Research Article

Development and Optimization of Gastro-Retentive Controlled-Release Tablet of Calcium-Disodium Edentate and its *In Vivo* Gamma Scintigraphic Evaluation

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ABSTRACT. Medical management of heavy metal toxicity, including radioactive ones, is a cause for concern because of their increased use in energy production, healthcare, and mining. Though chelating agents like EDTA and DTPA in parenteral form are available, no suitable oral formulation is there that can trap ingested heavy metal toxicants in the stomach itself, preventing their systemic absorption. The objective of the present study was to develop and optimize gastro-retentive controlled-release tablets of calcium-disodium edentate (Ca-Na₂EDTA). Gastro-retentive tablet of Ca-Na₂EDTA was prepared by direct compression method. Thirteen tablet formulations were designed using HPMC-K4M, sodium chloride, and carbopol-934 along with effervescent agents sodium bicarbonate and citric acid. Tablet swelling ability, *in vitro* buoyancy, and drug dissolution studies were conducted in 0.1 N HCl at 37±0.5°C. Ca-Na₂EDTA was radiolabeled with technetium-99m for scintigraphy-based *in vivo* evaluation. Formula F8 (Ca-Na₂EDTA 200 mg, carbopol 100 mg, avicel 55 mg, citric acid 30 mg, NaHCO₃ 70 mg, NaCl 100 mg, and HPMC 95 mg) was found to be optimum in terms of excellent floating properties and sustained drug release. F8 fitted best for Korsmeyer–Peppas equation with an *R*² value of 0.993. Gamma scintigraphy in humans showed mean gastric retention period of 6 h. Stability studies carried out in accordance with ICH guidelines and analyzed at time intervals of 0, 1, 2, 4, and 6 months have indicated insignificant difference in tablet hardness, drug content, total floating duration, or matrix integrity of the optimized formulation. Gastro-retentive, controlled-release tablet of Ca-Na₂EDTA was successfully developed using effervescent technique as a potential oral antidote for neutralizing ingested heavy metal toxicity.

KEY WORDS: calcium disodium EDTA; controlled-release tablet; gamma scintigraphy; heavy metal decorporation.

INTRODUCTION

Heavy metal poisoning remains a prevalent issue in many of the industrial nations, where exposure to heavy metals is not only confined to occupational workers but also to general population at large (1,2). The problem is accentuated by the fact that toxic biochemical and functional effects are being reported at lower levels of metal exposure than those that produce overt clinical signs and symptoms (3). Another area of concern is the increased use of radioactive metals (radionuclides) in areas like healthcare and energy production, which has enhanced the vulnerability to radionuclide accidents, in which case radiation toxicity becomes the more potent threat. The intentional use of a radiological device

being used by rogue states or terrorist organizations leading to significant radio-metal contamination of affected population is another concern that cannot be overlooked (4).

Ethylene diamine tetraacetic acid (EDTA) is an approved, safe, and frequently used drug for repeated parenteral administration for management of heavy metal poisoning. Use of EDTA and other chelating agents is the generally accepted treatment for lead and other heavy metal poisoning (5–7). However, clinical utility of EDTA and a related member of the family, diethylene triamine pentaacetic acid (DTPA), has been severely compromised by the need to administer them intravenously. In accidents involving radioactivity spillage, a lot of heavy radiometals are known to be liberated. Injectable DTPA is a known antidote drug for any such eventuality, which is given for many days/weeks for trapping heavy metals in the bloodstream after their systemic absorption. However, a better therapeutic option in such a scenario would be to somehow prevent the systemic absorption of these heavy metals altogether, thereby preventing any chance of their accumulation in respective target organs. The challenge of removing such contaminants from the body assumes greater significance considering that *in vivo* radio-metal deposition

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even in miniscule quantities may impart significant radiation dose to the body's target organs depending upon their half-lives.

Ingestion and inhalation are the two major routes through which radioactivity is internalized into the human body. Further, a major portion of the inhaled portion may get transported back to the oropharynx by mucociliary action and then get ingested. Thus, majority of the radioactivity available for systemic absorption is routed through the gastro-intestinal tract (GIT). We believe that this is a reason enough for having an oral chelating agent-based formulation to preempt or prevent absorption of ingested radiometallic ions. Unfortunately, no such oral formulation is presently available.

Further, for any decorporating formulation to be effective orally, the chelator should remain in the stomach continuously for maximum possible time. This is because of the fact that if a chelating agent is given as a routine tablet, then it will leave the stomach at the rate of peristalsis and any contaminated patient will have to take the chelator formulation probably every 2 h to ensure a continuous presence of chelating agent in the stomach. This is neither practical nor cost-effective. Thus, the objective of the present study was to develop a slow-release gastro-retentive calcium disodium-EDTA (Ca-Na₂EDTA) oral formulation.

Another highlight of the study is the use of gamma scintigraphy in the development and evaluation of this oral formulation, a technique which we have successfully used in the development of a few of our other drug formulations too (8–12). We have studied gastric retention time of the developed formulation by radiolabeling Ca-Na₂EDTA with technetium-99m (Tc-99m). Effervescent gastro-retentive tablets of Ca-Na₂EDTA having different excipient combinations were developed with an objective of achieving optimally prolonged gastric retention in comparison to conventionally made non-floating Ca-Na₂EDTA tablet.

MATERIALS AND METHODS

Reagents and Chemicals

Calcium-disodium ethylenediamine tetraacetic acid (Ca-Na₂EDTA) was procured from Merck India Ltd. (Mumbai, India). Hydroxy Propyl Methyl Cellulose-K4M (HPMC-K4M) was purchased from Otto Chemie Pvt. Ltd., Mumbai, India and Carbopol-940 was purchased from John Baker Inc. (CO, USA). Stannous chloride was purchased from Sigma Chemical Company, St. Louis, MO, USA, while technetium-99m pertechnetate was obtained from BRIT, BARC, India. All other chemicals and reagents used were of laboratory grade.

Preparation of Gastro-Retentive/Conventional Ca-Na₂EDTA Tablet

Thirteen tablet formulations containing Ca-Na₂EDTA were prepared by direct compression method using different grades and varying concentrations of the polymer as well as different concentrations of sodium bicarbonate and varying ratios of microcrystalline cellulose (Avicel PH-102). All the ingredients except magnesium stearate and talc were thoroughly mixed in a large-size poly bag using tumbling action.

Thereafter, magnesium stearate and talc were added and the contents were further mixed for additional 5 min. The blend was compressed using a ten-station rotary tablet punching machine (lab press; CIP Machinery Pvt Ltd, Ahmedabad, India) with 12-mm punches. The weight of the tablets was kept constant for all formulation combinations (650 mg). The compositions of all the formulations are tabulated in Table I.

The conventional, non-floating tablet of Ca-Na₂EDTA was prepared by the same method as described above with suitable modifications in the choice of excipients used. In this case, lactose and starch were used in place of carbopol, sodium bicarbonate, citric acid, and sodium chloride to make the conventional tablet of similar weight.

In Process Quality Control of Gastro-Retentive Tablet

All prepared tablets were evaluated for their uniformity of weight, hardness, friability, and thickness according to the Indian Pharmacopeia (IP) (13).

Tablet Hardness

The tablet crushing strength was tested by the commonly used Monsanto-type tablet hardness tester (IEC, Mumbai, India). Twenty tablets were randomly selected; each tablet was placed between the anvils and the crushing strength, which causes the tablet to break, was recorded.

Tablet Friability

This test is applicable to compressed tablets and is intended to determine the physical strength of tablets. According to the IP specifications (13), ten tablets were randomly selected and placed in the drum of a tablet friability test apparatus (Electrolab Friabilator, Delhi, India). The drum was adjusted to rotate 100 times in 4 min. The tablets were removed, dedusted, and accurately weighed. The percent weight loss was calculated as per the formula

$$\% F = \frac{(W_f - W_i)}{W_f} \times 100$$

Where

% F friability in percentage

W_i initial weight of tablets

W_f final weight of tablets after 4 min.

Tablet Weight Variation

Twenty tablets were randomly selected and weighed individually. The average weight was calculated. It was ensured that not more than two of the individual tablet weights deviated from the average weight by more than 5% and none deviated by more than twice that percentage (13).

Table I. Optimization of Tablet Formula at Different Compositions

Exp. no.	Tablet code	Amount of Ca-Na ₂ EDTA (mg)	Amount of carbopol (mg)	Amount of avicel (mg)	Amount of citric acid (mg)	Amount of NaHCO ₃ (mg)	Amount of NaCl (mg)	Amount of HPMC (mg)
1	F1	200	100	55	–	70	135	90
2	F2	200	100	55	–	70	90	135
3	F3	200	100	55	30	–	145	120
4	F4	200	100	55	30	–	120	145
5	F5	200	100	55	30	70	80	115
6	F6	200	100	55	30	70	85	110
7	F7	200	100	55	30	70	95	100
8	F8	200	100	55	30	70	100	95
9	F9	200	100	55	30	70	115	80
10	F10	200	100	55	30	70	90	105
11	F11	200	100	55	30	70	100	85
12	F12	200	100	55	30	70	130	65
13	F13	200	100	55	30	70	65	130

Tablet Thickness

Twenty tablets were randomly selected and their thickness was measured individually using Vernier calipers (Forbro Engineers, Mumbai, India). Results were expressed as mean values $\pm$ SD.

Tablet Swelling Ability

Swelling index of individual batch was carried out using USP dissolution apparatus-II (Paddle method; V Scientific, Delhi, India) in 900 ml of 0.1 N HCl, which was maintained at 37 $\pm$ 0.5°C and rotated at 50 rpm. The weight of the individual tablet was taken prior to the swelling study (W_i). The tablets were kept in a basket and observed at 1-h intervals up to 12 h after carefully removing excess water using a filter paper. The swollen tablets were re-weighed (W_f), and percent hydration (swelling index) was calculated as shown in the table below using the following formula:

$$\% \text{ Swelling Index} = \frac{(W_f - W_i)}{W_i} \times 1000$$

Where

W_i initial weight of tablet

W_f Final weight of the swollen tablet.

Tablet In Vitro Buoyancy Study

In vitro buoyancy studies were performed for all preparations in triplicate as per the method described by Rosa *et al.* (14) for determining the floating lag time. The randomly selected tablets from each formulation were placed in a 200-ml beaker containing simulated gastric fluid, pH 1.2 at 37 $\pm$ 0.5°C. The time taken for the tablet to rise to the surface and float was taken as floating lag time, and its buoyancy and total floating duration, “the time during which tablet remains buoyant”, were recorded.

Drug Content Uniformity

Ten tablets were randomly selected, weighed, and crushed. A quantity of powder equivalent to the mass of one tablet (650 mg) was extracted in 100 ml of 0.1 N HCl. The solution was filtered through a cellulose acetate membrane (0.45 μ m). Ca-Na₂EDTA content in filtrate was estimated by UV spectrophotometric method at λ_{max} =270 nm in 0.1 N HCl and coloring agent (15). A tablet is said to comply with the test if individual drug content is between 85 and 115% of the average content (label claim). On the other hand, a preparation fails to comply with the test if more than one tablet content is outside these limits or if any individual content is outside the limits as mentioned in IP-2010 (13).

Drug Release Study

In vitro drug-release studies of prepared gastro-retentive, controlled-release tablets (F1 to F13)/conventionally made tablet of Ca-Na₂EDTA were carried out in triplicate, in the dissolution test apparatus (USP Type II; V Scientific, Delhi, India) as per International Conference on Harmonization (ICH) guidelines (16). The paddle rotation was set to a speed of 100 rpm. The tablets were placed into 900 ml of 0.1 N HCl solution (pH 1.2). Aliquots of 5 ml were withdrawn from the dissolution apparatus at predetermined time intervals (0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 h) and filtered through a cellulose acetate membrane (0.45 μ m). The drug content was determined UV spectrophotometrically at a wavelength of 270 nm, as mentioned above. At each time of withdrawal, 5 ml of fresh medium was replaced into the dissolution flask to maintain sink condition. Cumulative percent drug release with time (h) was determined.

Analysis of Release Mechanism

In order to examine the release mechanism of Ca-Na₂EDTA from the prepared controlled-release tablets of the optimized formulation (F8), the results of the dissolution study were examined in accordance to the kinetic models. The regression coefficient, R^2 , value nearer to 1.0 indicated the model fitting of the release mechanism. The commonly adopted models for understanding the release of drugs from

matrices are zero-order equation, first-order equation (17,18), Higuchi equation (19), and Korsmeyer–Peppas simple exponential equation (20,21) models. These simple exponential equation models were used to elucidate the mode of release using Sigmaplot™ 10 software (Cranes Software International, Bangalore, India).

Physical Stability Studies

Physical stability studies were conducted according to ICH guidelines (16). Tablets of the optimized formula F8 were kept in polyethylene bottles at $25\pm 2^{\circ}\text{C}$ and $60\pm 5\%$ RH in a desiccator. The tablets were examined at different time intervals (0, 1, 2, 4, and 6 months) for any statistical difference in hardness values or friability using paired Student's *t* test. Differences were considered to be significant at $p < 0.05$. Tablet shelf-life was calculated as the time at which 95% one-sided confidence limit for the mean curve intersected the acceptance criterion of 90% label claim (drug remaining). It was determined using Sigmaplot™ 10 software (Cranes Software International, Bangalore, India) by plotting percentage label claim (% drug remaining) against time in months.

In Vivo Comparison of Floating Behavior with Conventional Tablet Using Scintigraphy

Gamma scintigraphy was used to study and compare the *in vivo* gastro-retentive, controlled-release behavior of optimized formulation vis-à-vis conventionally made non-floating tablet. $^{99\text{m}}\text{Tc}$ -Technetium pertechnetate (Tc-99m) was used as the radioactive tracer for the study.

Radiolabeling of Ca-Na₂EDTA

The surface of particulate was covalently complexed with Tc-99m using stannous-based reduction technology developed from our laboratory (4,22). Radiolabeling efficiency of Ca-Na₂EDTA with Tc-99m was characterized on the basis of serum and saline stability up to 24 h as per previously reported methods using a gamma counter (Capintec, USA) (10,23).

In order to establish that radiolabeled Ca-Na₂EDTA (*i.e.*, $^{99\text{m}}\text{Tc}$ -Ca-Na₂EDTA) could serve as a true marker for ascertaining the release characteristics of the developed formulation, *in vitro* dissolution of radiolabeled tablets was carried out. Both drug release and radioactivity release were measured in dissolution samples withdrawn at a regular interval of 1 h till 6 h using UV spectroscopy and radiometry (Gamma counter; Capintec, USA), respectively. The percentage drug release and percentage radioactivity release were plotted as a function of time.

In Vivo Animal Study

The *in vivo* transit behavior of optimized tablet was monitored in New Zealand white rabbits. The animal experiments were approved by the Institutional Animal Ethics Committee duly constituted for the purpose, *vide letter no. INM/IAEC/2009/06/009*, and conformed to general national guidelines on the care and use of laboratory animals. Six healthy New Zealand white rabbits weighing approximately 2–2.5 kg were

used for the study. The animals were kept in a fasting state for 24 h prior to experiment, but water was available *ad libitum*. The animals were given a 100-mg floating Ca-Na₂EDTA tablet compressed using a ten-station rotary tablet punching machine (lab press; CIP Machinery Pvt Ltd, Ahmedabad, India) with 8-mm punches by keeping the ratio of each component/excipient the same as that of the optimized formula F8. The optimized tablet was radiolabeled with 200 μCi of Tc-99m by a method previously reported from our laboratory and as mentioned above. The tablet was placed at the back of the animal's pharynx and was flushed with 5 ml of water to facilitate swallowing and to prevent the tablet from sticking to the animal's throat. Gamma scintigraphy images of animals' abdomen area were acquired at different time intervals (5 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 6 h) using a dual-head gamma camera (Symbia T2; Siemens, Erlangen, Germany) to observe movement of the tablet within the antrum or pyloric part of the stomach.

In Vivo Human Study

A total of six healthy volunteers were recruited for scintigraphy-based gastric emptying studies (age 22–45 years). Demographic data with respect to each human volunteer were recorded in the case report form at the time of enrollment. History of any disease, blood pressure, and heart rate were noted. It was ensured that the volunteers were not on any concomitant medicines for at least 1 week prior to the start of the study. Routine laboratory investigations for hematology, biochemistry, and ECG were done to make sure the volunteer was healthy. Informed consent form was obtained from the volunteers in vernacular language or English. The mean weight and height of the volunteers was 62 kg and 165 cm, respectively. The clinical protocol was approved by the Institutional Human Ethics Committee (Reg. No. INM/TS/IEC/011-07). Vital parameters were recorded before and 30 min post-administration of the tablet and again at the time of discharging the subject from the study center. Adverse events were monitored throughout the study. The studies were conducted on a dual-head gamma camera system (Symbia T2; Siemens, Erlangen, Germany). The volunteers were given the optimized effervescent, gastro-retentive Ca-Na₂EDTA tablet radiolabeled with 200 μCi of Tc-99m along with 240 ml of water.

Subjects were instructed not to lie down for the first 2 h after dosing except during gamma imaging. Dynamic scintigraphy imaging was performed for first 30 min followed by sequential static imaging at 1-h intervals till 6 h.

For comparison, the same volunteers were administered conventional non-floating tablet of Ca-Na₂EDTA on another day, and the scintigraphy procedure was repeated as described above.

Region of interest was drawn around the tablet area and whole stomach for obtaining count statistics. Comparison between gastric emptying rate of optimized Ca-Na₂EDTA tablet and conventionally made tablet was made on the basis of count rate in the initial and delayed scintigraphy images using the inbuilt software (Syngo; Siemens, Erlangen, Germany). Visual comparison was also done to ascertain the floating behavior of optimized tablet in stomach region vis-à-vis conventional tablet.

Table II. *In Vitro* Characterization of Different Batches of Formulation ($n=6$)

Exp no.	Tablet code	Drug content (%)	Weight (mg)	% friability (20 tab)	Hardness (kg/cm ²)	Thickness (mm)	Lag time (s)	Total floating time (h)
1	F1	97.01±1.80	648±2.5	0.53±0.08	4.50±0.24	6.28±0.22	35±2	≤8
2	F2	99.51±2.40	651±2.8	0.42±0.10	3.80±0.16	6.18±0.13	44±4	≤8
3	F3	98.01±2.54	652±3.5	0.48±0.05	4.15±0.22	6.16±0.18	56±5	≤18
4	F4	98.21±2.02	654±2.8	0.54±0.05	5.21±0.26	6.22±0.33	72±4	≤18
5	F5	99.05±1.98	654±4.2	0.64±0.09	5.02±0.25	6.11±0.38	19±2	≤24
6	F6	99.42±2.65	652±3.8	0.60±0.07	5.54±0.22	6.30±0.48	11±4	>24
7	F7	98.41±2.28	655±3.6	0.55±0.04	4.08±0.20	6.14±0.32	19±3	>24
8	F8	99.25±1.53	650±3.0	0.41±0.05	3.90±0.32	6.23±0.36	19±4	>24
9	F9	99.05±2.04	651±2.8	0.52±0.07	3.52±0.18	6.24±0.32	25±4	>24
10	F10	98.46±2.32	652±2.9	0.32±0.03	4.36±0.21	6.25±0.18	10±4	>24
11	F11	99.82±2.12	653±4.5	0.51±0.02	4.22±0.21	6.26±0.24	18±4	>24
12	F12	98.45±2.34	648±4.4	0.46±0.08	3.98±0.19	6.29±0.25	19±5	>24
13	F13	98.75±2.72	652±4.8	0.53±0.05	4.11±0.21	6.20±0.11	24±3	>24

Statistical Analysis

Data are expressed as mean±SD. Unpaired *t* test was applied for the calculation of significance at $p < 0.05$ using GraphPad Instat version 3.00 for Windows XP (GraphPad Software, San Diego, CA, USA).

RESULTS

Gastro-retentive, controlled-release tablets of Ca-Na₂EDTA were formulated through effervescent technique in 13 different batches (F1 to F13) using hydrophilic polymer (HPMC-K4M), diluent (sodium chloride), and hydrophobic polymer (carbopol-934) along with effervescing agents sodium bicarbonate and citric acid. Magnesium stearate was used as a lubricant, while talc was used as glidant. Although carbopol was found to have a negative effect on the floating behavior of tablets, it was used only for the drug release retardant characteristics. All the formulations were prepared by direct compression method. The prepared tablets (F1–F13) were evaluated for physical characteristics like tablet hardness, friability, weight variation, buoyancy lag time, total floating time, assay, and *in vitro* drug release.

For comparison, conventional, non-floating tablet of Ca-Na₂EDTA was prepared with suitable modifications in the choice of excipients used. In this case, lactose and starch were used in place of carbopol, sodium bicarbonate, citric acid, and

sodium chloride to make the conventional tablet of similar weight. Final composition of the conventional tablet was as follows: Ca-Na₂EDTA 200 mg, avicel 55 mg, lactose 200 mg, starch 100 mg, and HPMC 95 mg.

In Process Quality Control

The results of physicochemical characterization with respect to different batches are shown in Table II. The thickness of floating tablets was measured by Vernier calipers and was ranged between 6.11±0.38 and 6.30±0.48 mm. The hardness of the tablets was measured by a Monsanto-type tablet hardness tester (IEC, Mumbai, India) and was controlled between 3.52±0.18 and 5.54±0.22 kg/cm². The friability was below 1% for all the formulations. Weight variation in different formulations was found to be 648±4.4 to 655±3.6 mg. The percentage of drug content for different batches was between 97.01±1.8 and 99.82±2.1% of Ca-Na₂EDTA. The combination of sodium bicarbonate (NaHCO₃) and citric acid provides floating ability and was therefore selected for making the formulation. The tablet swelled radially and axially during *in vitro* buoyancy studies. Prepared formulations were also tested for buoyancy lag time (BLT) and total floating time (TFT). All tablets were mechanically stable and passed the weight variation test as the percent weight variation was within the Indian Pharmacopoeia limits of ±5% of the weight.

Fig. 1. Tablet swelling ability of F6, F8, and F10 formulations

Fig. 2. Photographs taken during *in vitro* buoyancy study of formula F8 in 200 ml 0.1 N HCl at different time intervals

Tablet Swelling Ability

Of all the test batches made using different excipient combinations, tablet swelling study up to 12 h was performed only on batches F6, F8, and F10 on the basis of obtained lag time and drug uniformity. A higher swelling ability was found for tablets of batches F8 and F10 (Fig. 1).

Tablet *In Vitro* Buoyancy Study

In vitro buoyancy experiments revealed the floating ability of all tablets made under different batches, with many of them able to maintain buoyancy up to 24 h (F6–F13) (Table II and Fig. 2). The prepared formulations contained NaHCO_3 as a gas-forming agent dispersed in a hydrogel matrix of HPMC-K4M and carbopol-934. The polymers formed a gel layer enabling efficient entrapment of NaHCO_3 for gas bubble generation, resulting in floating of the tablets on test medium (0.1 N HCl) for prolonged periods of time. These matrices

were fabricated in such a way that on arrival in the stomach region, carbon dioxide gas was liberated due to acidity of the gastric fluid and entrapped in the jellified hydrocolloid.

All the batches of tablets were found to exhibit short floating lag times due to the presence of carbopol, sodium bicarbonate, and citric acid. In the presence of citric acid, the tablets were found to float for longer duration (up to 24 h). Since pH of stomach gets elevated under fed conditions (~3.8), citric acid was therefore incorporated in the formulation to provide an acidic medium for sodium bicarbonate. Moreover, citric acid also acts as a stabilizer.

Drug Release Study

The data obtained from *in vitro* dissolution studies are shown in Table III and Fig. 3. Release of $\text{Ca-Na}_2\text{EDTA}$ from the effervescent controlled-release tablets was studied in 0.1 N HCl. Comparison study with non-floating control $\text{Ca-Na}_2\text{EDTA}$ tablet as well as with other test combinations

Table III. Dissolution Profile of Different Batches of Effervescent and Control (Conventional) $\text{Ca-Na}_2\text{EDTA}$ Tablets ($n=6$)

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	Control
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	6.23	5.78	6.28	7.21	5.46	5.32	4.98	4.28	5.28	5.86	4.42	5.88	5.64	10.28
0.50	9.24	9.38	7.48	9.28	8.28	8.42	8.04	7.24	11.28	10.24	8.82	11.48	12.62	20.58
1.00	18.34	16.79	15.34	16.28	18.28	16.48	19.26	15.38	18.24	20.32	18.20	19.42	28.26	42.25
2.00	25.24	24.20	20.12	32.28	38.42	32.54	29.42	24.28	28.28	26.42	32.34	38.45	35.28	65.28
4.00	58.65	46.46	47.51	48.65	49.63	49.57	48.81	44.41	46.31	49.56	50.31	49.74	51.60	99.28
6.00	71.23	56.59	58.32	68.24	60.23	62.29	58.32	52.12	54.86	62.12	65.29	68.23	66.26	99.38
8.00	81.21	69.97	70.65	87.25	72.21	74.51	70.65	64.81	69.28	72.89	73.36	82.42	78.36	99.89
10.00	90.18	91.16	92.48	98.34	80.28	83.38	82.21	76.24	79.22	86.32	89.42	86.42	84.54	99.90
12.00	94.23	97.43	99.23	99.93	91.37	90.60	92.05	83.25	87.11	90.22	91.26	91.48	93.40	99.91
20.00	99.23	99.81	99.80	99.87	99.82	99.24	99.21	99.61	99.81	99.34	99.42	99.34	99.35	99.92
24.00	99.43	99.88	99.90	99.87	99.98	99.82	99.65	99.81	99.85	99.82	99.68	99.56	99.89	99.92

Fig. 3. Plot of comparative dissolution profile of different batches of effervescent Ca-Na₂EDTA tablet and non-floating, control Ca-Na₂EDTA tablet

showed that the optimized formulation (F8) had better control over release rate in comparison to the conventional product. The conventional tablet released 99.28% of the drug in 4 h, whereas in the case of F8 the drug released in 4 h was only 44.41%. Besides, the optimized formulation F8 remained floatable in the stomach for longer duration and gave controlled and prolonged release up to 24 h (Fig. 4). Therefore, on the basis of release pattern obtained for various formulations, F8 was chosen as the final optimized formulation.

Analysis of Release Mechanism

Optimized formulation F8 was subjected to various simple exponential equation models to elucidate the mode of drug release. These included zero-order equation, first-order equation, Higuchi equation, and Korsmeyer–Peppas simple exponential equation models. The slope and regression coefficient (R^2) are shown in Table IV and Fig. 5. The optimized formulation F8 fitted best for Korsmeyer–Peppas equation with an R^2 value of 0.9930.

Physical Stability Studies

Stability studies for the optimized Ca-Na₂EDTA tablet carried out in accordance with ICH guidelines and analyzed at time intervals of 0, 1, 2, 4, and 6 months showed no significant difference in tablet hardness, drug

content, total floating duration, or matrix integrity of the optimized formulation (F8). Ca-Na₂EDTA degradation of only 1.07% in the optimized tablet was predicted at the end of 6 months. Tablet shelf-life analysis done using Sigmaplot™ 10 software (Cranes Software International, Bangalore, India) showed shelf-life of the formulation to be 56.3 months (Fig. 6).

In Vivo Comparison of Floating Behavior with Conventional Tablet Using Scintigraphy

Radiolabeling and Stability of Radiolabeled Ca-Na₂EDTA Particles

Ca-Na₂EDTA was covalently complexed with Tc-99m using stannous-based reduction technology developed from our laboratory (22). The radiolabeling efficiency for Ca-Na₂EDTA particles at the time of complexation was consistently >93%. Serum stability studies showed 95 and 94% radiolabeling efficiency of Ca-Na₂EDTA after 6 and 24 h, respectively, as per previously reported method from our laboratory (4), indicating stability of the labeled product.

Figure 7 shows the drug and radioactivity release profile corresponding to Ca-Na₂EDTA and ^{99m}Tc-Ca-Na₂EDTA, respectively. A similar release pattern for Ca-

Fig. 4. Comparative drug release profile of optimized formulation (F8) and conventional (control) formulation

Table IV. Kinetic Release Data of Different Models for Optimized Formulation (F8)

Model	Slope	R ² value
Zero order	4.436	0.8747
First order	-0.112	0.934
Higuchi matrix	23.218	0.9799
Korsmeyer and Peppas	0.662	0.9930

Na₂EDTA and ^{99m}Tc-Ca-Na₂EDTA was seen till 4 h with a slightly higher slope in the case of radiolabeled drug. Complete release was obtained at 6 h in both cases. The result suggested that ^{99m}Tc-Ca-Na₂EDTA and gamma scintigraphy could serve as efficient tools for ascertaining the release characteristics of the developed formulation *in vivo*.

In Vivo Animal Study

Intragastric behavior of optimized tablet (F8) in rabbits was observed in real time using gamma scintigraphy. Serial images were taken at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 6 h after oral administration of Tc-99m-labeled optimized effervescent, controlled-release tablet (F8)/conventionally made non-floating tablet. Changes in tablet impression were observed with time in rabbit stomach as radioactivity hot spot. In the case of optimized floating tablet, it did not adhere to gastric mucosa and was seen to float on the gastric fluid (Fig. 8). It was seen that the mean gastric residence time in rabbit was more than 4 h for the optimized floating Ca-Na₂EDTA tablet. At 6 h, the shape of the tablet was disturbed and a broad mass appeared in intestinal position.

In Vivo Human Study

Figure 9 shows scintigraphic comparison of intragastric behavior of optimized gastro-retentive, controlled-release tablet (F8) and control Ca-Na₂EDTA tablet at different time intervals in the same volunteer. Both F8 as well as control

tablets were seen to maintain their integrity in stomach. Not in a single case were they seen to break down into pieces upon swallowing. However, on entering the stomach, the control tablet settled down in the lower half of the stomach, in proximity of the pylorus, and with time radioactivity was seen to get released into the medium. The tablet was able to maintain its integrity up to 1 h. The released radioactivity slowly trickled into the upper small intestine through the duodenum. After 1 h, the tablet lost its identity and disintegrated completely.

On the other hand, the optimized gastro-retentive, controlled-release tablet (F8) was seen to initially settle down in the lower half of the stomach, after which it started moving in an upward direction (within 20–30 s) in the stomach due to initiation of floating behavior. The tablet integrity was maintained up to 4 h, which gave a clear indication of its floating nature in the stomach. Further, the optimized tablet appeared more or less at the same position in stomach for the first 4 h, after which small amounts of radioactivity were seen to get released from its matrix. Mean gastric retention period of optimized tablet was found to be approximately 6 h.

DISCUSSION

Ca-Na₂EDTA is a commonly used chelating agent against heavy metal poisoning including radioactivity decorporation (3,4). However, a suitable chelator-based oral formulation is required, which can bind to ingested heavy metals/radionuclides in the gastric milieu itself and prevent their absorption into the bloodstream. The present work was therefore undertaken to address this problem and present a viable option of oral dosage form having prophylactic ability as well. Another aim was to optimize a floating formulation of Ca-Na₂EDTA for controlled release so as to ensure its availability for optimum time in gastric environment for chelating action so that the frequency of dosage can be kept to a minimum to improve patient compliance. Since any incident involving incorporation of radionuclide(s) may impart continuous radiation dose to the body's target organs depending upon their half-lives, a gastro-retentive, controlled-release oral formulation of Ca-Na₂EDTA is desirable so as to

Fig. 5. Comparative plot of optimized formulation (F8)—**a** zero order, **b** first order, **c** Higuchi model, and **d** Korsmeyer and Peppas model

Fig. 6. Plot of percentage label claim (% drug remaining) against time for determining shelf-life of optimized formulation (F8)

prevent the systemic absorption of the radionuclide(s) and their subsequent accumulation in respective target organs.

Ca-Na₂EDTA is known to break down at acidic pH of the stomach, thereby showing its local chelator action (24). In the present work, effervescent floating tablets of Ca-Na₂EDTA using 13 different formulation combinations were prepared and the combination showing the best results (formula F8) with respect to floating lag time, total floating duration, swelling ability, and sustained drug release was chosen and compared with conventionally made non-floating tablet of Ca-Na₂EDTA for drug-release data. Effervescent oral drug delivery systems are known to be beneficial in the sense that they also reduce several of the local GIT side effects associated with administration of oral formulations, such as gastric irritation, nausea, and gastritis (25). Combination of sodium bicarbonate and citric acid was chosen as effervescent agent to provide the desired floating ability to the formulation. It was observed that the gas generated was trapped and protected within the gel formed by the hydration of polymer (Avicel PH-102), thus decreasing the density of tablet below one, making it buoyant. The tablet swelled

radially and axially during *in vitro* buoyancy studies. The observed increase in swelling property of the tablet with time can be attributed to the fact that hydrophilic polymer gradually absorbs water due to which the outermost hydrophilic polymer hydrates and swells and forms a gel barrier at the outer surface. As the gelatinous layer progressively dissolves and/or is dispersed, the hydration swelling release process is repeated towards new exposed surfaces, thus maintaining integrity of the dosage form (26,27).

It is worth mentioning here that though *in vitro* results had shown floating behavior of the optimized formulation for 24 h, *in vivo* results were more conservative. This could be due to peristalsis movement in the stomach wherein the tablet gets cleared from the stomach milieu with time. Further, the rate at which a tablet leaves the stomach is partly due to gastric emptying rate, which is influenced by the fasting/fed state of the stomach. However, *in vitro* conditions are different and more uniform. The results are consistent with previously reported findings involving controlled-release, gastro-retentive oral dosage forms (28–30). It may also be noted that although

Fig. 7. *In vitro* drug and radioactivity release profile corresponding to Ca-Na₂EDTA and ^{99m}Tc-Ca-Na₂EDTA, respectively ($n=5$)

Fig. 8. Gamma scintigraphy showing intragastric behavior of Ca-Na₂EDTA effervescent tablet (F8) at different time intervals in experimental rabbit (position of the tablet is pointed by an arrow)

F8 tablets were prepared using 12-mm punches, *in vivo* animal experiments were done with tablets compressed with 8-mm punches. This was done because it would have been extremely difficult, if not impossible, to administer a 650-mg tablet to rabbits. To circumvent this problem, a 100-mg tablet of the optimized formula (F8) was prepared for *in vivo* animal study by keeping the ratio of each component/excipient the same.

Physical stability studies for the optimized formula F8 have indicated that the developed formulation is quite stable as per ICH guidelines. The results have predicted a Ca-Na₂EDTA degradation of only 1.07% at the end of 6 months, with shelf-life of the formulation being 56.3 months. The results suggest that the developed Ca-Na₂EDTA formulation has the potential to be developed into a

Fig. 9. Gamma scintigraphy showing comparative intragastric behavior of Ca-Na₂EDTA effervescent floating tablet (F8) and control non-floating tablet represented at different time intervals (position of the tablet is pointed by an arrow)

prophylactic and/or therapeutic option for members of the rescue teams as well as victims in a radioactive fallout zone, and also for people working in mining and other such industries with high probability of heavy metal ion exposure.

CONCLUSION

A gastro-retentive, controlled-release tablet of $\text{Ca-Na}_2\text{EDTA}$ has been developed as a potential oral antidote for neutralization of ingested heavy metal toxicity. The tablet was prepared using effervescent technique. All in process quality control parameters confirmed to Indian Pharmacopoeia (IP-2010) guidelines. Among the various batches prepared using different excipient combinations, formula F8 showed satisfactory results with respect to floating lag time, total floating duration, swelling ability, and sustained drug release rate.

Conflict of Interest The authors declare that there are no personal or financial conflicts of interest with individuals or organizations.

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