Formulation and Evaluation of Ibandronate Sodium Buccal Patch for Antiresorption of Alveolar Bone and Teeth

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Abstract

This study aims at improving the buccal delivery of Ibandronate sodium as model highly water-soluble, low permeable ibandronate sodium. Two main strategies were combined; first ibandronate sodium was entrapped in liposomes, which were then formulated as mucoadhesive film.Ibandronte sodium loaded liposomes (LPs) containing Soya lecithin,Cholestrol were then incorporated into mucoadhesive film composed of SCMC and HPMC. Results showed prolonged release of Ibandronate sodium after 6 h from LP-film compared to control film containing ibandronate sodium.Mucoadhesive B3 formulation were assessed for pH, Weight Uniformity, Thickness, Swelling Index, Folding Endurance, Tensile Strength, Drug Content Uniformity,Water Permeability Test,Percent Moisture Loss. In vitro diffusion studies were conducted for 6 hours in phosphate buffer (pH 6.8) using cellophane membrane.studies was performed with 0.5 % of HPMC and 0.5 % of SCMC. From the above evaluation test formulation B3 is selected as best formulation because the In-vitro diffusion of the formulation B3 was obtained 58.88 % in 6 hours it is more than the other formulations. The drug content of the best formulation was obtained 94.04%, folding endurance is 185 times. No significant changes were observed on physical characteristics, drug content and on drug release of patches after keeping the patches for one month at 40 ± 2°C and 75 ± 5% RH. So, it was concluded that the prepared patches were stable under these stress condition.

1.Introduction

The buccal region of oral cavity is an attractive site for the delivery of drugs owing to the ease of administration. Buccal drug delivery involves the administration of the desired drug through the buccal mucosal membrane lining of the oral cavity. This route is useful for mucosal (local effect) and transmucosal (systemic effect) drug administration [1]. The buccal film is identical due to its small size and optimum thickness. Fast-dissolving films administer drugs via absorption in the mouth (buccally or sublingually) and/or via the small intestines (enterally). A film is prepared using hydrophilic polymers that rapidly dissolve on the tongue or buccal cavity, delivering the drug to the systemic circulation via dissolution when contact with liquid is made. [2].

The objective of this study is to create a sustained-release buccal (oral cavity) formulation for ibandronte sodium as has high water solubility and low permeability. This goal is achieved through a combination of two key strategies.Ibandronate sodium encapsulated within liposomes, which are lipid-based vesicles. This approach aims to enhance the permeability of drug, making it more readily absorbed through the buccal mucosa (the lining of the mouth).

The liposomal ibandronte sodium dispersion is then transformed into a mucoadhesive buccal film. This film is designed to stick to the buccal mucosa, thereby extending the time ibandronate remains in the mouth and controlling its release pattern. Mucoadhesive films are commonly used to improve drug absorption through mucous membranes.

Liposomal buccal patch capable of enhancing drug permeation, control drug release, and having mucoadhesive properties with characterised lipids.Soyalecithin make self-assemble into spherical or multilayered spherical vesicles to form liposomes .also provide optimum particle size ,rigidity , luidity, stability, and electrical charge are all significantly influenced by the lipid content [3,4,5].Cholesterol is the main steroid which utilised to increase the stiffness and stability of liposomes since it is integrated

into the lipid bilayer during the synthesis of liposomes in a ratio of less than 30% of the total lipids [6,7].

2. Material and method

2.1 Materials

Ibandronate sodium drug purchased From yarrow chemicals, Soya lecithin, Cholestrol, . Propylene glycol (PG), anhydrous ethanol were purchased from ALS Pharmaceutical Chemicals Co. Hydroxypropyl methyl cellulose (HPMC, molecular weight 4 kDa) and carboxymethyl cellulose sodium (SCMC) purchased from DP Traders.

2.2 Method

Liposomal formulations were crafted utilizing either the thin film hydration technique or the reverse phase solvent evaporation method. In this process, a lipid phase was generated by precisely measuring drug, lecithin (PC), and cholesterol (CHOL) quantities. Drug was constant 5mg for each formulation .Soya lecithin:Cholestrol ratios F1(95:5),F2(90:10),F3(85:15),F4(80:10),LF5(75:25),F6(70:30) [8,9]. These components were amalgamated within a chloroform-methanol mixture (2:1 v/v) within a 500ml round bottom flask. Connecting the flask to a rotary evaporator facilitated solvent removal at temperatures ranging from 45 to 50°C for duration of 15 to 30 minutes. This procedure yielded a fine lipid film across the flask surface.The desiccated lipid film was subsequently hydrated through exposure to a saline solution of phosphate buffer at pH 6.8. This hydration process took place at a temperature of 60±2°C. Following hydration, a 15-minute sonication procedure was implemented to yield unilamellar liposomes. The resulting dispersion was then allowed to stand undisturbed at room temperature for a period of 2 to 3 hours to ensure complete swelling of the lipid film. Subsequently, the dispersion was stored within the temperature range of 4 to 8°C for subsequent studies [10].

Figure 1. Preparation of liposomes

3. Characterisation of Liposomes

3.1 Determination of Drug entrapment

Ibandronate sodium liposomes formulations were subjected to centrifugation at 18,000 rpm for duration of 40 minutes at 4°C utilizing a refrigerated centrifuge. This procedure facilitated the segregation of liposomes from any unentrapped drug. The determination of free drug concentration was performed on the supernatant layer subsequent to centrifugation, and measurements were taken at 218 nm employing a UV-Visible Spectrophotometer. The calculation of the drug's Entrapment percentage within the Liposomes is executed through the utilization of the Subsequent formula.

$$\% \text{ drug entrapment} = (\text{Total drug} - \text{Drug in supernatant}) / \text{Total drug} \times 100$$

3.2 Measurement of particle size and Zeta Potential

The vesicles size distribution of liposomal suspension was carried out using malvern Zetasizer.7.01(M/s Malven instruments Ltd ,Worcestershine ,UK)installed at university institute of Pharmaceutical Sciences (UIPS),Punjab University ,chandigarh. Polydispersity index (PdI), and zeta potential of 1 ml of liposomal suspension was diluted 10 times with distilled water.

3.3 Optical Microscopy

Photomicrographs was taken from the optical microscope at magnification 100× results was the vesicles were clearly visible having round shape of F3 [11,12].

4. Preparation of Liposomal Buccal Patch

The buccal patches containing Liposomal Ibandronate sodium was prepared by solvent casting technique**.**Casting solution was prepared by dissolving different concentration of HPMC & SCMC in distilled water with occasional stirring kept for 24 hr to get a uniform dispersion of the solution in a beaker and remove air bubbles.

Table 1. Formulation code of preparation of buccal patch

Formulation code	Polymers for buccal patch	Concentration of polymer
B1	HPMC	1%
B2	SCMC	1%
B3	HPMC:SCMC	0.5% :0.5%

5.Characterisation of liposomal Buccal Patch

5.1 Weight Uniformity

The individual weight of 2 patches from each batch was determined using an electrical weighing balance. Then the mean weight standard deviation of Patches was calculated. It is desired that patches should have nearly constant weight [13].

5.2 Thickness

A Thickness of Patch was calculated by using vernier calliper. Patch was measured at five positions i.e. central and four corners and the mean thickness was calculated. Variation in the thickness of the patches should be less than 5% and mean ±SD was calculated. This is essential to ascertain uniformity in the thickness of the patches that is directly related with drug content uniformity, it is necessary to ascertain uniformity in the thickness of the patch [14].

5.3 Surface pH Measurement

The surface pH of the prepared buccal patch was determined to check the possible irritation potential of the patches to the mucosa. The patch to be tested was placed in a petridish and was moistened with 2ml of phosphate buffer pH 6.8 and kept for 30 min. The pH was noted after bringing the electrode of the pH meter in contact with the surface of the formulation and allowing equilibration for 1 min. The average of three determinations for each formulation was done [15].

5.4 Swelling Index

The degree of swelling of bio-adhesive polymer is an important factor affecting the adhesion.The polymers have a tendency to absorb water and swell. Thus, swelling index study was performed to study the hydration characteristics of the patch. Patches were weighed separately (Initial weight= W1) and placed in petri plates containing 5mL phosphate buffer pH 6.8 and allowed to swell. The swollen patch were weighed individually after 90min. (Final weight = W2) [16]. Swelling index of each system was calculated using the following formula

$$\text{Swelling Index} = \frac{W2-W1}{W1} X\ 100$$

5.5 Folding Endurance

Folding endurance of patch is essential to study the elasticity of the patch during storage andhandling. Folding endurance of patch was measured by repeatedly folding one patch at same place till it break. The number of time the patch is folded without breaking is known as the folding endurance value. The number of times the patch could be folded at the same place without breaking gave the exact value of folding endurance. The folding endurance of prepared patches was measured in triplicate and average with SD was calculated [17].

5.6 Tensile Strength

The highest stress that may be applied to a strip specimen before it breaks is its tensile strength. Tensile strength was tested using specially made tensile strength measuring apparatus. The apparatus consists of a pan that was filled with weights. Between the two clips was positioned the patch whose tensile strength is being measured. It was observed how long the patch was at first. Until the patch cracks, weights were put to the pan. The tensile strength can be calculated using the formula.

$$\text{Tensile Strength} = \frac{[Break\ Force]}{a\ X\ b}\ \mathbf{X}\ \frac{[1+\Delta L]}{L}$$

Where,

a is the thickness of the film b is the width of the film

ΔL is the length of elongation

L is the length of the film [18].

5.7 Water Permeability Test

One patch from the each formulation were weighed (W 1) and exposed to the 75 % relative Humidity by using the Potassium chloride crystals in desiccator for a period of 24 hours. Patch was then weighed again (W2) [19]. Water permeability is indicated by the increase in weight and it was calculated by the following formula:

$$\text{Water permeability} = \frac{W2-W1}{W1}\ X\ 100$$

5.8 Percent Moisture Loss

One patch from each formulation were weighed (W1) were placed in desiccator containing silica crystal/ Calcium chloride for a period of 24 hours. Patch was then weighed again (W2). Percent moisture loss is indicated by the decrease in weight and it was calculated by the following formula [20]:

$$\%\ \text{Moisture Loss} = \frac{W1-W2}{W2}\ X\ 100$$

5.9 Drug Diffusion Studies

Drug diffusion studies were carried out of the prepared patches by using Keishary chein Diffusion cell with phosphate buffer 6.8 using dialysis membrane for a period of 6 hours. The cell membrane was mounted on a diffusion cell in between the donor and receptor compartment. The buccal patch was fixed on the membrane. Receptor compartment was filled with Phosphate buffer pH 6.8 [21]. The fluid was maintained at 37±2° and stirred continuously at 100±2 RPM. Aliquots of 1ml were collected at predetermined intervals for 6 hrs and suitably diluted, filtered through 0.22 µm filter and analysed by

UV spectrophotometer.Same Phosphate buffer pH 6.8, 1 ml was replaced in the receptor medium to maintain the sink condition[22].

5.10 Drug release kinetics of optimised liposomal buccal patch.

The mathematical framework was employed to assess the dynamics and process of drug liberation from the preparations. The information garnered from the in-vitro drug liberation investigation was matched with various kinetic patterns to ascertain the dynamics of Drug release [23].

- **Zero order release model**: It describe the systems where the drug release rate is independent of its concentration of its dissolved substances.
  Zero order equation: Qt = ko t
- **First order release model**: It describe that release is concentration dependent.This model has been also used to described absorption and elimination of drug.
  First order equation: log (Q0-Qt) = k1t [24]
- **Higuchi release model**: The Higuchi equation suggests that the drug release by diffusion mechanisms.
  Higuchi equation: **Qt = kH VT**

Here, Qt represents the percentage of total drug release up to time t, while Q0 stands for the initial quantity of the drug within the buccal patch. Additionally, ko, k1, and kH denote the rate coefficients for zero-order, first-order, and Higuchi's rate equation correspondingly [25].

6. Results

6.1 Characterisation of Liposomes

6.1.1 Determination of Drug entrapment

Table 2. Entrapment efficiency of all formulations

Sr.No.	Formulation Code	Percentage Entrapment Efficiency
1.	F1	44.52±0.04
2.	F2	52.34±0.02
3.	F3	67.02±0.01
4.	F4	72.58±0.05
5.	F5	64.29±0.03
6.	F6	61.42±0.01

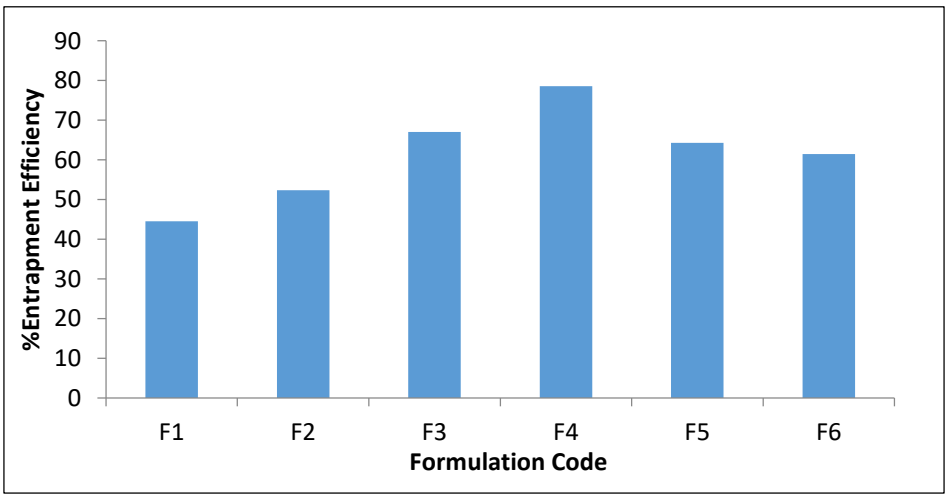


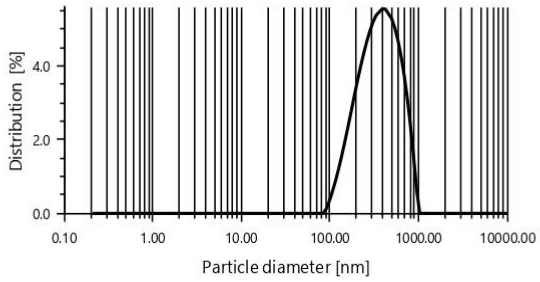
Figure 2. Entrapment efficiency graph of all formulations

6.1.2 Measurement of particle size and Zeta Potential

Measurement information

Measurement name	P1 dls	**User**	LENOVO
Method	-	**Time**	10-08-2023 11:37:56
Status	Succeeded	**Instrument type**	Litesizer 500
Measurement mode	Particle size		
		Filter opical density	1.198 (Automatic)
Measurement cell	Disposable	**Focus position**	-0.2 mm (Automatic)
Measurement angle	Side scatter (Automatic)	**Material**	Unknown material
Target temperature	25.0 °C	**Material refractive index**	-
Equilibration time	0h 00m 30s	**Material absorbance coefficient**	-
Analysis model	General	**Solvent**	Water
Cumulant model	Advanced	**Solvent refractive index**	1.3303
Processed runs	5 (Manual)	**Solvent viscosity**	0.8903 mPa.s
Time for each run	0h 00m 10s (Manual)		

Particle size distribution (intensity)



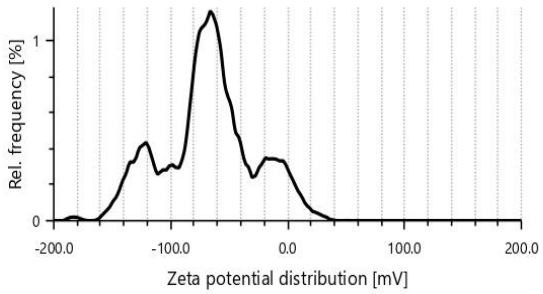
Result

Hydrodynamic diameter	396.5 nm	**Mean intensity**	286.5 kcounts/s
Polydispersity index	0.7 %	**Absolute intensity**	4518.2 kcounts/s
Diffusion coefficient	1.2 µm²/s	**Intercept g1²**	0.8813
Transmittance	79.8 %	**Baseline**	1.007

Particle size distribution peaks (intensity)

Peak name	Size [nm]	Area [%]	Standard deviation [nm]
Peak 1	397.2	100.00	209.7
Peak 2	-	-	-
Peak 3	-	-	-

Zeta potential distribution



Result

Mean zeta potential	-70.5 mV	**Mean intensity**	710.1 kcounts/s
Standard deviation	3.6 mV	**Filter opical density**	2.6938
Distribution peak	-65.5 mV	**Conductivity**	0.477 mS/cm
Electrophoretic Mobility	-5.4967 µm*cm/Vs	**Transmittance**	80.6 %

6.1.3 Optical Microscopy

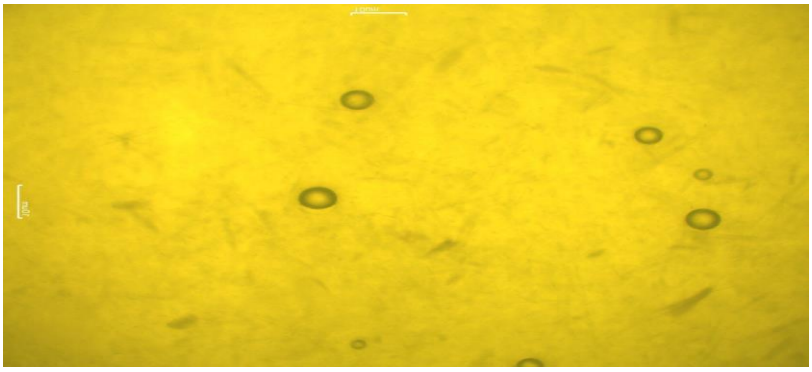


Figure 3. Optical microscopic studies

6.2 Characterisation of liposomal Buccal Patch

Table 3. Evaluation parameters of liposomal buccal patch

Evaluation Parameters	B1	B2	B3
Weight variation (mg)	82±1.32	76±2.43	63±1.132
Thickness (mm)	0.72±0.02	0.67±0.04	0.65±0.02
Surface pH	6.4±0.01	6.6±0.02	6.7±0.01
Swelling Index(%)	21.24±0.08	18.67±0.06	17.54±0.05
Folding endurance (Times)	176±4.682	182±3.612	185±4.251
Tensile strength(kg/mm^2)	1.364±0.012	1.632±0.008	1.812±0.054
% Drug Content	89.02±0.360	92.42±0.434	94.04±0.621
% Water permeability	4.82±0.302	3.85±0.242	3.21±0.221
% Moisture loss	8.12±0.02	7.70±0.12	6.15±0.04

6.2.1 Surface pH

The pH value of all the formulation (B1-B3) were determined in between 6.4 to 6.7.

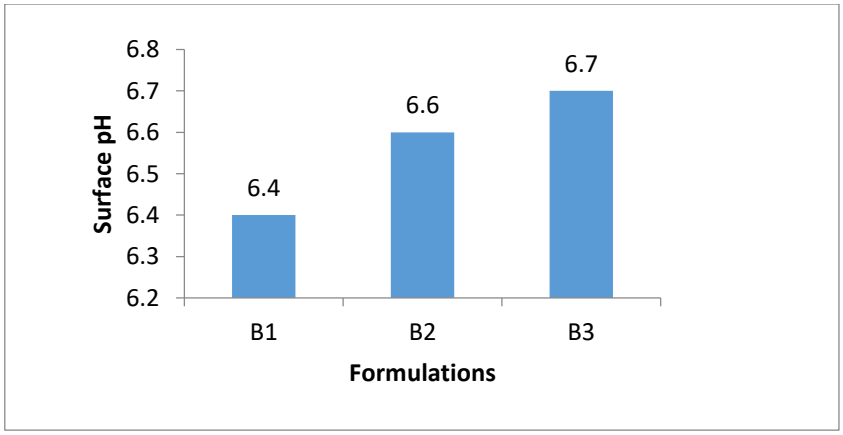


Figure 4. Graph of surface pH of formulations

6.2.2 Swelling Index

The swelling index of buccal patch of liposomal ibandronate sodium was found to be 17.54.

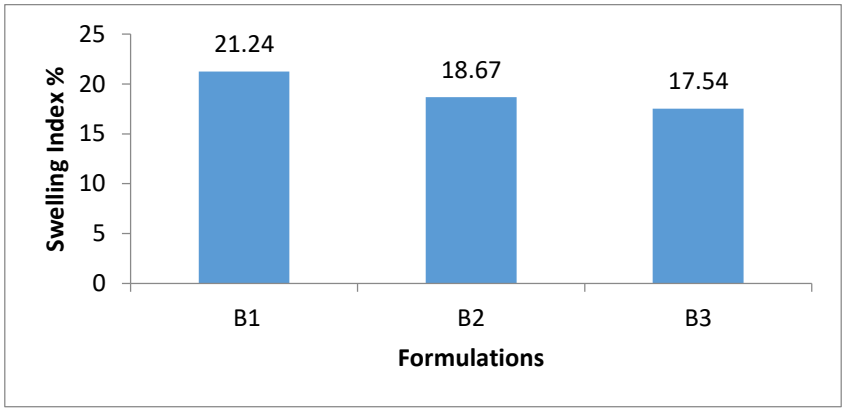


Figure 5. Graph of Swelling Index of formulations

6.2.3 Percent Moisture loss

The B3 formulation shows less moisture loss of 6.15 %

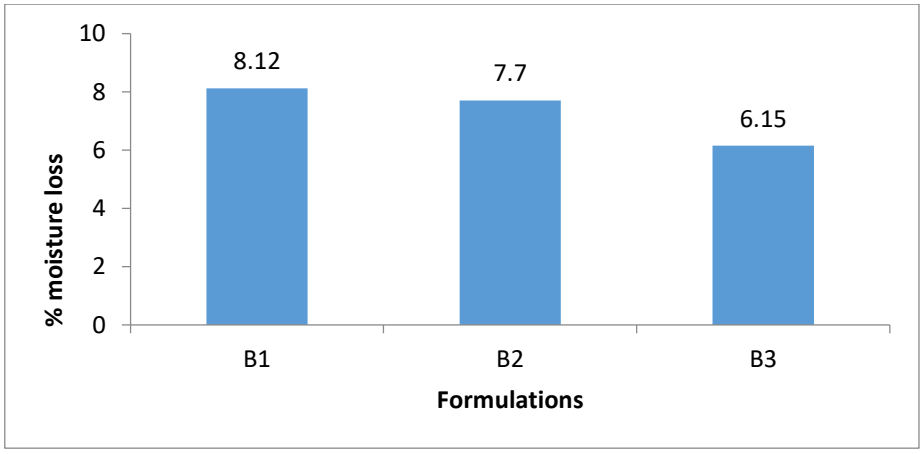


Figure 6. Graph of %Moisture loss of formulations

6.2.4 Precent water permeability

B3 has optimum water permeability

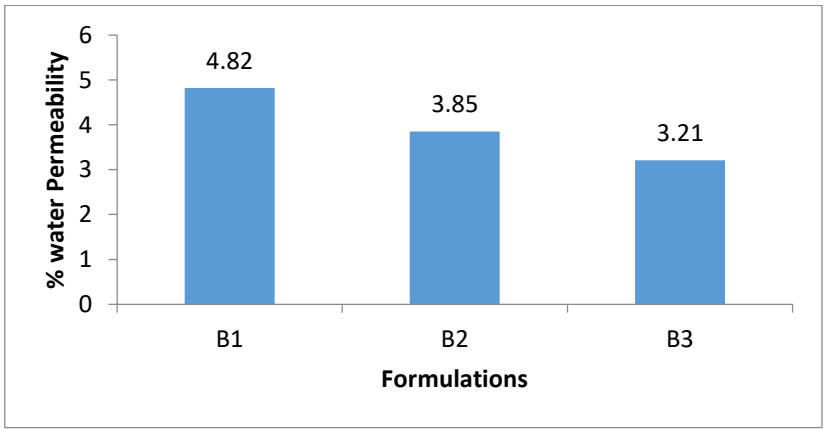


Figure 7. Graph of % Water permeability of formulations

6.2.5 Drug Diffusion Studies

Figure 8. Drug Diffusion Studies by Keishary chein Diffusion cell

Drug diffusion data

Table 4. Drug diffusion data of formulations

Time (hours)	B1	B2	B3
0.	0	0	0
1.	19.12 ± 0.21	21.08 ± 0.04	25.18 ± 0.11
2.	28.87 ± 0.11	30.35 ± 0.17	32.65 ± 0.09
3.	32.46 ± 0.58	34.25 ± 0.31	38.36 ± 0.02
4.	36.24 ± 0.35	38.78 ± 0.46	42.68 ± 0.16
5.	42.15 ± 0.22	46.65 ± 0.21	52.64 ± 1.23
6.	48.45 ± 0.13	54.45 ± 0.53	58.88 ± 0.04

6.2.6 Drug release kinetics

Table 5. In-Vitro Drug Release Profile of Ibandronate sodium from B1, B2, B3 liposomal buccal patches

Time (hours)	Square root of time	% cumulative drug diffused			Log % cumulative drug diffused			% cumulative drug retained			Log % cumulative drug retained		
		B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
0	0	0	0	0	0	0	0	100	100	100	2	2	2
1	1	19.12	21.08	25.18	1.281	1.323	1.401	80.88	78.92	74.82	1.90	1.89	1.87
2	1.141	28.87	30.35	32.65	1.460	1.482	1.513	71.13	69.65	67.35	1.85	1.84	1.82
3	1.732	32.46	34.25	38.36	1.511	1.534	1.583	67.54	65.75	61.64	1.82	1.81	1.78
4	2	36.24	38.78	42.68	1.559	1.583	1.630	63.76	61.22	57.32	1.80	1.78	1.75
5	2.236	42.15	46.65	52.64	1.624	1.668	1.721	57.85	53.35	47.36	1.76	1.72	1.67
6	2.449	48.45	54.45	58.88	1.685	1.735	1.769	51.55	45.55	41.12	1.71	1.65	1.61

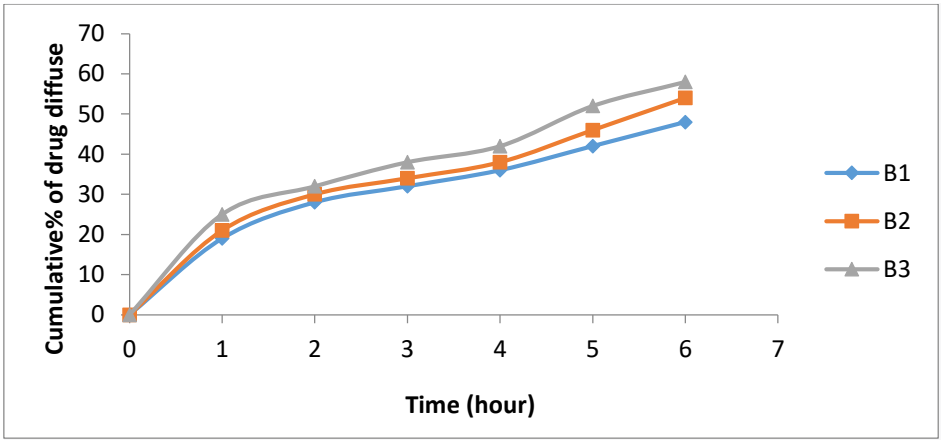


Figure 9. Kinetics release of drug in different formulations

Zero order release

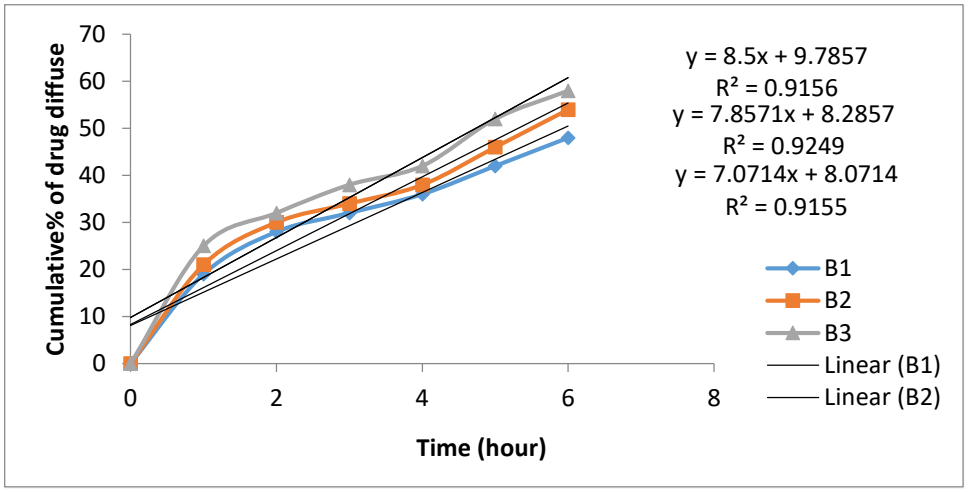


Figure 10. Zero order graphs of B1, B2, B3 Formulation if Liposomal Buccal Patch.

First order release

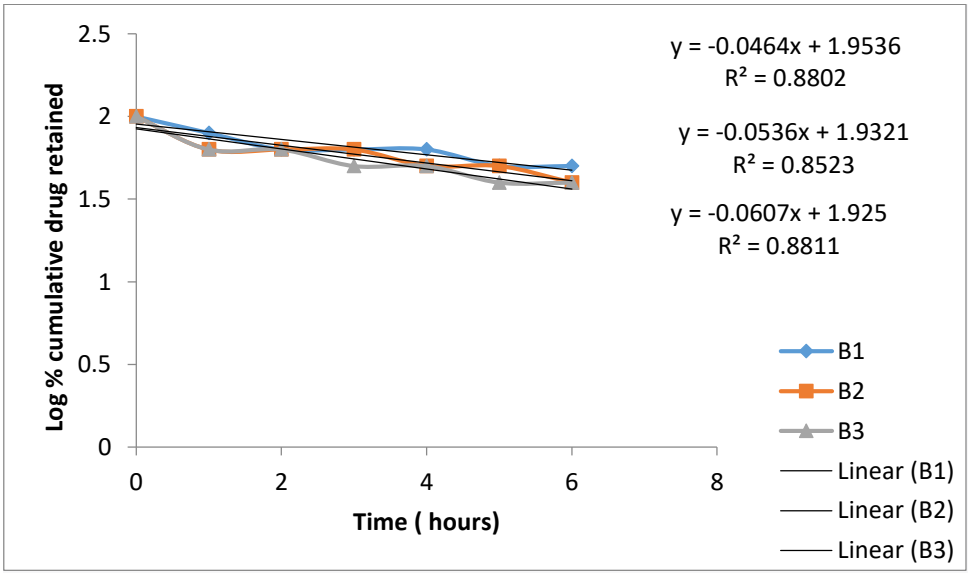


Figure 11. First order graphs of B1, B2, B3 Formulation if Liposomal Buccal Patch.

Higuchi order

Table 6. Higuchi order of B1, B2, B3 Formulation of Liposomal Buccal Patch

Formulation code	Zero order		First order		Higuchi model	
	r^2	k_0	r^2	k_1	r^2	k_H
B1	0.9155	7.0714	0.8802	-0.0464	0.9716	18.48
B2	0.9156	8.5	0.8523	-0.0536	0.9656	20.37
B3	0.9249	7.8571	0.8811	-0.0607	0.9750	22.25

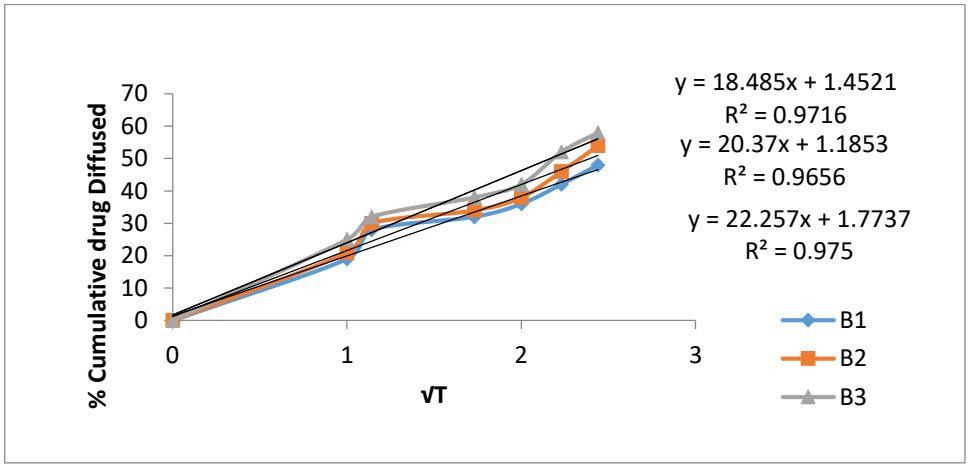


Figure 12. Higuchi order graph

The estimated regression coefficient for zero order, first order and Higuchi models.The Higuchi model (r^2=0.9750)was determined to be the best fit for the release data based on the determination cofficient.

2.6.7 Stability studies of optimised B3 Liposomal Buccal patch Formulation

Table 7. Evaluation data of F4 formulation during storage period

Time (days)	Real Time(30^0C/65%RH)				Accelerated(40^0C/75%RH)			
	Physical Appearance	Thickness (mm)	Drug Content (%)	Folding Endurance	Physical Appearance	Thickness (mm)	Drug Content (%)	Folding Endurance
0	Transparent	0.65	94.04	185	Transparent	0.65	94.04	185
7	Transparent	0.65	93.94	184	Transparent	0.65	93.82	181
14	Transparent	0.65	92.87	185	Transparent	0.65	92.83	180
21	Transparent	0.65	90.78	182	Transparent	0.65	90.75	178
28	Transparent	0.65	90.75	180	Transparent	0.65	89.14	175

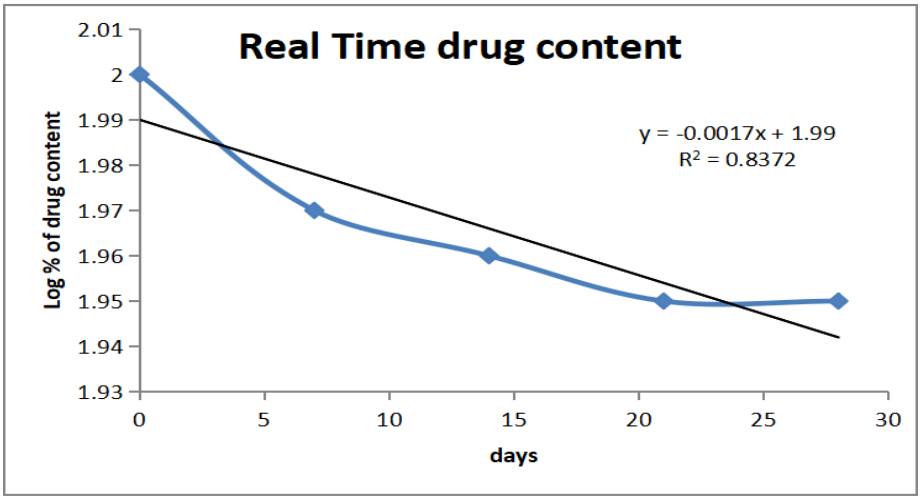


Figure 13. Real time drug content before storage

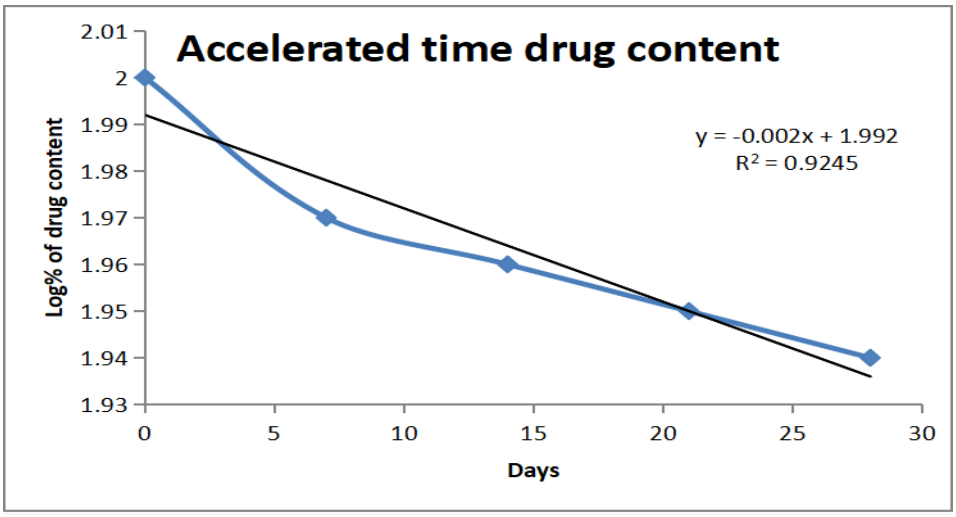


Figure 14. Accelerated time drug content after storage

7. Discussion

7.1 Determination of shelf life of formulated buccal patches of ibandronate sodium

Shelf life is the time period during which a drug product is expected to remain within the approved specification for use, provided that it is stored under the condition define on the container label. The half life and shelf life of the patches was calculated by using the equations:

$$t_{50\%} = \frac{0.693}{m}$$

$$t_{90\%} = \frac{0.105}{m}$$

M is the slope value obtained from the graphs plotted between log % drug content vs time.From the drug content data it was concluded that the $t_{50\%}$ (half-life) of the formulated buccal patches of prochlorperazine maleate was found 407 days in real time storage condition and 346 days in accelerated

time storage condition.Also, the $t_{90\%}$ of the buccal patches was found to be 61 days in real time storage condition and 52 days in accelerated time storage condition.

7.2 FTIR spectrum of Buccal patch B3

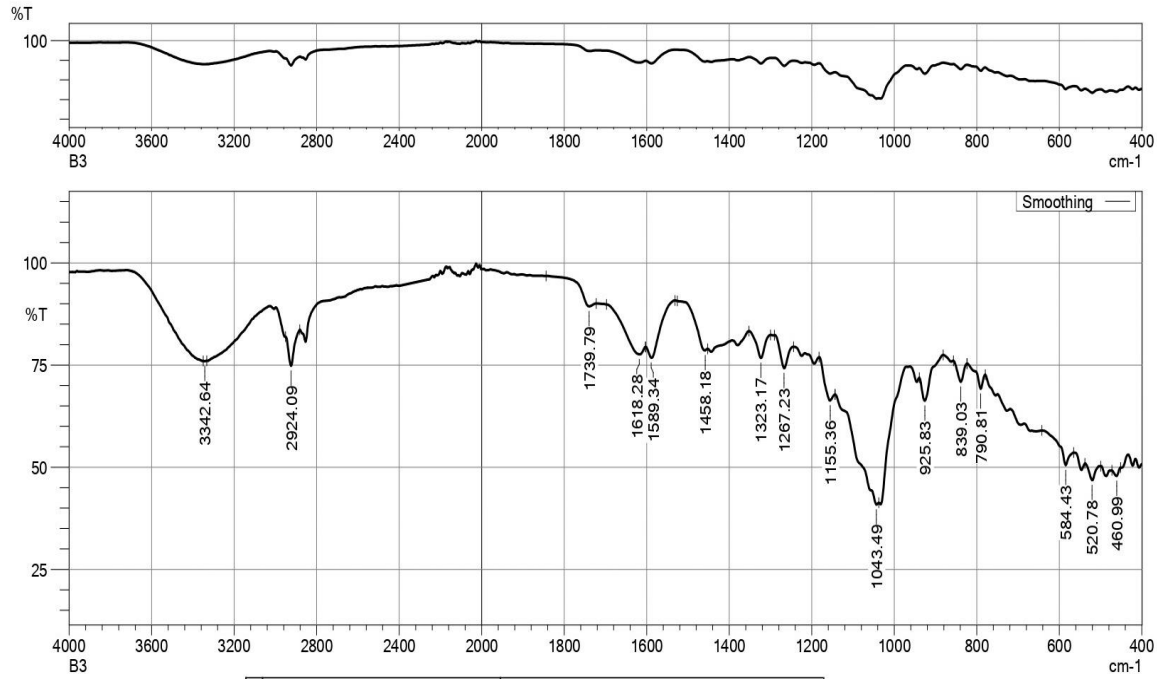


Figure 15. FTIR spectrum of Buccal patch B3

7.3 Interpretation of FTIR spectrum of Buccal Patch B3

Table 8. Interpretation of FTIR spectrum of Buccal Patch B3

Sr No.	Observed Peak (cM-1)	Functional Group
1	3342	O-H Stretching
2	2924	*CH3* Stretching
3	1589	N-H Stretching
4	1267	C=O Stretching
5	1043.49	P=O Stretching
6	925.83	P=O Stretching
7	839.03	C-H Stretching
8	790	C-H Stretching
9	520.78	C-Cl Stretching
10	460	C-Cl Stretching

Figure 15 and Table 8 Shows the FTIR spectrum of buccal patch IR absorption peaks of B3 was detected as stretching vibrations.

References

1. Abd El Azim, H., Nafee, N., Ramadan, A., Khalafallan, N., Liposomal Buccal Mucoadhesive Film for Improved Delivery and Permeation of Water-Soluble Vitamins, International Journal of Pharmaceutics, Vol. 488, No. 1-2, pp. 78–85, (2015).
2. Akbarzadeh A, Sadabady RR, Davaran S, Woo-Joo S, et al. Liposome: classification, preparation, and applications. Nanoscale Research Letters 2013, (8):102.
3. Berginc, K., Suljaković, S., Škalko-Basnet, N., Kristl, A., Mucoadhesive Liposomes as New Formulation for Vaginal Delivery of Curcumin, European Journal of Pharmaceutics and Biopharmaceutics, Vol. 87, No. 1, pp. 40-46, (2014).
4. Bozzuto G, Molinari A. Liposomes as nanomedical devices. International journal of nanomedicine. 2015 Feb 2:975-99.
5. Briuglia ML, Rotella C, McFarlane A, Lamprou DA. Influence of cholesterol on liposome stability and on in vitro drug release. Drug delivery and translational research. 2015 Jun;5:231-42.
6. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017 Mar 27;9(2):12.
7. Campisi G, Paderni C, Saccone R, Fede OD, Wolff A, Giannola LI. Human buccal mucosa as an innovative site of drug delivery. Current pharmaceutical design. 2010 Feb 1;16(6):641.
8. Dua JS, Rana AC, Bhandari AK. Liposome: methods of preparation and applications. Int J Pharm Stud Res. 2012 Apr;3(2):14-20.
9. Dymek M, Sikora E. Liposomes as biocompatible and smart delivery systems–The current state. Advances in Colloid and Interface Science. 2022 Aug 19:102757
10. Cipolla D, Wu H, Gonda I, Eastman S, Redelmeier T, Chan HK. Modifying the release properties of liposomes toward personalized pmedicine. Journal of pharmaceutical sciences. 2014 Jun 1;103(6):1851-62.
11. Combination of targeted daunorubicin liposomes and targeted emodin liposomes for treatment of invasive breast cancer. Journal of drug targeting. 2020 Mar 15;28(3):245-58.
12. Eroschenko VP, Di Fiore MSH. Di Fiore's Atlas of Histology with Functional Correlations. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2000.
13. Fathi S, Oyelere AK. Liposomal drug delivery systems for targeted cancer therapy: is active targeting the best choice?. Future medicinal chemistry. 2016 Nov;8(17):2091-112.
14. Grazia Calvagno M, Celia C, Paolino D, Cosco D, Iannone M, Castelli F, Doldo P, Fresta M. Effects of lipid composition and preparation conditions on physical- chemical properties, technological parameters and in vitro biological activity of gemcitabine-loaded liposomes. Current drug delivery. 2007 Jan 1;4(1):89-101.
15. Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, Deng Y. A review on phospholipids and their main applications in drug delivery systems. Asian journal of pharmaceutical sciences. 2015 Apr 1;10(2):81-98.
16. Lian T, Ho RJ. Trends and developments in liposome drug delivery systems. Journal of pharmaceutical sciences. 2001 Jun 1;90(6):667-80.
17. Lohe SW, Umekar MJ. Formulation and evaluation of liposomes of natural phospholipids for the treatment of inflammatory conditions. Latin American Journal of Pharmacy. 2023 Feb 16;42(1):19-35.
18. Munot NM, Shinde YD, Shah P, Patil A, Patil SB, Bhinge SD. Formulation and Evaluation of Chitosan-PLGA Biocomposite Scaffolds Incorporated with Quercetin Liposomes Made by QbD Approach for Improved Healing of Oral Lesions. AAPS PharmSciTech. 2023 Jun 28;24(6):147.
19. Nafee NA, Ismail FA, Boraie NA, Mortada LM. Mucoadhesive delivery systems. I. Evaluation of mucoadhesive polymers for buccal tablet formulation. Drug development and industrial pharmacy. 2004 Jan 1; 30(9):985-93.

20. Nakane S, Kameyama Y. Root resorption caused by mechanical injury of the periodontal soft tissues in rats. J Periodontal Res 2010;22(5):390-5.
21. Nakhaei P, Margiana R, Bokov DO, Abdelbasset WK, Jadidi Kouhbanani MA, Varma RS, Marofi F, Jarahian M, Beheshtkhoo N. Liposomes: structure, biomedical applications, and stability parameters with emphasis on cholesterol. Frontiers in bioengineering and biotechnology. 2021 Sep 9;9:705886.
22. Okafor NI, Ngoepe M, Noundou XS, Krause RW. Nano-enabled liposomal mucoadhesive films for enhanced efavirenz buccal drug delivery. Journal of Drug Delivery Science and Technology. 2019 Dec 1;54:101312.
23. Roopa Pai .S, Kusum Devi. V. Lamivudine liposomes for transdermal delivery Formulation, Characterization, stability and in-vitro evaluation. Int.J.Pharm. sci. Nanotech. 2009; 1 (4):317-26.
24. Sahoo SK, Labhasetwar V. Nanotech approaches to drug delivery and imaging. Drug discovery today. 2003 Dec 15;8(24):1112-20. V. Nanotech approaches to drug delivery and imaging. Drug discovery today. 2003 Dec 15;8(24):1112-20.
25. Srinivas Murthy BR, Vishakha K, Prakash P, et al. Formulation and in vitro evaluation of liposomes loaded with mupirocin. International Journal of Research in Pharmaceutical and Nano Sciences 2015, 4(3): 162-174.