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Original Article

Formulation and Evaluation of Aspirin-PLGA Microsphere for the Dental Stem Cell Stimulation

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ABSTRACT

Aim: According to WHO, dental caries is the most prevalent oral disease, and its progression leads to tooth loss. Clinical management of caries focuses on the severity and extent of disease with the main aim, i.e., the 'art' of creating a good restoration. Recently, it has been reported that aspirin can stimulate existing stem cells and regenerate damaged teeth. But, the therapeutic effectiveness of a drug depends on developing a suitable novel drug delivery system, to retain at the site and suitably release the drug to produce effective therapy. Therefore, the present investigation intends to develop Aspirin-Poly lactic-co-glycolic acid microspheres for the restoration of dentin. **Materials and Methods:** Aspirin- Poly lactic-co-glycolic acid microsphere was formulated by the double emulsion technique and evaluated for particle size, encapsulation efficiency, characterization (differential scanning calorimetry, X-ray powder diffraction), *in vitro* release, as well as irritation testing using the Hen's egg test-chorioallantoic membrane method. **Results:** The formulation exhibited good encapsulation efficiency (87.31±1.52%) and a particle size of 7.52 µm by Scaning Electron Micrscopy. *In vitro* release study exhibited sustained release (98.76±0.49%) for 16 days and triphasic release. This confirms that release is due to polymer erosion, swelling, and degradation. The *ex vivo* permeation study also confirmed sustained permeation and showed the significant partition and accumulation of the drug in the tissue. Further, the prepared formulation showed significantly low irritation compared to positive control by Hen's Egg Test-Chorioallantoic Membrane method. **Conclusion:** Thus, the above finding suggests that the formulation can stimulate stem cells for the regeneration of dental tissue.

Keywords: Stem cell, Dental caries, Sustained release, Aspirin, Dentinogenesis, Microsphere.

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INTRODUCTION

According to World Health Organization, oral health plays a crucial role in good health and well-being.[1] Improper oral care may lead to a severe threat to the oral cavity causing cavity to cancer and systemic diseases.[1] Dental caries is a multifarious infection that develops due to bacterial biofilm (dental plaque), leading to the demineralization of hard dental tissues.[2,3] If the disease is not treated properly, it will lead to the formation of a dental cavity and even permanent loss of the tooth. The main aim of the management of present diseases is to prevent the progression of the disease by pharmacological agents and also some non-therapeutic approaches like surgical intervention, and mechanical therapy, depending on the stage of the disease and person to person.[4] Although these therapeutic and non-therapeutically approaches provide satisfactory clinical efficacy in the control of infection, pain and seal the space in the cavity. But, these treatment modalities, may lead to tooth fracture, loss of the tooth, or even reinfection. However, till now no treatment is developed for the restoration of the decayed tooth. Hence, this remains a thrust area for pharmaceutical scientists to explore and develop a suitable method for the complete restoration of teeth.

Aspirin (Acetyl Salicylic Acid, ASA) most widely used non-steroidal anti-inflammatory drug (NSAIDs), is used to modulate a variety of disease conditions like pain[5] (toothaches, and headaches), cardiovascular disease,[5,6] arthritis[5,7] and anti-inflammatory.[8] Recent studies reported that Aspirin is capable of stimulating the various dental stem cells, this promotes the migration, proliferation, and differentiation of odontoblast cells responsible for the enhance the regeneration of dentin.[9-11] These findings strongly suggest that Aspirin has excellent potential in the management of several dental problems for

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which there is no clear-cut treatment strategy. Hence, Aspirin's therapeutic effectiveness depends on the design and development of a suitable novel drug delivery system for Aspirin.

MATERIALS AND METHODS

Materials

Aspirin was a gift from Alta Labs, India, also PLGA (75:25) was a gift sample from Evonik, India, and ethyl acetate was procured from S.D. fine chemicals. India, Poly (vinyl alcohol) (MW 1,30,000, 18-88% hydrolyzed) was obtained from Sigma-Aldrich. Analytical grade chemicals and solvents were used.

Methods

Formulation and optimization of Aspirin loaded microsphere

Aspirin-PLGA microspheres were formulated by the double-emulsion-solvent extraction method (w/o/w).[12,13] Aspirin in the aqueous solution (different volume) was dispersed into the ethyl acetate-ethanol solvent system containing PLGA (different concentration) resulting in a primary emulsion. For stabilization, the emulsion was homogenized using a probe homogenizer (Kinematica Polytron™ PT2100) and to the aqueous phase of polyvinyl alcohol (PVA, different ratios) the primary emulsion was injected and stirred continuously to form a W/O/W emulsion. Microspheres formed were hardened by pouring into a defined volume of water,[12] stirred overnight at room temperature to remove the solvent, separated by centrifugation (Remi Equipment Pvt. Ltd., India), and washed continuously. Schematic diagram of the formulation is represented in Figure 1.

Box-Behnken (BB) design (3-factor 3-level) was adopted to optimize the concentration of PLGA (X_1), Volume of the internal phase of primary emulsion (mL) (X_2), PVA concentration (%) (X_3) as the independent variable and encapsulation efficiency (Y_1, %), and mean particle size (Y_2, μm) as dependent variable. The level of the selected factors were fixed based on the preliminary trials conducted and applied in BB design (Table 1).

Characterization of Aspirin PLGA Microsphere

Powder X-ray Diffraction (PXRD)[14,15]

PXRD for the drug, polymer, physical mixture, and formulations were carried out using a D8 Advance BRUKER diffractometer. Samples were mounted on aluminum plates and measured at 2θ diffraction angle from 0 to 40° with a source of nickel-filtered and Cu-anode ceramic[14,15]

Differential Scanning Calorimetry Analysis

The thermograms of pure drugs, polymers, and formulations were recorded (Perkin Elmer Corporation, Mississippi, MA, USA). A aliquot amount sample was heated in a closed aluminum pan. Measurements were done at a scanning rate of 10°C/min between 30 and 350°C.[14,15]

Particle size and Polydispersity Index

The mean particle size of the formulated microspheres were calculated by using an optical microscope equipped with a camera. The measured mean particle size was used to study the Polydispersity Index (PDI).[16]

Surface morphology

The shape and the surface morphology of the optimized microsphere were examined with scanning electron microscopy (SEM, Joel JSM-6490la, Japan).

Encapsulation Efficiency

The amount of Encapsulated (EE) was calculated by the ratio of the difference between the amount of Aspirin used for microsphere formulation and the amount of non-entrapped Aspirin remaining in the external phase after microsphere formation to the amount of Aspirin used for the formulation.[17,18]

$$EE(\%) = \frac{(\text{mass ofdrug loaded in MS})^{*}100}{\text{mass of drug processed}}$$

In vitro release study

An aliquot amount of microsphere is filled into the dialysis tube (21 mm, Mw: 8000–14400 Da), fastened on both sides. The bag is suspended into 50 mL of the simulated salivary fluid at 50 rpm 37°C. At specified intervals of time, the sample was withdrawn, filtered, and analyzed at 265 nm to find out the amount of drug release.[19]

The release data were further analyzed to study the release mechanism by fitting into the five kinetic models: zero-order, first-order, Higuchi and Korsmeyer– Peppas, and Hixson-Crowell equation was evaluated by DD solver.

Ex vivo Permeation Studies

Ex vivo permeation studies were conducted using modified Franz diffusion cells with an excised porcine buccal mucosa.[20] The tissue was mounted on the cell and the microsphere was placed on the donor compartment. The receptor compartment was filled with simulated salivary fluid at 37±0.5°C and 50 rpm. The sample was withdrawn at a set time and were analyzed.[21,22]

Drug Deposition studies in Mucosa

After permeation studies, the buccal mucosal was sonicated with a known amount of ethanol and analyzed.[21]

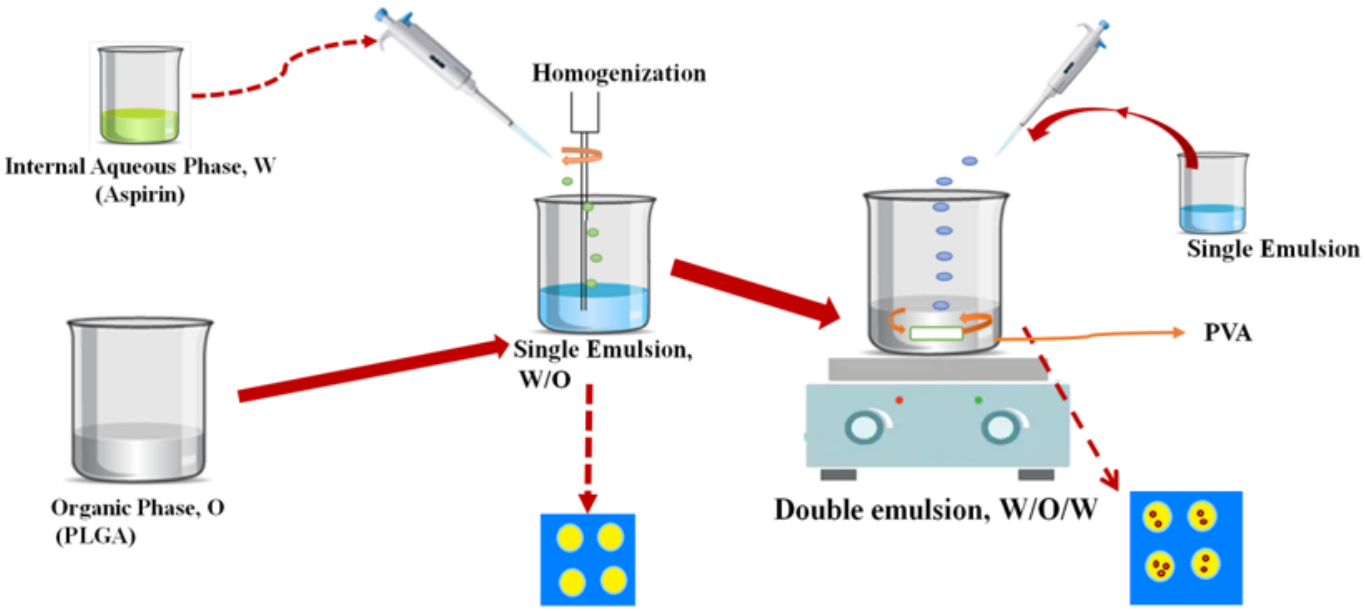


Figure 1: Schematic representation of formulation of Aspirin PLGA microsphere by double emulsion method.

Table 1: Independent and dependent Variable Levels in Box-Behnken design.

Independent Variables	Levels		
	Low (-1)	Center point (0)	High (+1)
X_1-PLGA(mg)	250	500	750
X_2-Volume of internal phase of primary emulsion(mL)	0.75	1.00	1.25
X_3 - PVA concentration(%)	0.5	1	2
Dependent Variables	Constraints		
Y_1- encapsulation efficiency (%)	Maximize		
Y_2-Particle size(μm)	Minimize		

Irritation testing

The optimized formulation was used to study the irritative reaction on the gingival tissue, which was performed on the hen egg using HET-CAM (Hen's egg test-chorioallantoic membrane) technique. The procured eggs were incubated at 37±0.5°C for ten days and rotated every day.[23,24] On the 10th day of incubation, non-viable eggs were discarded by the candling method. Selected egg shells were moistened with 0.9% sodium chloride and carefully shells were removed, and the Chorioallantoic Membrane (CAM) was exposed.

The eggs were divided into the following category

Positive control: Sodium Hydroxide (0.1 N)

Negative control: 0.9% sodium chloride

Test formulation(T1): Optimized aspirin PLGA microspheres

0.3 mL of the above formulations were applied on the CAM and was observed for the endpoint for 300s. Time for the Hemorrhage (H), Vascular Lysis (V), and Coagulation of Protein (C) to appear was recorded as the endpoint. The irritancy potential is calculated and expressed as an Irritation Score (IS), ranging from 0-21(Luepke scale).[24]

$$IS = \frac{5(301-H)}{300} + \frac{7(301-V)}{300} + \frac{9(301-C)}{300}$$

The average score IS is classified from non-irritant to strongly irritant.

RESULTS AND DISCUSSION

According to the recent study, aspirin aids in the enhancement of dental tissue regeneration by stimulating the stem cells.[10] So, a suitable drug delivery system is necessary to deliver the drug to the site of action and efficiently release the drug to increase its efficacy. PLGA polymer was selected due to its potential role in dental tissue regeneration, by inhibiting the early degeneration of gingival epithelium and connective tissues and enhancing regeneration by repopulating the denuded root surface with cells. This can step towards achieving mechanical stability and three-dimensional niches for the growth of new tissue.[25] Therefore,

Table 2: Results of process variables on dependent variable.

Formulation	Concentration of PLGA(mg)	Volume of internal phase(mL)	PVA concentration(%)	Encapsulation Efficiency(%)	Particle size(μm)
1	250	0.75	1	48.75±2.31	39.45±1.24
2	500	1	1	60.87±1.32	40.51±2.34
3	500	0.75	0.5	66.67±0.67	22.75±0.56
4	250	1	2	62.24±1.56	19.73±1.25
5	500	0.75	2	81.3±2.3	15.7±1.34
6	750	1	2	89.97±1.34	30.23±2.3
7	750	0.75	1	85.56±1.25	41.38±0.78
8	500	1.25	2	83.09±1.34	34.41±0.89
9	750	1	0.5	67.25±1.56	38.44±1.23
10	250	1	0.5	42.17±2.34	39.89±2.3
11	250	1.25	1	47.16±1.67	68.2±2.5
12	500	1.25	0.5	68.71±1.56	58.24±2.3
13	750	1.25	1	74.78±2.78	75.61±2.67

*Mean±SD, n=3

PLGA is selected for the formulation of the aspirin microsphere, to achieve collective synergistic regeneration of the dental tissue in dental conditions like dental caries. Aspirin-loaded PLGA microsphere was formulated by the double emulsion-solvent extraction method, and preliminary screening revealed that concentration of PLGA, volume of internal phase and PVA concentration had significant effect on the entrapment efficiency and hence to optimize and examine the effect and the interaction of various factors, the Box Behnken design was ultilized. The variables are concentration of PLGA (X_1), Volume of the internal phase of primary emulsion (mL) (X_2), PVA concentration(%) (X_3) at constant homogenization time, stirring speed for double emulsion, emulsification and flow rate, the experimental runs were employed to maximize encapsulation efficiency (Y1) and minimize the mean particle size (Y2). The effect of independent variables on dependent variables was evaluated and contour plots were developed and represented in Table 2.

Effect on Encapsulation efficiency

The encapsulation efficiency of developed microspheres was found to be between 42.17±2.34% -89.97±1.34%.

The given polynomial equation was projected by the model for the effect of independent variables on the encapsulation efficiency

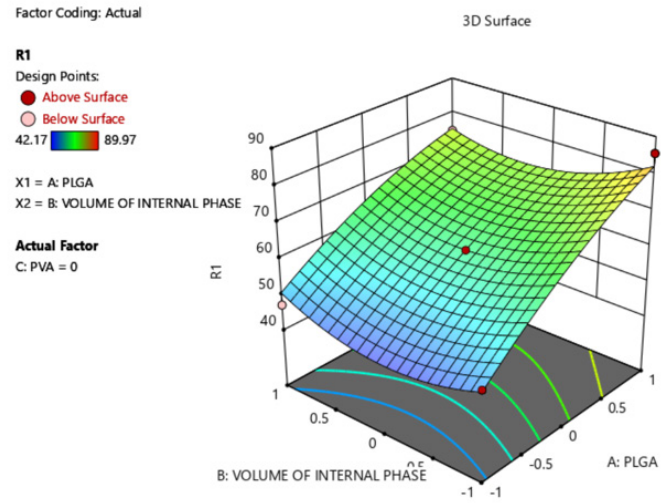
$$\text{Encapsulation Efficiency} = 60.87+14.65X_1-1.0675X_2+8.975X_3-2.2975X_1{*}X_2+0.6625X_1{*}X_3 -0.062497X_2{*}X_3-3.17125X_1^2+6.36375X_2^2+7.70875X_3^2$$

The model was considered significance with F-value = 12.37, $p<0.05$. The developed model's R^2 value of 0.9976 demonstrated strong correlation between experimental and predicted values. Analyzing the model, PLGA concentration (X1), PVA concentration (X3) had positive effect on the entrapment efficiency and was significant, whereas the volume of internal phase (X2) had negative effect and exhibited no significant effect (Figure 2).

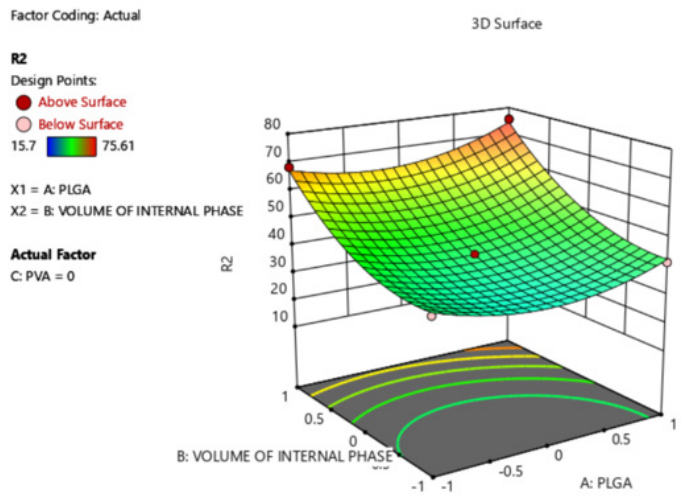
It is clear from the results that the encapsulation of aspirin significantly depends on PLGA and PVA concentration, indicating increase in PLGA and PVA concentration enhances the encapsulation efficiency. The increased PLGA concentration will lead to increased polymer concentration in the orangic phase, thereby enhances rate of polymer precipitation and solidification at constant temperature and homogenization. This prevents the drug from diffusing from the organic phase, increasing encapsulation efficiency.[12,26]

The second parameter that direct relation on encapsulation efficiency is PVA. This can be accounted for by the fact that viscosity increases with PVA concentrations. Furthermore, PVA stabilizes the double emulsion, contributing to higher resistance to aspirin diffusion out of the polymeric phase, resulting in higher encapsulation efficiency in microspheres prepared with more PVA.[12]

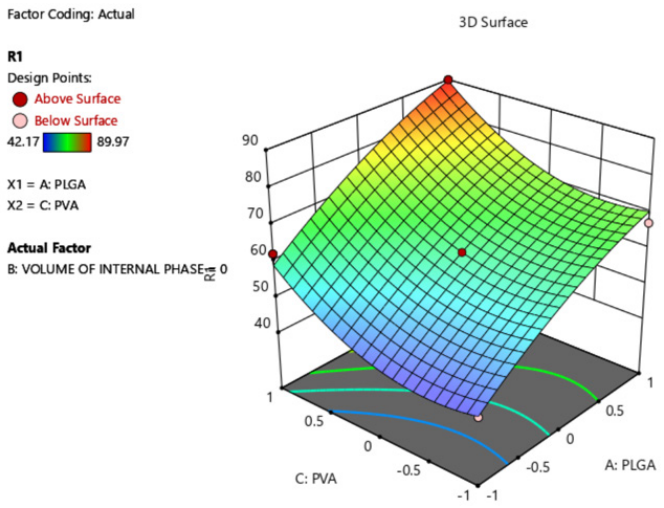
Whereas the volume of the internal phase exhibited negative encapsulation efficiency, this may be to the fact that an increase in the concentration of the aqueous phase may lead to a higher rate of leaching, physical instability of the primary emulsion, and hence leads to a decrease in encapsulation efficiency.[27,28]



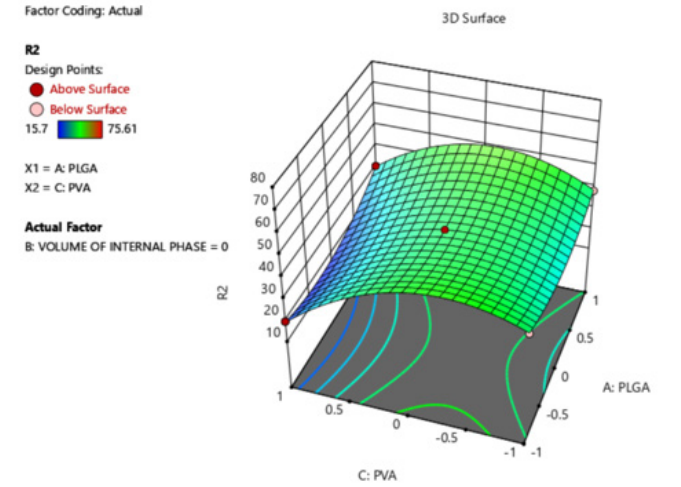
A. PLGA and volume of the internal phase



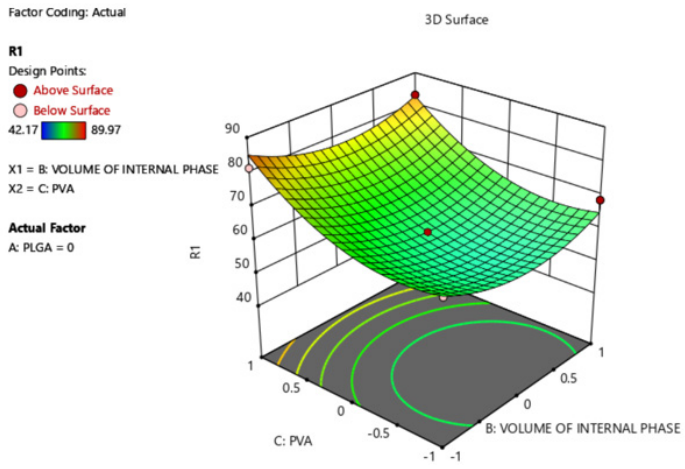
A. PLGA and volume of internal phase



B. PLGA and PVA concentration

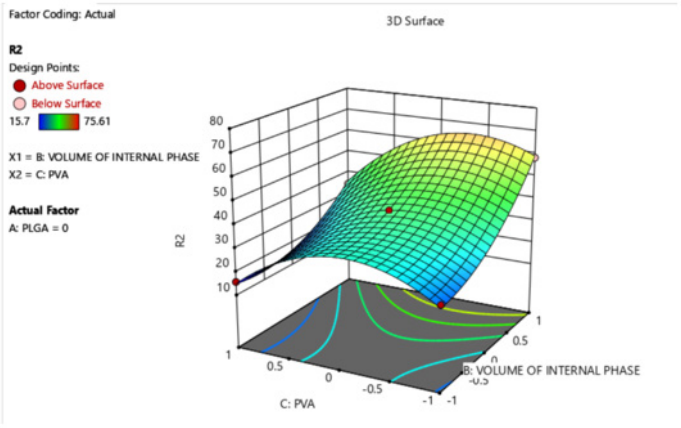


B. PLGA and PVA concentration



C. Volume of internal phase and PVA concentration

Figure 2: 3D surface plot for the effect of process parameter on encapsulation efficiency.



C. Volume of internal phase and PVA concentration

Figure 3: 3D surface plot for the effect of process parameter on particle size.

Effect on Particle size

The effect PLGA concentration (X1), the volume of internal phase (X2), and PVA concentration (X3) on particle size was analyzed and found between 15.75 µm and 75.61 µm.

$$\text{Particle Size} = 40.51 + 2.29875X1 + 14.6475X2 - 7.40625X3 + 1.37X1*X2 + 2.9875X1*X3 - 4.195X2*X3 + 7.47375X12 + 8.17625X22 - 15.91125X32$$

The model was considered significance with *F*-value = 121.86, $p<0.05$. The developed model's R^2 value of 0.9973 demonstrated strong correlation between experimental and predicted values. Analyzing the model linear terms of PLGA (X1), volume of internal phase(X2), PVA (X3), and interaction between PLGA and PVA concentration (X_1*X_3) and volume of internal phase*PVA concentration (X_2*X_3) are significant in 95% confidence level, whereas PLGA concentration and volume of internal phase (X_1*X_2) is non-significant (Figure 3).

The results shows that as PVA concentration increases, the particle size decreases. This accounts that PVA (stabilizer) can orient at the interface of organic and the aqueous phase. Therefore, reduces the interfacial tension by enhancing the net shear. Thus, results in smaller particle sized microsphere.[12,29]

Whereas, as mentioned PLGA concentration and volume of the internal phase positive impact. This could be as a result of the fact that when the volume of the internal phase increases, the droplet size raises and results in increased size of microspheres.[26-28] Furthermore, when PLGA concentration increases, viscosity increases. This decreases the shear force and forms a larger globule size.[29]

Optimization of process parameter

The independent variables were optimized by employing Design-Expert software, after identifying the relationship between the main effects (PLGA, Volume of internal phase, and PVA concentration) on both encapsulation efficiency and particle size. The levels of independent variables that spontaneously produced the highest enacapsulation efficiency and smallest particle size was established using desirability function. Based on the generated model, microspheres mean 22.51 µm sized with 89.43% encapsulation efficiency were calculated with minimum errors, 3.8% and 1.9%, respectively represented in Table 3 and Figure 4.

The optimized microspheres were evaluated for particle size, polydispersity index, and SEM. Obtained results reveal that the particle size is 4.49±0.8 µm, the polydispersity index is 1.0025, and SEM results indicate that the microsphere is smooth and has spherical surface morphology. Particle size, particle size distribution, and SEM of the optimized microsphere were given in Figure 5.

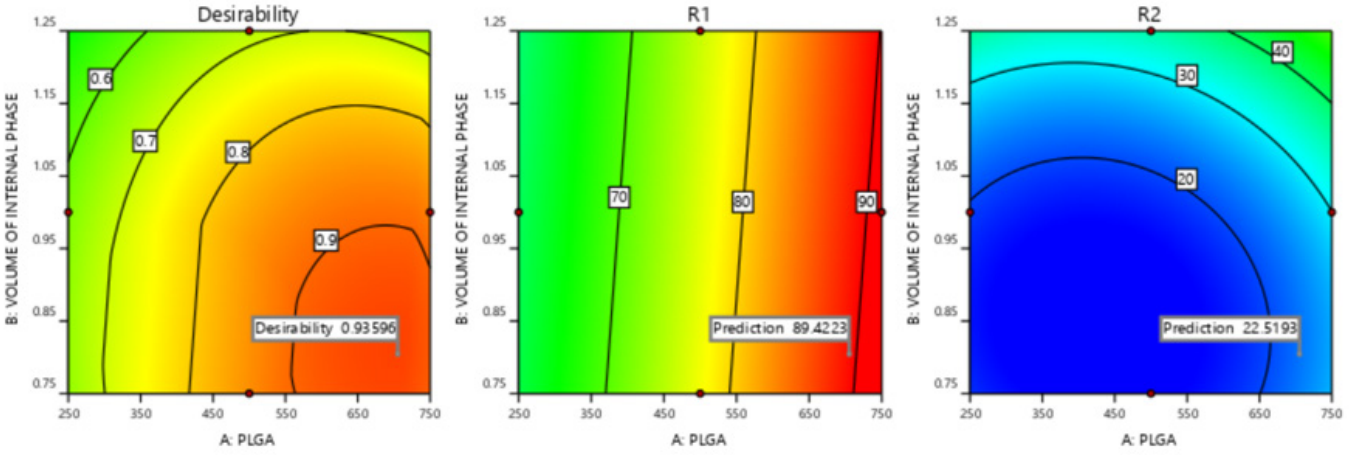


Figure 4: Desirability plot of optimized formulation.

Table 3: Predicted and experimental response of optimized formulation.

Factors			Desirability	Model		Experiment		PDI
Concentration of PLGA(mg)	Volume of internal phase(mL)	PVA concentration(5)		EE(%)	Particle size (µm)	EE(%)	Particle size(µm)	
705.29	0.8	2.00	0.935	89.43	22.51	87.65±1.8	4.49±0.8	1.0025

*Mean±SD, $n=3$

Table 4: *In vitro* Release study of the optimized formulation and drug.

Time(hr)	% Cumulative drug release (optimized formulation)	% Cumulative drug release (Aspirin)[a]
0	0.00	0
0.25	0.31±0.11	12.9±1.23
0.50	1.66±0.35	22.7±0.87
0.75	2.33±0.08	46.6±1.09
1	3.01±0.13	56.9±0.98
2	3.73±0.4	69.9±1.34
3	4.79±0.46	79.9±1.87
4	5.79±0.39	98.7±0.98
5	6.97±0.49	
6	7.96±0.73	
24	10.57±0.32	
48	13.44±1.35	
72	15.69±2.69	
96	18.36±4.2	
120	21.06±5.35	
132	26.65±4.89	
144	33.83±5.84	
168	39.44±5.96	
192	46.11±4.90	
216	53.42±5.18	
240	62.97±5.2	
264	70.85±4.21	
288	77.44±4.42	
312	84.12±4.36	
336	90.66±3.5	
360	96.10±2.97	
384	98.76±0.49	

*Mean±SD, *n*=3

Characterization of Optimized Microsphere

The raw materials and optimized formulation was characterized by XRD and DSC thermograms. The XRD patterns of Aspirin, PLGA, and Aspirin-loaded PLGA microspheres were recorded and aspirin exhibited a sharp and well defined peaks at 15.9◦, 17.1◦, 21.3◦, 22.8◦, 27.3◦ indicates crystalline nature (Figure 6). Similar peaks were reported in the literature.[14,15] Whereas, no distinct and intense peak in the diffractogram of PLGA was observed demonstrating the amphorous nature. Aspirin peaks were not observed in the XRD pattern of the Aspirin microsphere, exhibiting that aspirin is uniformly dispersed in the polymer matrix, revealing the amorphous form.

Similarly, the DSC studies are an important thermoanalytical tool to confirm the physical nature and the stability of the drug during in the formulation. In the present study, Aspirin exhibited endothermic peak at 144. 82°C (sharp) with 170.98 J/g of enthalpy of fusion (H)[14,15] represents its melting point (Figure 7). Another endothermic peak was also observed at 172.24°C, which may be partly superimposed to the melting one and is ascribable to the thermal degradation of aspirin, this confirms the crystalline nature of the drug. In PLGA (75:25), no peak observed for pure PLGA polymer, confirming its amorphous nature. Whereas, the drug's peak in the thermogram of aspirin PLGA microsphere has disappeared, while a wide peak was observed near 36.76°C.

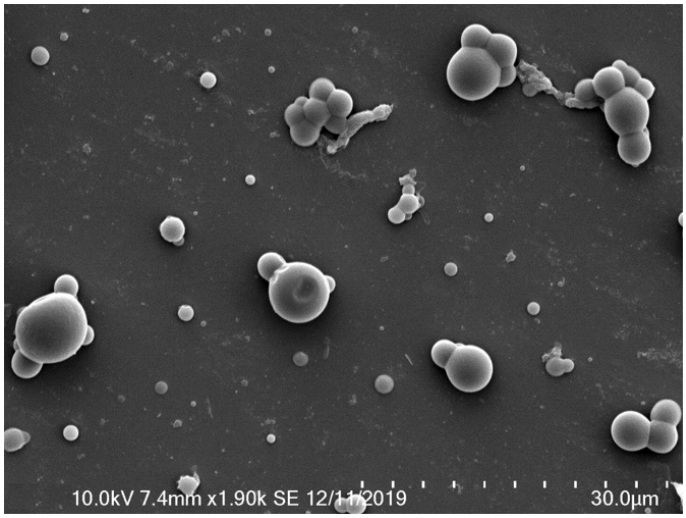


Figure 5: Scanning electron microscopy of optimized Aspirin PLGA microsphere.

In vitro release study

The release study PLGA microsphere is represented in Figure 8 and Table 4, it is clear that aspirin tended to release a lesser amount of drug ~10.57±0.32% initial 24 hr, with relatively 98.76±0.49% drug being released within 16 days. The release pattern indicates a triphasic release profile[30,31] i.e. an initial slight burst phase (0-1d), subsequently steady lag phase (1-5d), and a rapid burst release phase(5d ~ 16d). Literature reveals that release is due to diffusion, hydrolysis, and erosion. The initial burst release may account for the diffusion of drug molecules trapped on the surface of the microsphere, followed by a second phase (lag phase), which may be controlled by polymeric erosion. Further, the second burst effect is due to the degradation of polymeric matrix.[31,32]

The above-mentioned drug release data was used to determine the kinetics necessary to know the pattern and mechanism of release and is recorded in Table 5. Formulation followed the zero-order release model as compared to Hixson Crowell followed by the Higuchi model. The '*n*' values from the Korsmeyer-Peppas model were found to be 0.619 which follows between $0.45 < n = 0.89$, indicates the release in non-Fickian transport. Hence the study confirms that release is due to polymer erosion, swelling, and degradation.[32]

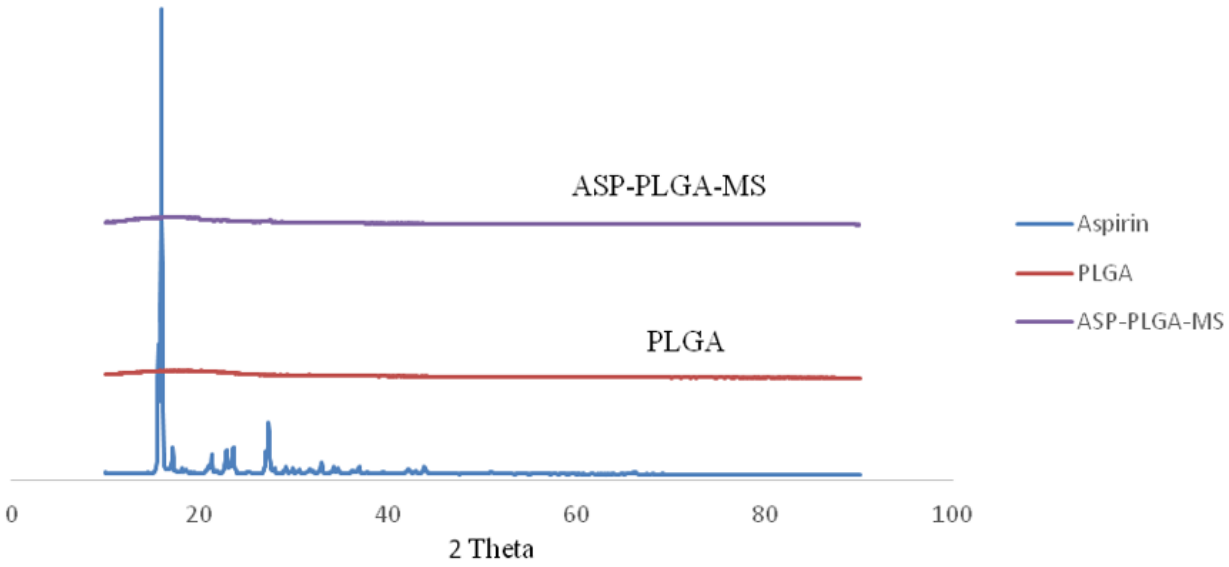


Figure 6: X-ray Diffractation pattern of Aspirin, PLGA, and Aspirin-loaded PLGA microspheres.

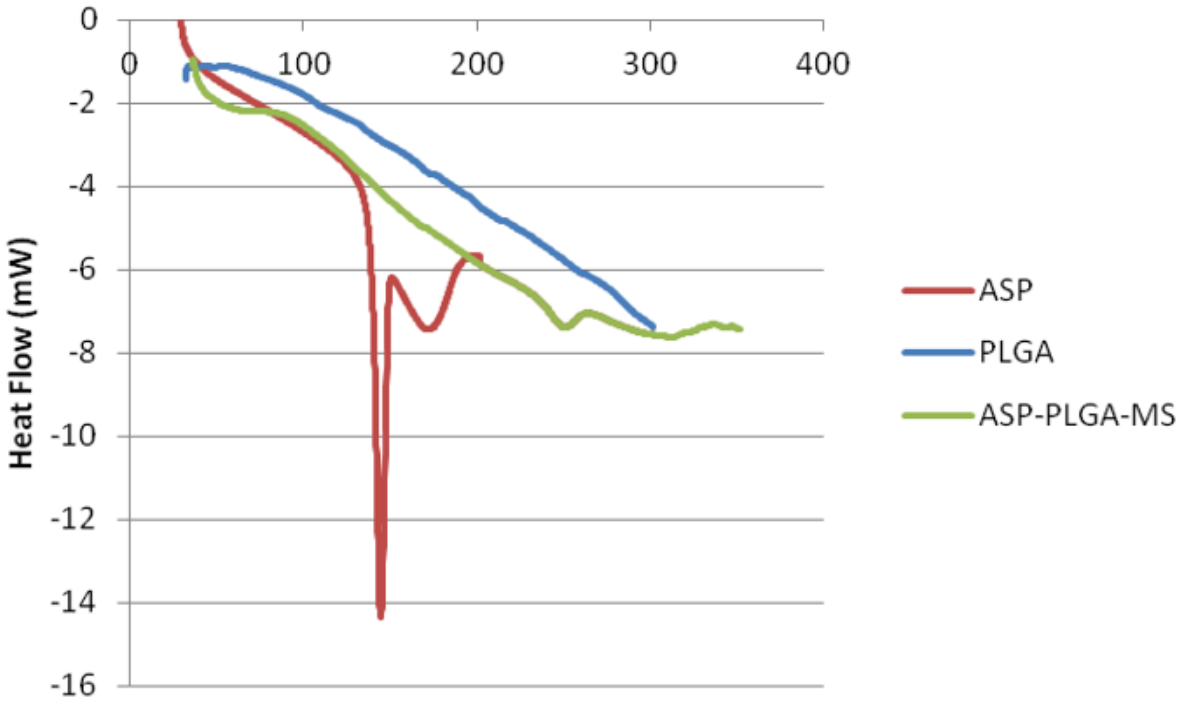


Figure 7: DSC thermogram of Aspirin, PLGA, and Aspirin-loaded PLGA microspheres.

Table 5: *R*²′ **values of various kinetic models and value of 'n'.**

Kinetic model	Zero order R^2	First order R^2	Higuchi R^2	Hixson Crowell R^2	Korsmeyers-peppas	
					R^2	n
Optimized microsphere	0.984	0.780	0.889	0.901	0.939	0.619

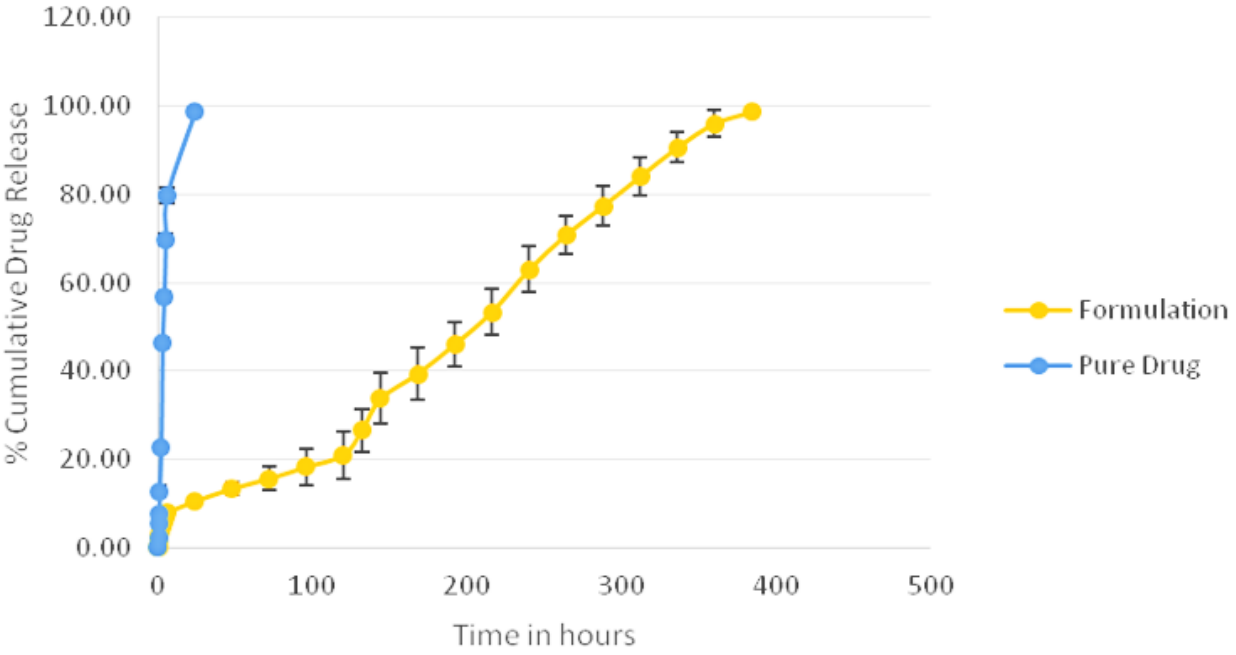


Figure 8: *In vitro* release pattern of Aspirin from PLGA Microsphere.

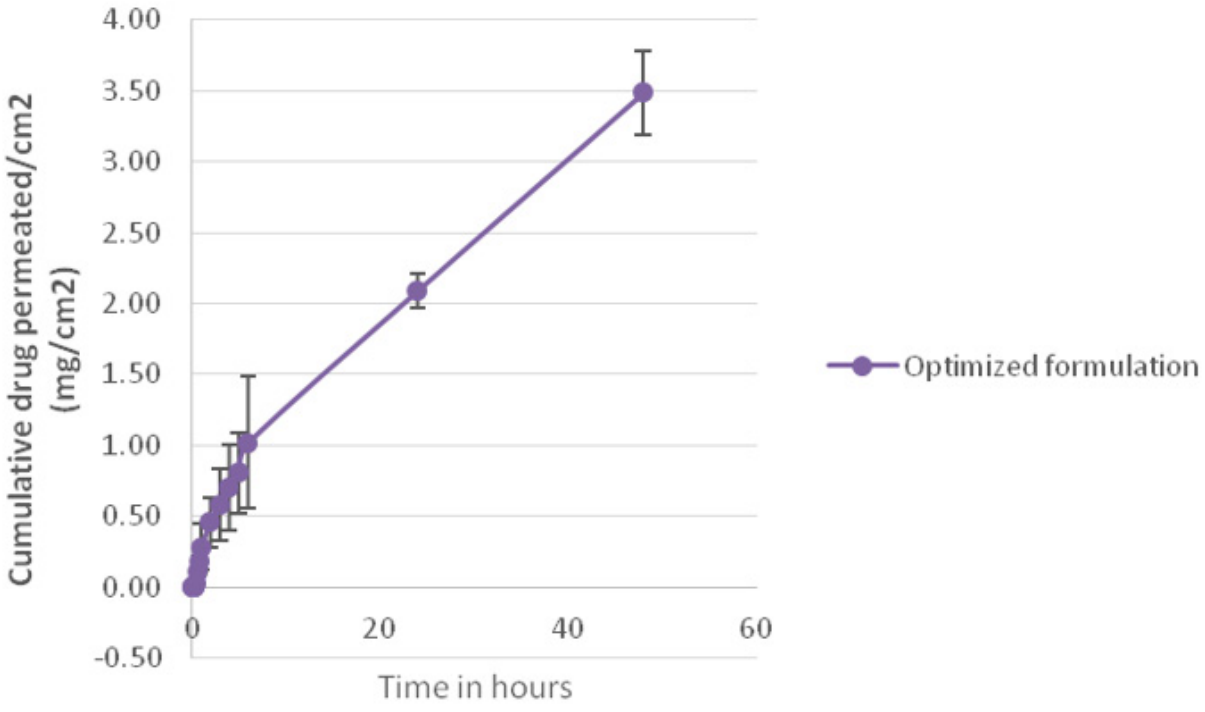


Figure 9: *Ex vivo* permeation studies of Aspirin PLGA Microsphere.

Ex vivo permeation study

Ex vivo permeation study was conducted through porcine buccal mucosa due to the following reason: human and porcine buccal mucosa were non-keratinized,[21] with the cell nucleus observed in the superficial layers, permeation behavior through porcine mucosa was significantly higher compared to that of bovine mucosa, and also the thickness buccal mucosa of porcine mucosal was almost same to human.[33] The permeation study was carried out for 48 hr with 26.05±2.21% drug release and illustrated in Figure 9. Permeation study reveals that permeation flux, permeability coefficient (Kp), diffusion coefficient and Partition coefficient are calculated and recorded in Table 6. Also, the study shows that the microspheres are partitioned into the

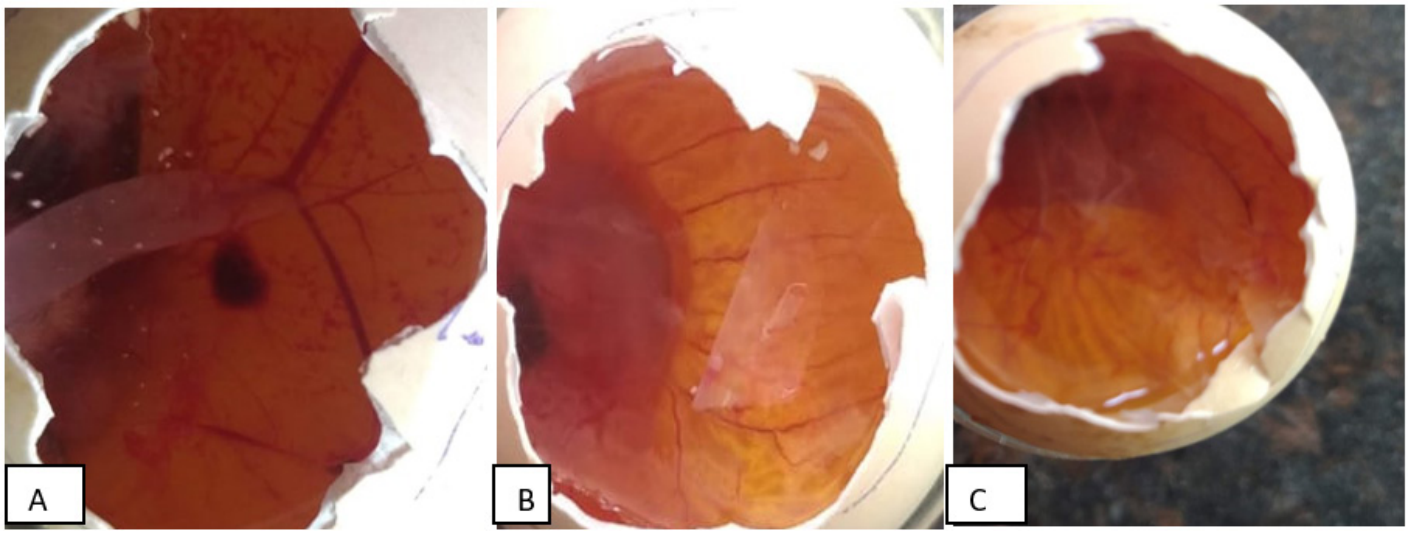


Figure 10: HET-CAM Method for A: Positive control, B: Negative Control, C: Optimized PLGA microsphere.

Table 6: Permeation Parameter of *ex vivo* permeation studies.

Parameters	Optimized formulation
Time (days)	2
% Cumulative drug permeated(%)	26.05±2.21
Flux (mg/cm^2/h)	0.073
Permeability coefficient, Kp cm/h x10^{-3}	1.7
Lag Time(Hr)	0.33
Diffusion coefficient(D)	2.02
Partition coefficient(Km)	1.68

Table 7: Irritation study by HET-CAM study.

	Time in seconds									Irritation Score
	Hemorrhage			vascular lysis			coagulation			
Positive Control	80	100	165	94	110	145	83	105	151	12.58±0.103
Negative Control	295	297	300	295	298	300	294	299	300	0.205±0.012
Test Formulation(T1)	286	283	300	273	288	300	278	283	300	0.778±0.056

*Mean±SD, n=3

mucosal lining and release the drug in a sustained manner. So, the result support that aspirin accumulates in the layer below the site of application, and releases the drug in the defined manner. This property can account for the activation of stem cells of the dental cavity and hence regenerate the tooth structure.

Irritation Study

The irritation study results are recorded in Table 7 and Figure 10. The irritation potential of aspirin PLGA microsphere was assessed using the *in vitro* HET-CAM method. The irritation score for PLGA microsphere was 0.778±0.056. The study revealed that the formulation didn't irritate mucous membranes. The isotonic sodium chloride and NaOH(0.1N) solution scores were 0.205±0.012 and 12.58±0.103, respectively. The outcomes demonstrated that the formulation has a much lower irritation value than the positive control, hence appropriate to retain in the oral cavity for a longer duration of time.

CONCLUSION

Aspirin loaded PLGA microsphere was prepared by double emulsion technique using PLGA (75:25) and ethyl acetate-ethanol as solvent system and optimized using Box-Behnken method. Synthesized microsphere with maximum encapsulation efficiency (87.65±1.8%) and minimum particle size (4.49±0.8µm) at optimum formulation conditions; 705.29 mg PLGA concentration, 0.8mL of Volume of internal phase and 2.00% PVA concentration were obtained. Aspirin loaded PLGA microsphere

released the drug in the sustained manner for a period of 16 days. Drug release mechanism showed triphasic release, with initial burst release phase subsequently lag phase and nearly complete release at the end of this time course. This confirm that release is due to polymer erosion, swelling and degradation. The drug release kinetic of *in vitro* release and permeation showed zero order kinetics. *Ex vivo* permeation study through porcine mucosa confirmed sustained permeation and also shows the significant partition and accumulation of drug in the mucosa. This property signifies that the formulation has the potential to stimulate the stem cells, hence it can help in the regeneration of dental tissues. Hence, further study needs to be carried out in dental stem cells to find the mechanism of regeneration.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PLGA: Poly Lactic-co-Glycolic Acid; **PVA:** Polyvinyl Alcohol; **XRD:** X-ray Diffraction Study; **DSC:** Differential Scanning Calorimetry; **SEM:** Scanning Electron Microscopy; **HET-CAM:** Hen's egg test-chorioallantoic membrane; **IS:** irritation score.

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