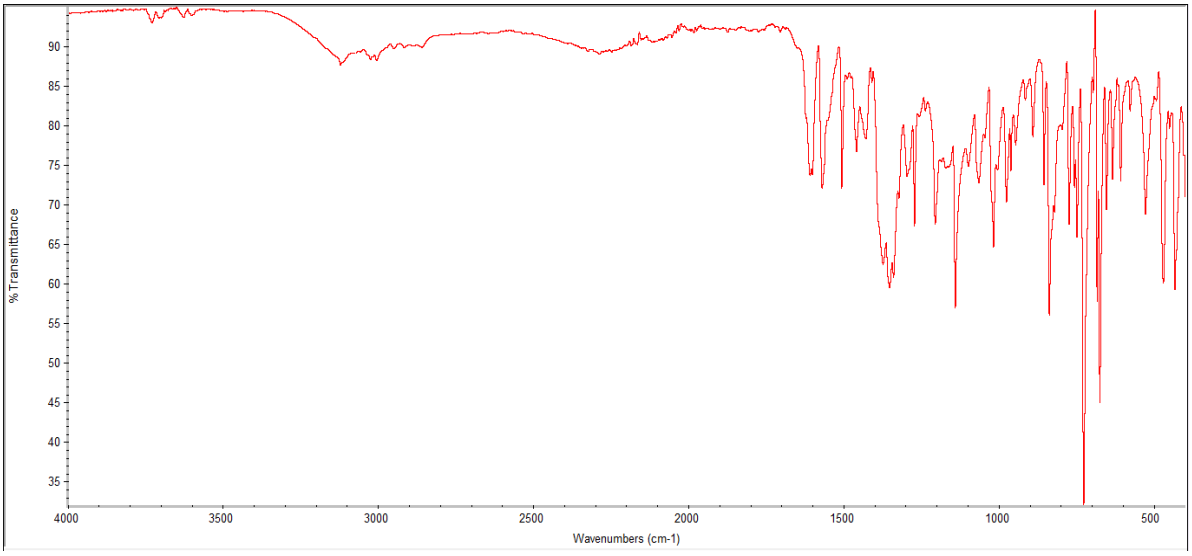

Figure5: FTIR spectra for pure rizatriptan benzoate.

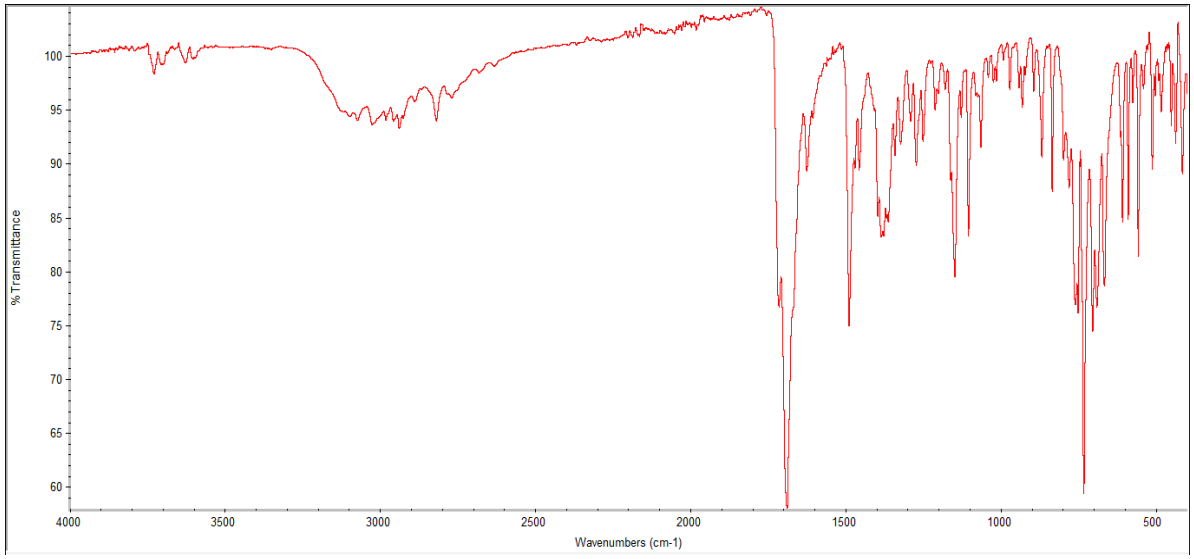

Figure 6: FTIR spectra for pure domperidone.

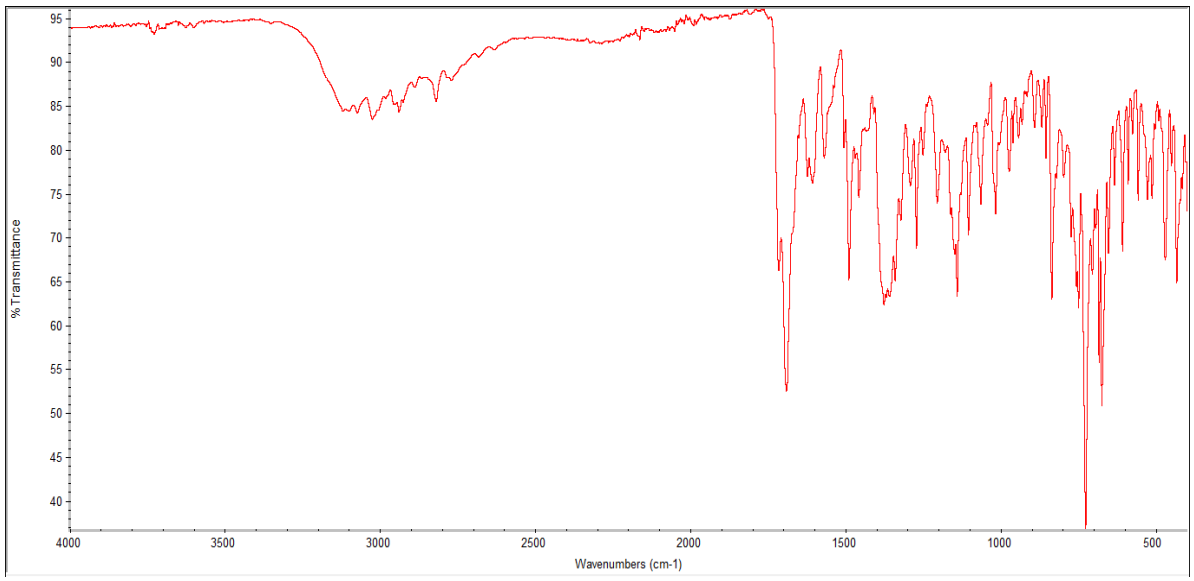

Figure 7: FTIR spectra for physical mixture of RIZ + DOM.

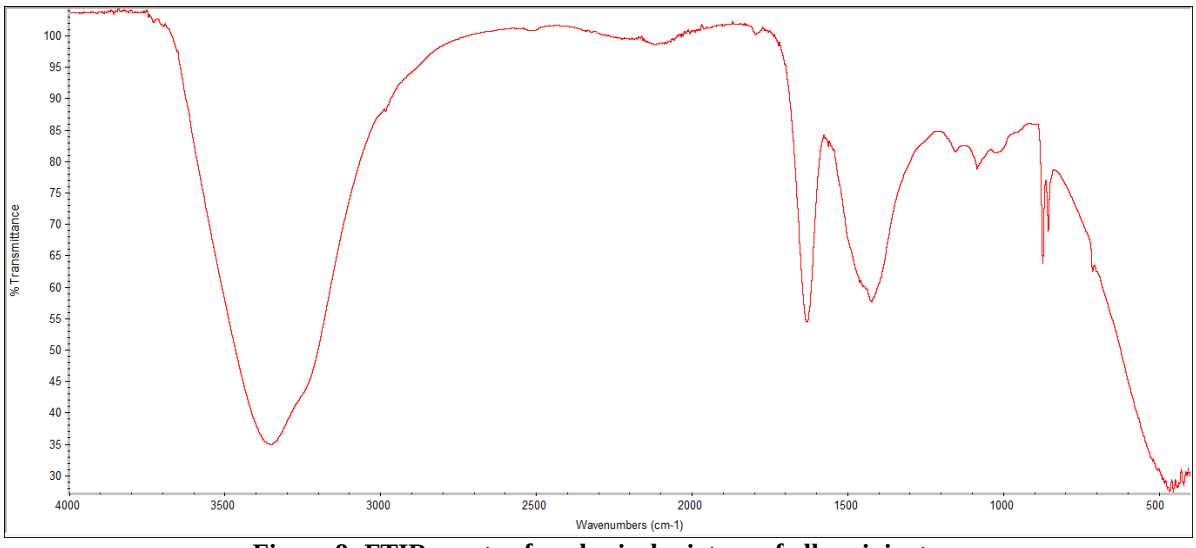

Figure 8: FTIR spectra for physical mixture of all excipients.

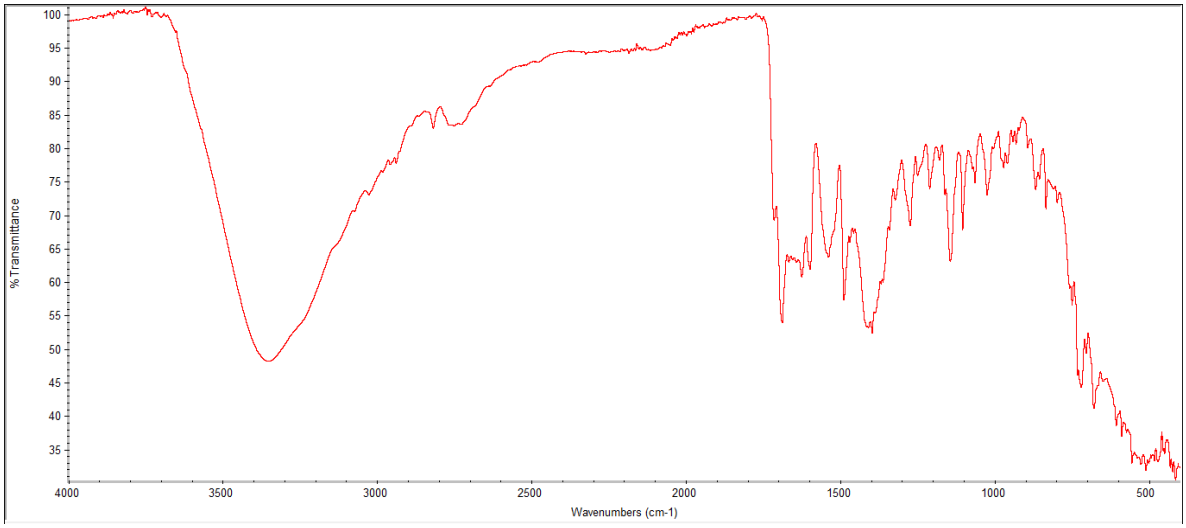

Figure 9: FTIR for physical mixture of RIZ +DOM+excipients.

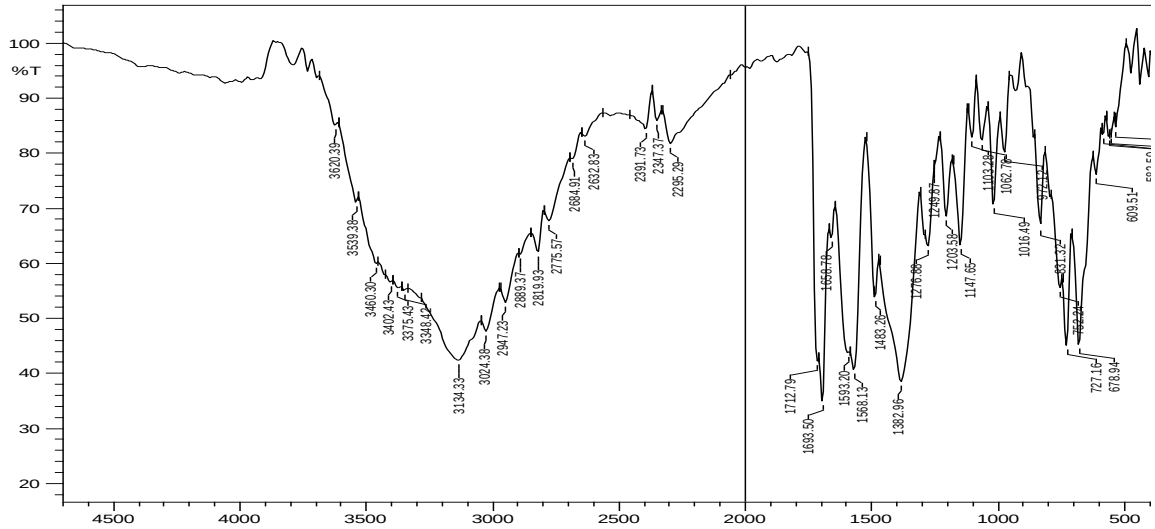

Figure 10: FTIR spectra for physical mixture of RIZ +DOM+ glacial acetic acid.

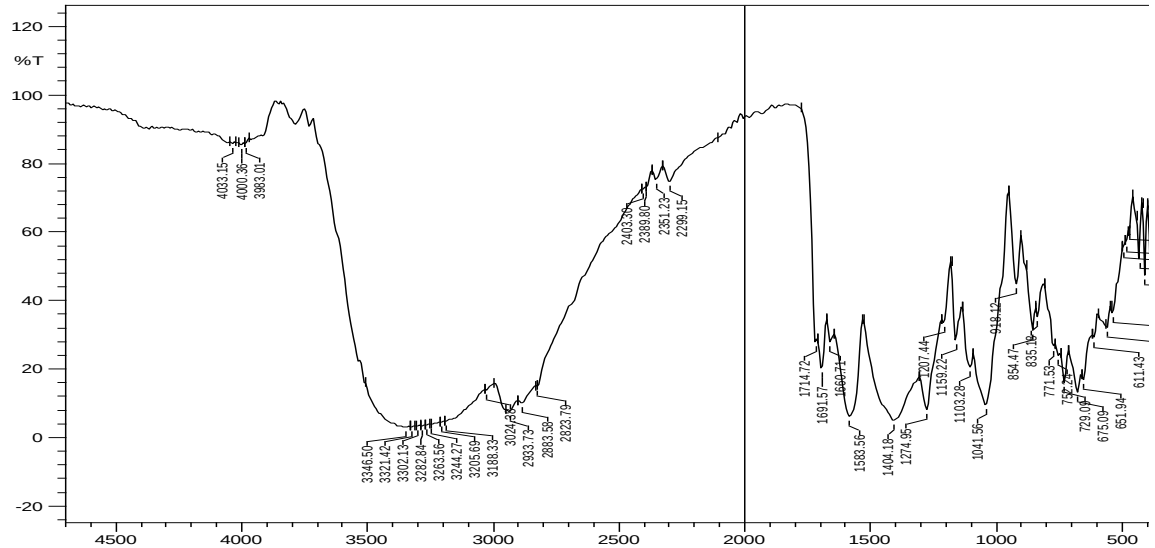

Figure 11: FTIR spectra for physical mixture of RIZ + DOM+ sodium alginate.

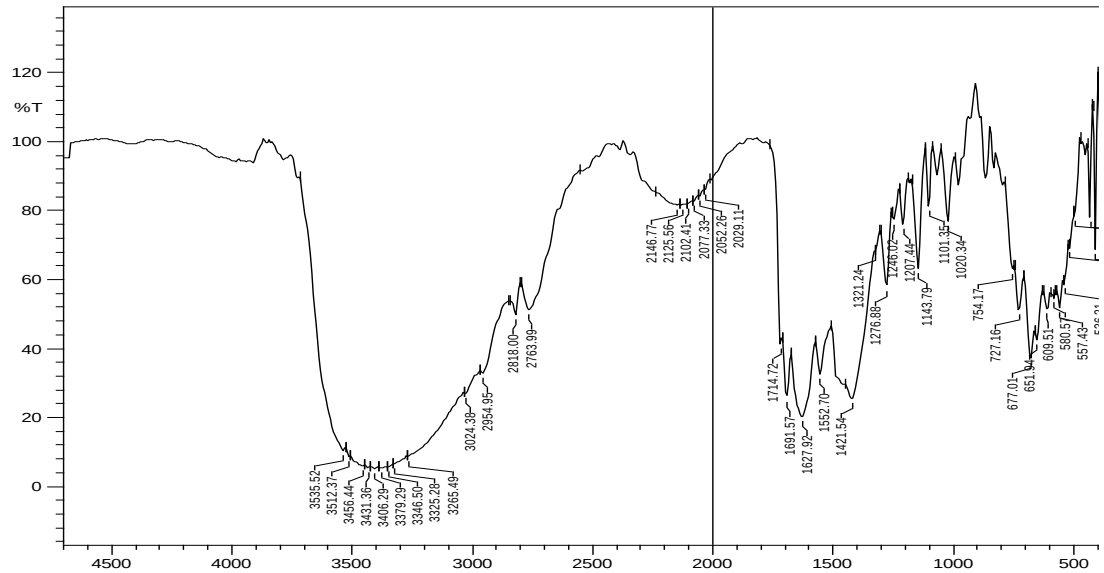

Figure 12: FTIR spectra for physical mixture of RIZ+DOM+ calcium chloride.

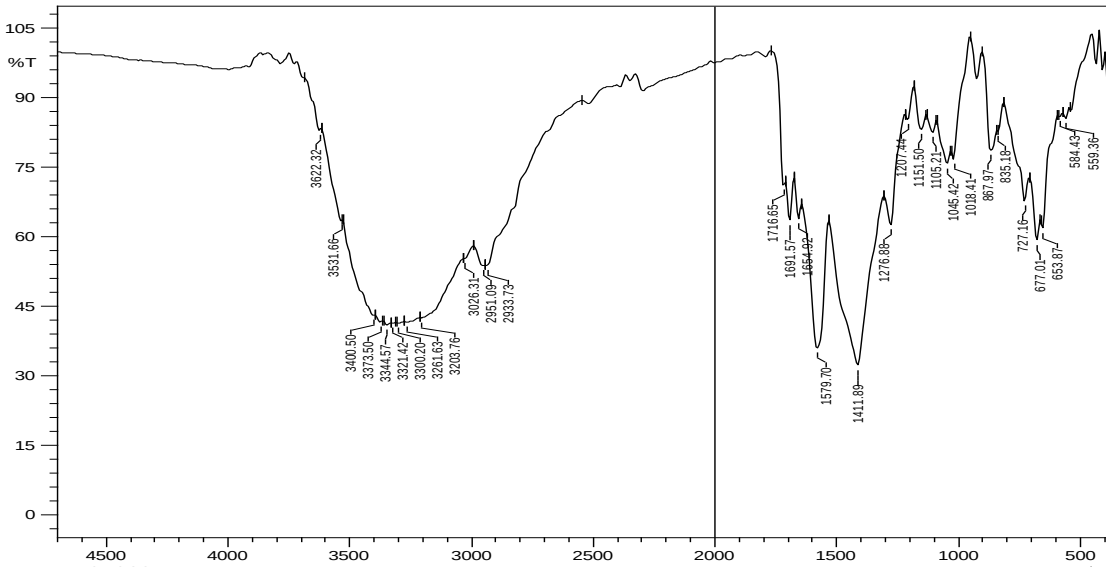

Figure13: FTIR spectra for physical mixture of RIZ+DOM+ calcium carbonate.

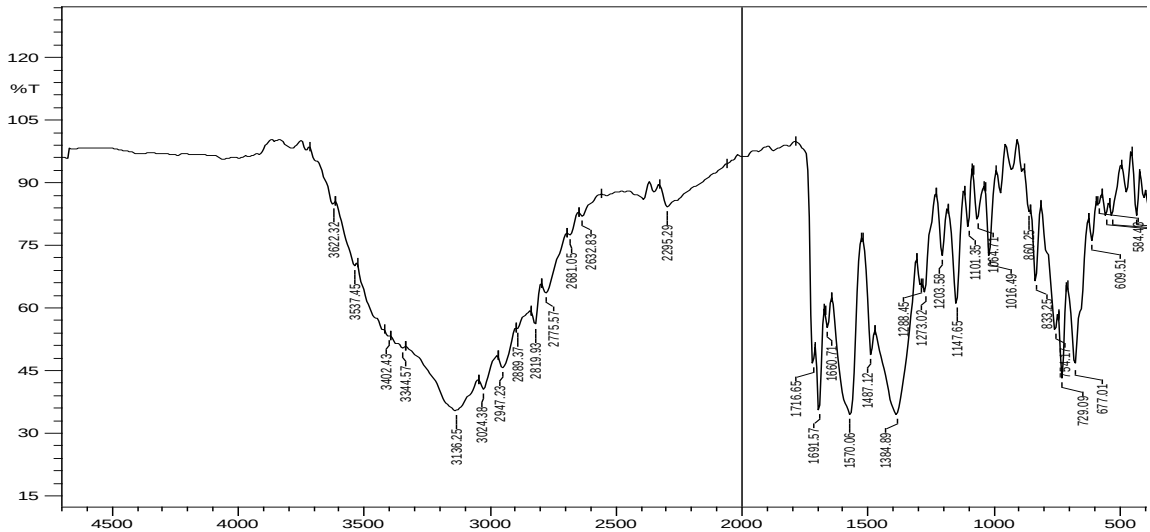

Figure14: FTIR spectra for physical mixture of RIZ + DOM + chitosan.

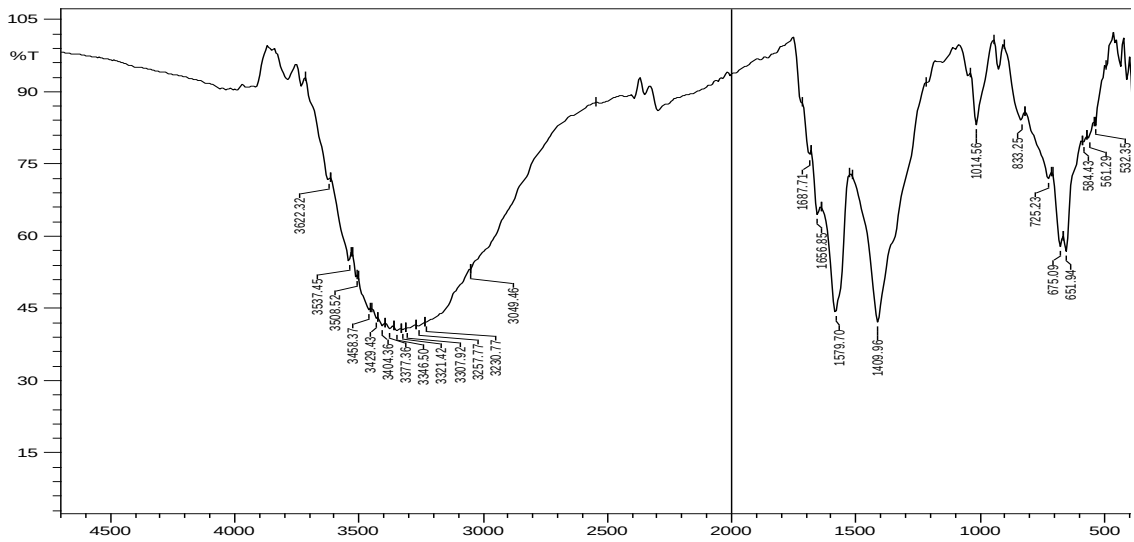

Figure15: FTIR spectra for physical mixture of RIZ + DOM+ sodium bicarbonate.

Interpretation

FTIR of rizatriptan benzoate and domperidone individually showed all the peaks corresponding to the functional groups present in the structure. There was no additional peak in the spectrum taken with combination of these drugs which clearly indicates compatibility of both. So it is possible to conclude that there was no drug-drug interactions which can be clearly understood from the peak values of pure drugs obtained and its comparison with characteristic peak values. The data are depicted in the **Table12** and **13**. The combination spectrum of drugs and excipients showed no change in the peak values corresponding to functional groups of drugs when combined with excipients. Thus it is clear evident to the compatibility of drugs with excipients.

Table 12: Data for interpretation of rizatriptan benzoate.

Rizatriptan benzoate			
Sl.no	**Functional groups**	**Characteristic absorptions (cm^{-1})**	**Peak values in cm^{-1}**
1.	N-H stretching of amide	3400 – 3200	3200
2.	C-H stretch	3000 – 2840	2938,2888
4.	C=C stretch (alkenyl)	1680 – 1620	1608
5.	CH bend	1439-1399	1451,
6.	CH_3 bend	1390-1370	1377
7.	R-CN stretch	2260-2240	2110,2188,2190
8.	R-C=O-O (carboxylate ion)	1600-1590	1589
9.	-N=N stretch (azobenzene)	1576-1429	1568,1427
10.	CH bend (aromatic)	860 – 680	720,853,676

Table 13: Data for interpretation of domperidone.

Domperidone			
Sl.no	**Functional groups**	**Characteristic absorptions (cm^{-1})**	**Peak values in cm^{-1}**
1.	N-H stretching	3350-3180	3180,3220
2.	C=O stretching (ketone)	1685-1666	1685
4.	=C-O-C (ether)	1275-1200	1250
5.	C-Cl stretch	850-750	745

IN-VITRO EVALUATION OF MICROBEADS

Determination of viscosity of sodium alginate solution

It affects both size and shape of formulations. The viscosity data is depicted in **Table14**. The viscosity of sodium alginate solution increases with the increase in its concentration.

Table 14: Data for viscosity of sodium alginate solution.

Formulation code	Viscosity (cps) at 100 rpm
F_1	1180
F_2	2568
F_3	5466
F_4C	1245
F_5C	2678
F_6C	5587

Physical characterization of microbeads

Table15: Physical evaluation of microbeads.

Formulation code	Colour	Appearance
F_1	Light brown	Flattened
F_2	Brown	Spherical
F_3	Brown	Spherical
F_4C	Light brown	Flattened
F_5C	Brown	Spherical
F_6C	Brown	Spherical

Micromeritics study

The results of the all micromeritics properties obtained from all the batches of formulation were mentioned in the **Table16**.

The bulk density and tapped density values were lies in between 0.475 to 0.745 g/cm^3 and 0.593 to 0.855 g/cm^3 indicates good packing. The values of % compressibility, hausner ratio and angle of repose of formulations were found to be in the range of 12.8 % to 19.89 %, 1.14 to 1.24 and 19.85º to 32.20º respectively indicates acceptable flow property and also good packing ability.

Table 16: Micromeritics properties of microbeads.

Sl. no	Formulation code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's Index (%)	Hausner's ratio	Angle of repose (ɵ)
1	F_1	0.475	0.593	19.89	1.24	32.20
2	F_2	0.566	0.675	16.14	1.19	19.85
3	F_3	0.665	0.782	14.96	1.17	22.65
4	F_4C	0.695	0.807	13.87	1.16	20.55
5	F_5C	0.745	0.855	12.86	1.14	28.16
6	F_6C	0.585	0.727	19.55	1.24	23.65

PRODUCTION YIELD

The percentage yields of all the batches of microbeads were given in **Table17**. It was found to be in the range of 80.68 to 97 %. Formulation F_5C shows maximum percentage yield that may be due to correct proportion of drug and polymer concentrations.

Table 17: Production yields of formulations.

Formulation code	Production yield (%)
F_1	81
F_2	90.56
F_3	94.96
F_4C	80.68
F_5C	97
F_6C	89

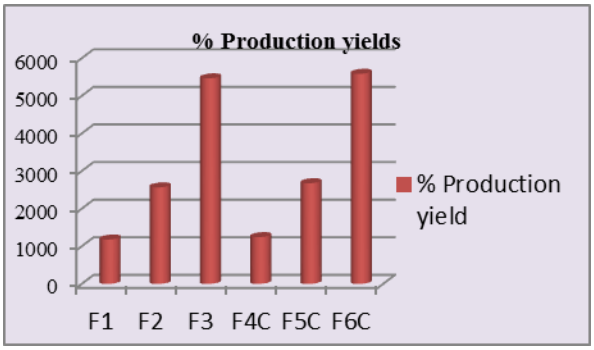

Figure 16: Production yields of all batch formulations.

ESTIMATION OF DRUG CONTENT

The result of drug content of rizatriptan benzoate estimated at 225 nm and domperidone at 284 nm is given in **Table18**. The drug content was found to be in the range of 20 mg to 24.7 mg and 20.5 mg to 24.5 mg.

The drug content was more in the case of formulation F_5C. Overall, it was evident that the drug was properly distributed in the polymer matrix.

Table 18: Data for drug content.

Formulation code	Drug content (mg)	
	at 225nm	at 284nm
F_1	20	20.5
F_2	21.50	21
F_3	24.5	24.5
F_4C	20.55	20.25
F_5C	24.7	24.2
F_6C	24.5	24.5

ESTIMATION OF DRUG ENCAPSULATION EFFICIENCY

The drug encapsulation efficiency of all the formulation batches was in the range of 80 % to 97 %. The results are shown in **Table19**. The drug encapsulation efficiency of microbeads increases with increase in the concentration of chitosan and sodium alginate concentration.

Table 19: Data for drug encapsulation efficiency.

Formulation code	Drug encapsulation efficiency (%)	
	at 225nm	at 284nm
F_1	80	82
F_2	86	84
F_3	95	96.12
F_4C	82	81
F_5C	96.53	97
F_6C	95.68	96

DRYING RATE STUDY OF MICROBEADS

The data for drying rate of each formulation batch were given in **Table20**. The drying rate is increased in the formulation with increased concentration of sodium alginate and less concentration of coating polymer.

Table 20: Data for drying rate of microbeads.

Formulation code	Drying rate at 55 $^{\circ}$ C (h)
F_1	6
F_2	4
F_3	4
F_4C	6
F_5C	4
F_6C	5

PARTICLE SIZE ANALYSIS

The particle size of prepared microbeads was determined to be in the range of 640 µm to 798 µm. The size analysis data are given in **Table21**. The results indicated that the sizes were within the microparticulate range. As the polymer concentration was increased the viscosity of solution also increases, thus the mean particle size was also increased clearly shown in **Figure17.**

This significant increase is due to the increase in the viscosity of droplets along with the increase in the concentration of polymer solution. As the size is increased it is expected to be showing more encapsulation efficiency.

Table 22: Size analysis data.

Formulation code	Microbead size (µm)
F_1	640
F_2	787
F_3	791
F_4C	654
F_5C	796
F_6C	798

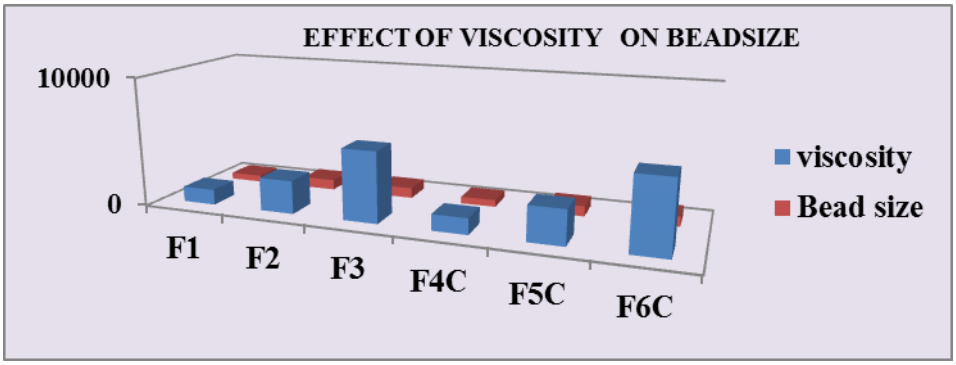

Figure 17: Effect of viscosity of polymer solution on bead size.

SCANNING ELECTRON MICROSCOPY (SEM)

The morphology of microbeads (F_5C, F_6C) was investigated by SEM analysis. The photographs of formulations are shown in **Figure18** and **Figure19**.

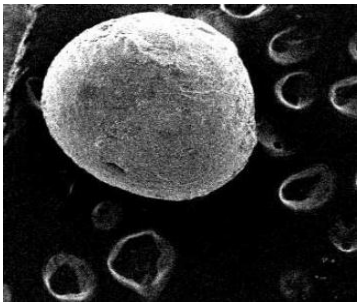

Figure 18: SEM of optimized batch F_5C.

Figure 19: SEM of batch F_6C.

The surface characteristics observed for F_5C showed spherical structure with smooth appearance where as F_6C showed tear shaped structure with irregular appearance.

The SEM photograph shown in **Figure20** remarkable erosion which evidenced the drug release with combined mechanism of diffusion and erosion.

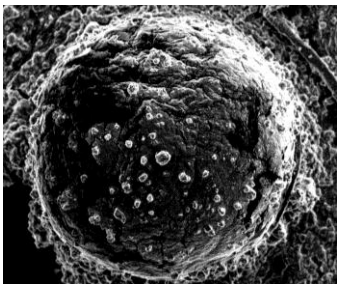

Figure 20: SEM photograph of optimized batch microbead picked from dissolution medium at the end of 6 h.

SWELLING STUDY

The release of the entrapped drug from the microbeads depends on the swelling behaviour, because swelling is directly proportional to the drug release. The dynamic swelling study was carried out in 0.1N HCL of pH 1.2, phosphate buffer pH 6.8 and pH 7.4 and the results are depicted in **Table23**.

The result shows that swelling ratio is decreased in stomach pH (pH 1.2), whereas it is increased in the intestinal pH. The swelling ratio of F_5C at pH 1.2 was found to be lesser among other formulations were illustrated in **Figure21**.

So it was evident from the results that F_5C can consider as optimized formulation for acid resistant drug delivery.

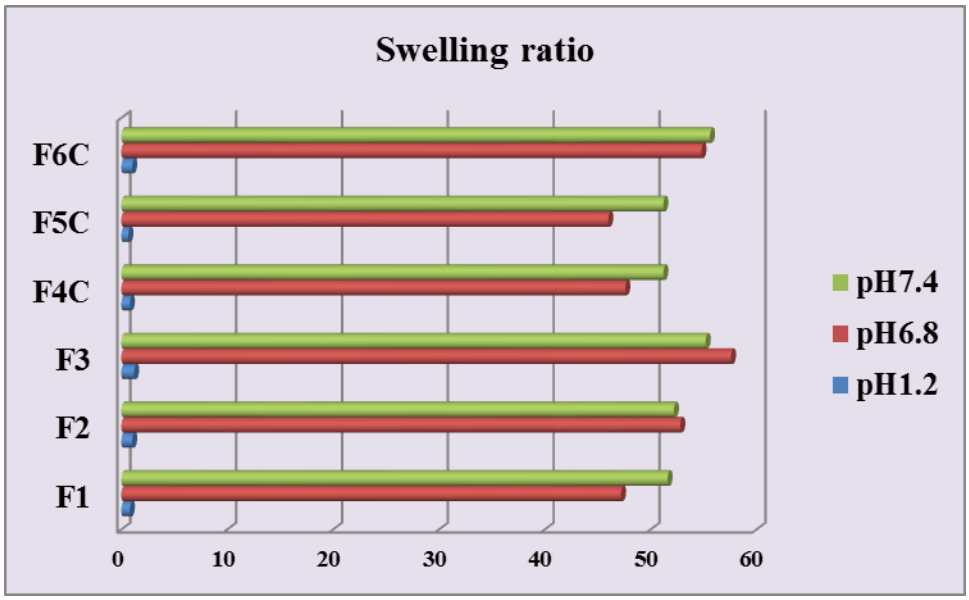

Figure 21: Effect of pH on swelling ratio.

Table23: Data of swelling ratio.

Formulation code	Initial wt of microbeads (mg)	Weight of swollen microbeads (mg)			Swelling ratio (%)		
		at pH 1.2	at pH 6.8	at pH 7.4	at pH 1.2	at pH 6.8	at pH 7.4
F_1	250	252	368	379	0.8	47.2	51.6
F_2	250	252.56	382	380	1.02	52.8	52.2
F_3	250	253	394	388	1.2	57.6	55.2
F_4C	250	252	369	378	0.8	47.6	51.2
F_5C	250	251.66	365	378	0.66	46	51.2
F_6C	250	252.55	387	389	1.02	54.8	55.6

BUOYANCY TEST

The floating ability of the prepared microbeads was evaluated and shown in the **Table 24**. The beads containing NaHCO3 (F_1 to F_3) sank in few hours in 0.1 N HCL, while the beads containing CaCO3 (F_4C to F_6C) demonstrated instantaneous and excellent floating ability which was illustrated in **Figure 5.24.** The mechanism of floating is due to the fact that beads upon contact with acidic medium, NaHCO3 or CaCO3 effervesces, releasing CO2. In this case the released CO2 was most likely entrapped in the beads gel net work produced by the reaction of calcium ion present in the gellation medium with alginate medium.

Table 24: Buoyancy behaviour of microbeads at pH 1.2.

Formulation code	Buoyancy lag time (sec)	Buoyancy duration (h)	Buoyancy (%)
F_1	8	7	93
F_2	6	9	93
F_3	9	9	94.56
F_4C	8	11	95
F_5C	5	12	97.18
F_6C	10	12	96.99

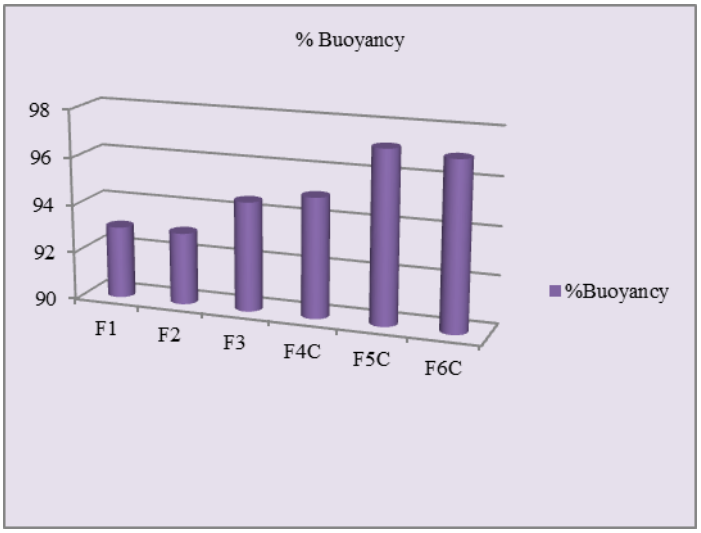

Figure 22: Effect of gas forming agent on % buoyancy.

IN- VITRO MUCOADHESION TEST

The percentage mucoadhesion was evaluated and depicted in the **Table25** and the mucoadhesive property of formulated microbeads were shown in **Figure23.** It was observed from the result that as the concentration of mucoadhesive polymer chitosan increased, mucoadhesion also get increased. This can be due to availability of more polymer chains for entanglement with the mucus. Also it is evidenced from the data that increase in the concentration of cross linking agent decreases the percentage mucoadhesion. The effect of cross linking agent on mucoadhesion is represented in **Figure24**.

Table 25: % Mucoadhesion at different time intervals at pH 1.2.

Formulation code	Mucoadhesion (%)					
	2h	4h	6h	8h	10h	12h
F_1	89	86	85	84.45	84	84
F_2	96.45	96	95.67	95	93.13	92
F_3	85	84.89	83.32	82	80	79
F_4C	89	87	86	86	85.68	85
F_5C	98.66	98	98	97.12	97	97
F_6C	86.12	86	85.10	84	80	80

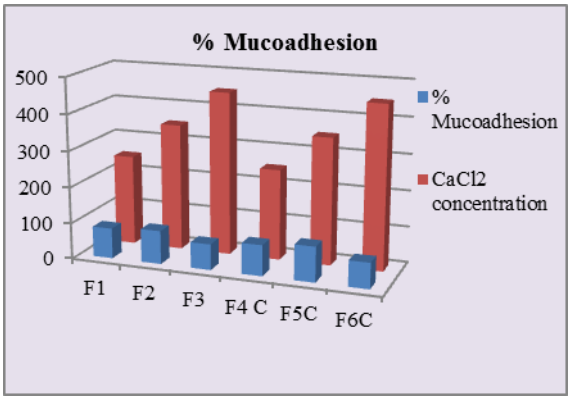

Figure 24: Effect of cross-linking agent

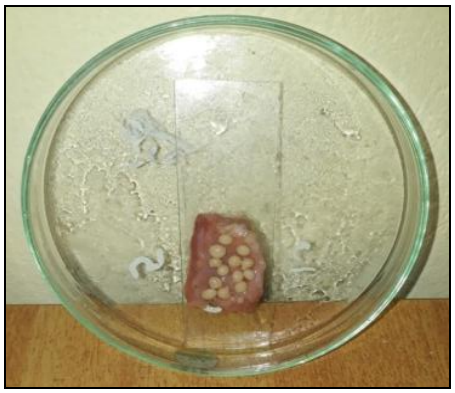

Figure 23: Mucoadhesion of microbeads

LOOSE SURFACE CRYSTAL STUDY (LSC)

The loose crystal study was performed and data were shown in **Table26**. This study indicates the amounts of drug released at 15 min for immediate action are not properly entrapped.

Table 26: Loose surface crystal study of drug loaded microbeads.

Formulation code	LSC (%)	
	at 225 nm	at 284 nm
F_1	4.9	4.5
F_2	3.5	4
F_3	0.5	0.5
F_4C	4.5	4.75
F_5C	0.3	0.8
F_6C	0.5	0.5

IN-VITRO DISSOLUTION PROFILE OF OPTIMIZED BATCH F_5C

The formulation F_5C containing sodium alginate, calcium carbonate and chitosan ratio of 1:0.06:0.14%w/w was found to be optimized batch. It showed sustained release of drug over 12 h. The % CDR data is depicted in the **Table27** and graphically represented in **Figure25.**

Table 27: % CDR profile data optimized batch at pH 1.2.

Sl.no	Time (h)	% CDR of rizatriptan benzoate	% CDR of domperidone
1	1	27.60	27.62
2	2	32.99	32.89
3	3	37.99	36.99
4	4	45.76	45.79
5	5	52.68	52.68
6	6	59.10	59
7	7	65.98	68.98
8	8	79.60	79.97
9	9	89.95	89.95
10	10	94.54	94.54
11	11	95.89	95.89
12	12	96.40	96

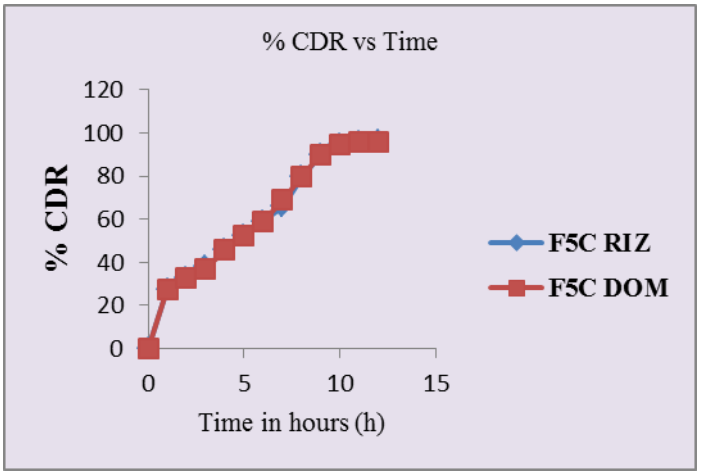

Figure 25: %CDR profile data of optimized batch at pH 1.2

KINETIC MODELLING OF OPTIMIZED FORMULATION

The drug release data of optimized batch F_5C was subjected for mathematical treatment to check whether the release is following first order or zero order kinetics. The F_5C showed most satisfactory zero order release which is illustrated in **Figure26** & **30** which describes that the drug release rate is independent of its concentration where as dependant on the composition of microbead. The kinetic modelling analysis data are depicted in **Table28** & **29**. The co-efficient of correlation values are shown in **Table 30** & **31.**

Table 28: Kinetic modelling of F_5C for rizatriptan benzoate.

Time (h)	% CDR	% drug remaining	square root time	log cumu % drug remaining	log time	log % CDR	cube root of % drug remaining (wt)
0	0	100	0.000	2.000	0.000	0.000	0.000
1	27.60	72.4	1.000	1.860	0.000	1.441	4.168
2	32.99	67.01	1.414	1.826	0.301	1.518	4.062
3	37.99	62.01	1.732	1.792	0.477	1.580	3.958
4	45.76	54.24	2.000	1.734	0.602	1.660	3.785
5	52.68	47.32	2.236	1.675	0.699	1.722	3.617
6	59.10	40.9	2.449	1.612	0.778	1.772	3.445
7	65.98	34.02	2.646	1.532	0.845	1.819	3.240
8	79.60	20.4	2.828	1.310	0.903	1.901	2.732
9	89.95	10.05	3.000	1.002	0.954	1.954	2.158
10	94.54	5.46	3.162	0.737	1.000	1.976	1.761
11	95.89	4.11	3.317	0.614	1.041	1.982	1.602
12	96.40	3.6	3.464	0.556	1.079	1.984	1.533

Table 29: Kinetic modelling of F_5C for domperidone.

Time (h)	% CDR	% drug remaining	square root time	log cumu % drug remaining	log time	log % CDR	cube root of % drug remaining (wt)
0	0	100	0.000	2.000	0.000	0.000	4.642
1	27.62	72.38	1.000	1.860	0.000	1.441	4.167
2	32.89	67.11	1.414	1.827	0.301	1.517	4.064
3	36.99	63.01	1.732	1.799	0.477	1.568	3.979
4	45.79	54.21	2.000	1.734	0.602	1.661	3.785
5	52.68	47.32	2.236	1.675	0.699	1.722	3.617
6	59	41	2.449	1.613	0.778	1.771	3.448
7	68.98	31.02	2.646	1.492	0.845	1.839	3.142
8	79.97	20.03	2.828	1.302	0.903	1.903	2.716
9	89.95	10.05	3.000	1.002	0.954	1.954	2.158
10	94.54	5.46	3.162	0.737	1.000	1.976	1.761
11	95.89	4.11	3.317	0.614	1.041	1.982	1.602
12	96	4	3.464	0.602	1.079	1.982	1.587

Table 30: Model fitting data of rizatriptan benzoate for in-vitro release kinetic parameters of optimized batch.

Formulation code	Zero order (R^2)	Higuchi matrix (R^2)	Kors-peppas (R^2)	First order (R^2)
F_5C	0.996	0.967	0.899	0.979

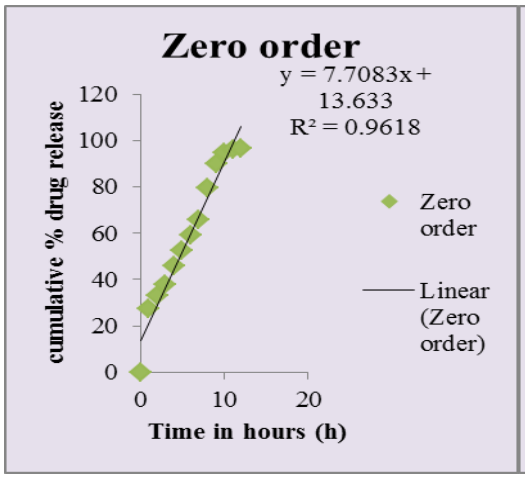

Figure 26: Zero order kinetics

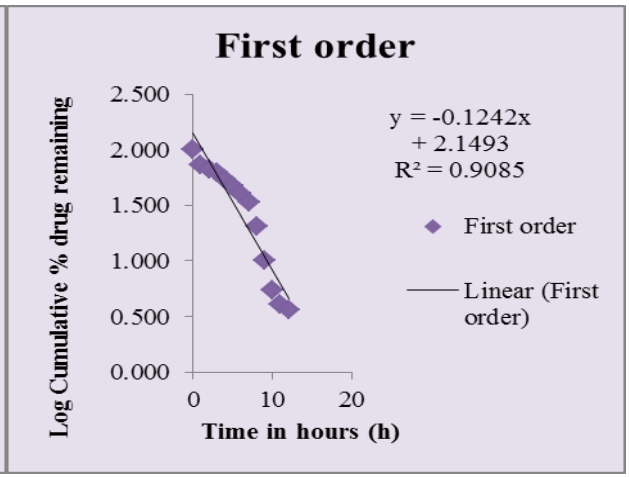

Figure 27: First order kinetics

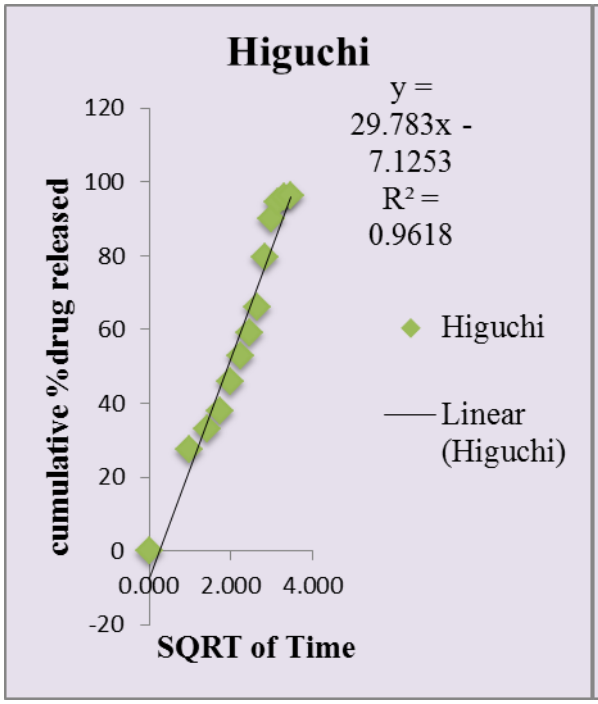

Figure 28: Higuchi model

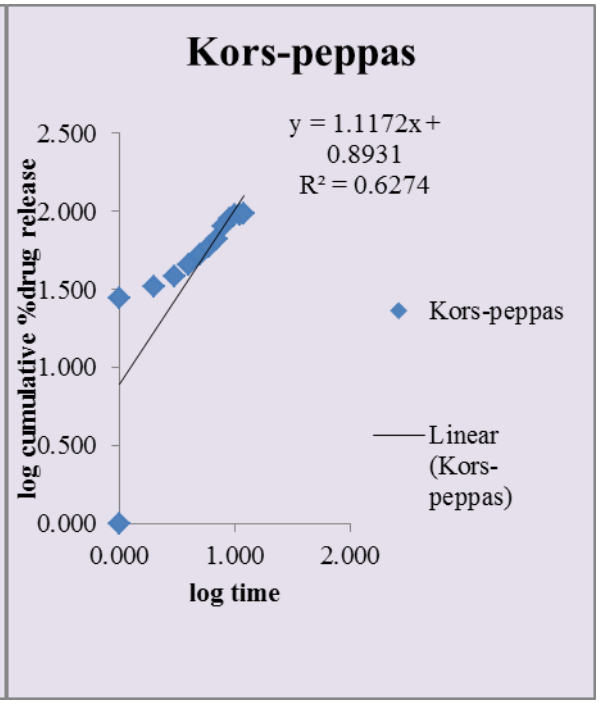

Figure 29: Kors-peppas model

Table 31: Model fitting data of domperidone for in-vitro release kinetic parameters of optimized batch.

Formulation code	Zero order (R^2)	Higuchi matrix (R^2)	Kors-peppas (R^2)	First order (R^2)
F_5C	0.996	0.967	0.899	0.978

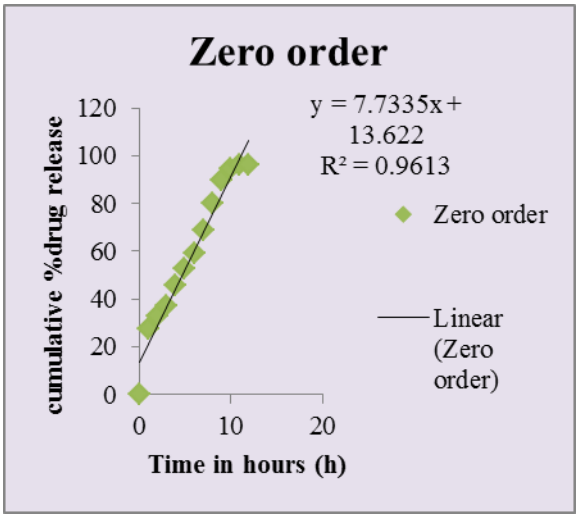

Figure 30: Zero order kinetics

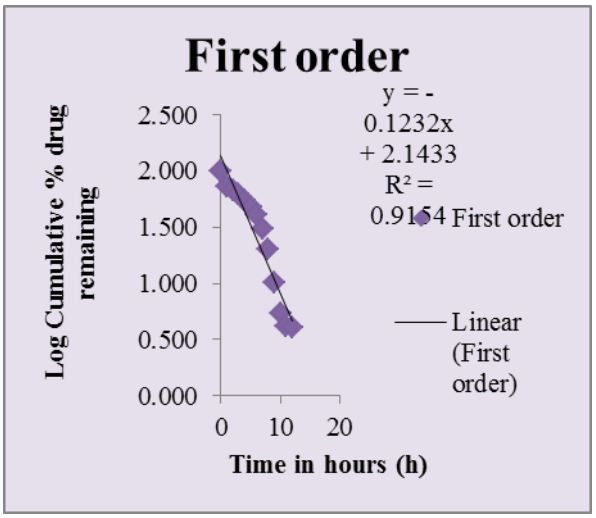

Figure 31: First order kinetics

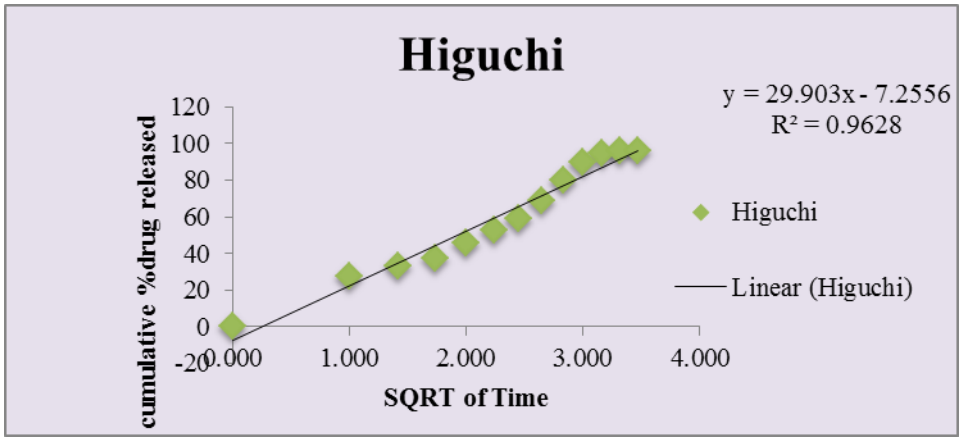

Figure 32: Higuchi model.

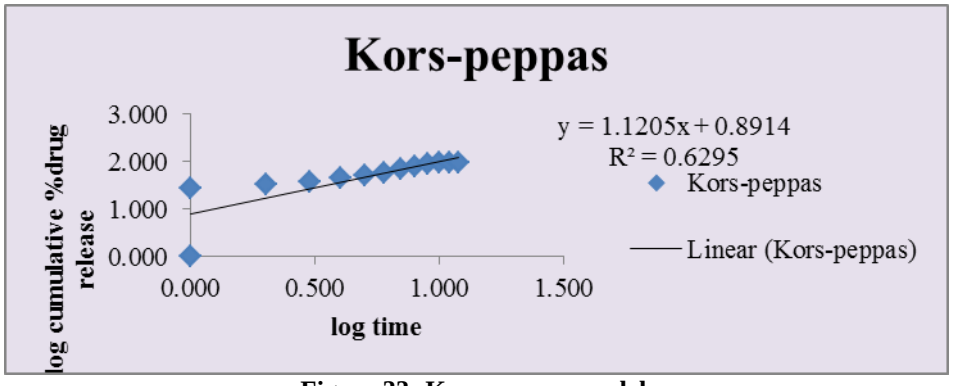

Figure 33: Kors-peppas model.

The values of co-efficient of correlation were found to be best fitted to Higuchi models as shown in **Table 5.31 & 5.32.** Higuchi model describes the release of drugs from an insoluble matrix as a square root of a time- dependent process based on Fickian diffusion. The release constant was calculated from the slope of the appropriate plots, and the regression coefficient was determined. It was found that the in vitro drug release of drug from microbeads of optimized batch was best explained by zero order model as it showed the highest linearity (R^2 = 0.996), followed by Higuchi model (R^2 = 0.967).

CONCLUSION

In conclusion, ionotropic gelation technique can be used for the preparation of buoyant microbeads of rizatriptan benzoate and domperidone using sodium alginate and natural coating polymer chitosan as drug release retardant and mucoadhesive agent. The buoyancy of formulations was obtained by effervescent agents such as sodium bicarbonate (F_1 to F_3) or calcium carbonate (F_4C to F_5C). Release of drugs from microbeads was influenced by alginate, coating polymer, effervescent and cross-linking agent concentration. SEM analysis of

formulation showed spherical and smooth surface for F_5C. From the FTIR studies it was clear that it didn't reveal any significant drug-drug as well as drug- polymer interactions. The study revealed that $CaCO_3$ is a better and effective gas-forming agent than $NaHCO_3$ by producing superior buoyant microbeads. The wash off test for in vitro mucoadhesive strength determination of various formulations showed that microbeads formulation (F_5C) exhibits greater mucoadhesive strength than other formulations. Similarly, the F_5C formulation prepared with calcium carbonate as gas forming agent showed buoyancy duration of 12 h with control drug release rate having maximum of 96.40 % for rizatriptan benzoate and 96 % for dompridone. Drug entrapment efficiency can be increased by increasing alginate concentration and decrease by increasing carbonate ratio. The results of release and release in-vitro kinetic indicated sustained release and exhibited zero order kinetic. Therefore, one can assume that the rizatriptan benzoate and domperidone microbeads are promising pharmaceutical dosage forms by providing controlled release drug delivery systems and improving bioavailability.

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