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Natural polymers composed mucoadhesive interpenetrating buoyant hydrogel beads of capecitabine: Development, characterization and *in vivo* scintigraphy

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ABSTRACT

The present research deals with the optimization and preparation of aluminium ion cross-linked buoyant interpenetrating polymeric network (IPN) microbeads (MBs) of locust bean gum (LBG) and sodium alginate (NaAlg) carrying Capecitabine (CAP). The formulation was prepared by ionotropic gelation method. The optimization was done by Response Surface Methodology based Box Behnken Design (BBD) involving a significant changes or effect on responses, particle size, drug entrapment and buoyancy on varying the concentration of polymeric blend (LBG:NaAlg), cross-linker (Aluminum chloride; $AlCl_3$) and pore former (sodium bicarbonate; $NaHCO_3$). Finally, the best optimized batch of buoyant IPN MBs of CAP obtained through BBD was examined through different evaluations that exhibited particle size of 402.12 ± 1.9 μm, drug entrapment 82.45 ± 2.5% and % buoyancy 85.03 ± 1.1% with total floating time of greater than 10 h. The formed IPN MBs showed good buoyancy in gastric pH and better mucoadhesion in intestinal pH. The results of pharmacokinetic study displayed improved systemic circulation, bioavailability and extended plasma half-life of encapsulated CAP within polymeric shell than free CAP. To ensure the gastroretention of formed buoyant IPN MBs, the gamma scintigraphy study performed revealed the gastroretention of MBs for more than 6 h suggesting the designed and developed optimized batch is effective for gastroretention with improved bioavailability.

1. Introduction

Oral drug delivery is an acceptable and ideal route of administration of the therapeutics as improves patient compliance and comfort [1]. However, in cancer, this route has been replaced by chemotherapy that involves administration of mixture of anticancer agents through intravenous route. Chemotherapy is a complex process involving excess exposure of drugs to the patients in different rounds of rotations thus causes severe pain, trauma and several major side effects [2]. 5-Fluorouracil (5-FU) is an extensively used drug against colon cancer, administered intravenously in both bolus and continuous infusion form [3,4] however, its highly inflexible schedule and severe adverse effect provided the pharmaceutical engineers a strong rationale to develop oral fluoropyrimidines [5]. Capecitabine (CAP), a prodrug of 5-FU is extensively utilized not only in the first line treatment of colorectal cancer but also in advanced and metastasis breast cancer in combination with docetaxel [6]. The drug is commercially available as an immediate release tablet Xeloda® with high dose (1250 mg/m^2 b.i.d.) and short elimination half-life (1–1.5 h). Once it is taken it converts into its active agent 5-FU by following trienzymatic series pathway [7]. The conversion of CAP to 5-FU occurs rapidly and approximately within 6 h, 5- FU eliminates completely thus the prescribed twice daily dosing creates a dosing exposure gap of 6 h in between two subsequent doses. Therefore, for getting continuous treatment effect the drug needs to be given four times a day, which is a patient non-compliant dosing schedule. Present study is an effort towards a preparation of cross-linked gastroretentive buoyant interpenetrating polymeric network (IPN) system in order to extend the systemic circulation and biological half-life of CAP. IPNs are the multiparticulate system and are considered as an 'alloys' of cross-linked polymeric network where at least one polymer is either cross-linked or synthesized in the presence of another polymer [8]. IPNs have always been considered as a potential delivery vehicle in controlling the release of therapeutics. For instance, cefadroxil (CF), an antibiotic drug has a short elimination half-life of approximately 2 h at dose of 0.5 and 1.5 g. *Rao* et al. prepared a pH sensitive CF loaded IPN MBs of chitosan and acrylamide grafted polyvinyl alcohol cross-linked with glutaraldehyde. The prepared formulation showed entrapment efficiency of CF 95% with 95% release in

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alkaline pH for 10 h and lower release at acidic pH suggesting it to be suitable for colon delivery [9]. Another work done by Mohamed et al. reported the extended release of doxorubicin from IPN hydrogel prepared by blending the polymers gelatin and divinyl ester. The preparation showed initiall burst release for 36 h followed by controlled release of drug approximately 86% for 6–10 days *in vitro* [10]. IPN is a multiparticulate drug delivery system. Other drug delivery systems such as pellets, microparticles, macrobeads etc. are also there however IPNs are different from these delivery system with respect to its denser cross-linked network and extent of swelling mechanism that alters with change in pH that's why called as intelligent drug delivery system also [11]. Pellets are free flowing spherical particulates prepared by agglomerating fine powders or granules with numerous excipients [12]. This dosage form preparation methods for instance extrusion spheronization or layering techniques involves multiple steps with numerous excipients, however, the same is not true with IPNs. Their method of preparation, requires least excipients and practical applicability makes them superior from other multparticulate dosage form. Another multiparticulate dosage form is microparticle. Microparticles (generally composed of single polymers) offers several advantages such as limited fluctuation in *in vivo* profile, controlled release and less side effects. However, due to their low mechanical stability it occupies entire volume of water and behave like typical liquid molecule thus rapid disintegration of polymers occurs. IPNs can be differentiated from simple mixture of polymers in terms of solubility i.e. they do not dissolve but swells, as cross-linkers maintains the rigidity of the polymeric chain network that reinforces the swelling rather than solubility. Secondly, having an advantage of two polymers they modify the drug release and with respect to cross-linking exhibits rigid and dense network that helps the drug to entrap inside the polymeric matrix firmly thus, excellent entrapment of drugs [13]. For a system to be a buoyant and to release the drug for extended time the attributes such as swelling, floating and mucoadhesion all three are required. With the passage of time as the stomach empties the floating capacity of the dosage form reduces as well as formulation also passes towards other parts of GI tract. At that time the property of mucoadhesion can provide the formulation to remain in intimate contact with mucosal wall of the GI tract, thereby enhancing the bioavailability and prolong the release of the drug from the delivery vehicle. Sodium alginate (NaAlg) is suitable polymer and used frequently in the preparation of IPN due to its most important property of chelation with di-valent and tri-valent cations. Sodium alginate is composed of α-L guluronic (G) and β-D mannuronic (M) acid and in between these two-unit, unit G is capable of gel formation. The divalent or trivalent cations attaches with the G unit and forms a three dimensional immobile network [14]. Besides these advantages, one drawback associated with NaAlg is its rapid solubility that leads to the low drug entrapment, burst release and sometimes low buoyancy also appears [15,16]. To minimize this, another polymer locust bean gum (LBG) is utilized in the present work. LBG is a natural non-ionic polymer composed of β-1,4-D mannose and α-1,6-D- galactose. The mannose to galactose ratio of LBG is 4:1 whereas for NaAlg it is 0.53:1. The high value of mannose implies for more swellability and viscosity of the polymer which is necessary for a floating system [7,17]. In previously performed experiment IPNs composed of natural polymers, LBG and NaAlg loaded with CAP were found to be sufficient in extending the release [18]. However, to further sustain the release of such high dose drug, the concept of dual mechanism i.e. combination of floating and mucoadhesion would be an ideal approach for further prolonging its release. Presently, we have prepared a novel gastroretentive trivalent cation cross-linked IPN of CAP by using response surface methodology (RSM) based three factors three level box behnken design (BBD). The factors polymer blend ratio by weight (A), amount of cross-linker by weight (B) and amount of sodium bicarbonate (C) by weight were selected as independent variables and corresponding to this, particle size (μm); drug entrapment (%); and buoyancy (%) were used as dependent variables. Design- Expert® Version 11 (Stat-Ease Inc. USA) was employed for the optimization and evaluation of the statistical analysis. And finally, optimized batch was evaluated for *in vitro* and *in vivo* evaluations.

2. Materials and methods

2.1. Materials

Capecitabine was procured from Cipla Ltd. Bangalore India; sodium bi-carbonate, sodium alginate (yellowish brown powder; 240 kDa) were purchased from Central Drug House, India; locust bean gum (white to yellowish cream colour; 300 k Da) and calcium chloride were procured from Sigma Aldrich; all other chemicals and buffer salts utilized throughout the study were of analytical grades.

2.2. Methods

2.2.1. HPLC analytical method development and validation

The bioanalytical method validation of CAP was carried out on HPLC (Shimadzu, corporation, Kyoto, Japan) comprising UV–Visible detector (SPD-20 A), pump (LC-20AD), rheodyne annual injector (SIL-20A). Estimation of the drug was done by injecting the samples in a C-18 reverse phase column (250 mm × 4.6 mm, 5μ Enable). The analysis was performed under an isocratic condition with mobile phase methanol and phosphate buffer (pH 6.8) (70:30) at a flow rate of 1 mL/min at wavelength 232 nm. The % recovery of the drug was determined by spiking equal amount of drug into the blank plasma sample and was calculated by comparing the peak areas of CAP The standard curve of CAP in plasma was constructed with six different concentrations in the range of 10–500 μg/mL and validated for precision, linearity, accuracy, limit of detection (LOD) and limit of quantification (LOQ).

2.2.2. Preparation of gastroretentive (buoyant) sodium alginate and locust bean gum IPN microbeads (IPN MBs) carrying capecitabine (CAP)

The buoyant IPN MBs of LBG and NaAlg containing CAP were formulated by ionotropic gelation method [19]. The polymeric blend of LBG and NaAlg (0.50 g each i.e. LBG: NaAlg(1:1) was prepared by dispersing both the polymers in 50 mL of distilled water followed by stirring at 100 rpm. Once the polymer blend was prepared, CAP (50 mg) and sodium bicarbonate ($NaHCO_3$; 100 mg), a gas forming agent were added in the above formed aqueous polymeric mixture while the stirring was continued. The formed homogenous solution then poured drop-wise through 23G into another aqueous solution of trivalent aluminium chloride ($Al^{3+}/AlCl_3$) containing hydrochloric acid solution (HCl) (1%v/v). The formed suspended IPN MBs were left in the solution for approximately 20 min to enhance the mechanical strength of the MBs. Incorporation of HCl in the solution of cross-linker was done for the liberation of carbon-di-oxide (CO_2) occurs which can be released only in the presence of acidic media (HCl) in order to make the formulation float. The scheme of release of CO_2 is shown in Fig. 1. The prepared buoyant IPN MBs were finally collected, rinsed with double distilled water and finally dried at room temperature.

2.2.3. Experimental design and statistical analysis

Design of experiment and its statistical validation was done by Design Expert® software Version 11. Box-Behnken Design (BBD) was used for the optimization purpose [21]. The effect of three independent factors polymer ratio (A), amount of cross-linker (B) and amount of $NaHCO_3$ (C) on size of particle (Y_1), drug entrapment (Y_2) and buoyancy (Y_3) were studied. The polynomial equation generated for the BBD is shown below:

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + B_{33}C^2$$

Where Y is the response; b_0 is the intercept; b_1, b_2, b_3, b_{12}, b_{13}, b_{23},

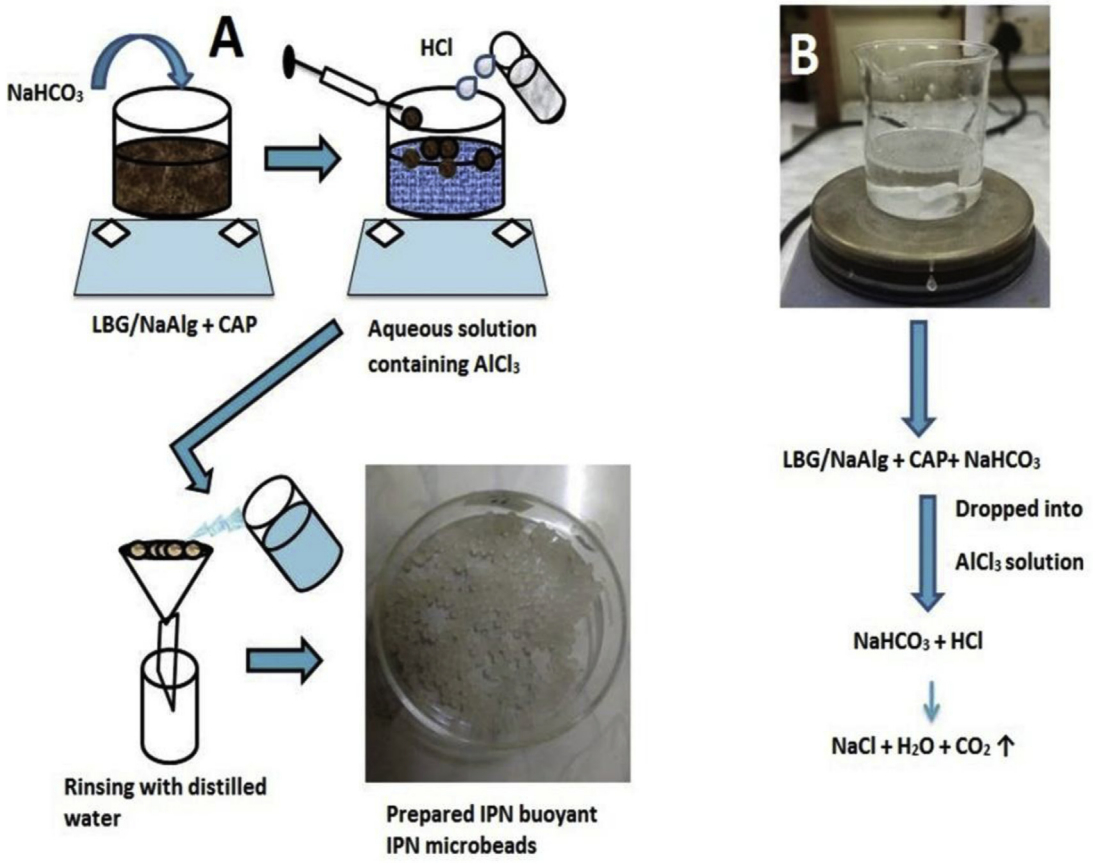

Fig. 1. Process development of buoyant IPN MBs (A) steps involved in the preparation; (B) chemical reaction involved during pore former (modified figure [20]).

Table 1
Experimental plan for the preparation of buoyant cross-linked IPN MBs.

Formulation Batch	Polymer ratio (by weight)	Cross-linker amount (by weight)	$NaHCO_3$ Amount (mg)	Particle size (μm)	Drug entrapment (%)	Buoyancy (%)
1	1:1	2	10	434.39	72.65	60.71
2	1:3	3	10	350.09	61.55	65.28
3	1:1	3	55	428.76	76.38	72.18
4	1:1	4	100	342.78	80.11	79.51
5	1:3	4	55	536.12	64.85	82.31
6	3:1	2	55	421.56	88.06	54.53
7	1:3	2	55	357.62	57.85	80.69
8	1:1	3	55	426.87	74.89	73.98
9	3:1	3	100	527.59	91.23	50.38
10	1:1	2	100	432.29	74.25	76.58
11	3:1	4	55	520.05	94.69	47.64
12	3:1	3	55	425.31	72.19	74.62
13	1:3	3	100	348.51	60.02	85.66
14	3:1	3	10	530.45	90.75	31.48
15	1:1	4	10	420.48	79.59	59.05

b_{11}, b_{22}, b_{33} are the regression coefficient; A, B and C are the independent variables. The terms AB, AC and BC and the terms A^2, B^2 and C^2 represents interaction and quadratic terms respectively. The dependent and independent variables along with their levels (low, medium and high) are exhibited in Table 1 and a total of 15 experimental runs generated using three factors at three levels of each response are shown in Table 2. Analysis of Variance (ANOVA) was used for the statistical analysis. All the responses were fitted into linear, quadratic and two factor interaction model and evaluated by statistical significance of the coefficient and R^2 values. Based on the selected model, polynomial equations were generated for each response and based on the dependent variable constraints an optimized batch was selected [22].

2.2.4. Determination of particle size

Estimation of particle size of buoyant IPN MBs were done by the earlier method reported [23]. The size was estimated by optical microscope (Dewinter, optical Inc, India), involving calibration of eye piece micrometre with stage micrometre. Approximately 150 prepared MBs from the optimized were selected randomly and their average particle size was calculated using Dewinter biowizard software.

2.2.5. Scanning electron microscopy (SEM)

Surface morphology of dried buoyant IPN MBs was determined by

Table 2
Optimal parameters with their level, constraints, predicted value, experimental value and summary of ANOVA.

Levels				
Factors	Low (−1)	Medium (0)	High (+1)	BBD results
A = Polymer ratio (by weight)	1:3	1:1	3:1	LBG:NaALG 1:1
B= Cross-linker (%w/v)	1	3	4	3
C= $NaHCO_3$ (mg)	10	55	100	100

Dependent Variables	Constraint	Predicted value	[a]Experimental value
Y_1= Particle size (μm)	Minimum	426.98	402.12 ± 1.9
Y_2 = Drug entrapment (%)	Maximum	79.88	82.45 ± 2.5
Y_3= Buoyancy (%)	Maximum	77.54	85.03 ± 1.1; TFT 12 h

Summary of the ANOVA

Model	R^2	Adjusted R^2	Predicted R^2	F-value	*p*-value	Remarks
Response: Particle size (Y_1)						
Quadratic	0.9998	0.9994	0.9980	2695.98	<0.0001	Significant
Response: Drug entrapment (Y_2)						
Linear	0.9887	0.9856	0.9841	320.06	<0.0001	Significant
Response: % Buoyancy (Y_2)						
Quadratic	0.9945	0.9847	0.9358	101.06	<0.0001	Significant

SEM (Supra 40, Zeiss, Japan). The samples for the examination were mounted on the double adhesive tapes and were accelerated at 10 kV under high vacuum.

2.2.6. Drug-interaction study

To determine the compatibility between pure drug and optimized formulation, FTIR (Shimadzu, 8400, Japan) study was performed. Small quantity of pure CAP and optimized buoyant IPN MBs were mixed individually with potassium bromide (KBr) and converted into pellets employing KBr disk method. The scanning of samples was done between 500 and 4000 cm^{-1}.

2.2.7. X-ray diffraction (XRD) study

Samples for XRD evaluation was done on X-ray diffractometer (Rigaku, Japan). For the analysis, CAP and the optimized buoyant IPN MBs were exposed to CU-K radiation at 45 kV with 25 mA current. The diffraction patterns were recorded at a step size of 0.01° with scanning between 5° and 60° at angle 2θ with 2° min^{-1} scanning speed.

2.2.8. Drug entrapment

Entrapment of CAP in buoyant IPN MBs was determined by the method reported earlier [18]. 100 mg of IPN MBs were poured into 20 mL simulated gastric fluid (SGF pH 1.2) followed by sonication for 10 min. Filtrate was examined on UV–Visible spectrophotometer (Shimadzu 1800, Japan) at 239 nm. Entrapment of CAP was estimated by the following equation:

$$Drug\ entrapment\ (\%) = \frac{actual\ amount\ of\ drug}{theoretical\ amount\ of\ drug} \times 100$$

2.2.9. Swelling study

Swelling of MBs was measured in terms of % weight gain by the method reported previously [24]. Initially, 50 mg of MBs were immersed in 100 mL of SGF (pH 1.2). Swollen beads, removed periodically at time interval of 1 h from media were blotted softly in order to remove the adhered droplet. The increase in % weight gain was recorded on the electronic weighing balance (Axis® India). The study was done for 5 h and calculated by the following equation:

$$Swelling\ index\ (\%) = \frac{weight\ of\ the\ swollen\ IPN\ MBs - weight\ of\ the\ dried\ IPN\ MBs}{weight\ of\ the\ dried\ IPN\ MBs} \times 100$$

2.2.10. In vitro buoyancy study

Floating ability of buoyant IPN MBs were determined by the procedure reported by Singh et al. [25]. Briefly, 350 mg of IPN MBs were placed in 100 mL of SGF. Due to buoyant in nature the beads appeared initially on the surface of the media. Afterwards the media containing IPN MBs was stirred by magnetic stirrer at 100 rpm for about 12 h. After 12 h both the floated as well as settled MBs were collected and dried separately followed by weighing of each proportion to calculate buoyancy by using the equation given below. The time taken by MBs to come at the water surface and the time to remain float on the surface of the media were evaluated in terms of floating lag time (FLT) and total floating time (TFT) respectively were also evaluated.

$$Buoyancy\ (\%) = \frac{weight\ of\ the\ floated\ MBs}{weight\ of\ the\ floated\ MBs - weight\ of\ the\ settled\ MBs} \times 100$$

2.2.11. Ex-vivo mucoadhesion

Mucoadhesive property of optimized formulation was performed by wash off method [26]. The fresh excised piece of intestinal mucosal membrane of goat (2 × 2cm), procured from local slaughterhouse was rinsed with physiological saline to remove off the debris adhered to the membrane. Briefly, 50 buoyant IPN MBs were initially scattered on the membrane that was further adjusted to the USP tablet disintegration apparatus (Electrolab, Mumbai, India) in such a way so that at every up and down movement the mucosal membrane remains in contact with the test fluids. At the end of 5 h and at periodical time interval of 1 h the number of MBs adhered to membrane were counted. The entire study was carried out in gastric pH (0.1 N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 6.8) and calculated by using the formula

$$Mucoadhesion\ (\%) = \frac{microbeads\ taken - nonadherent\ microbeads}{microbeads\ taken} \times 100$$

2.2.12. Drug release study

In vitro drug release of optimized buoyant IPN MBs was performed on USP type II dissolution apparatus (Electrolab, Mumbai, India). An equivalent weight of MBs corresponding to 500 mg of CAP was placed in 900 mL of SGF (pH 1.2) for 8 h followed by pH 6.8 until complete drug release. The dissolution apparatus was maintained at 37 ± 0.5 °C and operated at 50 rpm. The aliquot of 5 mL withdrawn at regular time interval were filtered and analysed on UV spectrophotometer at 239 nm.

2.2.13. In vivo pharmacokinetic study

Male Albino Wistar rats (160–200 g) procured from Institute of Medical Science Banaras Hindu University were used for the *in vivo* experimental study. The study followed the rules proposed by Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA approval no. Dean/2017/CAEC/712) as well as the rules given in ARRIVE guidelines following the U.K. Animals (scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Rats were kept in poly propylene cage provided with healthy diet, water and proper animal house condition. The experimental animals were grouped into two as pure CAP (Group I; n = 6) and drug loaded optimized buoyant IPN MBs (Group II; n = 6). Before commencement of study, all the animals were fasted overnight, though accessed with sufficient water. The solution of pure CAP and suspension of optimized buoyant IPN MBs were given orally (209.4 mg/kg) to the respective groups of animals marked as Group I and Group II respectively [27]. Serial blood samples were collected from retro orbital plexus at different time interval into heparin containing eppendorf tubes. The blood samples were then centrifuged at 4000 rpm at 4 °C for 20 min. The separation of drug from the plasma was done by liquid –liquid extraction (protein precipitation) method. Plasma was deproteinized using methanol and centrifuged at 13000 rpm at 4 °C for 5 min. The supernatant was collected and injected into reverse phase column (C_{18}) of HPLC system (Shimadzu, Corporation, Kyoto, Japan) for estimation of drug. The mobile phase used was methanol: phosphate buffer (70:30) and the flow rate of drug was set in isocratic manner at 1mL/min. The quantification of CAP was made by UV–Visible detector (SPD-20 A) at a wavelength 232 nm attached with the HPLC system. The pharmacokinetic (PK) parameters were calculated by non-compartmental analysis using Kinetica 5.1™ (Lancaster, CA), software.

2.2.14. In vivo gamma (γ) scintigraphy imaging

The study was performed to determine the location and extent of transit time through gastrointestinal tract of the optimized gastroretentive IPN MBs after its administration to the mice through oral route [28]. For the study the Al^{3+} cross-linked buoyant IPN MBs were initially prepared as discussed in subsection 2.2.1. Afterwards, the formed beads were radiolabelled with ^{99m}TC (Technetium) following the procedure by Halder et al. [29]. Briefly, to 2 mci/mL ^{99m}TC solution, 0.2 mL of stannous chloride solution (1 mg/mL) was added and mixed homogenously. Both the aqueous solutions were prepared in nitrogen purged water. The pH of the solution was adjusted to 7.5 with 0.5 M sodium bicarbonate solution. Finally, to the above formed radiolabel containing solution approximately weighed 5 mg of formed trivalent ion cross-linked buoyant IPN MBs were added and incubated for 15 min at room temperature the imaging was done by administering 1 mL of preparation orally to the mice of 18–20 g. The images were captured at a pre-set time of 2 min/view with large field view γ camera (Varicam, Panasonic, Japan) after dose administration at 0.5, 2, 4 and 6 h.

3. Results and discussion

3.1. HPLC analytical method development and validation

The HPLC calibration curve of CAP exhibited linear graph with 0.990 as correlation coefficient value. The precision of 10, 250 and 500 μg/mL was found to be 72.2%, 99.02% and 99.82% respectively. In case of both repeatability and inter-day precision, the % RSD (Relative Standard Deviation) was below 15% thus, reveals the method to be reproducible and satisfactory. The values of LOD and LOQ were found to be 0.01 μg/mL and 0.025 μg/mL respectively.

3.2. Formation of trivalent cross-linked buoyant IPN MBs

The trivalent (Al^{3+}) cross-linked floating IPN MBs loaded with CAP were prepared by organic solvent free, ionotropic gelation method [19]. LBG and NaAlg were selected as a polymer to achieve mucoadhesiveness and swelling whereas $AlCl_3$ was chosen as cross-linker. The trivalent cation tends affinity to bind with the G unit of the alginate chains. Aluminium ion carries one more extra positive charge thus, its possibility to bind with one more alginate molecule and forms a three dimensional “egg-box” structure leads to the formation of firm cross-linked MBs [30]. Finally, to make the system buoyant, pore forming agent $NaHCO_3$ present within cross-linked IPN MBs reacted with the solution of cross-linker $AlCl_3$ containing HCl formed pores in the beads whereas liberation of CO_2 enables the beads to float.

3.3. Optimization and statistical analysis

3- Independent variables, 3- responses and 15 experimental runs were generated by Design Expert ® software version 11. Polymer ratio, amount of cross-linker and amount of $NaHCO_3$ were selected as independent variables whereas particle size, drug entrapment and buoyancy as their corresponding response. The interaction of the factors with responses were further exhibited by means of contour and 3-dimensional plots (Fig. 2) and the statistical analysis of each responses were done by fitting the data in the model (linear, quadratic and two factor interaction) investigated by ANOVA shown in Table 2.

3.3.1. Fitting of the model and analysis of the polynomial equation

3.3.1.1. Effect on particle size. The quadratic model was suggested for the above response. The model F-value also known as Fisher's F-value 2695.98 indicating it to be significant whereas Lack of fit F- value 20.83 suggesting it as non-significant. The value of the predicted R^2 i.e. 0.9980 is in the agreement with the adjusted R^2 0.9994 which means the model is adequate to fit to the experimental data. To measure the signal to noise ratio adequate precision of the model is required that should be greater than 4. For particle size the ratio of signal to noise was found to be 144.369 indicating the model to be adequate and can be used to navigate the design space. The polynomial equation generated for particle size is given below:

$$(Y_1) = 426.98 + 89.40\ A - 6.97\ B - 0.7175\ C - 0.3075\ AB - 0.32\ AC + 0.725\ BC + 12.10A^2 + 0.0562B^2 + 0.07C^2$$

The codes A, B, C, AB, AC, BC A^2, B^2 and C^2 included in the model also helped in the interpretation of relation between independent variable and factors. Among all the coded terms only the polymeric ratio (A) had positive value indicating a synergistic effect on the particle size whereas the value of amount of cross-linker (B) having highest coefficient value of 6.97 had most significant and antagonistic effect on particle size. A graphical presentation of the effects of variables on the responses is exhibited through response plot in Fig. 2 depicting that as the polymer ratio is increasing from 1:1 to 3:1 (LBG:NaAlg), particle size is increasing due to increase in the polymer blend ratio however, on other hand on increasing the amount of cross-linker from 2% w/v to 4%

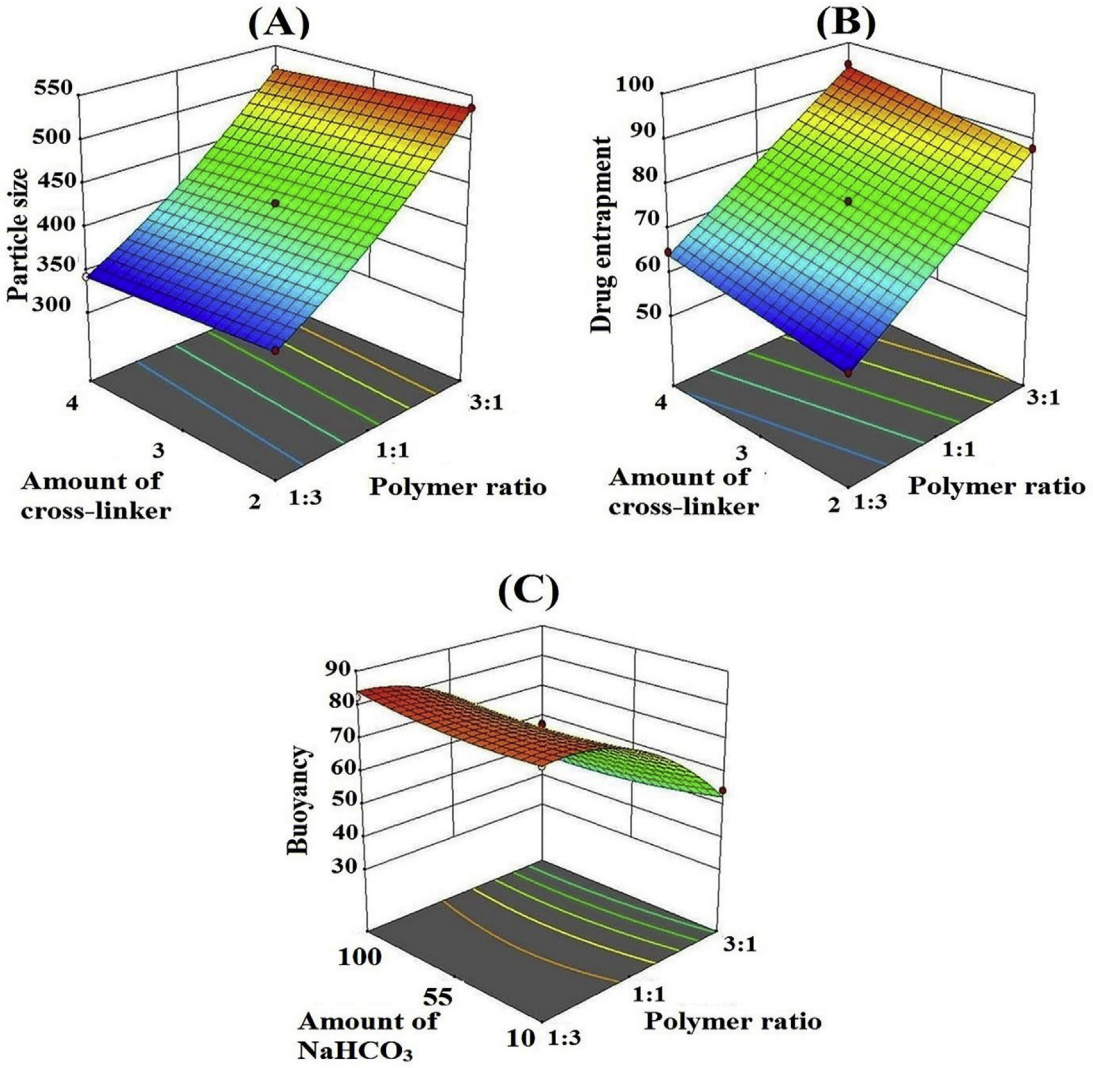

Fig. 2. Three-dimensional contour plots exhibiting relationship and influence of independent variables on (a) particle size; (b) % drug entrapment and (c) % buoyancy.

w/v particle size reduced because cross-linker constrict the polymeric network that lead to the decrease in the particle size.

3.3.1.2. Effect on drug entrapment. Linear model was suggested for the above response. Model F- value of 320.06 implies the model is significant. The Lack of Fit F- value of 0.33 suggesting it as non-significant relative to the pure error. The Predicted R^2 of 0.9841 is in practicable agreement with the Adjusted R^2 of 0.9856. The ratio of 50.536 indicates an adequate signal suggesting the model to be used to navigate the design space. The polynomial equation for drug entrapment is given below:

$$(Y_2) = 75.5933 + 15.0575\ A + 3.30375\ B - 0.13375C$$

The coded model terms A and B have positive value of coefficient representing a favourable synergistic influence on the drug entrapment while negative value of C showing an antagonistic relationship. To understand the influence of polymer blend ratio and amount of cross-linker on drug entrapment an interaction plot is also presented in Fig. 2 that is clearly indicating that the viscous property of polymer blends and good affinity of cross-linker toward polymers retained the drug within IPN network thus, increased % drug entrapment.

3.3.1.3. Effect on buoyancy. Quadratic model was suggested for the above response. The Model F-value of 101.06 implies the model to be fit and significant. The Lack of Fit F-value of 3.11 indicates the model to be insignificant. Further, value of Predicted R^2 0.9258 is in rational agreement with the adjusted R^2 0.9847 whereas signal to noise ratio of 33.035 is indicating an adequate signal proposing the model to be useful in navigation of design space. The polynomial equation for buoyancy is given below:

$$(Y_3) = 73.5933 + 9.6025A - 0.5B + 9.45125C - 2.1275\ AB - 0.37\ AC + 1.1475\ BC - 9.03167\ A^2 + 1.73083\ B^2 - 6.36167$$

The positive values of the independent factors A and C represents polymer ratio and amount of $NaHCO_3$ respectively. The response plot (Fig. 2) obtained is implying that buoyant lag time increased with increase in the amount of $NaHCO_3$ whereas decreased with the increase in amount of polymer blend.

3.4. Determination of particle size and surface morphology

Optical microscopy and SEM studies showed porous and smooth surface spherical IPN MBs (Fig. 3). The appearance of pores was due to the generation of CO_2 as when basic $NaHCO_3$ reacted with acidic HCl an effervescent reaction occurred leaving CO_2 to liberate which released by penetrating the polymeric surface of the IPN MBs.

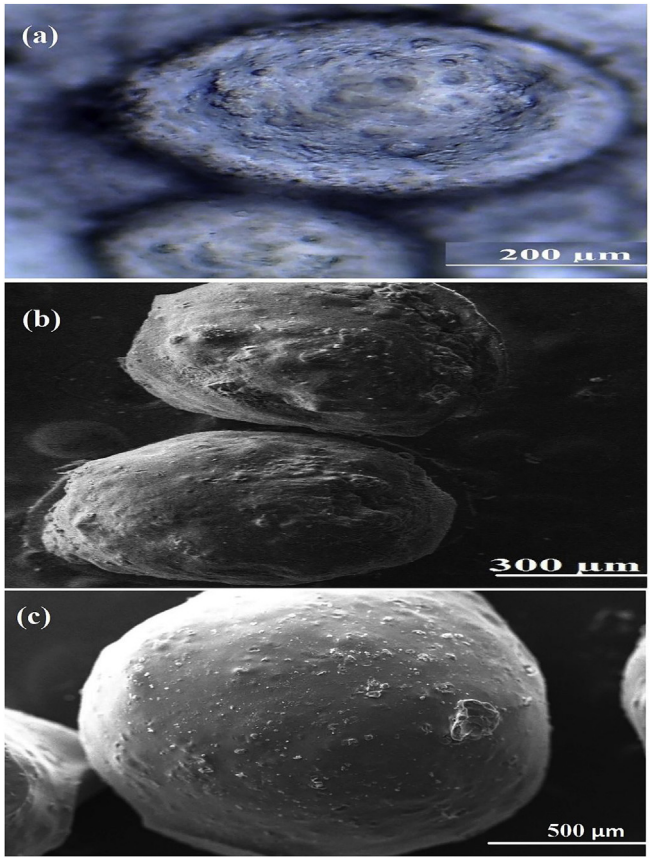

Fig. 3. (a) Represents optical microscopic image (b) scanning electron microscopic image showing spherical shape with smooth surface (c) closer look of the surface of the buoyant IPN MBs flooded with numerous pores showing the evidence of liberation of CO_2 leaving holes on it.

3.5. FTIR

FTIR analysis was done to investigate possible chemical interaction of pure drug (CAP) with other excipients utilized in preparation of optimized formulation (CAP IPN MBs). The model drug CAP displayed absorption bands at 3432 cm^{-1}, 3234 cm^{-1},1684 cm^{-1},1721 cm^{-1} and 1042 cm^{-1}due to –OH, –NH, carbonyl, urethane carbonyl and C–F stretching bands, presence of imide stretching and appearance of tetrahydro furan ring were observed at 1758 cm^{-1} and 1202 cm^{-1} respectively Fig. 4 (a). The optimized batch CAP IPN MBs also exibited all important characteristic peaks of CAP Fig. 4 (b) that confirmed absence of chemical interaction between the pure CAP and other components involved in preparation of optimized formulation. Moreover, with reference to cross-linking in case of optimized formulation there observed drifting of carboxyl group absorption band towards higher wavenumber. This shifting may be due to the interaction between Al^{3+} and guluronic acid of sodim alginate [31]. Sodium alginate is well known for its chelation causing property. Al^{3+} consist three positive charge thus binds more strongly with three units of guluronic acid indicating cross-linking of aluminum ion with polymer.

3.6. DSC

DSC thermal curves of CAP, LBG, NaAlg and CAP IPN MBs are shown in Fig. 5. CAP showed two sharp peaks at 123.76 °C (melting peak) and other peak at 156.80 °C (thermal decomposition) [32]. Further, the biopolymers LBG and NaAlg also exhibited their corresponding melting peaks. Absence of CAP melting peaks at 123.76 °C in optimized formulation, suggesting dispersion of CAP in amorphous form.

3.7. XRD

XRD pattern recorded for CAP, and buoyant CAP IPN MBs are illustrated is Fig. 6. The diffractogram obtained for pure CAP (Fig. 6(a)) exhibited corresponding 2θ peaks at 10.08°, 10.55°, 10.64°, 15.20°, 17.88°, 18.7°, 20.02°, 20.26°, 20.83°, 24.73°, 25.46°, 26.40°, and numerous minute peaks up to 45° suggesting crystalline property of drug. The most prominent peak was appeared at 20.26°. In contrast, CAP IPN MBs (Fig. 6(b)) did not exhibit any intense peaks that were present in spectrum of pure drug indicating that the pure drug has now converted into amorphous form on getting entrapped within the polymeric matrix. Moreover, the result of XRD study can also be correlated with thermal study DSC interpreting the dispersion of pure CAP to amorphous state.

3.8. % Drug entrapment

The entrapment of CAP in buoyant IPN MBs ranged from 57.85 ± 1.6% to 94.69 ± 2.34% (Table 1). It was observed that the drug entrapment in the IPN MBs increased with increase in the ratio of LBG: NaAlg and the concentration of cross-linker. Increase in the drug entrapment with an increase in the ratio of LBG: NaAlg was due to the more viscous and less hydrophilic nature of LBG. The property of solubility in polysaccharides is governed by mannose component whose value is reported as 4 in comparison to NaAlg which is 1. Thus, LBG showed limited solubility and maximum volume expansion and holds the drug firmly in the polymeric mixture [33]. Other factor that increased the amount of drug to be entrapped was the concentration of cross-linker. More the cross-linker more the intricated network was formed within the polymeric matrix. As the cross-linker used was a trivalent Al^{3+}, so it bound with three guluronic unit of NaAlg and associates firmly thus prevent the leaching of drug into the external media.

3.9. Swelling study

The swelling behaviour of LBG and NaAlg of IPN MBs loaded with CAP was studied in pH 1.2 as a function of time (Fig. 7). Three different batches of IPN (i.e. LBG:NaAlg 1:1; F-1, F-3 and F-15 with 2% w/v cross-linker; LBG:NaAlg 1:3; F-7, F-2 and F-5 with 3% w/v cross-linker and LBG:NaAlg 3:1; F-6, F-9 and F-11 with 4% w/v cross-linker) having varying polymeric ratio and cross-linker were examined. All the batches of IPN MBs showed good swelling however it was observed that maximum swelling was observed in case of batch containing more part of LBG than NaAlg. LBG is relatively more viscous than NaAlg thus, instead of solubilizing, more retention of water was observed in batch 3:1 in comparison to 1:1 and 1:3 resulting into more swelling. Variation in the cross-linker concentration also affected the extent of swelling. It was found that all the batches with 2% w/v $AlCl_3$ showed highest swelling value in comparison to 3% w/v and 4% w/v. Cross-linker provides rigidity to the polymeric matrix as well as reduces the polymeric network. Thus, IPN MBs with 2% w/v $AlCl_3$ concentration showed maximum swelling index value whereas 4% w/v exhibited the minimum.

3.10. In vitro buoyancy study

The result of *in vitro* % buoyancy, floating lag time (FLT) and total floating time (TFT) is shown in Table 3. The buoyancy of the MBs was found to be dependent on MBs diameter and amount of pore former ($NaHCO_3$). Both played a permissive role in influencing the buoyancy [24]. The formulations with higher content of LBG (with respect to viscosity) showed more bead size and absorbed more water inside the matrix that resulted into increased weight of the formulation thus, less number of MBs appeared on the surface and showed greater onset of time to reach on the surface of the media [34]. Another factor $NaHCO_3$

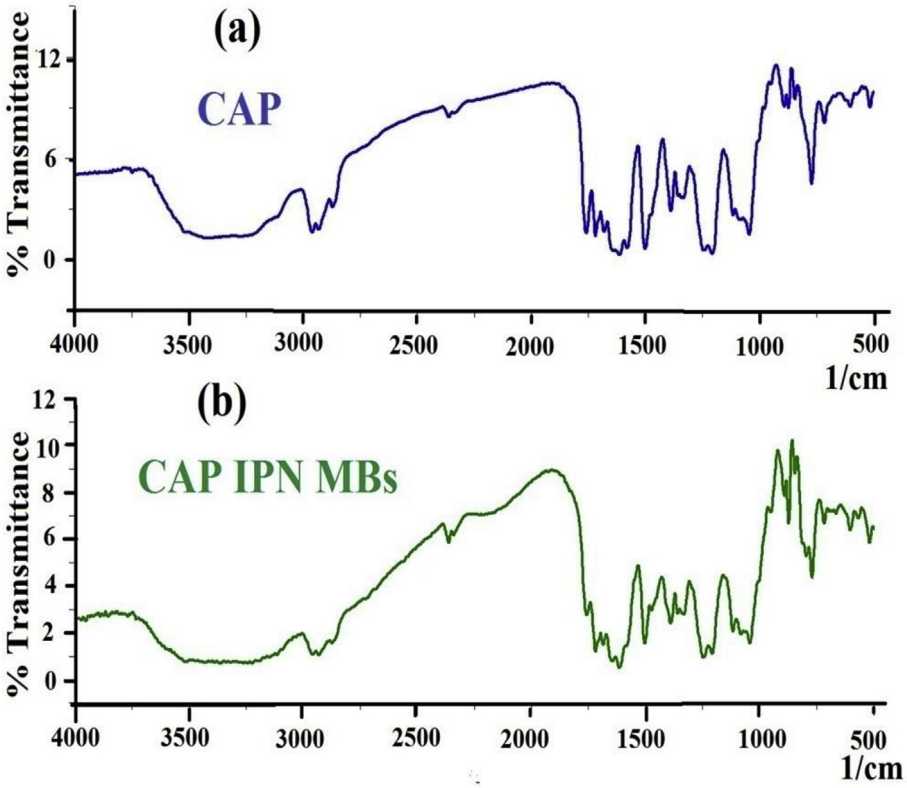

Fig. 4. FTIR spectra of (a) pure CAP and (b) CAP IPN MBs.

enhanced the floating time of the formed IPN MBs. This can be explained as when $NaHCO_3$ came in contact with acidic media (pH 1.2 0.1 N HCl) it liberated carbon dioxide (CO_2) and the generated gas got entrapped within the polymeric gel layer between LBG and NaAlg thus decreased the density of the IPN MBs and the beads became buoyant.

3.11. Ex vivo mucoadhesion

The result of wash off test performed to evaluate the mucoadhesion of the MBs at two different test fluids is exhibited in Table 4. The percentage of mucoadhesion of the MBs in intestinal fluid at pH 6.8 was observed in the range of 86 ± 3.43% to 38 ± 1.22% and 34 ± 1.01% to 02 ± 2.1% in gastric fluid at pH 1.2. The polymers used in the preparation of buoyant IPN MBs are mucoadhesive in nature. The viscous property of the polymers is making them to stick to the mucosal. LBG and NaAlg are polysaccharides and contains galactomannans, a combination of galactose and mannose (M/G). The viscous property is governed by mannose component, higher the mannose content greater will be the viscosity. The polymer LBG possess M/G 4 whereas NaAlg 0.53 this indicates that LBG is more viscous than alginate. Thus, it imparts less solubility and more adhesive strength [35]. Thus, the batch containing more proportion of LBG has shown better mucoadhesion. Further, from the result it is clear that maximum % of mucoadhesion was observed in intestinal pH 6.8 because with the passage of time floating capacity of the dosage form reduces as well as formulation also passes towards other parts of GI tract. At that time the property of mucoadhesion can provide the formulation to remain in intimate contact with mucosal wall of the GI tract.

3.12. Drug release study

In vitro drug release of the optimized buoyant trivalent ion cross-linked MBs was carried out in 0.1 N HCl (pH 1.2) for 8 h followed by 900 mL phosphate buffer (pH 6.8) for remaining 20 h in USP type II dissolution apparatus (Fig. 8(a)). The optimized MBs showed 10% drug release for the first 2 h and 44% drug release for remaining 6 h followed by the extended release of drug from polymeric matrix for the next 12 h at pH 6.8. Drug release from the hydrophilic polymeric matrix is considered as a combined phenomenon of swelling and diffusion [36]. At pH 1.2 a constant sustained release of CAP was observed from the buoyant IPN MBs this may be due to the combined effect of swelling, diffusion and presence of pores in the MBs. LBG is a highly viscous and low water soluble biopolymer in comparison to NaAlg as discussed in section 3.11 [37]. At pH 1.2 a constant sustained release of CAP was observed from the buoyant IPN MBs this may be due to the combined effect of swelling, diffusion and presence of pores in the MBs. When the formulation was poured in simulated gastric fluid (SGF) due to protonation less swelling occurred resulted in the slow drug release however presence of pores allowed multiple entries for the media to enter inside the matrix that resulted into maximum but diffusion of drug from the polymeric matrix till 8 h. Further, at pH 6.8 extended and maximum drug release of 50% was observed. At this pH more number of ions in the buffer media appears, leading to the increase in the osmotic force that results into continuous and notable extended release. Apart from diffusion and pore formation another factor that played an additive effect in extending the release till 20 h was CAP's amorphous rapid gel forming property which is formed when it comes in contact with water due to its low glass transition temperature [38]. Zero order release kinetics was observed for the above formulation showing a maximum R^2 value of 0.991.

3.13. In vivo pharmacokinetic study

Pharmacokinetic studies estimate the drug efficacy and response as a function of drug concentration [39]. The mean plasma concentration time profile of CAP and buoyant IPN MBs is exhibited in Fig. 8(b) and their calculated pharmacokinetic parameters are shown in Table 5.

The results of *in vivo* study of the optimized formulations showed

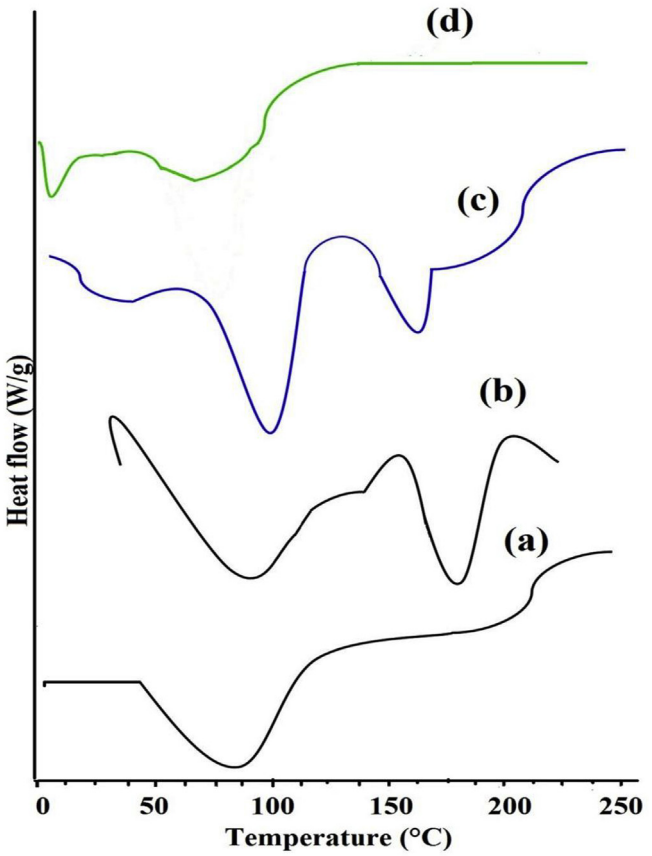

Fig. 5. DSC curves of polymers (a) LBG and (b) NaAlg showing melting peaks at 92.55 °C and 187.14 °C respectively. (c) Pure CAP showed its crystalline nature by exhibiting a sharp melting peak at 120.68 °C followed by its thermal decomposition peak at 157.76 °C. (d) CAP loaded IPN MBs exhibited flat and abraded peaks indicating its conversion to amorphous form.

well promising results when compared to the pure CAP in solution form. At all-time points a significant difference was observed between optimized formulation and pure CAP in solution form. The AUC of optimized formulation (17.23 ± 0.29 μg-h/mL) was increased 6.346 folds whereas MRT of the optimized formulations (20.177 ± 0.94 h) increased 10.40 folds in comparison to the pure drug. Also, an increased $t_{1/2}$ of 13.393 ± 2.88 was observed when compared to pure drug that was 0.557 ± 0.12 h revealing the slower elimination and longer half-life. Thus; the overall data of the pharmacokinetic study illustrates that a buoyant system of IPN could be a potential dosage form for prolonging the systemic circulation of CAP.

3.14. In vivo gamma (γ) scintigraphy imaging

Gamma scintigraphy study was performed for monitoring the gastroretentive behavior of optimized aluminium ion cross-linked buoyant IPN MBs in mice. Direct radiolabelling method was employed for labelling the optimized formulation with Technetium −99 (^{99m}Tc) that involved mixing of acidic stannous chloride ($SnCl_2$) to reduce ^{99m}TC from higher (heptavalent) oxidation state to lower oxidation state in order to assess the formation of complex of radiolabelled metal substance with the functional groups such as hydroxyl, carboxylic groups etc. present on the surface of MBs. The entire process was performed at pH 7.4 from using sodium bicarbonate solution [40]. The radiolabelling efficiency of the radiolabelled complex buoyant IPN MBs was estimated by instant thin layer chromatography (ITLC) by varying the concentration of stannous chloride (10–40 μg/mL) using silica gel as

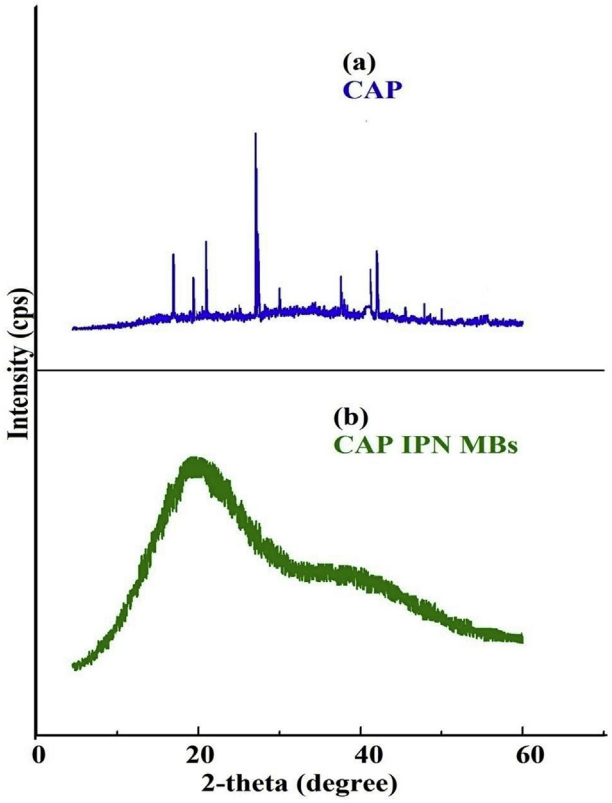

Fig. 6. XRD curves of (a) crystalline CAP exhibiting several sharp peaks and (b) Buoyant CAP IPN MBs containing abridged and diffused peaks indicating conversion of crystalline CAP into amorphous state.

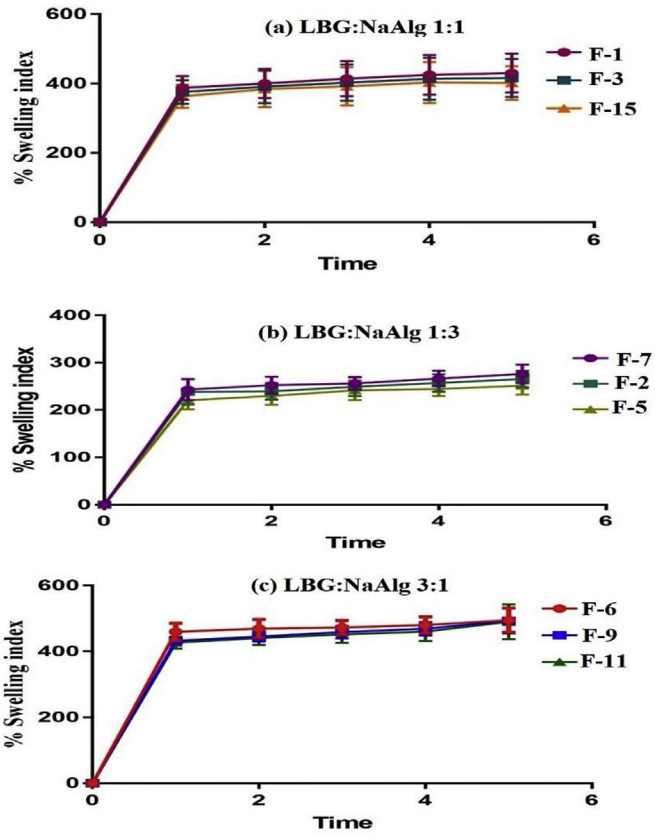

Fig. 7. Swelling index value of batches F-1, F-3 and F-15 (LBG:NaAlg 1:1); F-7, F-2 and F-5 (LBG:NaAlg 1:3); and F-6, F-9 and F-11 ((LBG:NaAlg 3:1).

Table 3
Estimated density, diameter, floating lag time and total floating time of formed buoyant IPN MBs.

Batch	Diameter(mm^3)	Density	FLT	TFT
1	1.60	0.59	3.07	10
2	1.36	0.90	5.28	> 10
3	1.52	0.69	3.20	> 8
4	1.33	0.95	5.40	> 12
5	1.92	0.47	2.10	> 8
6	1.44	0.79	4.45	> 10
7	1.39	0.88	5.18	> 10
8	1.49	0.72	4.35	> 10
9	1.70	0.55	2.18	8
10	1.57	0.66	3.15	> 8
11	1.65	0.61	3.10	> 8
12	1.47	0.76	4.40	10
13	1.35	0.92	5.30	> 10
14	1.85	0.50	2.15	8
15	1.42	0.82	5.10	> 10

Table 4
% Mucoadhesion of the formed buoyant IPN MBs at two different pH 1.2 & pH 6.8.

Batch no	LBG:NaAlg (% w/v)	Cross-linker (% w/v)	% Mucoadhesion[a] (%)	
			pH 6.8	pH 1.2
1	1:1	2	70 ± 3.8	10 ± 2.5
2	1:3	3	40 ± 4.2	06 ± 4.4
3	1:1	3	49 ± 1.9	09 ± 4.9
4	1:1	4	58 ± 2.4	08 ± 5.7
5	1:3	4	38 ± 4.4	02 ± 2.0
6	3:1	2	86 ± 1.3	34 ± 1.9
7	1:3	2	54 ± 1.8	06 ± 2.6
8	1:1	3	42 ± 3.9	07 ± 5.5
9	3:1	3	80 ± 2.8	29 ± 1.8
10	1:1	2	65 ± 3.1	07 ± 1.8
11	3:1	4	75 ± 3.9	14 ± 1.9
12	3:1	3	82 ± 4.2	32 ± 2.8
13	1:3	3	44 ± 2.9	04 ± 3.1
14	3:1	3	78 ± 2.4	25 ± 5.5
15	1:1	4	61 ± 1.3	08 ± 1.8

[a] Mean ± S.D.; n = 3.

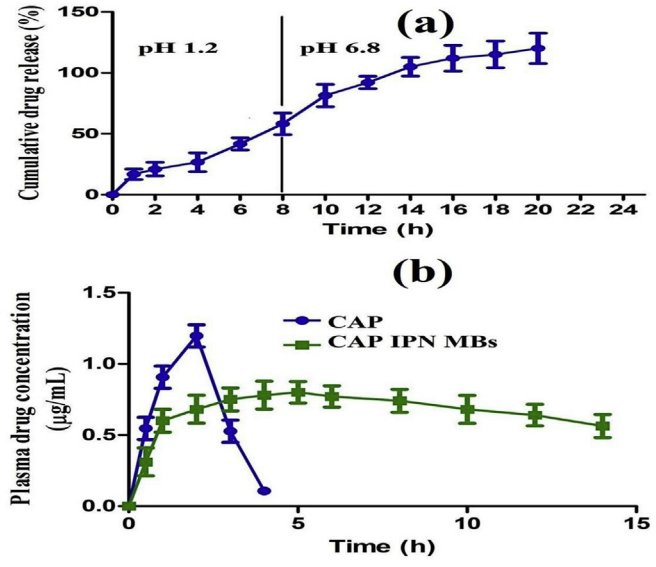

Fig. 8. (a) *Drug* release profile of CAP from buoyant IPN MBs at acidic and alkaline pH; (b) plasma drug concentration profile of CAP loaded buoyant IPN MBs against time.

Table 5
Calculated PK parameters for CAP and buoyant CAP IPN MBs.

PK Parameters	[a]Pure drug	[a]Optimized formulation
C_{max} (μg/mL)	1.2 ± 0.25	0.90 ± 0.19
T_{max} (h)	2.0 ± 0.0	4.0 ± 0.0
$AUC_{(0-t)}$ (μg-h/mL)	2.715 ± 0.435	17.23 ± 0.29
MRT (h)	1.940 ± 0.61	20.177 ± 0.94
$t_{1/2}$ (h)	0.557 ± 0.12	13.393 ± 2.88

[a] Mean ± S.D.; n = 6. Pharmacokinetic parameter AUC ($p < 0.0001$), MRT ($p < 0.05$) and $t_{1/2}$ ($p < 0.005$) of the groups, compared by one-way ANOVA.

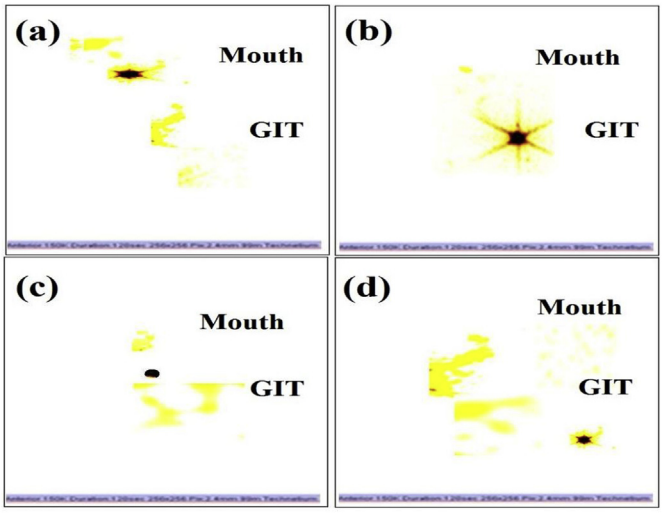

Fig. 9. γ-Scintigraphy images showing gastroretention of buoyant IPN MBs at time points (A) 0.5 h, (B) 2 h, (C) 4 h, (D) 6 h.

stationary phase and acetone as mobile phase that was found to be 95.69 ± 0.10% at 30 μg/mL [41]. Afterwards the radiolabelled buoyant IPN MBs were orally administered to the mice and the images of the mice by laying them at anterior position were captured with scinti camera (gamma camera) of low energy and high resolution connected with computer. While recording the images, only the area of interest i.e. stomach was focused which was recorded at 0.5 h, 2 h, 4 h and 6 h of post administration. The scintigrams obtained exhibited in Fig. 8 were analysed. Due to small size of mice it was difficult to discriminate the organs in the GIT however a marked change in the position of administered MBs at 0.5 h (Fig. 9 (a)) and 2 h (Fig. 9(b)) can be observed suggesting the movement of MBs from mouth to stomach region that remained at the same position till 4 h (Fig. 9 (c)and (d)). However, at the 6 h the position of MBs was at the lowest position that interprets that it has been moved from its present position i.e. from stomach to downwards i.e. to the intestine region.

Thus, the optimized buoyant IPN MBs showed its gastroretention till 4 h which is the proof of concept that the formed mucoadhesive buoyant IPN MBs of natural polymers is capable of enhancing the gastric residence of short elimination half-life capecitabine.

4. Conclusion

The CAP loaded buoyant IPN MBs composed of natural polymers were successfully prepared and showed good result against colon cancer. The optimized batch of the IPN exhibited minimum particle size, maximum drug entrapment, good buoyacy and prolonged controlled release these obtained results were also found to be in close agreement with the predicted results by softwrae. The morphological study showed smooth and spherical shape of IPN MBs along with appearance of pores due to presence of pore forming agent in formulation. Results of solid-state characterization techniques confirmed the

dispersion of pure CAP into amorphous form when encapsulated with polymer matrix. *In vivo* pharmacokinetics performed in Albino Wistar rats showed enhanced systemic bioavailability and elimination half-life of the drug with higher plasma drug concentrations maintained for longer duration as compared to pure drug. The *in vitro* buoyancy study performed in case of buoyant IPN MBs showed total floating time of 12 h. The *in vivo,* γ scintigraphy study showed gastoretention of the IPN MBs more than 6 h indicating it to be an effective delivery system for CAP. The result of mucoadhesion study performed in two different pH media, SGF and SIF in intestinal goat mucosa by wash off method showed good result in SGF in comparison to SIF proving the ability of the IPN MBs prepared of natural polymers to stay in the stomach for longer time with prolonged release potential of the formulation. Thus, the outcomes revealed that the formed IPN MBs could be effective in enhancing the systemic bioavailability of the drug.

Declaration of competing interest

The authors assures no conflict of interest in this article.

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