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Research paper

Formulation of multiparticulate systems as lyophilised orally disintegrating tablets

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ABSTRACT

The current study aimed to exploit the electrostatic associative interaction between carrageenan and gelatin to optimise a formulation of lyophilised orally disintegrating tablets (ODTs) suitable for multiparticulate delivery. A central composite face centred (CCF) design was applied to study the influence of formulation variables (gelatin, carrageenan and alanine concentrations) on the crucial responses of the formulation (disintegration time, hardness, viscosity and pH). The disintegration time and viscosity were controlled by the associative interaction between gelatin and carrageenan upon hydration which forms a strong complex that increases the viscosity of the stock solution and forms tablet with higher resistant to disintegration in aqueous medium. Therefore, the levels of carrageenan, gelatin and their interaction in the formulation were the significant factors. In terms of hardness, increasing gelatin and alanine concentration was the most effective way to improve tablet hardness. Accordingly, optimum concentrations of these excipients were needed to find the best balance that fulfilled all formulation requirements. The revised model showed high degree of predictability and optimisation reliability and therefore was successful in developing an ODT formulation with optimised properties that were able deliver enteric coated multiparticulates of omeprazole without compromising their functionality.

1. Introduction

Orally disintegrating (dissolving) tablets (ODTs) are solid dosage forms that are placed in the mouth, rapidly disintegrate/dissolve when in contact with the saliva and then easily swallowed without the need for water [1]. The fast disintegrating behaviour of the ODT in the mouth limits the active ingredients that can be incorporated to drugs that exhibit good taste, stability in gastric conditions and have long half-life. Bitter tasting drugs can cause discomfort to patients and consequently reduce their compliance, whereas incorporating drugs that suffer from instability in gastric fluids reduces the efficacy of the dosage form (bioavailability). On the other hand, delivering active drugs that have short half-life in ODTs compromise the practicality of the dosage form as more frequent administration is required. To address these issues, a great deal of interest has been directed towards incorporating multiparticulate drug delivery system in ODT formulations [2].

The multiparticulate drug delivery system comprises of drug particles encapsulated or coated by one or more layers of polymers that control the release of the drug. The polymer can be selected to provide extended, delayed or pulsed drug delivery, allowing the rate of release of the drug to be tailored as required. Therefore, multiparticulate drug delivery systems can mask the unpleasant taste of active drugs, protect acid-labile drugs from possible degradation in the stomach and extend the drug release over several hours. Moreover, they provide many advantages over single-unit dosage forms because of their multiplicity and small sizes including reduced risk of systemic toxicity, enhanced bioavailability, reduced risk of local irritation and reduced patient to patient variability as a result of their more predictable gastric emptying [3]. Accordingly, the formulation of multiparticulate into ODTs can extend their application to more challenging drugs (e.g. acid sensitive) by overcoming restrictions imposed by the nature of these drugs and combine the benefits of ODTs and multiparticulate drug delivery system [2].

The compression of multiparticulate into ODT formulations has attracted substantial attention in both academia and industry and resulted in many scientific publications and patent applications [4]. However, to produce a tablet with good structural integrity, relatively high compression pressures are required. These high pressures can cause damage to the polymer layers of the multiparticulate system, and, as a result, compromise their release controlling properties [5].

Freeze drying is an alternative technique to produce ODTs without application of any compaction force, which could be useful in the formulation of multiparticulate into ODTs. However, three major requirements need to be addressed in order to ensure development of a successful formulation. Firstly, the need for high viscous liquid formulation that is able to suspend the multiparticulate long enough to complete formulation and freezing without

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compromising the disintegration performance. Secondly, minimum interaction between the liquid formulation and the multiparticulate that may lead to unwanted changes in the original properties of the multiparticulate such as early drug leakage. For example, for multiparticulate coated with hydrophobic polymers, the use of thick hydrophilic environment in the formulation reduces premature drug release, whereas for enteric coated multiparticulate, the use of acidic formulation ensures multiparticulate integrity. Thirdly, physical protection against possible damage during freezing and annealing step as a result of ice crystal growth.

The current study aimed to optimise ODT formulations suitable for multiparticulate delivery based on gelatin, carrageenan and alanine. The selection of these excipients can potentially benefit the formulation in many ways. Firstly, the choice of these excipients is based on exploiting the electrostatic associative interaction between the anionic sulphate groups of carrageenan polymer and the positive net charge of gelatin (below its isoelectric point) to produce highly viscous solution at relatively low concentration of both polymer [6], which ensures fast disintegration property and shorter freeze drying cycle [7]. Also, carrageenan has cryoprotectant activity which might be useful to protect the multiparticulate integrity during freezing and annealing steps [8]. Additionally, previous research from our laboratory has shown that gelatin and alanine have superior properties as matrix supporting agents in ODT formulations [9].

Successful development of new pharmaceutical formulations requires extensive and comprehensive research to determine the significant factors that influence formulation, understand their effects (individually and collectively) and optimise them to obtain high quality products. For lyophilised ODTs, traditional experimentation approach can be time and material consuming and consequently is associated with high cost, due to the existence of multiple factors that influence the formulation performance and manufacturing process. Recently, design of experiment (DoE) supported by statistical software has been reported as an efficient and powerful tool in the development and optimisation of pharmaceutical dosage forms [10]. The design evaluates the influence of various formulation parameters and their interaction with the lowest number of experiments, hence reducing the cost and time of the work [11]. Moreover, design of experiment is considered an essential part of quality by design paradigm (QbD), which is recommended by the FDA as a new regulatory requirement for approval of generic drugs [12].

Response surface modelling (RSM) was applied in this study to evaluate the influence of varying the concentration of the selected excipients (independent variables) on four crucial responses: disintegration time, hardness, viscosity and pH. Quantitative estimation of the significant model terms (linear, polynomial and interactive) was used to build statistical model for each response that can describe the relationship between the dependant and the independent variables. These models were used to optimise the concentration of the excipients that maximise the quality of the formulation. Furthermore, ODTs containing therapeutic dose of enteric coated pellets of omeprazole were prepared based on the optimised formulations and fully characterised to evaluate their feasibility as drug delivery system. Omeprazole was chosen as a model drug that is challenging to formulate as ODTs due to its acid labile nature and, therefore, needs to be incorporated in enteric coated pellets in order to tolerate the formulation of ODTs.

2. Materials

Gelatin from bovine skin, type B (Bloom strength ~ 75), lambda carrageenan and L-alanine (C3H7NO2, Reagent plus™ ⩾99%) were all purchased from Sigma–Aldrich Chemicals (Pool, UK). Enteric coated pellets of omeprazole (8.5% omeprazole, batch number: OME-020907) were supplied by MKPPL (Pune, India). Concentrated hydrochloric acid (specific gravity of 1.80), acetonitrile, phosphate buffered saline tablets (PBS) and standard solutions at pH 4.0 and 7.0 were all purchased from Fisher Scientific (Loughborough, UK). All the materials were used as received.

3. Methods

3.1. Design of experiment

The statistical experimental design in this study was performed using MODDE software version 8 (Umetrics Inc., NJ, USA). The top RSM (response surface modelling) design choice suggested by the software was a central composite face centred (CCF) that composed of 34 experiments in total, 15 fractional factorial runs in duplicate (15×2) and four replicated centre points. The concentration of gelatin (X_1), carrageenan (X_2) and alanine (X_3) was selected as independent variables at three levels. The three factorial levels for each independent factors, low, medium and high, were coded as -1, 0 and 1, respectively. The disintegration time (Y_1), hardness (Y_2), viscosity (Y_3) and pH (Y_4) were investigated as dependant variables (responses).

3.2. Preparation of ODTs for RSM experiments

A required amount of gelatin was solubilised in 100 ml double distilled water at about 40 °C to obtain a concentration of 3%, 4% and 5% (w/v). Carrageenan was added slowly in small portions under continuous stirring to the solution at concentration of 0.2%, 0.5% and 0.8% (w/v). After obtaining clear solution, alanine was added at concentration of 2%, 3.5% and 5% (w/v) and the formulations were kept under stirring until alanine dissolved completely. A constant mass of 1.50 g of the formulation was poured in a tablet mould with internal diameter of 13.50 mm, frozen at −80 °C for about 60 min, annealed in −20 °C a pre-cooled freezer for 12 h and then transferred back to the −80 °C freezer. The frozen formulation was freeze-dried (ADVANTAGE Freeze-dryer, VirTis Inc., USA) according to an optimised regime (primary drying for 48 h at a shelf temperature of −40 °C and secondary drying for 10 h at a shelf temperature of 20 °C and under constant vacuum of 50 mTorr throughout primary and secondary drying). The optimised formulation was prepared by the same method and the observed (experimental) and the predicted (from the model) values for the responses were compared with evaluate the validity of the model.

3.3. Viscosity and pH measurements

The viscosity of the formulation was measured using a Brookfield viscometer (LVT, Stoughton, MA, USA) with its spindle number 3 rotating at speed of 20 rpm at room temperature in a 100-mL beaker with the spindle guard.

The pH was measured using pH meter (MP230, Mettler Toledo, Ohio, USA). The pH meter was calibrated using standard solutions at pH 4.0 and 7.0.

3.4. Disintegration time

Disintegration time is the time required for ODTs to disintegrate completely without leaving any solid residue. In vitro disintegration time for lyophilised ODTs was evaluated using US pharmacopoeia monograph (USP General Chapter 2008, ⟨7 0 1⟩ disintegration). A disintegration tester (Erweka ZT3, Heusenstamm, Germany) was used in this study as a disintegration appa-

ratus and distilled water (800 mL) as disintegration medium; the disintegration medium temperature was maintained at 37 °C by a thermostat. At each time, one tablet was placed in the basket rack assembly and covered by transparent plastic disc. The disintegration time was taken as the time required for ODTs to disintegrate completely without leaving any solid residue. The results were average of three measurements.

3.5. Mechanical property

The mechanical properties of the freeze-dried tablet (hardness) were investigated with a texture analyser (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated by standard weight of 500 g and 5 kg. The tablet was placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1 mm penetration of 5 mm diameter probe at a speed of 6 mm/min. The results were average of three measurements.

3.6. Density and diameter of the enteric coated pellets

The density of the pellets was determined on 2 g of the pellets using Multipycnometry (MVP-D160-6, Quantachrome, UK) with 4.25 cm^3 sample cup at 22 °C. Prior to analysis, the helium pycnometry was calibrated against a standard steel ball. Each determination included 10 purges at 19.5 psi and 10 analytical runs at 19.5 psi with an equilibration rate of 0.0050 psi/min. The results were average of three measurements.

The diameter of 50 randomly chosen pellets was measured using a digital caliper (Whiteworth, CA, USA).

3.7. Drug content and HPLC analysis

Fifty milligrams of omeprazole pellets was dissolved in 50 ml of a mixture of acetonitrile: phosphate buffered saline (PBS) mobile phase (28:72) and transferred immediately to an amber container. After good shaking, the solution was filtered through a 0.45 μm nylon filter (CHROMACOL LTD., Herts, UK) in autosampler vials for HPLC assay.

HPLC analysis of was carried out using Reverse phase HPLC (Dionex AS 50 autosampler with GP50 gradient pump HPLC System: Dionex, UK) at room temperature using a Gemini 5 μm, 4.6 × 150 mm, column (Phenomenex La Luna: Phenomenex, Torrance, USA). The column was placed in a digital HPLC column heater (Model 605, chromtech, MN, USA) at a fixed temperature of 35 °C. The mobile phase was a mixture of pH 6 USP phosphate buffer (0.05 M): acetonitrile (72:28). The mobile phase flow rate was 1 ml/min, the injection volume was 20 μl and the UV absorbance was at 280 nm [13]. Under these conditions, the retention time was 3.31 min. The concentration of omeprazole was determined by reference to a calibration curve constructed from dilutions of a stock solution (1 mg/mL), using the mobile phases, in a concentration range between 5.0 and 200.0 μg/mL. The calibration curve was performed in triplicate and resulted in a linear correlation in the studied concentration range (r^2 = 0.99).

3.8. Dissolution studies

The dissolution profiles of 120.5 mg unprocessed pellets (10 mg omeprazole) and prepared ODTs that contained therapeutic doses of omeprazole pellets (10 mg omeprazole) were evaluated using the USP dissolution apparatus (Erweka DT 600, Heusenstamm, Germany) with baskets at a rotational speed of 50 rpm, in 900 mL dissolution medium at 37 °C. Acidic dissolution medium 0.1 N HCl was used during the first 2 h, followed by 1 h in phosphate buffer saline (pH 6.8). At fixed time intervals, 5 ml samples were withdrawn and immediately 1 mL of 0.25 N NaOH was added. The samples were replaced with fresh medium (37 °C). The samples were filtered through a 0.45 μm nylon filter (CHROMACOL LTD., Herts, UK) in autosampler vials for HPLC assay.

4. Results and discussion

4.1. Design of experiment

The aim of this work was to optimise formulation parameters for incorporation of enteric coated multiparticulate (pellets) of omeprazole into lyophilised ODTs. In theory, a successful formulation should keep the pellets stable and suspended during and after the formulation process, exhibit adequate mechanical strength in the dry state and disintegrate quickly upon hydration. Suspending the pellets in the binder solution for enough time can be controlled by the viscosity of the solution, whereas the stability of the pellets is linked with the pH of the surrounding environment, due to the presence of enteric coating around the pellets. Therefore, the crucial responses that were selected as dependant variables were disintegration time (Y_1), hardness (Y_2), viscosity (Y_3) and pH (Y_4).

Gelatin, carrageenan and alanine were selected as main excipients. Gelatin was used as matrix forming agent which gives shape and provides mechanical strength to the tablets [7]. Moreover, it forms thermo-reversible gels upon hydration with melting points around 35–37 °C (just below body temperature), which provides smooth feeling in the mouth after disintegration. Our previous study [7] suggested that gelatin at stock solution concentration between 2% and 5% (w/v) is most suitable for developing lyophilised orally disintegrating tablets. Carrageenan was added as viscosity modifying agent that drastically increases the viscosity of gelatin stock solution, due to the formation of complex coacervates (associative interaction) between the two polymers [14]. Preliminary studies (see Appendix A) showed that concentrations from 0.2% to 0.8% (w/v) of carrageenan were capable of increasing the viscosity of gelatin stock solutions (2–5% w/v). Alanine was used as a matrix supporting/disintegration enhancing agent. Previous study [9] suggested that inclusion of 2–5% (w/v) of alanine in ODT formulation based on gelatin as a binder was able to cement the porous structure of the lyophilised tablets and accelerate the disintegration at the same time. Moreover, alanine showed tendency to crystallise in the frozen formulation and consequently stabilise the formulation against possible collapse [9]. Accordingly, the influence of varying these three formulation (independent) variables, at three concentration levels within their pre-optimised ranges (see above), on the selected responses was studied using response surface modelling (RSM).

The Central Composite Design (CCD) is one the most widely used type of experimental designs because of its high efficiency and flexibility with respect to the number of runs required [15]. Three main varieties of CCD are available: central composite circumscribed (CCC), central composite inscribed (CCI) and central composite face centred (CCF). In this investigation, the CCF was employed as it provides relatively high quality predictions over the entire design space and does not require using points outside the original factor range, when compared to CCC and CCI [16]. The top RSM design choice suggested by the software was central composite face centred (CCF), which was composed of 34 experiments in total, 15 fractional factorial runs in duplicate (15 × 2) and four replicated centre points. The full worksheet is presented Table 1.

The results (Table 1) showed that the disintegration time of the tablets varied from 8 to 341 s, the hardness from 5.42 to 21.38 N, viscosity from 92.3% to 453.1% and pH from 5.5 to 5.8. The wide variation in the disintegration time, hardness and viscosity values

Table 1
The CCF design worksheet and characterisations data.

Exp name	Run order	Gelatin% (w/v)	Carrageenan% (w/v)	Alanine% (w/v)	Disintegration time (s)	Hardness(N)	Viscosity(m Pa s)	pH
N1	32	3	0.2	2	15	6.36	98.8	5.6
N2	5	5	0.2	2	12	15.45	159.4	5.5
N3	14	3	0.8	2	23	5.42	153.3	5.8
N4	17	5	0.8	2	286	16.23	391.9	5.7
N5	10	3	0.2	5	27	8.44	113.9	5.6
N6	18	5	0.2	5	24	21.38	156.4	5.5
N7	20	3	0.8	5	63	10.5	134.1	5.8
N8	21	5	0.8	5	339	19.53	448.5	5.7
N9	22	3	0.5	3.5	22	8.84	92.3	5.7
N10	1	5	0.5	3.5	44	16.91	400.3	5.6
N11	19	4	0.2	3.5	32	12.06	211.9	5.6
N12	6	4	0.8	3.5	229	15.26	239.1	5.7
N13	34	4	0.5	2	28	11.27	210.3	5.6
N14	26	4	0.5	5	91	15.36	213.4	5.6
N15	23	4	0.5	3.5	93	17.36	216.9	5.6
N16	11	4	0.5	3.5	100	17.84	270	5.6
N17	16	4	0.5	3.5	97	17.74	237.3	5.6
N18	9	3	0.2	2	14	6.63	105.8	5.6
N19	31	5	0.2	2	8	15.94	170.5	5.5
N20	24	3	0.8	2	23	5.56	142.3	5.8
N21	30	5	0.8	2	81	16.71	381	5.7
N22	27	3	0.2	5	28	8.79	97.5	5.6
N23	15	5	0.2	5	36	19.14	181	5.6
N24	12	3	0.8	5	66	11.16	144.8	5.8
N25	28	5	0.8	5	341	20.5	453.1	5.7
N26	25	3	0.5	3.5	26	7.85	108.1	5.7
N27	3	5	0.5	3.5	43	16.63	420.1	5.6
N28	2	4	0.2	3.5	31	9.67	142	5.5
N29	4	4	0.8	3.5	232	16.25	236.7	5.7
N30	33	4	0.5	2	28	10.56	190.3	5.6
N31	7	4	0.5	5	93	15.09	203.4	5.6
N32	29	4	0.5	3.5	93	16.56	237.1	5.6
N33	13	4	0.5	3.5	100	16.64	224.2	5.6
N34	8	4	0.5	3.5	102	18.02	206.9	5.6

for different formulations and the high degree of reproducibility (Fig. 1) suggested that these responses are strongly dependent on the selected independent factors. In case of pH, although small variations were noticed between different formulations, the results seemed to be systematic and repeatable, which may suggest dependency on the studied factors.

4.2. Analysis of variance (ANOVA)

A quadratic statistical model incorporating interactive and polynomial terms was used to evaluate the influence of the studied factors (independent factors) on the responses (dependent variables).

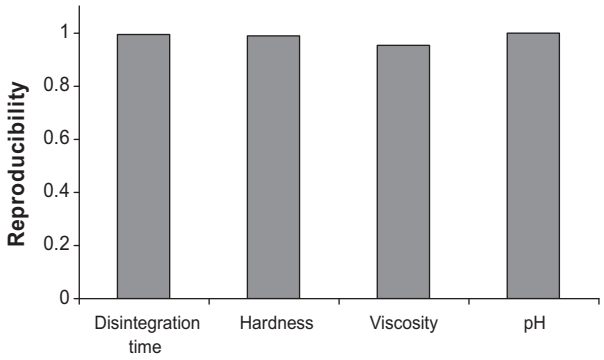

Fig. 1. Reproducibility of the results for all four responses. Reproducibility: is the variation of the response under the same conditions (pure error) compared with the total variation of the response. Reproducibility = 1 − (MS(Pure error)/MS(total SS corrected)). A reproducibility value of 1 represents perfect reproducibility.

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{123}X_1X_2X_3$$

where Y_i is the response (dependent variable), b_0 is the arithmetic mean response of the 34 trials, b_i is the estimated coefficient for the relevant model terms, X_1 is gelatin concentration, X_2 is carrageenan concentration and X_3 is alanine concentration. The main effects (X_1, X_2 and X_3) represent the average result of changing one factor at a time from its low to high value while keeping the other factors at their canter point. The interaction terms (X_1X_2, X_1X_3, X_2X_3 and $X_1X_2X_3$) show the change in the response when factors are varied simultaneously. The polynomial terms (X_1^2, X_2^2 and X_3^2) express non-linear correlations with the response.

Analysis of variance (ANOVA) was performed to evaluate the significance of the quadratic models (linear, interactive and polynomial) on the responses and to estimate their quantitative effects. Table 2 summarises the effects of the model terms and associated p values for all four responses. At a 95% confidence level, a model was considered significant if the p value < 0.05. The sign and value of the quantitative effect indicate trend and magnitude of the term's influence on the response, respectively. Positive signs indicate an increase in the response value, while negative signs demonstrate a decrease in the response value. The results indicate that the disintegration time of the tablets was significantly influenced by the linear models of gelatin (X_1), carrageenan (X_2) and alanine (X_3), in addition to the interactive model of gelatin-carrageenan (X_1X_2) and carrageenan–alanine (X_2X_3). Quantitative estimation of the significant models indicated that carrageenan and gelatin had the prime influence on the disintegration time linearly and interactively, suggesting that increasing carrageenan and/or gelatin concentration in the formulation increases the disintegration time drastically (Fig. 2). The deteriorating effect of X_1 and X_2

Table 2
The quantitative factor effects and associated p value for the responses.

	Disintegration time		Hardness		Viscosity		pH	
Term	Effect	p Value	Effect	p Value	Effect	p Value	Effect	p Value
X_1	33.9755	<0.0001	3.8395	<0.0001	77.3120	<0.0001	−0.0348	<0.0001
X_2	55.9070	<0.0001	0.5089	0.1318	50.9857	<0.0001	0.0707	<0.0001
X_3	26.9802	0.0008	1.5766	<0.0001	3.3790	0.5917	0.0028	0.5007
X_1^2	−16.8769	0.1220	−0.7425	0.1431	7.2172	0.4477	0.0201	0.0032
X_2^2	20.8862	0.0590	−0.4343	0.3844	−11.5445	0.2291	0.0097	0.1273
X_3^2	−4.2117	0.6923	−0.5014	0.3166	−9.7039	0.3098	0.0021	0.7388
X_1X_2	34.5981	<0.0001	−0.0416	0.8845	31.4688	<0.0001	−0.0041	0.2580
X_1X_3	9.9296	0.1162	0.0541	0.8504	5.2006	0.3461	0.0036	0.3149
X_2X_3	14.5584	0.0252	0.1815	0.5283	2.5914	0.6363	−0.0044	0.2303
$X_1X_2X_3$	6.9345	0.1566	−0.2460	0.2763	3.9755	0.3547	−0.0031	0.2727

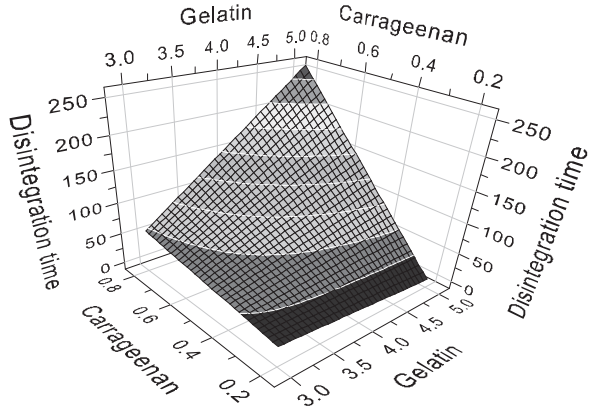

Fig. 2. Surface response plot showing the influence of varying gelatin and carrageenan concentrations (%w/v) on the disintegration time of the ODT at constant concentration of alanine (3.5% w/v).

on disintegration could be explained by the associative interaction between gelatin and carrageenan upon hydration which forms a strong complex and consequently more resistant to disintegration in aqueous medium. Similar behaviour was reported by Bonferoni et al. [17] where much adhesive systems based on carrageenan and gelatin showed high resistance to erosion in an aqueous environment (lachrymal fluid) as a result of their associative interaction. Moreover, the formation of viscous solution upon hydration as a consequence of this interaction might limit the movement of water [18] inside the tablet and consequently reduce rate of penetration of the disintegration medium and therefore result in longer disintegration time. The large positive coefficient (34.5981) of the interactive term (X_1X_2) suggested that detrimental effect of gelatin and carrageenan on the disintegration of the tablet is synergised by increasing the concentration of both polymers simultaneously, which might be explained by the existence of more polymer chains available for complexation and consequently stronger interaction resulting in viscous environment upon hydration. On the other hand, increasing alanine concentration showed significant increase in disintegration, linearly (X_3) and interactively with carrageenan (X_2X_3) but to a lower degree when compared to gelatin and carrageenan. The inclusion of high concentration of alanine decreases the porosity of the tablets [9] and increases the probability of forming complex with carrageenan due to the presence of positive amino group on alanine that can form a complex with the negative sulphate group of carrageenan.

For hardness (Y_2), ANOVA results (Table 2) suggested that gelatin concentration (X_1) and alanine concentration (X_3) were the only significant terms with a p value < 0.00001. Increasing gelatin concentration was the most effective way to improve the hardness as indicated by its large positive coefficient (3.8395), possibly due to the formation of more extensive 3D networks of gelatin fibres [7]. Increasing alanine concentration also enhances the hardness significantly, which could be as a result of cementing the porous structure of the tablet, increasing the contact points between the excipients, and enhancing the intermolecular bonding forces within the tablets [9]. See Fig. 3.

The viscosity (Y_3) was significantly influenced by gelatin concentration (X_1), carrageenan concentration (X_2) and their interactive term (X_1X_2), with a p value of <0.0001 and positive large coefficients for all the terms, suggesting that increasing carrageenan and/or gelatin concentration in the formulation increases the viscosity drastically. This could be explained by the attractive electrostatic interactions between gelatin and carrageenan which depends on the concentration and ratio of both polymers [19]. Accordingly, the results suggested that, at the investigated concentration ranges for both the polymers, the interaction was enhanced by increasing the total concentration of the polymers individually and more effectively by simultaneous increase in concentrations of both the polymers (Fig. 4).

For the fourth response Y_4 (pH), significant terms were identified as X_1 (gelatin concentration) X_2 (carrageenan concentration) and X_1^2 (polynomial model of gelatin concentration). The results (Table 2) suggested that increasing gelatin concentration decreases the pH of the formulation (Fig. 5). However, this decrease is limited as indicated by the significant influence of the positive coefficient of the polynomial model of gelatin concentration (X_1^2). Similar effect of gelatin on the pH was reported in literature [20]. Carrageenan concentration (X_2) had a positive coefficient, suggesting that increasing its concentration raises the pH of the formulation (Fig. 5).

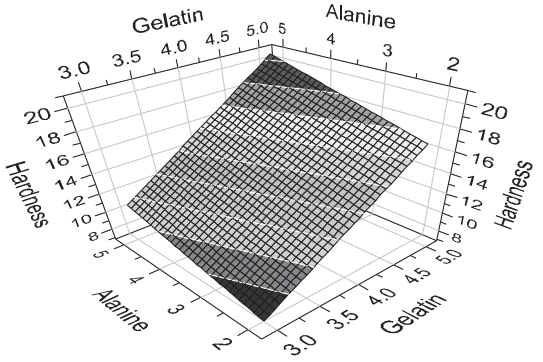

Fig. 3. Surface response plot showing the influence of varying gelatin and alanine concentrations on the hardness of the ODT.

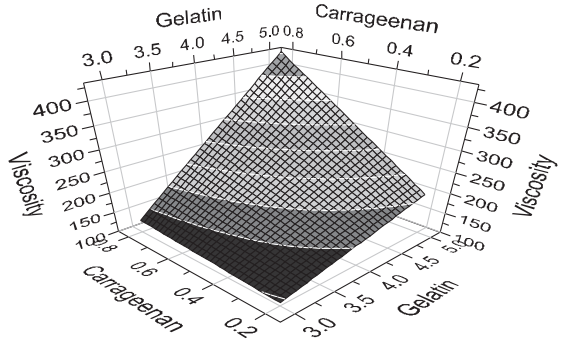

Fig. 4. Surface response plot showing the influence of varying gelatin and carrageenan levels on the viscosity of the stock solution.

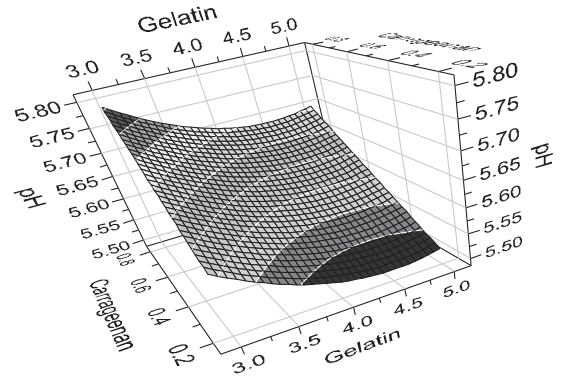

Fig. 5. Surface response plot showing the influence of varying gelatin and carrageenan levels on the pH of the stock solution.

4.3. Revised models and surface response plots

The resulting equations for all four responses, Y_1 (disintegration time), Y_2 (Hardness), Y_3 (viscosity) and Y_4 (pH), are presented below:

$$Y_1 = +84.4118 + 35.3049X_1 + 56.6747X_2 + 22.9657X_3 + 33.1818X_1X_2 + 12.5X_2X_3$$

$$Y_2 = +13.7544 + 3.84851X_1 + 1.54766X_3$$

$$Y_3 = +217.429 + 76.7327X_1 + 50.1198X_2 + 32.1477X_1X_2$$

$$Y_4 = +5.60919 - 0.0354952X_1 + 0.070379X_2 + 0.0268962X_1^2$$

Statistical analysis for testing the validity of the models is summarised in Table 3. p Values for all the simulated responses were well below the significant level (<0.05), suggesting that all the revised models were significant in predicting their response values. The correlation coefficients (R^2) for all four responses indicated good fits to the raw data (observed) in the revised model. However, lower correlation coefficients were obtained for hardness (0.803) and disintegration time (0.824). This might be explained as the influence of the same freezing and annealing conditions on the internal structure of different formulation is expected to show some variations. Previous research showed that freezing and annealing conditions control the internal structure of lyophilised tablets and consequently the hardness and disintegration time [21]. Moreover, the qualitative nature of the disintegration test that depends on the visual evaluation in addition to the fact that few seconds' inaccuracy in evaluating the disintegration time can result in big error [7].

Based on the revised equations, the software was used to generate response surface plots (three dimensional) that simulate the influence of the independent factors on each response individually. The graphs for disintegration time, hardness, viscosity and pH are presented in Figs. 2–5, respectively. The plots can provide uninterrupted visual assessment of the change in the response surface as a function of varying the independent factors, individually and simultaneously, which is valuable to further understand the system and optimise the formulation.

4.4. Optimum ODTs formulation

Based on the response surface plots, the software was used to perform hot spot analysis to obtain optimum formulation variables (gelatin, carrageenan and alanine concentrations) to produce ODTs with desired characteristics. The request was to minimise the disintegration time, and maximise the hardness and viscosity of the formulation, whereas the pH was excluded from the optimisation due to its limited variation in response to the studied factors. The optimal formulation was determined as 4.7% (w/v) gelatin, 0.02% (w/v) carrageenan and 3% (w/v) alanine. The observed response values of the optimised formulation compared with the predicted values are presented in Table 4. The closeness between the experimental (observed) and calculated (predicted) values of the responses can add further experimental verification to the validity of the established statistical models.

4.5. Inclusion of enteric coated pellets of omeprazole

The characterisation of the enteric coated pellets of omeprazole used in the study is presented in Table 5. The results showed that the pellets were able to withstand the gastric condition (0.1 N HCl) for 2 h with less than 10% of the total drug amount being released, which complied with the USP specification for enteric coated pellets. The dissolution profile after transferring the pellets to a pH

Table 3
Summary of results for testing validity of the revised models. DF indicates: degrees of freedom; SS: sum of squares; MS: mean of square; F: Fischer's ratio; p: probability; R^2: regression coefficient.

	DF	SS	MS (variance)	F	p	R^2
Disintegration time						
Regression	5	219302	43860.3	20.4012	<0.0001	0.824
Lack of Fit	9	39007.7	4334.19	3.88643		
Viscosity						
Regression	3	322,215	107,405	77.5712	<0.0001	0.886
Lack of Fit	11	35393.4	3217.59	9.94932		
Hardness						
Regression	2	567.807	283.903	63.3272	<0.0001	0.803
Lack of Fit	12	129.304	10.7754	21.1665		
pH						
Regression	3	0.221275	0.0737584	134.959	<0.0001	0.931
Lack of Fit	11	0.0063957	0.000581427	1.10471		

Table 4
Observed and predicted responses and residual values for the optimised formulation. The observed results are means, $n = 3$.

Response	Observed	Predicted	Residual
Disintegration time (s)	14 ± 3	15	−1
Hardness (N)	17.22 ± 0.74	16.17	1.05
Viscosity (m Pa s)	172.40 ± 10.25	181.26	−8.86
pH	5.5 ± 0.0	5.5	0

Table 5
Characterisations of omeprazole enteric coated pellets. Results are mean ± SD, $n = 3$.

Drug content% (w/w)	Drug recovery%	Density (g/cm^3)	Diameter (μm)
8.27 ± 0.29	91.24 ± 1.22	1.439 ± 0.006	710 ± 40

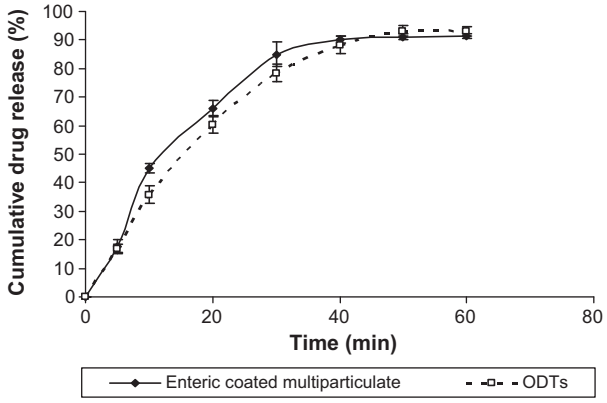

Fig. 6. Cumulative percent of omeprazole released in phosphate buffer (pH 6.8) after 2 h of gastric resistance study in 0.1 N HCl. Results are mean ± SD ($n = 3$).

6.8 phosphate buffer is shown in Fig. 6. Based on the optimised formulation, lyophilised ODTs containing 120.5 mg of enteric coated pellets of omeprazole (10 mg dose of omeprazole) was prepared using 18 mm diameter mould. The solution was able to suspend the pellets long enough before transferring the formulation to the freezer with no obvious settling or aggregation of the pellets. Moreover, no degradation or colour change was noticed throughout mixing, freezing and lyophilisation stages.

Characterisation of the tablets is summarised in Table 6. The tablets disintegrated in less than 19 s and had an average hardness of 17.24 ± 0.74 N ($n = 3$). The disintegration time and hardness of the prepared tablets were not significantly different when compared to the optimised formulation without the pellets (Table 5), which suggested that the pellets did not compromise the tablets properties. The results showed no significant decrease in drug recovery after 2 h in gastric condition compared with the original pellets, suggesting that the formulation and manufacturing process did not interfere with the integrity of the pellets. The dissolution profile after transferring the pellets to a pH 6.8 phosphate buffer is shown in Fig. 6.

5. Conclusion

The central composite face centred (CCF) design applied in this study was used to provide details of the influence of independent variables on the responses. The results of analysis of variance (ANOVA) showed that all three independent variables had significant effect on the selected response. The revised model showed high degree of reliability and resulted in the development of ODT formulations with optimum properties. Work presented in this article has been filed for patent protection.

Table 6
Characterisations of orally disintegrating tablets containing omeprazole pellets. Results are mean ± SD, $n = 3$.

Disintegration time (s)	Hardness (N)	Viscosity (m Pa s)	Drug recovery (%)
16 ± 3	17.2 ± 0.74	172 ± 21.3	93.14 ± 1.22

Acknowledgement

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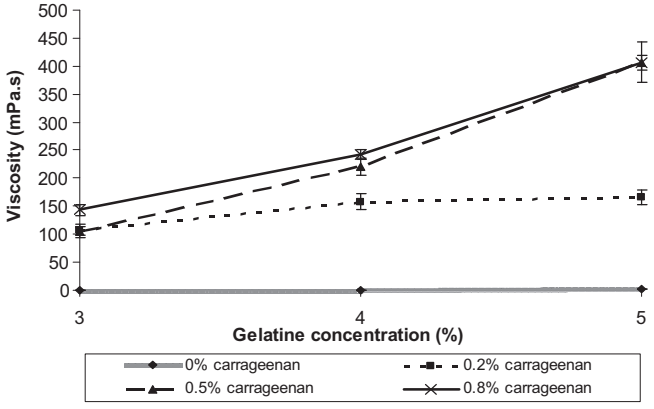
Appendix A



Preliminary experiments to study the viscosity of different solution of gelatin (3%, 4% and 5%) before and after the addition of 0.2%, 0.5% and 0.8% carrageenan. The results show substantial increase in the solution viscosity after addition of small concentration of carrageenan as a result of their associative interaction. Addition of 0.2% carrageenan to for 5% gelatin solution increased the viscosity by more than 100-fold, from 1.5 ± 0.1 m Pa s for 5% gelatin alone to 165.8 ± 13.7 m Pa s upon addition of 0.2% carrageenan. Results are mean ± SD, $n = 3$.

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