International Journal of Pharmacognosy and Chemistry

Research Article

FORMULATION DEVELOPMENT AND IN VITRO CHARACTERIZATION OF ATORVASTATIN BUCCAL PATCHES

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Abstract

The present investigation is concerned with the development of the Atorvastatin buccal films, which were designed to prolong the buccal residence time, to increase penetration through buccal mucosa and thus increase the bioavailability. Various formulations were developed by using release rate controlling film-forming polymers like Gellan Gum, HPMC E5, and HPMC E50 in various combinations using plasticizer PEG 400. The prepared films were evaluated for the number of parameters like physical appearance and surface texture, weight uniformity, the thickness of the films, folding endurance, swelling index, tensile strength, drug excipients interaction study, content uniformity, in-vitro drug release study. The FTIR studies indicate that Atorvastatin showed complete entrapment within the polymer carrier bonding is suggested, and there was no chemical interaction. From all the formulations, F8 shows 98.24% of drug release at the ends of 8hr and chosen as optimized formulation and which follows zero-order release with non-fickian transport.

Keywords: Atorvastatin, PEG 400, HPMC, FTIR, Gellan Gum.

Article Info

Received: 05-04-2020

Revised: 25-05-2020

Acepted: 30-05-2020

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INTRODUCTION

Since the early 1980s, there has been renewed interest in theuse of bio adhesive polymersto prolong contact timein the various mucosal routes of drug administration.The ability to mainta in a delivery systemat aparticular location for an extended period has great appeal for both local as well assystemic drug bioavailability [1-4]. Drug absorption through a mucosal surface is efficient because mucosal surfaces are usually rich in blood supply,providing rapid drug transport to the systemic circulation and avoiding degradation by gastrointestinal enzymes and first-pass hepatic metabolism [5].

ORALTRANSMUCOSALDRUGDELIVERY

Within the oral cavity, the delivery of the drug is classified into the categories. Due to its excellent accessibility and reason able patient compliance, the oral mucosal cavity offers an attractive route of drug administration. Within the oral mucosal cavity delivery of the drug is classified into the following categories:

a) Sublingual delivery which is systemic delivery of drug through the mucosal membrane lining the floor of the mouth.
b) Buccal delivery & local delivery for the treatment of conditions of the oral cavity. The oral cavity is the for emostpart of the digestive system of the human body.It is also referred to as a "buccalcavity."It is accountable for various primary functions of the body. The careful examination of various features of the oral cavity canhelpin the development of a suitable mucoadhesive drug delivery system[6].

Atorvastatin is a lipid-lowering drug included in the statin class of medications. By inhibiting the endogenous production of cholesterol in the liver, statins lower abnormal cholesterol and lipid levels, and ultimately reduce the risk of cardiovascular disease. More specifically, statin medications competitively inhibit the enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA) Reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid. This conversion is a critical metabolic reaction involved in the production of several compounds involved in lipid metabolism and transport, including cholesterol, low-density lipoprotein (LDL) (sometimes referred to as "bad cholesterol"), and very-low-density lipoprotein (VLDL). Prescribing statins is considered standard practice for patients following any cardiovascular event, and for people who are at moderate to high risk of developing cardiovascular disease 5.

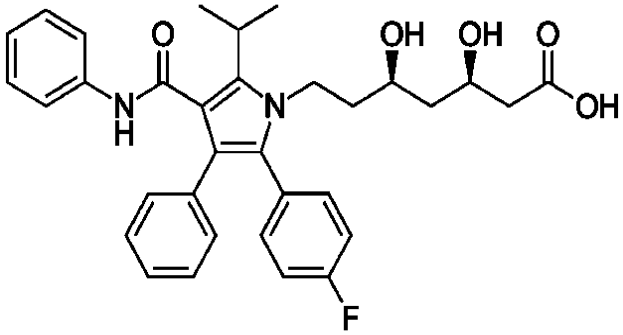

Fig 01: Chemical structure of the

ATORVASTATIN

The main and objective of the research work focuses on the formulation and development of Atorvastatin buccal patches to improve bioavailability and to avoid extensive first-pass metabolism. Based on the literature conclude that the formulation and development of new excipients were used in the formulation of Atorvastatin buccal patches to avoid the extensive first pass.

EXPERIMENTAL WORK

MATERIALS AND METHODS

The chemicals Atorvastatin purchased for BMR chemicals, Hyderabad, HPMC E5, HPMC E50 Loba Chemical Pvt. Ltd., Mumbai, PEG 400, Methanol, Gellan Gum Purchased AR Grade form the Merck Pvt.Ltd. And instruments and equipment's used in the formulations UV Visible spectrophotometer T60, PG Instrument, Digital balance BT 220H, Shimadzu Corporation, pH Meter Systronics pH system 361, Sonicator Remi Equipments, Mumbai, Thickness tester Screw Gauze, Tensile Strength tester Tinius Olsen (HT400), FTIR like Jasco FTIR410.

PREFORMULATION STUDIES [55-63]

Preformulation testing is the initial phase in the improvement of dose types of a drug substance. It is one of the critical essentials being developed of any drug delivery system. It tends to be characterized as an examination of physical and synthetic properties of a medicament substance alone and when joined with excipients.

Characterization of the medicament is an essential advance at the preformulation period of item improvement taken after by concentrate the properties of the excipients and their similarity. The general goal of preformulation testing is to produce data valuable to the formulator in creating steady and bioavailable measurements frames, which can be mass-produced. The following are the various preformulation studies.

SOLUBILITY

The solubility of Atorvastatin was determined in Methanol, Ethanol, 0.1N HCl, pH 7.4, and pH 6.8 phosphate buffers. Solubility studies were performed by taking an excess amount of Atorvastatin in different beakers containing the solvents. The mixtures were shaken for 48hrs in a rotary shaker. The solutions were centrifuged for 10mins at 1000 rpm, and the supernatant was analyzed at 247nm.

DRUG-EXCIPIENTS INTERACTION STUDY OF FILMS

There is always a possibility of drug-excipients interaction in any formulation due to the irintimate contact. The technique employed in this study to knowdrug-excipients interactions is IR spectroscopy; IR spectroscopy is one of the most powerful analytical techniques which offer the possibility of chemical identification. Infra-red spectra of pure drug Atorvastatin and formulations were scanned by using Jasco FTIR 410, by a thin film method.

ANALYTICAL METHODS FOR THE ESTIMATION OF ATORVASTATIN

PREPARATION OF REAGENTS

A. Potassium Dihydrogen Phosphate0.2M)

27.218gm of potassium dihydrogen phosphate is dissolved in distilled water and make up to 1000 ml with thesame.

B. SodiumHydroxideSolution (0.2M)

8 gm of sodiumhydroxide was dissolved in 1000mlof distilled water.

C. Phosphatebuffer pH of 6.8

50 ml of 0.2M of potassium dihydrogen phosphate solution and 22.4ml of 0.2M sodium hydroxide solution weremixed andmadeup to 200 ml with distilled water.

Determination of λmax for Atorvastatin

10mg of Atorvastatin was dissolved in 3ml of methanol and made up to 10ml with 6.8 pH buffers so as to get a stock solution of 1000 µg/ml concentration. From the solution, take 1ml solution was withdrawn and diluted to 10ml with the same to get a concentration of 100µg/ml (SS-II). From this stock solution, pipette out 1 ml of the solution and make up the volume to 10ml using the same buffer to get the concentration of 10µg/ml concentration, this solution was scanned under UV Spectroscopy using 200-400nm.

Preparation of standard calibration curveof Atorvastatin

The standard calibration curve for Atorvastatin was prepared using pH6.8 phosphate buffer.

STANDARD SOLUTION

10mgofAtorvastatinwasdissolved in 3ml of methanol and made up to 10 ml with pH 6.8 phosphatebufferto give a concentration of 1000 µg/ml.

STOCK SOLUTION

From the standard solution, take 1ml of the solutionin a 10 ml volumetric flask. The volume was made up to mark with pH 6.8 phosphate buffer to produce a concentration of 100µg/ml of Atorvastatin, respectively. From the working standard solution take 0.2, 0.4, 0.6, 0.8, 1.0, & 1.2 ml of the solution and make up to the mark with 6.8 ph buffer to get the concentrations of 2, 4, 6, 8, 10 & 12 µg/ml. The absorbancedatafor the standard calibration curve and plotted graphically. The standard calibration curve yield sastraight line, whichs hows that the drugobeys Beer's law in the concentration range of 2-12 µg/ml.

Atorvastatin Buccal Patches [64-72]

Initially, the polymer was dissolved in methanol under constant stirring until the clear solution was obtained. Then to this solution, propylene glycol was added. To this solution, Atorvastatin was added by stirring. The resultant solution was then poured on the petri dish of area 44.156 sq.cm and allowed to dry undisturbed at room temperature. The dried film was cut into discs of 2x2 cm (4sq.cm of the area) diameter. The compositions of films are reported in Table.

Table 01: Formulation Details of Drug Incorporated Buccal Films.

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Atorvastatin	110	110	110	110	110	110	110	110	110
Gellan Gum	100	200	300	-	-	-	-	-	-
HPMC E50	-	-	-	100	200	300	-	-	-
HPMC E5	-	-	-	-	-	-	100	200	300
PEG 400 (%w/v)	15	15	15	15	15	15	15	15	15
Methanol (ml)	5	5	5	5	5	5	5	5	5

Note 4 sq.cm buccal films containing 10 mg of Atorvastatin.

Evaluationoffilms [70-80]

Evaluation of Atorvastatin buccal films

The Atorvastatin buccal films were evaluated for the following properties:

PHYSICAL APPEARANCE ANDSURFACE TEXTURE OF THE FILM

This parameter was checked simply with a visual inspection of film sand evaluation of texture by feel or touch.

WEIGHT UNIFORMITY OF FILMS

Three films of the size 4sq.cm were weighed individually using digital balance, and the average weights were calculated.

THE THICKNESS OF FILMS

The thickness of the films was measured using a screw gaugeat different spots of he films.The thickn ess was measured at three different spots of the films, and the average was taken.

FOLDING ENDURANCE OF FILMS

The flexibility of film scan be measured quantitatively interm sofwhat is known as folding endurance. Folding endurance of the films was determined by repeatedly folding filmsat the sameplacetillit broke. The number of times films could be folded at the same place, without breaking gives the value of folding endurance.

SWELLING INDEX OF FILMS

The swelling index of the films was determined by immersing pre weighed film of size in 50 ml water. The films were taken out carefullyat0.5,1, 2up-to 3hrs. Intervals blotted with filter paper and weighed accurately.

The swelling index calculated by,

$$\% \text{ Swelling Index} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100$$

SURFACE PHOF FILMS

Surface pH was determined by thefilms that were allowed in contact with 1ml of distilled water. The surface pH was noted by bring in gaco mbinedglass electrodeor pH paper nearthe surface offi lm sand allowing equilibrate for 1min.

TENSILE STRENGTH OF FILMS

The tensile strength of the film was determined with a digital tensile strength tester(Tinius-Olsen). Thesensitivity range of themachineis1-10Newton's. It consists of two load cell grips. The lower one was fixed, and the upper one was movable. The test film of size (1x4 cm^2) was fixed between thesecellgrips, and force was applied untilit breaks. The tensile strength of thefilmwasdirectlytaken fromthe dial reading in Newton's, which wasconverted into kilograms.

Drug content uniformity study of films

The film were tested for drug content uniformity by the UV-Spectrophotometric method. Films of 4sq.cm were cut from three different places from the casted films. Each film was place din a 100ml volumetric flask and dissolved in pH6.8 phosphate buffer, and 1 mlistaken and diluted with pH 6.8 phosphate buffer up to 10 ml. The absorbance of the solution was measured at 247nmusing a UV/visible spectrophotometer (ShimadzuUV-1700). The percentage of drug content was determined using the standard graph,and the same procedure was repeated for three films.

Determination of Moisture Content and Moisture Absorption

The buccal patches were weighed accurately and kept in desiccators containing anhydrous calcium chloride. After 3 days, the patches were taken out and weighed. The moisture content (%) was determined by calculating moisture loss (%) using the formula:

Moisture Content (%) = Initial weight – Final weight/Initial weight*100

The buccal patches were weighed accurately and placed in the desiccators containing 100 ml of a

saturated solution of aluminum chloride, which maintains 76% and 86% relative humidity (RH). After 3 days, the films were taken out and weighed. The percentage of moisture absorption was calculated using the formula:

Moisture Absorption (%) = Final weight – Initial weight/Initial weight*100

***In-vitro* drug release of films:**

*In-vitro*releasestudieswerecarriedout by attaching the dialysis membrane,prepared buccal films containing drug was placed inside the donor compartment, which is agitated continuously using a magnetic stirrer at 50RPM, and then the temperature was maintained at 37 ± 0.5ºC. The receptor compartment consists of 40 mlof pH 6.8 phosphate buffer, a sample of 1 mlwas withdrawna tperiodic intervals from the receptor compartment and replaced with fresh pH6.8 phosphate buffer immediately. Drug release was analyzed spectrophotometrically at 247 nm. The release rate was studied for all prepared formulations.

DRUG RELEASE KINETICS [89,90]

In order to predict and correlate the release behavior of Atorvastatin from different patches, it is necessary to fit into a suitable mathematical model. The *in vitro* Atorvastatin release data from buccal patches were evaluated kinetically using various mathematical models like zero-order, first-order, Higuchi, and Koresmeyer–Peppas model equations.

Zero-Order Kinetics

$F = K_o t$, where F represents the fraction of drug released in time t, and K_o is the zero-order release constant.

First-Order Kinetics

$\ln (1 - F) = -K_1 t$, where F represents the fraction of drug released in time t, and K_1 is the first-order release constant.

Higuchi Model

$F = K_H t^{1/2}$, where F represents the fraction of drug released in time t, and K_H is the Higuchi dissolution constant.

Koresmeyer–Peppas Model

$F = K_p t^n$, where F represents the fraction of drug released in time t, K_p is the Koresmeyer–Peppas release rate constant, and n is the diffusion exponent.

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

SOLUBILITY STUDIES

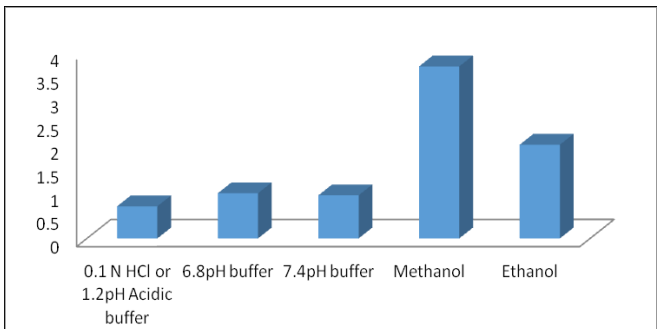

Table 01: Solubility studies of Atorvastatin

Fig 01: Solubility studies of Atorvastatin

Drug-excipients interaction studies of films

SOLVENTS	SOLUBILITY STUDIES (MG/ML)
0.1 N HCl or 1.2pH Acidic buffer	0.689
6.8pH buffer	0.968
7.4pH buffer	0.924
Methanol	3.69
Ethanol	2.01

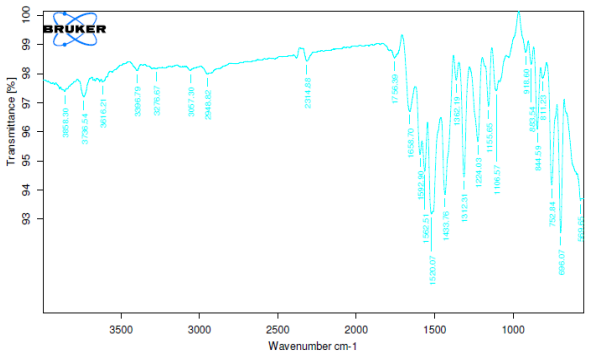

Fig 02: FTIR Spectra of Atorvastatin (pure drug)

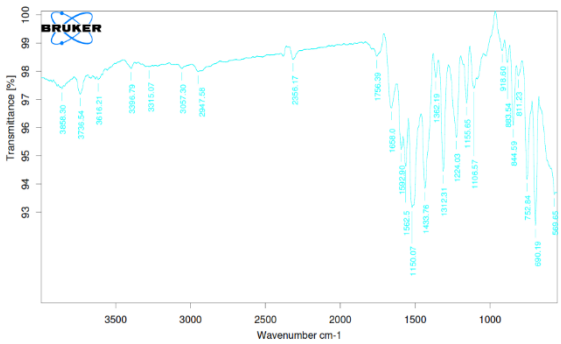

Fig 03: FTIR Spectra of optimized formulation

Table 02: Evaluations of Buccal Films

Formulation code	Thickness (mm)	Folding endurance	Surface pH	Weight variation(%)
F1	0.10	156	6.8	0.36
F2	0.16	124	6.2	0.45
F3	0.11	136	6.8	0.96
F4	0.14	152	6.5	1.02
F5	0.09	167	6.8	1.53
F6	0.13	169	6.8	0.75
F7	0.06	153	6.9	0.64
F8	0.01	105	6.7	1.20
F9	0.01	101	6.5	0.24

SWELLING INDEX OF FILMS

The swelling index of the films was determined by immersing preweighed film of size 10 mm in 50 ml water. The films were taken out from the Petri dish carefully at 0.5, 1, 2, up to 3hrs intervals, blotted with filter paper and weighed accurately, and the average swelling index of all films was given in Table. From allthesefilms, F8 formulation buccal filmfilmsshow a high percentswellingindex.

TENSILE STRENGTH OF FILMS

The tensile strength of all the films was evaluated by using standard tensile strength tester, and the average tensile strength of all films was given in Table.

In allthe cases, the calculatedstandard deviationvalues are very low, which suggests that the prepared filmsshow uniformtensile strength.

DRUG CONTENT UNIFORMITY OF FILMS

Atorvastatin buccal films prepared with various polymers were subjected to the evaluation for uniform dispersion of drugs through out the film. Ineach case, three films were used, and the average drug content was calculated, the results were shown inTable-03. The drug was dispersed in the range of 96 to 102%. They were suggesting that the drug was uni formly dispersed through out all prepared films.

Table 03: Evaluation of Atorvastatin Buccal Patches

FC	Avg. Swelling index (%)	Moisture content(%)	Moisture absorption (%)	Tensile strength	Drug content (%)
F1	32.18	1.06	3.06	3.85	96.13
F2	38.42	0.45	3.53	3.94	98.42
F3	41.05	0.96	4.10	5.13	97.63
F4	21.43	0.75	3.52	3.26	100.46
F5	28.72	1.05	3.06	4.53	101.78
F6	32.46	0.97	3.42	4.98	98.32
F7	12.43	0.86	2.96	2.05	99.42
F8	16.46	0.75	3.01	2.56	97.42
F9	14.16	0.62	2.87	3.01	96.28

In-vitro drug release of films

Thedetailed *in-vitro*drugreleasedatawereplottedbetweenpercentdrug released from the formulation and time, as shown in Fig. The present study indicates a good potential of erodible mucoadhesive buccal films containing Atorvastatin for systemic delivery with an added advantage of circumventing the hepaticfirst-pass metabolism. The result of the present study shows that therapeutic levels of Atorvastatin can bedelivered buccally. It may be concluded that the formulations F8 shows promising controlled drug release.

Table 04: Drug release data of Atorvastatin buccal films

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	13.28	10.36	8.41	35.18	30.46	26.35	43.15	40.36	36.19
1	23.56	16.49	19.86	43.16	39.86	36.15	49.82	45.26	42.53
2	29.75	22.15	22.35	55.89	47.82	42.18	56.75	52.36	49.16
3	35.13	28.43	28.64	66.52	57.43	49.63	64.82	59.83	53.85
4	39.46	35.17	35.16	76.32	63.85	54.12	75.82	65.46	58.72
5	45.36	42.38	39.85	84.15	69.43	59.82	86.49	73.46	65.43
6	56.32	53.19	45.32	90.53	73.49	65.43	95.06	81.72	72.63
7	65.43	59.75	50.36	97.52	77.12	71.53		90.82	79.82
8	72.53	64.15	59.83		81.76	76.32		98.24	84.18

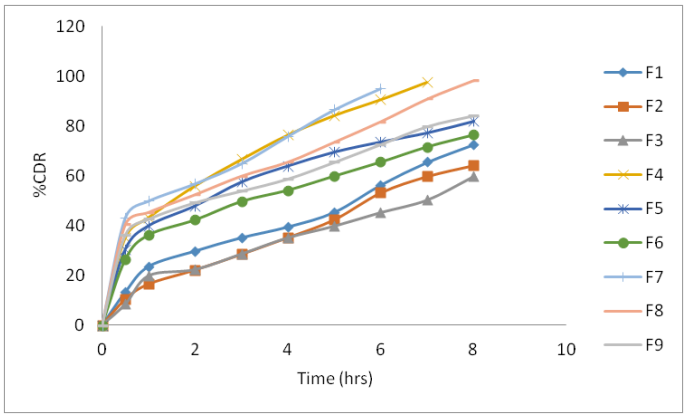

Fig 04: In-vitro Drug Release of Formulations (F1-F9)

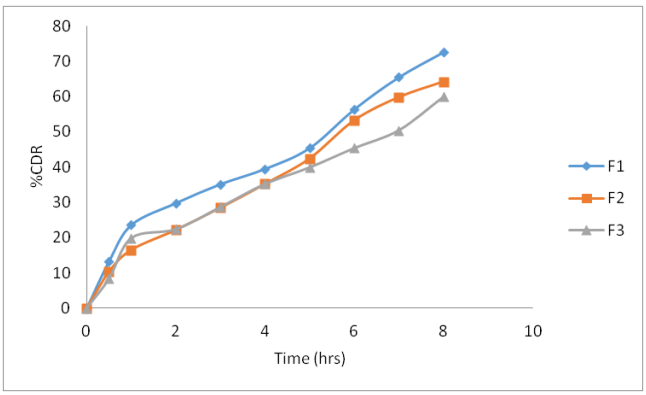

Fig05 : In-vitro Drug Release of Formulations (F1-F3)

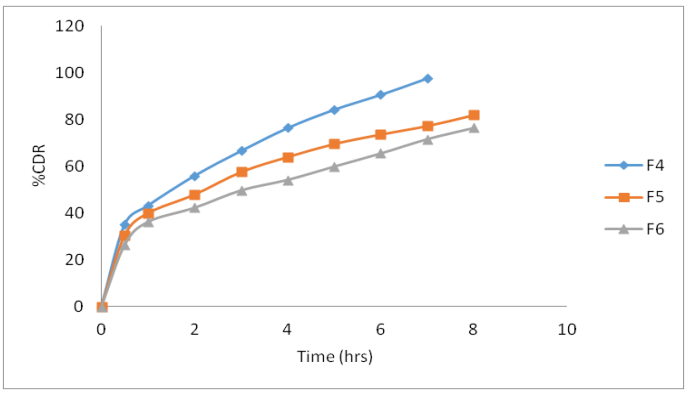

Fig 06: In-vitro Drug Release of Formulations (F4-F6)

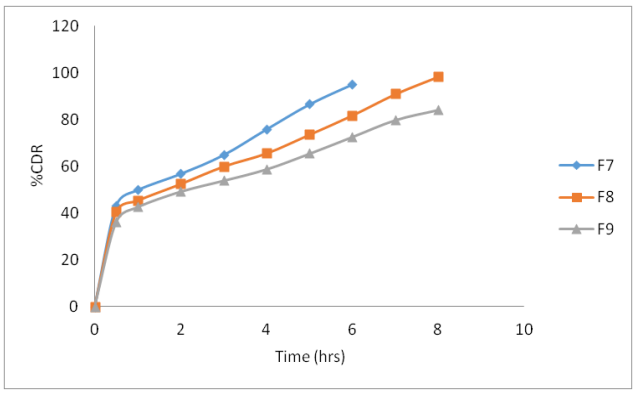

Fig 07: In-vitro Drug Release of Formulations (F7-F9)

Total Nine formulations was formulated by Gellan gum, HPMC E5 and HPMC E50 in three

different concentrations. From all the formulations, F8 shows 98.24 % of drug release at the ends of 8hr and chosen as optimized formulation. So the drug release kinetics were performed for the optimized formulation (F8).

Kinetic data of Atorvastatin mucoadhesive buccal films:

ZERO ORDER

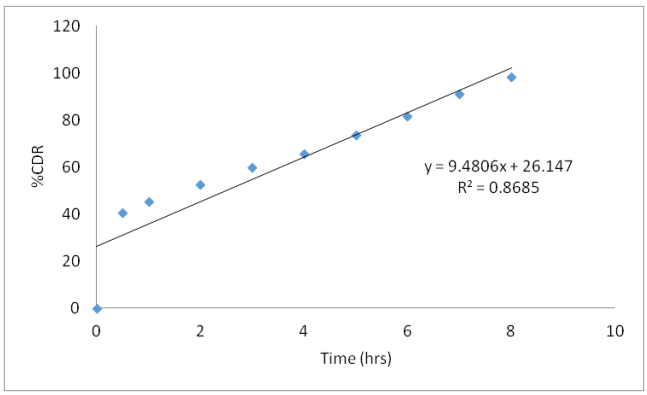

Fig 08: Zero-order of F8 formulation

FIRST ORDER

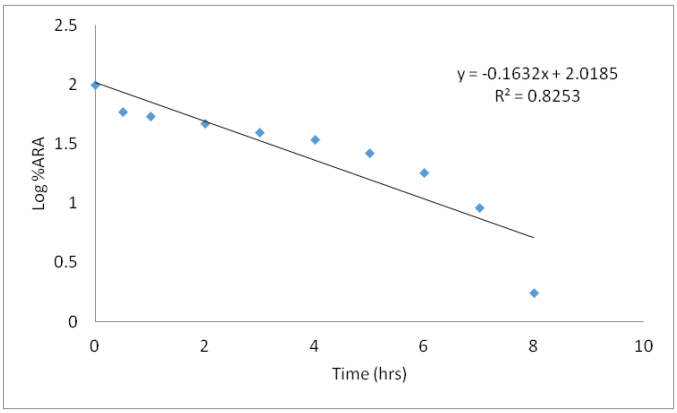

Fig 08: First order of F8 formulation

HIGUCHI PLOT

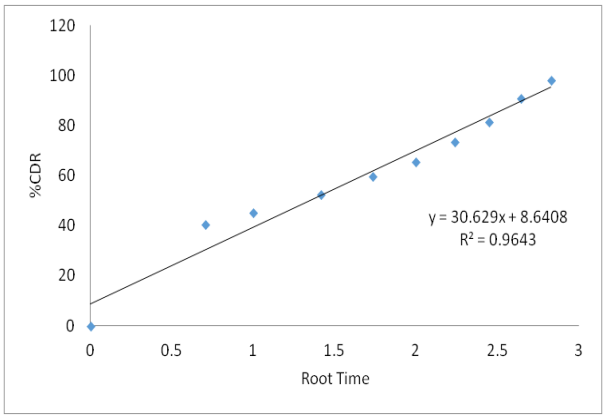

Fig 09: Higuchi plot of F8 formulation formulation

PEPPAS PLOT

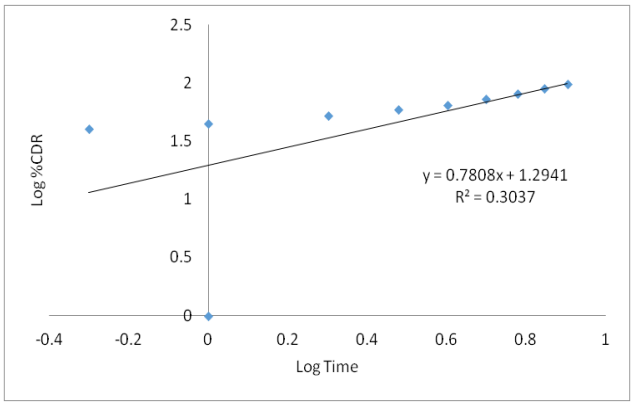

Fig 10: Peppas plot of F8

The in-vitro dissolution data for best formulation F8were fitted in different kinetic models i.e, zero order, first order, Higuchi, and korsemeyer-Peppas equation. Optimized formulation F8 shows R^2 value of 0.948. As its value nearer to the '1', it is confirmed as it follows the Zero-order release. The mechanism of drug release is further confirmed by the korsmeyer and peppas plot, if n = 0.45 it is called Case I or Fickian diffusion, 0.45 < n < 0.89 is for anomalous behavior or non-Fickian transport, n = 0.89 for case II transport and n > 0.89 for Super case II transport. The 'n' value is 0.780 for the optimised formulation (F8) i.e., 0.45 < n < 0.89 is for anomalous behavior or non-Fickian transport.

SUMMARY AND CONCLUSION

Recently the buccal patchh as been increasingly used for the administration of drugs mainly because of advantages like the drug is directly available to the systemic circulation,avoidance of first- pass metabolis mandeasy removal of patch from the site, etc.Among the various drug delivery systems, the development of a buccaldrug delivery system is one by which one can improve the bioavailability of the drug by avoiding first-pass metabolism. So, in the present research work, we have prepared Atorvastatin buccal films intending to improve its bioavailability.Atorvastatin buccal films were prepared by the solvent casting technique using Gellan gum, HPMC E50, and HPMC E5 and PEG 400. The detailed formulation compositions were shown in Table-1 The prepared films were evaluated for the number of parameters like physical appearance

R^2 values					n values
Formulation	**Zero order**	**First order**	**Higuchi**	**Korsmeyer - Peppas**	**Korsmeyer-Peppas (n)**
F8	0.868	0.825	0.964	0.303	0.780

and surface texture, weight uniformity, the thickness of the films, folding endurance, swelling index,tensile strength,drug excipients interaction study,content uniformity,*in-vitro* drugreleasestudy.

The results are quotedin a different section of the chapter-from theresultof various evaluation parameters, we can summarize:The films prepared were checked visually for its appearance and surface texture. All the prepared films were of smooth surface and elegant texture. The percentage weight variation of all the prepared films using different concentrations is between 0.24 to 1.53%. The films show thickness values between 0.01 to 0.16 mm. Thefilmsshow that folding enduranc evaluesare below 100 to 170. The films show swelling index values in between 12.43 to 41.05 %.

Similarly surface pH of all the films prepare disranging inbetween 6.2 to 6.9 pH. The tensile strength of all the films prepared is ranging from 2.05 to 5.13 Kg/cm^{2}respectively. The FTIR studies indicate that Atorvastatin showed complete entrapment within the polymer carrier bonding is suggested, and there was no chemical interaction.

Similarly,thefilms are also subjected to drug content uniformity study, and it lies in between 96 to 101 %, which suggests that uniform dispersion throughout the buccal films.

Finally,the*in-vitro* drug release study was carried out for all the films, and the release profile was subjected tovarious kinetic equations like the Higuchi diffusion equation and Peppas exponential equation. The regression coefficientvaluesof this kinetic equationareverynearertoone(1) suggesting that plots are fairly linear, and slope values of the Peppas equationare (0.45<n<0.89) suggest that the drug was released by Non-Fickian Transport. From the above results, it can be concluded that Atorvastatin can be delivered in the form of buccal films.The releasepatternof the drug from these films can be altered by using different formulation variables.

REFERENCES

1. JainN.K.ControlledandNovelDrugDelivery,1st edition,publishedby CBS Publishers and Distributors, New Delhi. 1997; 52-81.
2. ShojaeiAH.Asystemicdrugdeliveryviathebuccalmucosalroute, Pharm. Tech. 2001; 70-81.
3. NohaAN,FatmaAI,BoraieNA,MortadaLM.Mucoadhesivebuccal patchesofmiconazolenitrate:*in-vitro/in-vivo* performanceandeffectof aging. Int. J. pharm. 2003; 264: 1-14.
4. AmirH.Shojaei.Buccalmucosaasarouteforsystemicdrugdelivery:A review; J. Pharm. Sci.1998; 1 (1): 15-30.
5. https://www.drugbank.ca/drugs/DB01076
6. John, Asha & R, Sathesh&Goli, Divakar &Jangid, Manoj. (2010). Development and evaluation of buccoadhesive drug delivery system for Atorvastatin calcium. Journal of Current Pharmaceutical Research.
7. Ramani, Ketan & Singh, Saurabh &Mourya, Abhishek & Deshmukh, Dr Prashant &Chatap, Vivekanand. (2013). Design and Development of Atorvastatin Calcium BuccoadhesiveBilayered Devices for Oral drug delivery. Journal of Drug Delivery Science and Technology.
8. DR. ANNA BALAJI, B. KRISHNAVENI, VISHNUVARDHAN GOUD.; Formulation And Evalution Of Mucoadhesive Buccal Films Of Atorvastatin Using Natural Protein – Int J Pharm Pharm Sci, Vol 6, Issue 2, 332-337.
9. Hendriksen, B.A.; Felix, M.V.; Bolger, The composite solubility versus pH profi le and its role in intestinal absorption prediction. AAPS Pharm Sci. 2003, 5, E4.
10. Goodman Gilman's, in: Hardman, J.G.; Limbird, L.E.; The Pharmacological Basis of Therapeutics. A. Goodman Gilman (Eds.), h

ed., McGraw-Hill, New York, 2001, 10. 1009.

11. Dr. Safila Naveed. Simple UV Spectrophotometric Assay of Atorvastatin API Formulation and their Comparative Study. Global Journal of Medical Research 36Volume XIV Issue II Version I Year 2014.
12. Manoj SC, Abhiner G, Chakole RD. Simultaneous determination of atorvastatin calcium and telmisartan in pharmaceutical formulations by reverse phase-high performance liquid chromatography. Int J Pharm Chem. 2012;2:1–6.
13. Stanisz B, Kania L. Validation of HPLC method for determination of atorvastatin in tablets and for monitoring stability in solid phase. Acta Pol Pharm. 2006;63:471–6.
14. Zarghi A, Shafaati A, Foroutan SM, Khoddam A. A simple and rapid HPLC method for the determination of atorvastatin in human plasma with UV detection and its application to pharmacokinetic studies. Arzneimittelforschung. 2005;55:451 –4.
15. Bhanja S, Ellaiah P, Choudhury R, Murthy KV, Panigrahi B, Padhy S. Formulation, development and evaluation of mucoadhesive buccal patches of methotrexate. J. Adv. Pharm. Res. 2010; 1: 17- 25.
16. Viram PJ, Lumbhani AN, Vijaya Lakshmi P, Jha S. Formulation development and evaluation of buccal films of carvedilol. Int. J. Pharma. Sci. Res. 2010; 1 (8): 149-56.
17. Shah D, Gaud RS, Misra AN, Parikh R. Formulation of a water soluble mucoadhesive film of lycopene for treatment of leukoplakia. Int. J. Pharma. Sci. 2010; 2 (1): 6-10.
18. Attama AA, Akpa PA, Onugwa LE, Igwilo G. Novel buccoadhesive delivery system of hydrochlorothiazide formulated with ethyl cellulose- HPMC inter polymer complex. Sci. Res. Essay. 2008; 3 (6): 343-47.
19. Dharni S, Shayeda. Formulation and *in-vitro* evaluation of mucoadhesive buccal patches of ondansetron hydrochloride. Int. J. Pharm. Sci. Nano.2010; 3: 1-1.
20. Kumar GVP, Krishna RV, William GJ, Konde A. Formulation and evaluation of buccal films of salbutamol sulphate. Ind. J. Pharm. Sci.2005; 67(2): 160-64.
21. Deshmane SV, Joshi UM, Channawar MA, Biyani KR, Chandewar AV. Design and characterization of carbopol-HPMC-ethyl cellulose based buccal compact containing propranolol Hcl. Ind. J. Pharm. Educ. Res.2010; 44 (3): 253-58.
22. Koland M, Sandeep VP, Charyulu NR. Fast dissolving sublingual films of ondensetron hydrochloride effect of additives on *in-vitro* drug release and mucosal permeation. J. Young Pharm. 2010; 2 (3): 216-22.
23. Biswajit B, Garala K, Thimmasetty J. Formulation and evaluation of pimozide buccal mucoadhesive patches. Int. J. Pharm. Sci. and Nano.2010; 2 (4): 739-46.
24. Patel RS, Poddar SS. Development and characterization of mucoadhesive buccal patches of salbutamol sulphate. Current Drug Delivery. 2009; 6:140-44.
25. Mona Semalty, Semalty A, and Kumar G. Formulation and characterization of mucoadhesive buccal films of glipizide. Ind. J. Pharm. Sci. 2008; Jan-Feb; 43-48.
26. Khairnar A, Jain P, Baviskar D, Jain D. Development of mucoadhesive buccal patch containing aceclofenac*in-vitro* evaluations. Int. J. Pharm. Tech. Res. 2009; 1 (4): 978-81.
27. Wong CF, Yum KH, KokkhiangPeh. Formulation and evaluation of controlled releaseeudragit buccal patches. Int. J. Pharm. 1999; 178:11-22.
28. Supriya SS, Nilesh S, Saindane, Sutar S, Kadam V. Mucoadhesive bilayered patches for administration of sumatriptan succinate. AAPS Pharm. Sci. Tech. 2008; June; 909-16.
29. Kapil KP, Manoj KJ, Asha SJ, Shivanand K. Formulation and evaluation of timolol maleate buccal mucoadhesive patches. J. Pharm. Res.2010; 3 (8): 2031-35.
30. ArtusiM, Santi P Colombo P, JungingerHE. Buccal deliveryof thiocolchicoside:*in-vitro*and*in-vivo*permeationstudies.Int.J.Pharm.2003; 250: 203-13.
31. LuanaP,ValeriaA,FaustaA,MaurizioR,Stefano G,MarinellaC, Carlo R. Development of mucoadhesive patches for buccal administration of ibuprofen. J. Cont Rel. 2004; 99: 73-82.
32. IsabelDiaz,FrancoiseF,RichardHG,YvesJ.*Ex-vivo*evaluationof bioadhesivefilms for buccal delivery of fenatyl. J. ContRel.2007;

1-15.

33. PunithaS,GirishY.Polymersinmucoadhesiveb uccaldrugdelivery system. Int. J. Res. Pharm. Sci. 2010; 1(2): 170-86.
34. JosephA,Niclazzo,BarryLR,BarrieCF.Buccalpe rmeationenhancers how they really work. J. Cont Rel. 2005; 105: 1-15.
35. SevdaS,AtillaAH.Drugpermeationenhanceme ntviabuccalroute: possibilities and limitations. J Cont Rel. 2001; 72: 133-44.
36. SilviaRossi,SandriG,CarmellaCM.Buccaldrugd elivery:Achallenge already won. Drug discovery today tech. 2005; 1(2): 59-65.
37. YajamanSudhakar,KetousetuoKuotsu,Bandy opadhyayAK.Buccalbio adhesivedrug delivery-apromising optionfor orallyless efficientdrugs. J. Cont Rel. 2006; 114: 15-40.
38. EouaniC,PiccerellePH,PrinderreP,BourretE,J oachimJ.*In-vitro* comparative study of buccal mucoadhesive performance of different polymeric films. Eur. J. Pharm. Bio. 2001; 52: 45-55.
39. AnthonyC.Moffat,M.DavidOsselton,andBrian Widdop.Clarke's analysis of drugs and poisons, 3rd edition.2004; 1714.
40. Higuchi T.Mechanism ofsustained action medication. Theroetical analysis of rateof release of solid drugs dispersed in solid matrices. J PharmSci 1963; 51: 1145-.
41. PeppasNA.Analysis ofFickianand non-fickiandrug release from polymer. PharmActaHelv 1985; 60: 110-11.