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FORMULATION DEVELOPMENT OF LYOPHILIZED FLOATING BEADS CONTAINING GINGER EXTRACT AND NIGELLA SATIVA OIL

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ABSTRACT: Lyophilized floating beads have been investigated as a possible gastro-retentive dosage forms containing Ginger (*Zingiber Officinalis*) extract (ZOE) and Nigella Sativa oil (NSO). Emulsion gelation method was used in preparation of ZOE-NSO entrapped floating alginate beads. Drug content and floating properties of the beads were found to be satisfactory. The results revealed that lyophilized beads showed better floating behaviour (> 8 hrs). The formulations containing sodium alginate at 3% w/v concentration with 30 % w/v calcium chloride for cross-linking and 30 min for curing time presented with optimum drug release (8 hrs) and floating time (>24 hrs). The optimized formulation was subjected to storage at 40 and 50° C / relative humidity of 60% for a period of three months. *In vitro* release data of the optimized batch presented Higuchi-diffusion and Korsmeyer Peppas models with Non-Fickian release. The data proposed that the prepared lyophilized NSO and ZOE hydrogel beads delivered their active ingredients by diffusion and erosion mechanisms.

INTRODUCTION: Gastric retention of the dosage form is beneficial when drug is absorbed from stomach and/or needs localization in the stomach for better local as well as systemic effect. Gastric retention can be made possible with several approaches such as floating drug delivery, mucoadhesion, and superporous hydrogel system ¹⁻³. Floating drug delivery systems are classified depending on the formulation approaches, i.e., effervescent and non-effervescent systems. Effervescent system of floating dosage incorporates one or two gas generating agents (Eg: Sodium Bicarbonate, citric acid, tartaric acid) with polymeric swellable matrix (Eg: Guar gum, Chitosan, Hydroxy propyl cellulose etc.).

The single or multiple unit dosage form with effervescent base when come in contact with gastric fluid generates CO₂ which gets entrapped into the swollen polymeric matrix and thus provides buoyancy to the dosage form ⁴. Floating systems having low density have sufficient buoyancy to float over the gastric contents and remain in the stomach without getting affected by the gastric emptying rate. While the system floats over the gastric contents, the drug is released slowly at the desired rate and reduces fluctuation in plasma drug concentration. ⁵ Different approaches have been studied like chitosan based lyophilized dosage form, use of sodium bicarbonate as an effervescent agent, inert oil entrapped alginate beads ⁶⁻⁸. The present study involves formulation of floating beads containing *Zingiber officinale* extract and *Nigella sativa* seed oil as a drug candidate, which remain in stomach or upper part of GIT for prolonged period of time, therefore maintaining the maximum drug release at desired site.

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The ginger extract composes of oleoresin containing volatile oils of gingerols, shogaols, α -zingiberene, β -bisabolene, β -sesquiphellandrene and *ar*-curcumene.⁹ Gingerols has been considered as a main substance responsible for the pungent smell of fresh ginger. There are some reports stating that the gingerols is responsible for many pharmacological effects of ginger. Ginger has been used to treat diseases related to gastrointestinal tract such as flatulence, indigestion, nausea and vomiting.¹⁰

N. sativa seed oil has chemical constituents like thymoquinone, dithymoquinone, thymohydroquinone and thymol. *N. sativa* seed oil has been shown to be gastroretentive in animal model studies. Active compound is considered, to be thymoquinone and protective mechanism is believed to be the free radical scavenging and antioxidant activity of the oil and specifically that of thymoquinone.¹¹

The preparation of alginate hydrogel beads described here is based on the cross linking properties of this polymer with CaCl_2 . The calcium ion is believed to interact with five different oxygen atoms of two adjacent guluronate units in intra-chain binding. In addition it makes an 'egg-box model' through inter-chain binding of calcium to two or more alginate chain. The enclosed drug is released from the alginate matrix at a controlled rate.¹²

Further lyophilization technique was used with an aim to give porosity to the beads so that the buoyancy of the formulations can be increased. As compared to unit dosage form multi particulate system would cover larger area and hence better localized effect of drug can be achieved.¹³

Keeping the above facts in focus the study was aimed to formulate gastro-retentive lyophilized floating drug delivery system using ionotropic gelation of sodium alginate with CaCl_2 .

MATERIALS AND METHODS:

Zingiber officinale extract were received as a gift sample from Konark Herbs and Health Care, Dabhel, Nani Daman, India. *N. sativa* seed oil was extracted with ether in laboratory. Tween 80 was

obtained from Microlab Instruments, Mumbai, India. Sodium alginate and calcium chloride were obtained from Thomas Baker (Chemicals) Pvt Ltd, Bharat Mahal, Mumbai, India.

Extraction of *N. sativa* seed oil:

About 100 g of powdered *Nigella sativa* seeds were extracted with 600 ml n-hexane (ratio 12:1) using distillation unit. The temperature of the bath was kept at 100°C. Chilled water was circulated from the condenser. Total extraction time required was approximately 6 hours. The final extract was filtered using muslin cloth and then evaporated to remove the organic solvent (n-hexane). The remaining oily extract was further used for the formulation development.

Lyophilized Alginate Beads containing NSO and ZOE:

The present formulation development is based on lyophilized calcium alginate beads containing NSO and ZOE to enhance the retention time of these drugs into the stomach. The drug loaded calcium alginate beads were prepared by dissolving finely powdered sodium alginate in 100 ml double distilled water containing 1% w/v Tween 80 and 3% v/v NSO with slow agitation. To this above mixture ZOE (1% w/v) was dispersed and stirred till a homogeneous dispersion was obtained.

The homogeneous dispersions were then added drop wise through nozzle (21 gauge) at a rate of 5 ml/min into a solution of calcium chloride with gentle agitation at room temperature. The resultant beads were allowed to remain in the calcium chloride solution for 2 h for complete curing and then filtered through thin muslin cloth. After washing twice with double distilled water resultant beads were subjected to lyophilisation process. Total 3 cycles of 5 hours lyophilization were given after its 12 hours pre-freezing at -40 degree (LED-100A, Labnics Equipments, USA).

Table 1 depicts the detailed formula of total six batches. All six batches were subjected to pharmacotechnical characterization, i.e, diameter of beads, floating time, floating lag time, drug content, in-vitro drug release studies and stability studies as per the ICH guidelines¹⁴. The lyophilization process gives porosity to the beads

and entrapped NSO also reduces the density of the calcium alginate beads, therefore buoyancy is improved. These beads provide a sustained release formulation for 8-12 hours.^{15, 16}

Characterizations of lyophilized NSO-ZOE Floating Beads:

Particle size and shape:

The mean diameter and shape of 50 drug loaded lyophilized beads was determined by optical microscopy. Average of triplicate batch is summarized in **Table 1**.

Buoyancy:

The floating ability was determined using modified USP dissolution test apparatus. Beads were introduced in the vessels and stirred using magnetic stirrer in 200 ml of 0.1N HCl maintained at 37 ±0.5 °C for 24 h. The floating ability of the beads was measured by visual observation. The preparation was considered to have buoyancy only when all beads floated on the test solution immediately or within a lag time of 2 min. The results are shown in **Table 1**.

Drug Content:

The drug content in each formulation was determined by triturating and then soaking 50 mgs of lyophilized beads for 2 hours. The above sample was then sonicated for another 1 hours to rupture the calcium alginate matrix to ensure the complete drug release into the media (0.1N HCL). The resulting solution was filtered using sintered glass funnel and the percent drug content was obtained by the simultaneous analysis method developed at our laboratory using UV/Visible spectrophotometer (Shimatzu UV1800, Japan) at 256 nm for *N. sativa* seed oil and 282 nm for *Zingiber officinale* extract. The results are mentioned in **Table 1**.

In vitro drug release studies:

The *in vitro* drug release study of formulations was carried out for F-2 and F-5 only as they exhibited good floating properties.

The *in vitro* dissolution study of the lyophilized hydrogel beads was carried out using USP dissolution test apparatus II (Basket Type)¹⁷. Lyophilized beads (500 mg) sample was put into the basket and the basket was covered with thin

muslin cloth. The basket was immersed into 900 ml 0.1N HCl filled into the dissolution bowl maintained at 37 ±0.5 °C and rotated at 50 RPM. An aliquot of 5 ml of the solution was withdrawn at predetermined time intervals of 1, 2, 3,4,5,6 hours and replaced by fresh dissolution medium. The withdrawn samples were analyzed for *Zingiber officinale* extract and *N. sativa* seed oil content spectrophotometrically at 282nm and 256 nm respectively. The percentage drug released was plotted against time to determine the release profile. Samples were run in triplicate.

Drug Release Kinetic study:

The drug release data obtained from the *in vitro* release studies were fitted to different mathematical equations to describe the kinetic and the mechanism of the drug release from the prepared beads. The kinetic models applied were; zero order, Higuchi and Korsmeyer Peppas models. The goodness of fit was evaluated based on coefficient (R²) values¹⁸⁻²¹

Stability studies:

The selected formulation F5 was subjected to storage at 40 and 50° C / relative humidity of 60% for a period of three months²². At the end of the storage period, the formulation was tested for any change in the physical appearance, drug content, *in vitro* dissolution studies and floating behaviour. The dissolution profiles of both fresh and stored beads were compared.

RESULTS AND DISCUSSION:

Preparation and characterization of lyophilized floating beads:

The present investigation was aimed at developing lyophilized alginate hydrogel beads containing NSO and ZOE. This formulation was intended for gastric retention for the treatment of stomach ulcers, flatulence, nausea and other gastric disorders. The present formulation is a lyophilized multi particulate device, which has benefit of being porous and its ability to give better localized effect. These dual advantages make the formulation more suitable for drugs that needs localized as well as systemic effect in the stomach.

Nigella Sativa Oil was entrapped into the calcium alginate matrix. The beads containing ZOE and

NSO were easily manufactured without any sophisticated equipment. Tween 80 was added to disperse the oil properly and to keep it dispersed during production of beads.

The initial screening for the selection of the percent sodium alginate revealed that the concentration as low as 1% w/v was enough to obtain good spherical gel beads. The reason for requirement of lower level sodium alginate may be due to resin content of ZOE which imparts viscosity to the solution. Hence, for the present study, only 1%, 3% and 5 % w/v sodium alginate was used. It was observed that the lower viscosity of polymer solution (less than 1% sodium alginate in presence of ZOE) led to oblong, tailed beads, while higher than 5% w/v concentration of polymer was difficult to handle. All the formulations contained 3% v/v NSO and 1% w/v ZOE.

A total of six formulations were prepared to study the effect of concentration of sodium alginate (1%, 3% and 5% w/v) and calcium chloride (20% and 30% w/v). The curing time was kept constant (1hr). All the formulations prepared yielded spherical beads with the mean diameter ranges between 1.78 and 1.97 mm. For complete drying all the batches required three lyophilization cycles of 5 hr after overnight pre-freezing.

All the formulation beads were softer and porous in nature. Batch F2 and F5 showed a good buoyancy and floating behavior (>24hrs). From these results it was concluded that sodium alginate level plays

an important role in floatability and buoyancy of the formulations. The calcium chloride as a cross linking agent showed its effect on drug release. Batch F2 and F5 contains 3% w/v sodium alginate with 20% and 30 % w/v calcium chloride. In vitro dissolution study was carried out for batch F2 and F5 only.

In vitro drug release studies and release kinetics

In vitro drug release studies were carried out in 0.1 N HCl as a dissolution medium using USP Type I (Basket Type) apparatus. It is evident from the figure 1 and 2 that ZOE was released faster as compared to NSO. This is due to water solubility of ZOE while NSO had to partitioned with an aqueous media which led to delayed release pattern for NSO. Also, oil remained entrapped in to the polymer matrix of the beads. The drug has to first diffuse from the oil pockets into the polymer matrix and then it transports out of the polymeric matrix into the dissolution medium. Batch F2 showed comparatively higher release of NSO at 6th hr.

This may be due to lower concentration of cross linking agent (Calcium chloride). There are possibilities of incomplete cross linking of sodium alginate with calcium ion. The increase in to the cross linking agent may lead to better ion exchange of sodium with calcium and hence formation of insoluble hydrophilic calcium alginate matrix. The stiffer polymeric matrix has capacity to retain drug in its matrix.

TABLE 1: LYOPHILLIZED HYDROGEL BEADS FORMULATIONS AND ITS CHARACTERISTICS.

Batch No	Formulations Variables*		Floating behavior	Average size (mm)
	% sodium Alginate	% Calcium Chloride		
F1	1	20	Non-Floating	1.97
F2	3	20	Floating	1.93
F3	5	20	Non-floating	1.89
F4	1	30	Non Floating	1.78
F5	3	30	Floating	1.81
F6	5	30	Non Floating	1.91

*All the batches contains 1% w/w ZOE and 3% v/v NSO in 1% v/v Tween solution,

To determine the drug release kinetics, the in vitro drug dissolution data were treated using PCP Disso Software, Pune, India. Dissolution data were fitted to zero, Higuchi and Korsmeyer-Peppas models. The in vitro release of NSO and ZOE from the lyophilized calcium alginate beads presented drug release coefficients (R^2) in favor of Higuchi-

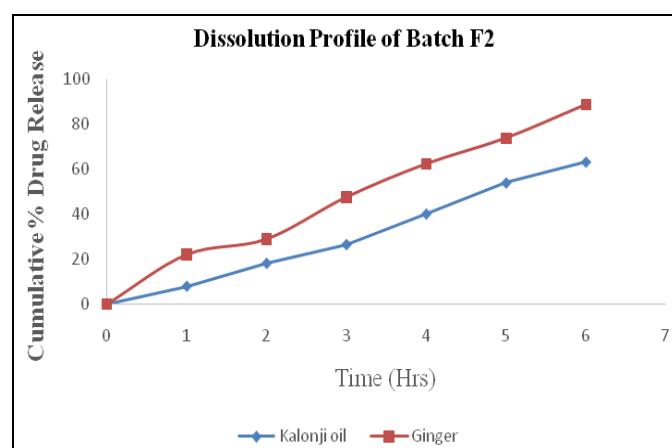
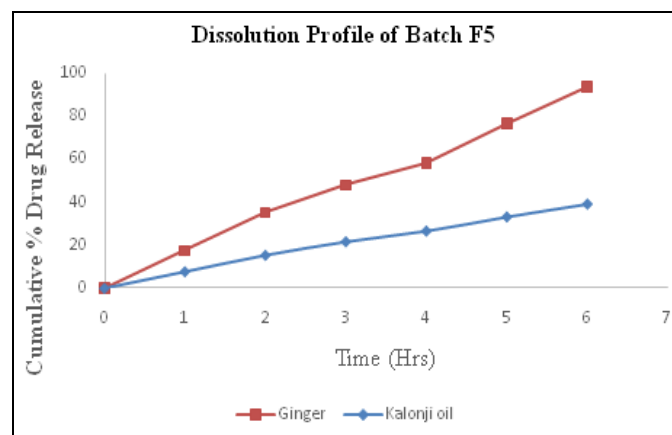
diffusion kinetics and Korsmeyer Peppas model. The values of diffusion exponent (n) was found to be 0.59 indicating non Fickian (anomalous) for batch F5. Based on R-square values it was proposed that these formulations delivered their active ingredient by coupled diffusion and erosion.

TABLE 2: RESULTS OF MODEL FITTING OF BATCH F5

Model fitting parameters	Zero Order	Higuchi	Korsmeyer/ Peppas
F-value	15.45	3.35	1.28
R-square	0.954	0.996	0.995.
Diffusion exponent (n)			0.59
Mechanism of Drug release		Non-Fickian (anomalous) diffusion	

TABLE 3: COMPARATIVE IN-VITRO DRUG RELEASE OF NSO AND ZOE

Time (hr)	Cumulative % Drug Release			
	Batch F2		Batch F5	
	NSO	ZOE	NSO	ZOE
0	0	0	0	0
1	7.87	22.01	7.69	17.52
2	18.05	28.91	15.3	35.19
3	26.35	47.55	21.54	48
4	39.92	62.36	26.52	58.04
5	53.73	73.85	33.08	76.74
6	62.97	88.66	38.99	93.71

**FIG 1: DISSOLUTION PROFILE OF BATCH F2****FIG 2: DISSOLUTION PROFILE OF BATCH F5****Stability studies:**

The Batch F5 was selected for stability studies as per the ICH guidelines. The stability study was carried out at 40°C and 50°C for 3 months at 60% RH in to an aluminium sealed container. The stored beads did not change any of the physical

parameters as well as drug release pattern, floating time and floating lag time.

CONCLUSION: In conclusion, the lyophilized alginate beads were successfully prepared using ionic gelation technique to provide a gastroretentive drug delivery system for NSO and ZOE. The optimized beads were porous, spherical beads with narrow size distributions with good drug entrapment efficiencies. All the selected formulations variables showed their effect on floating behavior, physicochemical parameters of beads and the release of NSO and ZOE.

The mechanism of drug release was observed to be coupled mechanism, i.e, diffusion and erosion. The lyophilized beads tend to be porous which led to diffusion type of mechanism for the drug release. The hydrophilic calcium matrix based multiparticulate system after coming in contact with biological fluids shows an intermediate erosion pattern during drug release. Hence, it can be concluded that the drugs like, Nigella Sativa and Ginger extract with their anti-ulcer and anti-flatulent activity can be delivered in multi-particulate system for better local as well as systemic absorption.

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