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Development, characterization and optimization of polymeric mucoadhesive microcapsules containing anticancer agent using response surface method: in vitro and in vivo study

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ABSTRACT

The present research work covenants the preparation, characterisation and optimisation of mucoadhesive microcapsules containing paclitaxel through ionic gelation method using 3^2 statistical factorial designs. The effect of mixing proportion of primary polymer sodium alginate to copolymer (X_1) and speed of magnetic stirrer (X_2) on the microcapsules size (Y_1), efficiency of paclitaxel encapsulation (Y_2), and percentage yield (Y_3) was optimised. The morphology of microcapsules was characterised and evaluated by in vitro and in vivo tests to study the swelling characteristics, mucoadhesion and drug release characteristics, followed by MTT assay on human HT-29 colon cancer cell lines. The size of prepared microcapsules was within the range of 361 $\pm$ 4.50 to 931 $\pm$ 22.41; encapsulation efficiency (%) was within the range of 42.72 $\pm$ 0.43 to 98.12 $\pm$ 0.43 %. The in vitro paclitaxel released over 24 hours were in a range of 82.15 $\pm$ 3.43 % to 96.75 $\pm$ 2.41 %. The controlled release pattern of paclitaxel was observed from the in vitro drug release study of microcapsules. The prepared microcapsules that showed better mucoadhesion were in the range of 73.66 $\pm$ 1.42 to 97.85 $\pm$ 1.08 % for a period of 6 h. The in vivo pharmacokinetic study conducted in rats resulted in high T_{max}, the area under the curve and mean residence time for microcapsules as compared to that of the marketed formulation. It could be concluded that the microcapsules containing povidone polymer showed superior results.

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INTRODUCTION

Intestinal carcinoma is life-threatening and is diagnosed extensively in the world, which accounts for ten per cent of all types of cancers (Rawla *et al.*, 2019). Colon cancer cases around fifty-four per cent of total cancer were diagnosed in New Zealand and Australia, with its widespread among seniors with a median age of seventy years (Chawla *et al.*, 2013). Almost around five lakhs sixty thousand people are losing their lives to colon cancer across the globe per year. Cytotoxic drugs are deleterious to carcinoma cells that can rapidly grow and divide and undergoes metastasis (Chaffey, 2003). Paclitaxel, as a part of the regular procedure, administered intra-

venously in liquid form but produced many adverse events (Boulanger *et al.*, 2014). When compared to other drugs, paclitaxel lack specificity in their systemic delivery of its effects which in due course results in side effects by attacking both cancerous and healthy cells as well (Fujita *et al.*, 2015). According to past reports, paclitaxel has emerged as potent second-line drugs for colorectal cancer (Ki and Rotstein, 2008). Paclitaxel is generally administered as intravenous therapy, directly into the vein; conversely, intravenous treatment exposes the patient to the risk of bacterial infection through the puncture of the skin (Huang *et al.*, 2017).

Further, intravenous therapies have not proven to be most efficacious to treat colon cancers as the administered drug is responsible not only for the death of cancerous cells but also the healthy and vital cells in the body, which results in severe and life-threatening conditions (Mishra *et al.*, 2013). The most common treatment option for colorectal cancer is radiotherapy which helps to decrease the size of the colon cancer tumour volume by inducing the process of DNA fragmentation during the cell division cycle and inhibits intracellular membranes by causing cell apoptosis (Amidon *et al.*, 2015). Therefore, the oral drug delivery systems were designed to reduce toxicity and improve on the specific delivery to the cancerous colon area.

MATERIALS AND METHODS

The drug under investigation, paclitaxel was obtained from Neon Laboratory as a gift sample. Sodium alginate and acacia were purchased from Sigma–Aldrich, macrogol and povidone were purchased from a local chemical supplier. The other reagents used were of pharmaceutical grade.

Design of Experiment

A 3-level 2-factor full factorial design was implemented for the preparation and optimisation of microcapsules. One factor was polymer to copolymer ratio at a level of low (1:1), medium (1:1.5) and high (1:2). The other factor was the speed of magnetic stirrer at a level of low (500 rpm), medium (750 rpm) and high (1000 rpm). The prepared microcapsules were optimised to get the smallest particle size with high entrapment efficiency and yield.

Preparation of Microcapsules

Paclitaxel encapsulated microcapsules were prepared by ionic gelation technique using the polymer (Yadav *et al.*, 2016). Paclitaxel was dissolved in methanol followed by purified water to make 5 mL of drug solution in methanol. Sodium alginate and copolymers were dispersed in 50 mL distilled water according to the formula given in Table 1 by using a stirrer. The drug solution was added into the polymer dispersion with continuous stirring. Then the drug-polymer dispersion was added dropwise from a height of 5 cm from the surface of the 100 mL of 10 % w/v calcium chloride solution through syringe manually at the rate of 1 mL/ min. Further, the medium was stirred for 20 min to complete the crosslinking. The microcapsules obtained were washed and dried at room temperature for 24 h and stored in desiccators.

Solid-state Characterisation of Microcapsules

Fourier Transform Infra-Red (FTIR) Spectroscopic Studies

FTIR spectrum of the drug-loaded microcapsules, pure paclitaxel and polymers were recorded. Approximately 2 mg of each sample was mixed with preheated moisture-free potassium bromide with a weight ratio of 1:50. The mixture was pulverised into a fine powder and compressed by an IR compressor at 3000 psi. The potassium bromide disks were measured by FTIR over the range of 3500-700 cm^{-1} to obtain the spectra (Nadigoti *et al.*, 2011).

Differential Scanning Calorimetry (DSC) Studies

Differential Scanning Calorimetry analysis was carried out to characterise the thermal behaviour of prepared microcapsules, pure paclitaxel and polymers using differential scanning calorimeter (DSC). Samples weighed around 4 mg were scanned in crimp sealed aluminium pans heated to 400 °C from 40 °C at a heating rate of 10 °C per minute under persistent purging of dry nitrogen at 20 mL/min, and the thermograms were collected (Mitra and Dey, 2011).

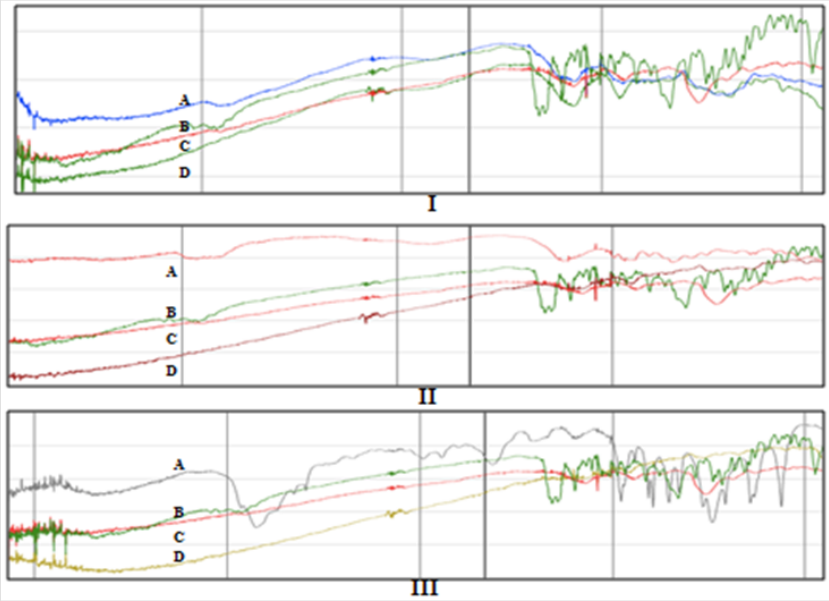
Microcapsule Morphology

The surface characteristics of the prepared microcapsule from different batches were studied using a scanning electron microscope (SEM). The procedure to prepare samples, the microcapsules were sprinkled lightly on a dual-sided adhesive tape fixed to a 10x10 mm brass knock and coated with a mixture of palladium and gold using a sputter coater to a thickness of 200 to 500 angstroms. The instrument was operated at a fixed voltage of 20KV voltage, WD 12 mm, 18 mA current and 10^{-2} bar pressure under an argon atmosphere and resolution were set to A: x 27, 500 mm; B: x 85, 200 mm; C: x 50, 500 mm; D: x 1000, 10 mm (Wang *et al.*, 2011).

Evaluation of Prepared Microcapsules

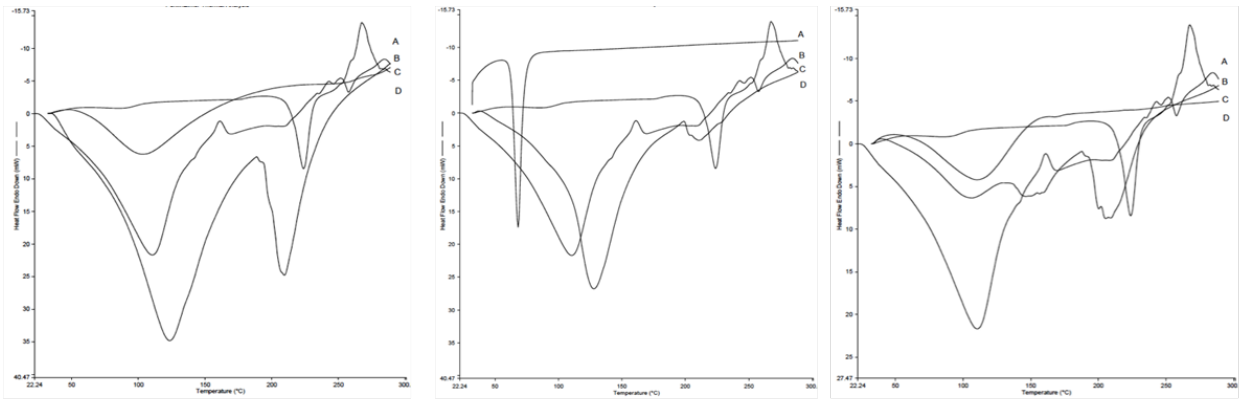
Size of Microcapsules

The diameter of the microcapsules was determined



I: APSA Batch (A: Microcapsules, B: Sodium alginate, C: paclitaxel, D:Acacia); II: CPSP Batch(A:Microcapsules, B: Sodium alginate, C: paclitaxel, D:Povidone); III: BPSM Batch(A:Microcapsules, B: Sodium alginate, C: paclitaxel, D:Macrogol)

Figure 1: FTIRspectra of microcapsules



overlay for I. APSA Batch; (A: Acacia, B: Sodium alginate, C: Paclitaxel, D: APSA microcapsules); II. BPSM Batch; (A: Macrogol, B: Sodium alginate, C: Paclitaxel, D: BPSM microcapsules); III. CPSP Batch; (A: Sodium alginate, B: BPSM microcapsules, C: Paclitaxel, D: Povidone)

Figure 2: DSC Thermogram

Table 1: Formula for experimental batches

Code	Drug (mg)	SA (g)	APSA Batch (Acacia) (g)	Stirrer (rpm)	speed
APSA1	50	1	1	500	
APSA2	50	1	1.5	500	
APSA3	50	1	2	500	
APSA4	50	1	1	750	
APSA5	50	1	1.5	750	
APSA6	50	1	2	750	
APSA7	50	1	1	1000	
APSA8	50	1	1.5	1000	
APSA9	50	1	2	1000	
Code	Drug (mg)	SA (g)	BPSM Batch (Macrogol) (g)	Stirrer (rpm)	speed
BPSM 1	50	1	1	500	
BPSM 2	50	1	1.5	500	
BPSM 3	50	1	2	500	
BPSM 4	50	1	1	750	
BPSM 5	50	1	1.5	750	
BPSM 6	50	1	2	750	
BPSM 7	50	1	1	1000	
BPSM 8	50	1	1.5	1000	
BPSM 9	50	1	2	1000	
Code	Drug (mg)	SA (g)	CPSP Batch (Povidone) (g)	Stirrer (rpm)	speed
CPSP1	50	1	1	500	
CPSP2	50	1	1.5	500	
CPSP3	50	1	2	500	
CPSP4	50	1	1	750	
CPSP5	50	1	1.5	750	
CPSP6	50	1	2	750	
CPSP7	50	1	1	1000	
CPSP8	50	1	1.5	1000	
CPSP9	50	1	2	1000	

using an optical microscope. This instrument was fitted with an ocular micrometre and a stage micrometre. The diameters of 50 microcapsules were measured randomly. Edmondson's equation was used to calculate the average particle size.

$$D\_mean = \frac{\sum nd}{\sum n}$$

Where n = Number of microcapsules observed; d= mean size range

Yield During Process

The dried microcapsules of all batches were accurately weighed, and the total percentage yield of microcapsules was calculated using the following equation,

$$Percentage \quad Yield = \frac{Dry\ Weight\ of\ microcapsules}{Dry\ weight\ of\ paclitaxel + Dry\ weight\ of\ polymers} \times 100$$

Drug Loading and Encapsulation Efficiency

The formulated microcapsules of paclitaxel were crushed and dissolved in 5 mL of methanol and drug was extracted into of phosphate buffer pH 7.0 by evaporating methanol. The mixed solution was filtered through membrane filter paper (0.45 μ porosity), suitably diluted, and the absorbance was analysed by Shimadzu UV 1800 spectrophotometer at 228 nm. The amount of drug encapsulated into the microcapsules was calculated.

Table 2: Evaluationof particle size, Encapsulation efficiency and Yield

Formulation	Particle size (μm)	Encapsulation efficiency (%)	Yield (%)	Swelling index	Mucoadhesion
APSA 1	656 ± 21.34	70.29 ± 0.11	82.11 ± 0.01	91.20 ± 1.22	79.36 ± 1.11
APSA 2	721 ± 10.71	68.24 ± 0.29	71.32 ± 0.05	93.83 ± 2.14	73.66 ± 1.42
APSA 3	891 ± 20.23	61.75 ± 0.20	69.33 ± 0.11	92.58 ± 1.14	76.48 ± 1.35
APSA 4	631 ± 30.33	52.11 ± 0.25	75.33 ± 0.03	95.12 ± 2.15	75.40 ± 1.36
APSA 5	399 ± 20.46	62.02 ± 0.27	76.33 ± 0.02	94.32 ± 2.45	81.03 ± 1.30
APSA 6	561 ± 10.53	69.33 ± 0.41	65.05 ± 0.03	91.16 ± 2.12	79.36 ± 1.34
APSA 7	531 ± 8.44	42.72 ± 0.43	61.03 ± 0.05	94.16 ± 1.15	77.50 ± 1.31
APSA 8	632 ± 22.37	68.32 ± 0.13	72.44 ± 0.07	91.55 ± 1.14	78.26 ± 1.22
APSA 9	411 ± 11.23	63.24 ± 0.27	73.44 ± 0.13	93.62 ± 1.32	75.83 ± 1.25
BPSM 1	589 ± 11.33	50.29 ± 0.11	82.11 ± 0.01	73.78 ± 1.41	87.00 ± 1.12
BPSM 2	659 ± 21.30	78.24 ± 0.29	71.32 ± 0.05	70.43 ± 1.13	89.16 ± 1.06
BPSM 3	721 ± 10.61	71.75 ± 0.30	69.33 ± 0.11	72.17 ± 1.13	86.08 ± 1.15
BPSM 4	891 ± 16.33	82.11 ± 0.25	85.33 ± 0.13	74.56 ± 1.43	90.86 ± 2.02
BPSM 5	831 ± 13.43	72.02 ± 0.37	56.33 ± 0.21	83.68 ± 1.12	89.13 ± 1.12
BPSM 6	799 ± 15.36	79.33 ± 0.41	55.05 ± 0.03	74.68 ± 1.60	89.83 ± 1.03
BPSM 7	861 ± 21.50	62.12 ± 0.43	61.03 ± 0.05	75.43 ± 2.61	86.66 ± 1.02
BPSM 8	931 ± 22.41	78.32 ± 0.13	82.44 ± 0.07	76.31 ± 1.13	85.39 ± 1.2
BPSM 9	832 ± 10.47	73.24 ± 0.27	83.44 ± 0.13	73.78 ± 1.41	86.66 ± 0.12
CPSP 1	513 ± 9.48	80.29 ± 0.11	81.11 ± 0.01	95.24 ± 3.21	90.90 ± 2.17
CPSP 2	401 ± 9.20	83.24 ± 0.29	87.32 ± 0.05	95.86 ± 2.23	95.16 ± 1.12
CPSP 3	411 ± 8.15	79.75 ± 0.30	83.93 ± 0.11	89.57 ± 2.13	94.76 ± 2.15
CPSP 4	491 ± 11.23	82.51 ± 0.25	85.33 ± 0.13	97.64 ± 2.15	95.00 ± 1.19
CPSP 5	631 ± 7.49	92.02 ± 0.37	86.33 ± 0.21	87.90 ± 3.12	97.85 ± 1.08
CPSP 6	499 ± 6.26	89.33 ± 0.41	85.05 ± 0.03	87.54 ± 2.09	97.57 ± 1.03
CPSP 7	361 ± 4.50	98.12 ± 0.43	91.03 ± 0.05	95.62 ± 2.13	95.23 ± 2.02
CPSP 8	382 ± 6.41	88.32 ± 0.13	81.44 ± 0.07	93.51 ± 3.26	95.73 ± 1.22
CPSP9	530 ± 1.47	93.24 ± 0.27	83.44 ± 0.13	87.92 ± 2.71	93.66 ± 1.17

Table 3: Pharmacokinetic parameters of different paclitaxel formulations (n=3)

Pharmacokinetic parameter	Intravenous Injection	PSP-MC	PSM-MC
Cmax (μg /ml)	3.158	2.912	2.194
$t_{\frac{1}{2}}$ (h)	3.8	7.2	9.2
Ke (h-1)	0.182	0.096	0.07
Tmax (h)	4	6	8
AUC0-∞ (μg h/ml)	11.23	14.26	16.47
AUMC0-∞(μg h2/ml)	3.48	20.11	75.33
MRT(h)	0.30	1.41	4.57
Relative Bioavailability %	-	126.98	146.67

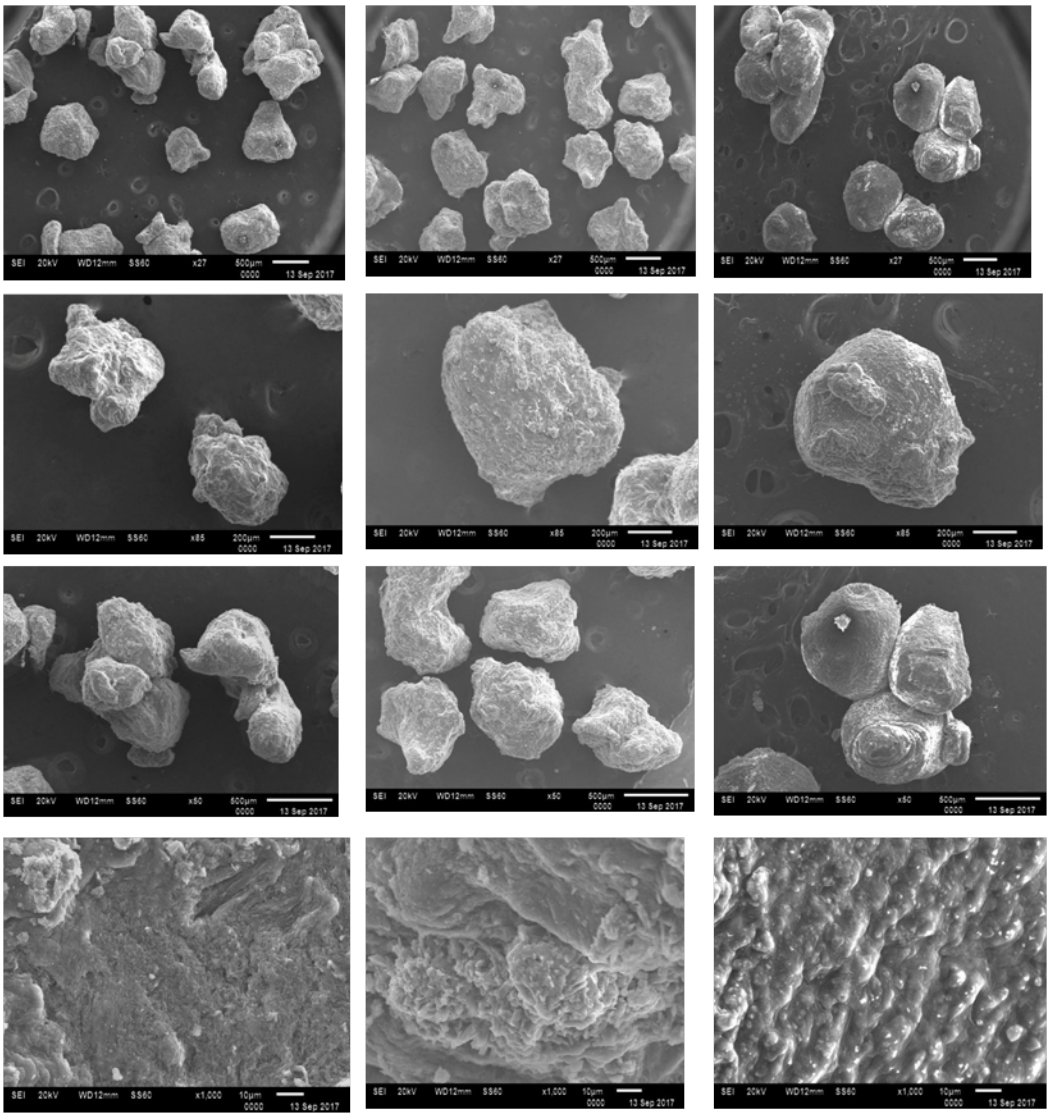


Figure 3: SEM of microcapsules; I: APSA Batch, II: BPSM Batch,III CPSP Batch; A: x 27, 500 mm; B: x 85, 200 mm; C: x 50, 500 mm; D: x 1000, 10 mm

$$Percentage\ drug\ loading = \frac{amount\ of\ drug\ in\ microcapsules}{Total\ weight\ of\ microcapsules} \times 100$$

$$Encapsulation\ efficiency = \frac{Percentage\ drug\ loading}{theoretical\ percentage\ of\ drug\ loading} \times 100$$

Swelling Index

The weighed microcapsules were allowed to swell in phosphate buffer pH 7.0. The weight of microcapsules was determined after 12 hours of swelling. The swelling index was calculated using formula,

$$Swelling\ index = \frac{Weight\ of\ Swelled\ microcapsules - Weight\ of dried\ microcapsules}{Weight\ of\ dried\ microcapsules} \times 100$$

Ex vivo Mucoadhesion Study

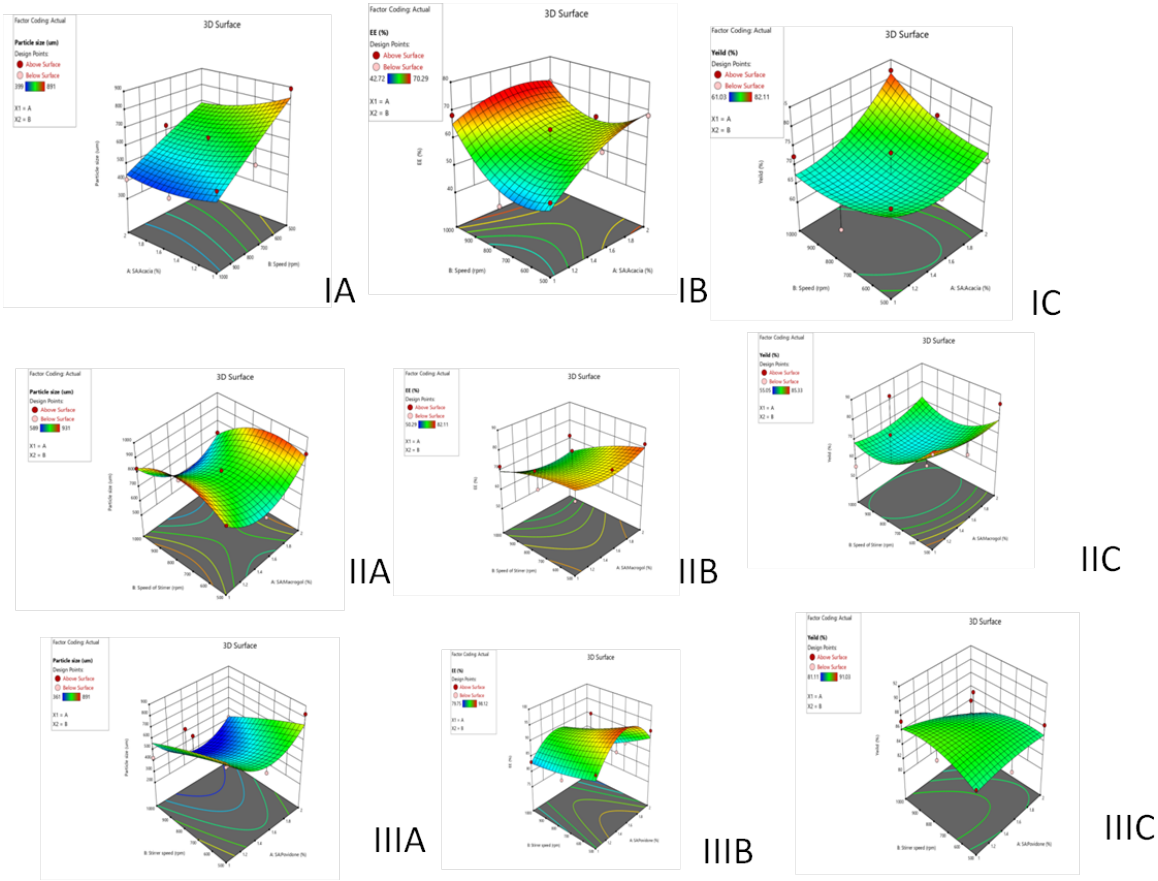
The microcapsules were premeditated on rat's intestinal mucosa for mucoadhesion characteristics by using phosphate buffer (Sabitha *et al.*, 2010). Microcapsules were accurately weighed and spread onto tissue specimen rinsedRingers Lactate solutionand tangled onto the arm of a USP tablet disintegrating test machine. The specimen was given an up and down movement inside phosphate buffer maintained at 37 °C. The weight of microcapsule leached out after specified time was measured. The percentage mucoadhesion was calculated by the following equation

$$Mucoadhesion(\%) = \frac{W_0 - W_t}{W_0} \times 100$$

Where, W_0 was the weight of microcapsule initially taken; W_t was the weight of microcapsule leached out after time t.

In vitro Drug Release Studies

Paclitaxel loaded microcapsule were investigated for determining the percentage drug release in a period of 24 h with help of USP Dissolution apparatus type I (rotating basket).



I: APSA batch (IA: Particle size; IB: EE%; IC: Yield); II: BPSM batch (IIA: Particle size; IIB: EE%; IIC: Yield); III: CPSP batch (IIIA: Particle size; IIIB: EE%; IIIC: Yield)

Figure 4: 3Dsurface response plot

The studies were carried out in phosphate buffer (PB) with 0.5 % Sodium lauryl sulphate (pH 7.0) as medium.

The dissolution media was maintained at 37±0.5°C and operated at a paddle stirring speed of 50 rpm (Borgmann, 2008).

Microcapsule containing about 10 mg of paclitaxel were taken in the dissolution media. The amount of drug released and percentage cumulative drug released at different time intervals were calculated by measuring the absorbance at 228 nm in UV spectrophotometer.

Drug Release Kinetics

The results obtained from the in vitro release study were tailored to different equations and kinetic models to explain the release pattern of paclitaxel from the microcapsules.

The zero order, first order, Hixon crowel, Korsmeyer-peppas and Higuchi kinetic models were used to find out the best fit model that have the value of correlation coefficient near to one.

MTT Assay on Human HT-29 Colon Cancer Cell Lines

Cells were seeded at 2 x 10^4 cells / well in 96 well microtitre plate and incubated for 24 h during which a partial monolayer forms. The cells were then exposed to various concentrations of microcapsules in 2 groups, BPSM and CPSP batches respectively. The third group received pure drug paclitaxel. The fourth group was control group where the wells were received only maintenance medium. The plated were incubated with 5 % carbon dioxide at 37 ºC, 75 % RH for 24 hours (Bahuguna *et al.*, 2017). The morphological changes were observed for microcapsules exposed plates using a microscope and compared with that of pure paclitaxel.

In vivo Animal Study

Wistar rats were used to study the pharmacokinetic properties of prepared microcapsules in comparison with the marketed formulation. Male rats weighing around 100 gram and age between 4-5 weeks were purchased. These rats were grouped into six groups (6 rats in each group).

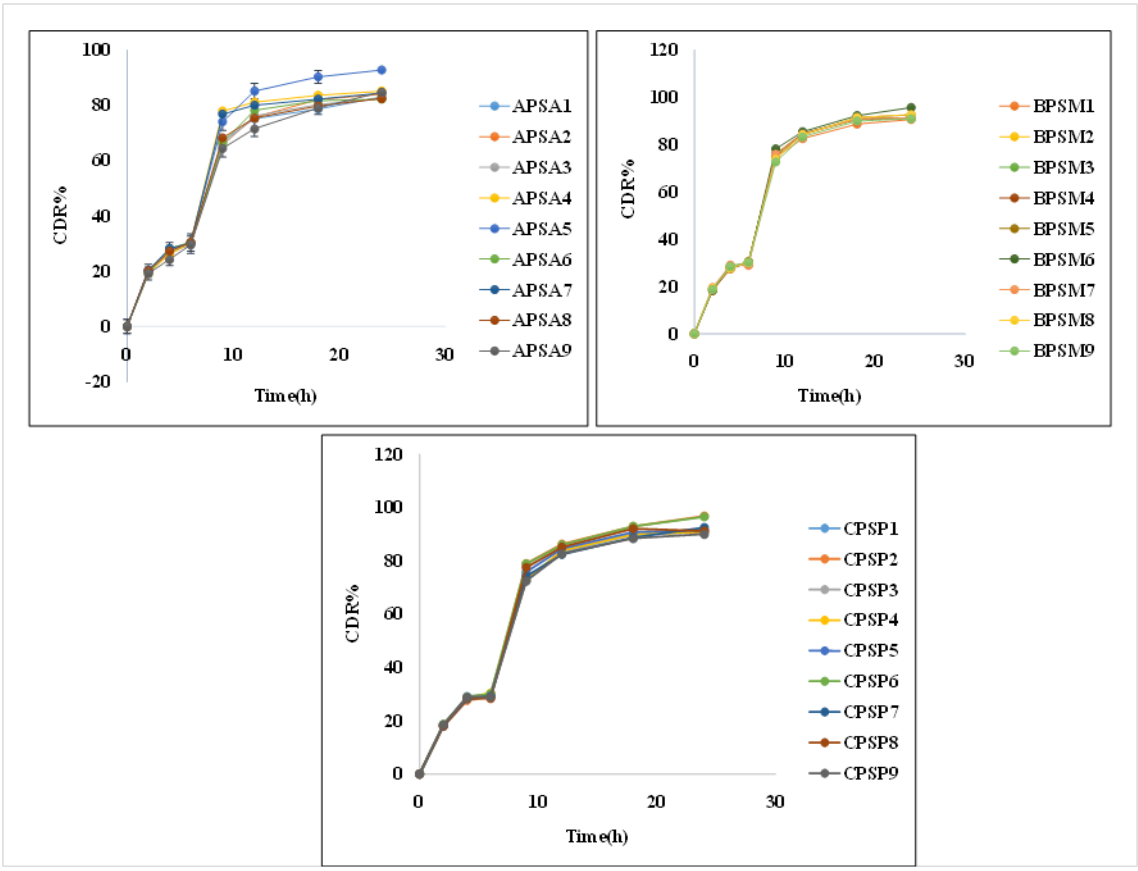


Figure 5: In vitro drug release characteristics of APSA, BPSM and CPSP microcapsules

Group, I was control (received normal saline), group II was induced for colorectal cancer, group III treated with a marketed tablet, group IV treated with BPSM batch, group V treated with CPSM batch and group VI normal rats naturally grown without any intervention.

Rats were taken care of during the study, in compliance with the Principles of Laboratory Animal Care and Guidance for the Care and Use of Laboratory Animals. The experimental protocol was prepared, and approval was obtained from the Institutional Animal Ethical Committee before conduct the study.

The concentration of paclitaxel in rat plasma was estimated using HPLC (Rahman *et al.*, 2008).

Pharmacokinetic Studies

Animals were administered appropriate formulation containing the equivalent of 10.0 mg of paclitaxel peroral route. In order to minimise the stress effects animals were randomised before collection of the blood sample.

The measured volume of blood around 1 mL was collected by retro-orbital bleeding under light anaesthesia at 0, 1, 2, 4, 6, 12 and 24h after administration of the prepared sample (Parasuraman *et al.*, 2015; Zhang *et al.*, 2010b).

The collected blood at each interval was centrifuged at 3000 rpm for 10 min, and the serum was collected and measured for paclitaxel concentration by HPLC at 228 nm. Pharmacokinetic (PK) parameters were analysed by using PKsolver software.

Area under the plasma paclitaxel concentration with time curve up to the last time (t) (AUC $_{0-t}$), area under the curve extrapolated to infinity (AUC$_{0-\infty}$), and area under the first moment curve extrapolated to infinity AUMC$_{0-\infty}$ were calculated using the linear trapezoidal rule. The calculated AUMC/AUC gives the mean residence time (MRT).

RESULTS AND DISCUSSION

A design matrix was constructed with nine experimental runs for each batch (APSA, BPSM and CPSP).

The linear computer-generated quadratic model for responses like microcapsule particle size (Y_1), encapsulation efficiency (EE %) (Y_2), Percentage yield (Y_3). The quadratic equations for various polymeric batches were given as below.

APSA Batch

Y_1 (Particle Size) = 39.05 + 0.750 X_1 + 3.10 X_2 - 1.18 X_1X_2 - 6.65 X_1^2 - 2.20 X_2^2

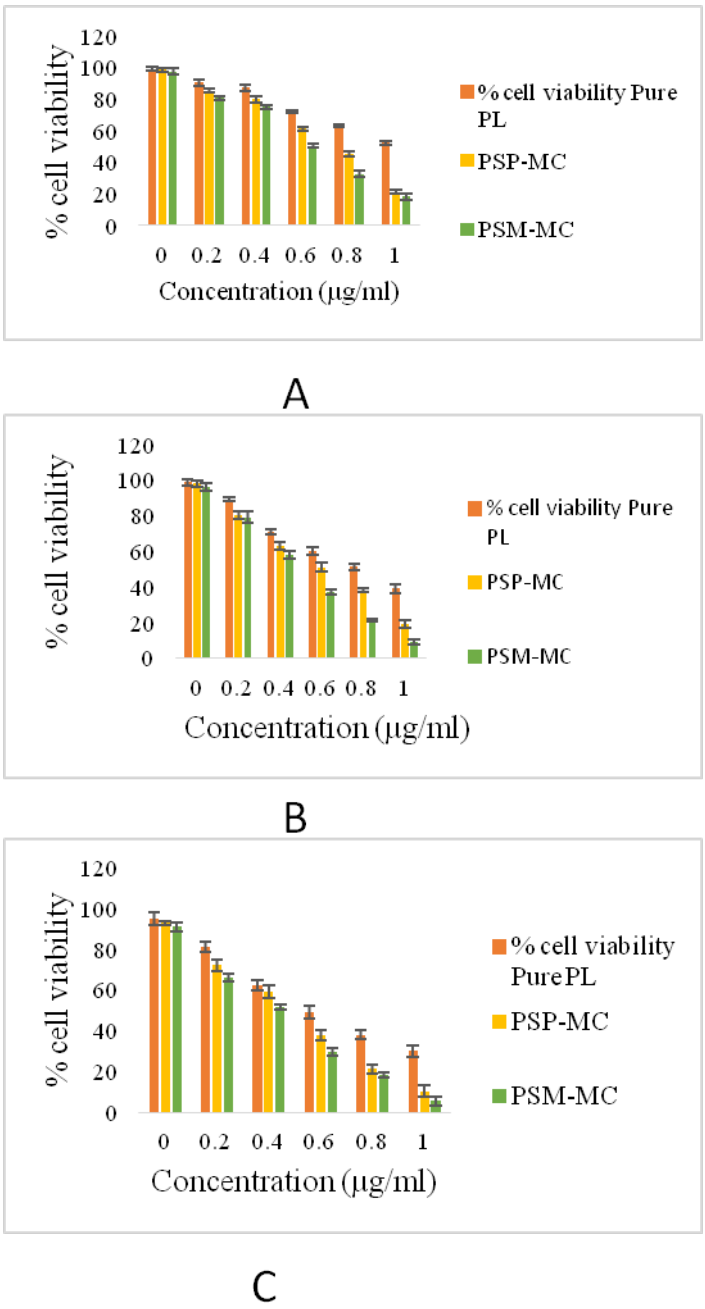


Figure 6: H-29 cells viability; A: after 24 hours incubation, B:after 48 hours incubation, C: after 72 hours incubation.

Y_2 (EE %) = 24.46 + 0.044 X_1 + 0.0185 X_2 - 0.1542 X_1X_2 - 0.2652 X_1^2- 0.0046 X_2^2

Y_3 (Percentage Yield) = 28.01 + 0.64 X_1 + 0.75 X_2 - 0.0512 X_1X_2 - 0.0652 X_1^2- 1.32 X_2^2

BPSM Batch

Y_1 (Particle Size) = 62.01 + 0.32 X_1 + 2.10 X_2 - 3.18 X_1X_2 - 0.065 X_1^2– 0.120 X_2^2

Y_2 (EE %) = 32.78 + 0.044 X_1 + 0.187 X_2 - 0.02542 X_1X_2 - 0.0652X_1^2- 0.146 X_2^2

Y_3 (Percentage Yield) = 48.01 + 0.04 X_1 + 0.65 X_2 - 0.0212 X_1X_2 - 0.1652 X_1^2- 2.42 X_2^2

CPSP Batch

Y_1 (Particle Size) = 79.05 + 0.620 X_1 + 3.10 X_2 - 1.18 X_1X_2 - 6.65 X_1^2 - 12.20 X_2^2

Y_2 (EE %) = 56.32 + 0.044 X_1 + 0.0185 X_2 - 0.1542 X_1X_2 - 0.2652 X_1^2- 0.0136 X_2^2

Y_3 (Percentage Yield) = 38.01 + 0.034 X_1 + 0.175 X_2 - 0.0412 X_1X_2 - 0.0652 X_1^2- 1.32 X_2^2

Where, X_1 and X_2 represent the independent variables which cause the main effects on responses Y_1, Y_2 and Y_3 in all the equations. X_1X_2, X_1^2, and X_2^2 are interactive terms that stand for the non-

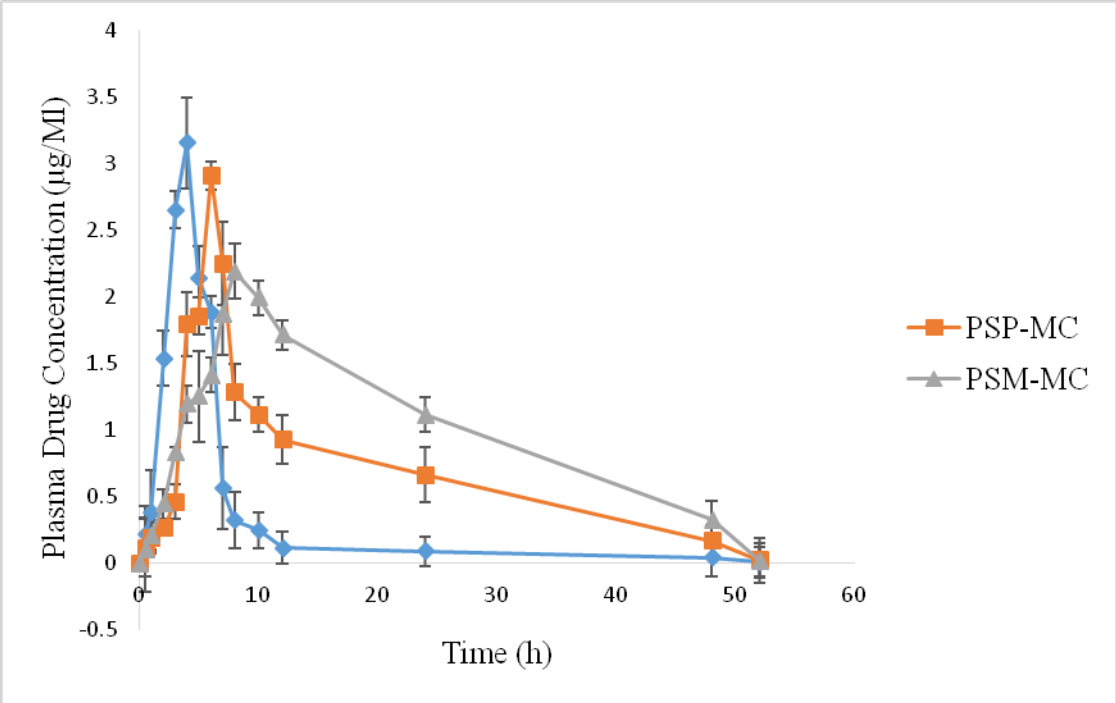


Figure 7: Plasma concentration ofpaclitaxel vs time curve for the paclitaxel immediate release intravenous injection, PSP-MC, PSM-MC microcapsules

linear relationship between the responses. The positive sign in the equation symbolises synergistic effects, and the negative sign indicates antagonistic effects on the responses. This equation states that two independent variables (X_1 and X_2) have a positive effect on the responses. Similar results were reported by (Sathyamoorthy *et al.*, 2017) while optimising the poly (ε-caprolactone) containing paclitaxel nanoparticles.

FTIR Study

The result from FTIR study revealed that there is no significant shift of the FTIR spectra of the prepared microcapsules (APSA, BPSM and CPSP batch) showed characteristic peaks for paclitaxel and polymers with no significant shift in peak positions indicated that there was no chemical interaction befell between paclitaxel. They used polymers, thus confirming the drug was compatible with the polymers. The spectra were presented in Figure 1.

DSC Study

As per the thermograms obtained from the DSC study, it was perceived that there is no significant change in the peak position of prepared microcapsules as compared with pure drug and polymers. The thermograms overlay for batches APSA, BPSM and CPSP were presented in Figure 2.

Morphology of Microcapsules SEM Study

SEM photographs of paclitaxel loaded microcapsule at A: x 27, 500 mm; B: x 85, 200 mm; C: x 50, 500 mm; D: x 1000, 10 mm; for batches APSA, BPSM and CPSP were given in Figure 3. The surface of microcapsules was seen to be uneven with parallel crinkles on the surface. The microcapsules were almost spherical and irregular. The roughness detected on the surface was contemplation to be advantageous in improved mucoadhesion.

Evaluation of Microcapsules

The properties evaluated for microcapsules such as particle size, yield, encapsulation efficiency, swelling index and percentage mucoadhesion were presented in Table 2.

Particle Size (μm)

The size of prepared microcapsules varied from a lowest of 361 $\pm$ 0.50 μm to a highest of 931 $\pm$ 0.41 μm. The order of decrease in size of microcapsules was BPSM > APSA > CPSP. Increase in the size of paclitaxel microcapsule was noticed with the rise in the concentration of copolymer in every batch that could be compared with similar findings reported by (Nayak *et al.*, 2012). The increase in viscosity of the drug and polymer mixture leads to generate the big size droplet while dropping out from the needle. On the other hand, with the increase in stirring speed, the particle size of microcapsules reduces. This could be due to breakdown of droplets by the high speed of vortex.

Encapsulation Efficiency (%)

The encapsulation efficiency was observed in a

range of 42.72 ± 0.43 % to 98.12 ± 0.43 %. The order of increase in encapsulation efficiency of microcapsules was APSA < BPSM < CPSP. An increase in encapsulation efficiency was observed with the decreasing of primary polymer to copolymer ratio. The more proportion of sodium alginate may lead to more crosslinking with paclitaxel and prevented drug leaching into the calcium chloride solution.

Yield (%)

The percentage yield was observed in a range of 55.05 ± 0.03 to 91.03 ± 0.05. For a fixed concentration of polymers, the percentage yield increases from BPSM < APSA < CPSP. Per cent yield was found high in all the three batches, and no significant difference was found between the three batches and within the formulations of the individual batch. The concentration of blend of polymer used for preparing microcapsule in each batch was found to be sufficient enough to give good yield with minimising the manufacturing process loss.

Swelling Index

The microcapsules got swelled as the time proceeded. It was observed that the swelling index was increased with an increase in weight gain by microcapsule. The swelling index of the APSA Batch was found between 91.16 ± 2.12 to 95.12 ± 2.15. The swelling index in BPSM batch ranges between 70.43 ± 1.13 to 83.68 ± 1.12. In CPSP batch it showed batter swell ability as compared to the other batches it ranges from 87.54 ± 2.09 to 97.64 ± 2.15. This might be due to an increase in the concentration of povidone in the formulation.

Mucoadhesion Studies

The batches under investigation showed good mucoadhesion, i.e. For APSA batches, batch 5 (81.03 ± 1.30 %), for BPSM batches, batch 4 (90.86 ± 2.02 %) and CPSP batch showed maximum mucoadhesion for batch 5 (97.85 ± 1.08 %) adhesion than the other. The results obtained from three batches were compiled in Table 3 to compare variation between different batches. For a fixed concentration of polymers, the swelling index increases from BPSM < APSA < CPSP and the percentage mucoadhesion increases from APSA < BPSM < CPSP. These finding could be comparable with the similar results reported by (Rao *et al.*, 2014), with working on simvastatin.

3D Surface Response Plots and Counterplots

Batch APSA

It was observed from the 3D surface plot and counterplot for the particle size of APSA, that to decrease the particle size the concentration of acacia should be increased, and speed of magnetic stirrer also required to be improved. Microcapsules of particle size around 500 mm could be obtained at 1: 1.8 ratio of SA: acacia at a speed of around 950 rpm. It was observed from the 3D surface plot and counterplot for encapsulation efficiency of APSA, that the encapsulation efficiency could be enhanced by increasing the concentration of acacia and speed of magnetic stirrer. Microcapsules with 70 % encapsulation efficiency could be obtained at 1:1.7 ratio of SA: acacia at a rate of around 950 rpm. It was observed from the 3D surface plot and counterplot for percentage yield of APSA, that to increase the yield, the concentration of acacia should be increased, and speed of magnetic stirrer also required to be increased. The percentage yield of 80 % could be obtained at 1:2 ratio of SA: acacia at a rate of around 1000 rpm.

Batch BPSM

It was observed from the 3D surface plot and counterplot for the particle size of BPSM, that to decrease the particle size the concentration of macrogol should be kept at the medium level, and speed of magnetic stirrer also required to be increased. Microcapsules of particle size around 700 mm could be obtained at 1: 1.6 ratio of SA: macrogol at a speed of around 900 rpm. It was observed from the 3D surface plot and counterplot for encapsulation efficiency of BPSM, that the encapsulation efficiency could be enhanced by increasing the concentration of macrogol and speed of magnetic stirrer. Microcapsules with 75 % encapsulation efficiency could be obtained at 1:1.5 ratio of SA: macrogol at a speed of around 800 rpm. It was observed from the 3D surface plot and counterplot for percentage yield of BPSM, that to increase the yield the concentration of macrogol should be decreased and the speed of magnetic stirrer also required to be reduced. The percentage yield of 80 % could be obtained at a 1:1.3 ratio of SA: macrogol at a speed of around 550 rpm.

Batch CPSP

It was observed from the 3D surface plot and counterplot for the particle size of CPSP, that to decrease the particle size the concentration of povidone should be kept at a medium level, and speed of the magnetic stirrer was required to be increased. Microcapsules of particle size around 300 mm could be obtained at 1: 1.5 ratio of SA: macrogol at a speed of around 1000 rpm. It was observed from the 3D surface plot and counterplot for encapsulation efficiency of CPSP. The encapsulation efficiency could be enhanced by increasing the concentration of povidone and speed of magnetic stirrer. Microcapsules with 90 % encapsulation efficiency could

be obtained at 1:1.5 ratio of SA: povidone at a speed of around 950 rpm. It was observed from the 3D surface plot and counterplot for percentage yield of CPSP, that to increase the yield the concentration of povidone should be kept at medium level and speed of magnetic stirrer also required to be decreased. The percentage yield of 85 % could be obtained at a 1:1.6 ratio of SA: povidone at a speed of around 800 rpm. The 3D surface response plots were presented in Figure 4. Similar observations were observed by (Aslan and Cebeci, 2007) while studying the response surface methodology.

In vitro Drug Release Study

The result from in vitro drug release from various batches insinuates that the concentration of copolymer affects the release profile of paclitaxel. APSA5 formulation showed release of 92.74 ± 2.51 % at 24 h. BPSM6 formulation showed a version of 95.63 ± 3.32 % at 24 h. The best uniform release profile of drug shown by CPSP2, i.e. 96.75 ± 2.41 % at 24 h. The results were presented in Figure 5. These finding could be comparable with the similar results reported by (Mahapatra *et al.*, 2020) while working with sodium alginate microcapsules.

Release Kinetics Study

In order to understand the in vitro release kinetics of drug from paclitaxel loaded microcapsule, the experimental data obtained in the drug dissolution studies were fitted into suitable models. The results for release rate constant K_s and correlation coefficients R^2 for different models were calculated. The APSA batch followed Higuchi release model (R^2 > 0.9944). BPSM batch followed Higuchi release kinetics (R^2 > 0.9760). CPSP batch followed first-order release kinetics (R^2 > 0.9172). These finding could be comparable with the similar results reported by (Zhang *et al.*, 2010a).

MTT Assay on Human HT-29 Colon Cancer Cell Lines

The cytotoxic activity of pure paclitaxel, sodium alginate- macrogol microcapsule (PSM-MC) and sodium alginate- povidone microcapsule (PSP-MC) were assessed by MTT assay. It was observed that incubation with increased concentration pure paclitaxel from 0.2 to 1 μg/mL showed an increase in the diminution of cell viability. The cell viability was reduced to 60.01 ± 0.01 % after 24 h. This could be compared with a similar report by (Ganguly *et al.*, 2016) while working with 5-fluorouracil. Cell viability after 48 h and 72 h for pure paclitaxel at the strength of 0.2 μg/mL was 97.03 ± 0.01 % and 96.03 ± 0.02 % respectively and at the strength of 1μg/mL. The cell viability after incubation of 48 h and 72 h were 42 ± 0.01 % and 38 ± 0.02 % respectively (Tekade *et al.*, 2013). The microcapsules batch PSP-MC The cell viability after 24 h, 48 h and 72 h were 21.03 ± 0.06 %, 19.07 ± 0.02 % and 10.32 ± 0.03 %. The microcapsules of batch PSM- MC were 18.22 ± 0.04 %, 9.22 ± 0.05 % and 5.47 ± 0.02 %. The result from the study could be deciphered as the prepared microcapsules increase cell viability as compared to pure drug. The strength of 0.5 μg/mL was found to be effective in producing inhibition of 50 % in the case of prepared microcapsules. The results were presented in Figure 6.

In Vivo Study

All the developed microcapsule containing paclitaxel PSP-MC and PSM-MC demonstrated a significantly high area under the curve (AUC), concentration maxima (C_{max}) and time to reach maximum concentration (T_{max}) when compared with paclitaxel intravenous administered paclitaxel. High T_{max} is in substantiation with sustained in vitro release of paclitaxel from the microcapsules. Formulated microcapsule also showing high mean residence time and thereby having enhanced bioavailability. The results were presented in Figure 7. The results obtained could be comparable with the similar findings reported by (Li *et al.*, 2015) while working with paclitaxel.

CONCLUSIONS

The study with 3^2 factorial design concluded that the proportion of primary polymer: copolymers has a positive effect on particle size, encapsulation efficiency and yield. Povidone containing microcapsules slowly swelled in the intestine and consequently adhered to the intestinal mucosa. This allowed more drug absorption by cells and overwhelmed the diffusion barriers by extending the intestinal transit time. The results from in vitro study established a good correlation between results perceived from in vivo study. Microcapsules of paclitaxel found successful in sustaining controlled release of drug for a more extended period. The results seen from MTT assay signposted the perpetuation of the cytotoxic effect of paclitaxel encapsulated mucoadhesive microcapsules. The novel polymeric microcapsule containing the anticancer drug was demonstrated to give promising oral paclitaxel delivery in the treatment of intestinal carcinoma. Clinical findings in humans are required to be further reconnoitred in order to ascertain the safety and therapeutic efficacy of paclitaxel microcapsules.

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Conflicts of Interest

The authors certify that they have no conflicts of interest in the subject matter or materials discussed in this manuscript.

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