International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 12 (C); December 2019; Page No.20767-20777
DOI: http://dx.doi.org/10.24327/ijcar.2019.20767-20777

Research Article

FORMULATION, DEVELOPMENT AND EVALUATION OF TASTE MASK ABACAVIR HCL USING CHITOSAN MICROBEADS IN FLAVOURED GEL

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A R T I C L E I N F O

Article History:
Received 15th September, 2019
Received in revised form 7th October, 2019
Accepted 13th November, 2019
Published online 28th December, 2019

Key words:

Microbeads, Abacavir HCl, chitosan, Gellan gum, Metered dose container.

A B S T R A C T

The purpose of this research was to mask the bitter taste of Abacavir HCl by formulating Microbeads. Taste masking was accomplished by initially formulating tasteless micro beads of abacavir HCl using chitosan, sodium alginate and gellan gum by polyelectrolyte complexation method. These microbeads was converted into gellan gum based flavoured gel. Gels were packed in metered dose squeeze container which can dispense the required amount of gel equivalent to the dose of drug. The gel can then be added to yoghurt, biscuits and breads or can be consumed as such. No significant changes in FTIR spectroscopy and DSC study. Practical yield was found to be 93.50%. The chitosan- gellan gum spherical microbeads formulations showed desirable drug entrapment efficiencies (60.10-91.30%) and invitro release (89.37%) in 24hrs. Formulation AG5 gel consisting of 0.3% gellan gum and 0.3% sodium citrate was considered as an optimum batch. Stability studies indicated no considerable changes in pH, viscosity and appearance of the optimised AG5 formulation. The product was finally dispensed in a metered dose container as well as in sachets. The results of the present study indicated that this novel oral gel containing microbeads of Abacavir HCL with an acceptable taste is feasible for paediatric patients.

INTRODUCTION

In recent years, biodegradable polymers have attracted attention as biomaterials particularly for tissue engineering, gene therapy, wound healing and controlled drug delivery systems (Motwani *et al*., 2008).Chitosan being a cationic polymer has been used for the production of microbeads and nanoparticles by ionotropic gelation with negatively charged polymers and there are many chitosan-polyanion complexes that have been investigated as drug delivery systems for drugs. (Kas, 1997) (Sezer, 1999).

Just a spoonful of sugar helps the medicine go down; in a most delightful way, one of the popular lines from childhood. More than 90% of pediatricians have reported that a drug's taste and palatability were the greatest barriers to completing treatment leading to non-compliance. (Smith, 2001).Many efforts have been focused for taste masking for paediatric patients on finding blockers for specific tastes, such as bitter, however, the fact there are around 25 putative bitter receptors makes it complicated to find a single, universal, bitter blocker (Schiffman, 2000). Besides this, microencapsulation, ion exchange resins, coating, solid dispersions, addition of flavourants and sweetners are also some methods (Sohi *et al*., 2004)

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Gellan gum (GG) has the characteristic property of thermo-reversible and cation ion induced gelation. Though GG can be complexed with any cationic substance, in the present study chitosan was used as it also requires an anionic substance for gelation, both materials are safe biocompatible and biodegradable. As the prepared beads are to be finally added into GG gel there would be no incompatibility issues. Gellan gum is widely used as a gelling agent in food products (Raymond *et al*.). Children like more intense sourness than adults, so lowering the pH increases the palatability, and can contribute to bitter taste masking and so citrate buffer was added. As well as, in the beads, colour, flavour and sweeetner can be incorporated. The presence of coloured beads in a transparent GG gel would appeal to paediatric patients. Finally dispensing the product in the form of metered dose pumping device would ensure accurate dosing can be easily squeezed out and added to yoghurts, on biscuits or breads.

Bitter taste of Abacavir HCl is felt when it comes in direct contact with mucous membranes of the tongue and taste buds, which reduce patient compliances causing the patients to feel unsatisfied. Taste masking of the drug as microparticles by the ionotropic gelation technique effect its release profile and other properties. In addition, chemical methods (after electrostatic reaction of cations with anions) such as altering the chemical structure of the drug itself have been used to remove the bitter taste (Jelvehgari *et al*.,2014).

MATERIAL AND METHOD

Abcavir Hydrochoride was obtained as gift sample from Lupin (P Ltd.) Pune, Sodium alginate, gellan gum, glacial acetic acid, calcium chloride, Methyl paraben, propyl paraben, and all other chemicals like citric acid, sodium citrate, purchased were of analytical grade.

Organoleptic properties

The sample of Abacavir HCl was checked for organoleptic characters such as colour, odour and appearance.

Fourier transforms infrared spectroscopic (FTIR) studies

The dry sample of Abacavir HCl was mixed with IR grade KBr in the ratio of 1:100. This mixture was compressed in form of a pellet by applying 10 tons of pressure in hydraulic press. The pellets were scanned over a wave number range of 4000 to 400 cm-1 in (Perkin Elmer, Spectrum BX, USA). FTIR instrument and spectral analysis was done (Perkin Elmer).

Physical Compatibility Test

Preformulation study was carried out with potential formulation polymers to determine drug-polymer interaction/compatibility. The physical mixture of Abacavir HCl with formulation excipients was placed in glass vials which were kept at room temperature. After 30 days, samples were observed for any physical changes and a chemical change was observed by infrared spectroscopy.

Differential Scanning Calorimetric (DSC) studies

The DSC thermogram of pure Abacavir HCl, and physical mixtures of drug excipients was recorded by using a Mettler Toledo DSC-823 system with a differential scanning calorimeter equipped with a computerized data station. All samples were weighed and heated at a scanning rate of 10°C/min between 40 and 360°C and 20 mL/min of nitrogen flow.

Analytical development (HPLC) method of Abacavir HCl (Kumari *et al*., 2007)

Preparation of standard stock solutions

Standard stock solution (1000µg/mL) of Abacavir HCl was prepared. 100 mg of drug was dissolved in 100 mL water (HPLC grade) in volumetric flask with shaking and then volume was made up to the mark with the same solvent.

Selection of analytical wavelength

By appropriate dilution of the standard stock solution with mobile phase, various concentrations of Abacavir HCl were prepared. Spectra were then obtained using double beam UV visible spectrophotometer in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. As per the literature review it was found out that λmax of Abacavir HCl is 218nm.

Chromatographic conditions

Column: 4.5×25cm Purospher star C18 HPLC column with 5µm (particles packing).
Mobile phase: a mixture of 50 mL of acetonitrile, 50 mL of phosphate buffer pH4.2
Flow rate: 1 mL per minute
The injection volume: 20µg/mL.
The elute was analyzed by a UV detector at 218 nm.

Preparation of Abacavir HCl microbeads from various polysaccharide gums by Ionotropic/Polyelectrolyte complex method. (K. Hemalatha *et al*., 2011 , Pathare *et al* 2013, Manjanna *et al*., 2013 , Reddy M C *et al* 2011, Santhi K *et al* 2013).

All microbeads were obtained by the ionotropic gelation/PEC method using Ca^{2+} ions. Abacavir HCl (0.30% w/v) + aqueous solution of alginate/ xanthan gum/ carragenan/ gellan gum (1% w/v)

Agitated to get complete dissolution.

chitosan was dissolved in glacial acetic solution (0.5% v/v) to which calcium chloride (Ca^{2+} ions) (1% w/v) were added and mixed using a magnetic stirrer.

The solution containing alginate/ xanthan gum/ carrageenan/ gellan gum and Abacavir HCl was then injected into the chitosan solution using a hypodermic syringe.

Microbeads formed rapidly which were left in the solution for 24 hours to ensure internal gelification. Finally, microbeads were filtered, washed and dried at room temperature.

Table 1 Microbeads formulation batches of chitosan-sodium alginate /gellan gum

Batches of Chitosan-sodium alginate microbeads							Batches of Chitosan-Gellan gum microbeads		
Formulation No.	Drug (mg)	Water (ml)	Acetic Solution (%0.5v/v) mL	$Cacl_2$ (gm)	Sodium alginate (gm)	Chitosan (gm)	Formulation No	Gellan gum (gm)	Chitosan (gm)
F1	300	100	100	1	1	0.3	**F13**	1	0.3
F2	300	100	100	1	1	0.5	**F14**	1	0.5
F3	300	100	100	1	1	0.7	**F15**	1	0.7
F4	300	100	100	1	1	0.8	**F16**	1	0.8

Evalution parameters for Microbeads

Percentage yield

The production yield of micro beads of various batches was calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of micro beads.

Percentage yield = Practical yield/ Theoretical yield ×100

Micrometric properties of the beads

The Angle of repose, flow properties, Compressibility index, bulk density, tap density Hausner's ratio and mean particle size of the microbeads was determined.

Swelling Ratio

Swelling ratio was studied by measuring the percentage water uptake by the beads. About 50 mg of beads from all prepared formulations were accurately weighed and placed in 100 ml of phosphate buffer pH 7.2. Beads were removed from their respective swelling media after 8 hr and weighed after drying the surface water using filter paper. The water uptake was calculated as the ratio of the increase in weight of beads after swelling to the dry weight.

Swelling ratio = Swollen weight -Initial weight × 100 /Initial weight

Drug content (mg)

100 mg microbeads were powdered and transferred into a 100 ml volumetric flask and the volume was made upto the mark with 7.2 pH phosphate buffer and kept aside for 12 hrs with occasional shaking. Then the absorbance was analyzed

spectrophotometrically at 289 nm. Three determinations were carried out for each formulation.

Drug entrapment efficiency

Abacavir HCl content in the microbeads was estimated by a UV-spectrophotometric method. Accurately weighed 100mg of microbeads (100 mg) were powdered and suspended in 7.2 pH phosphate buffer. The resulting solution was kept for 24hrs. Next day it was stirred for 20min using ultra sonicator. The solution was filtered through a 0.45 µm membrane filter, after suitable dilution if required, Abacavir HCl content in the filtrate was analysed at 287 nm using UV-Visible spectrophotometer. The obtained absorbance was plotted on the standard curve to get the exact concentration of the entrapped drug. Calculating this concentration with dilution factor the percentage of actual drug encapsulated in microbeads was calculated. The drug entrapment efficiency was determined using following relationship.

Drug entrapment efficiency =Actual drug content/Theoretical drug content×100

In Vitro Release Profile of Drug loaded beads

In vitro drug release studies of drug loaded beads were performed in PH 7.2 phosphate buffer (900 ml) using USP Type II Dissolution Test apparatus. Drug loaded beads equivalent to 100mg of drug were placed in dissolution jar. The dissolution medium was maintained at 37ºC ± 0.5ºC at 100 rpm. 10 ml sample was withdrawn after 1, 2, 4, 6, 8, 10, 12, 24hr and absorbance was measured at 287 nm employing Shimadzu-1700 UV/Vis spectrophotometer after suitable dilution of the samples.

In Vitro Release Kinetics study of Abacavir HCl microbeads

Determination of order of release of drug loaded batches was done by graphical method. From the dissolution data, a graph was plotted using % drug remaining Vs time. The slope of the curve was calculated to find out the release rate constant. Regression coefficient of the curve was determined to confirm the correlation between X and Y axis. In the second stage, from the same dissolution data, a graph was plotted through log % remaining Vs time. The slope and regression coefficient of the graph were also determined. In order to predict and correlate the release behavior of drug from the polymeric matrix, it is necessary to fit the in vitro release data in to a suitable model. Hence the dissolution data were fitted according to the well-known exponential equation, which is often used to describe the drug release behavior from a polymer system.

Formulation of Gellan gum based Flavoured gel including microbeads of Abacavir ***HCl*** Gohel *et al*., 2009, Bhoyar and Biyani, 2010, Dheivanai *et al*., 2012 , Lohani A)

Low acyl dry Gellan gum powder + 50 ml of distilled water
Stirring for 20 min at 95^0 to facilitate hydration of Gellan gum
Sucrose was added with continuous stirring at 80° then Citric acid, raspberry flavour and preservatives+ sodium citrate (0.3 %) was dissolved in 10 ml of distilled water added to the mixture.

The weight of the gel was monitored continuously and finally it was adjusted to 100 g with distilled water. The mixture was allowed to cool to room temperature (25±5°) to form a gel.

The prepared (undried or wet) Chitosan-sodium alginate/ Gellan gum beads were added to this Gellan gum gel and filled into metered dose pumping device for delivery of the product.

Table 2 Formulation of Gellan gum based Flavoured gel including microbeads of Abacavir HCl

Ingredient	Batch code					
	AG1	AG2	AG3	AG4	AG5	AG6
	Sodium alginate microbeads			Gellan gum Microbeads		
Abacavir HCl%	0.9	0.9	0.9	0.9	0.9	0.9
Gellan gum %	0.1	0.3	0.5	0.1	0.3	0.5
Citric acid %	0.05	0.05	0.05	0.05	0.05	0.05
Sucrose %	20	20	20	20	20	20
Sodium citrate %	0.3	0.3	0.3	0.3	0.3	0.3
Methyl paraben (mg)	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben (mg)	0.02	0.02	0.02	0.02	0.02	0.02
Orange flavor %	2	2	2	2	2	2
Water %, up to ml	50	50	50	50	50	50

Evalution of Abacavir HCl microbeads Flavoured Gel
Organoleptic properties

Oraganoleptic properties like colour, odour and texture of the gel were determined.

Determination of pH

The pH of the gels was determined using a calibrated pH meter. The readings were taken for average of three samples.

Rheological properties

Rheological studies were carried out using Brookfield Viscometer Model RVT (USA). Viscosity was measured using spindle number LV4 at the rotation of 50 rpm at room temperature. The viscosity measurements were made in triplicate using fresh samples each time.

Spreadability

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. Weight (50 g) was then added in instrumental fashion to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability (S).

Invitro Release of Abacavir HCl from gel (Costo and Lobo,2001)

An in vitro drug release study of the gel was performed in pH 7.2 phosphate buffer (900 ml) using USP Type II Dissolution Test apparatus. Gel equivalent to 150mg drug was placed in dissolution jar. The dissolution medium was maintained at 37ºC ± 0.5ºC at 100 rpm. 10 ml sample was withdrawn after 1, 2, 4, 6, 8, 10, 12, 24hr and absorbance was measured at 287 nm employing Shimadzu-1700 UV/Vis spectrophotometer after suitable dilution of the samples

Diffusion study of drug from beads

Beads equivalent to 3 doses of drug was added to the gellan gum gel (50 gms) to verify that there is no diffusion of Abacavir HCl from the beads into the gel base over a period of time. This was checked on 0, 1, 2….7 days and then on the 14^{th} day using UV spectrophotometer after suitable dilution of the samples.

Homogeneity of drug containing pellets (microbeads) in the prepared gel

Six different weights of gel containing microbeads were crushed using mortar pestle and then were extracted in water to obtain the drug. This solution was filtered through whatman filter paper. The filtrate was analyzed by UV/Vis spectrophotometer at wavelength 289 nm.

Filling of gel

The prepared gel containing microbeads formulation was filled in a suitable container namely a sachet or a metered dose container with actuator.

Calculation for Metered dose delivery

Accurately weighed amount of gel per actuation of pump was suspended in 7.2 pH phosphate buffer. The resulting solution was kept for 24hrs. Next day it was stirred for 20min using ultra sonicator. The solution was filtered through a 0.45 µm membrane filter. After suitable dilution if required, Abacavir HCl content in the filtrate was analysed at 287 nm using UV-Visible spectrophotometer. The obtained absorbance was plotted on the standard curve to get the exact concentration per actuation.

Taste Evaluation (Costo and Lobo 2001)

Six healthy Human volunteers participated in taste evaluation of gellan gum gel containing taste masked beads. One dose equivalent gel was given to every volunteer and they were told to keep the gel in mouth for 5 sec. The volunteers were instructed to not swallow gel. The volunteers were asked to comment on the bitterness, after taste, sweetness, mouth feel, and flavour of gel.

Stability studies of Gel (Q1A (R2, 2003)

The samples were kept at different temperatures (08°, 25±5°, 45±2°) for four weeks. The samples were observed for pH, viscosity and appearance at the interval of 2 weeks and 4 weeks for any changes.

Drug content by HPLC

In case of gel formulation product equivalent to 100mg Abacavir HCl was dissolved in 100mL water (HPLC grade) and kept under sonication for 30min to prepare 1000µg/ml of stock solutions. Further dilution was made to make 100µg/mL, 200 µg/mL test solution. The respective solution was sonicated and filtered through whatman filter paper (45µm). Drug content was then determined by reverse phase high performance liquid chromatography.

RESULT AND DISCUSSION

Characterization of Abacavir HCl

The sample of Abacavir HCl was found to be white crystalline powder having characteristic odour and bitter taste.

Fourier transforms infrared spectroscopic (FTIR) studies

The FTIR spectrum is shown in figure 1 and interpretation of FTIR spectra is given in Table 3. FTIR spectrum of Abacavir HCl showed all the peaks corresponding to the functional groups present in the structure of Abacavir HCl.

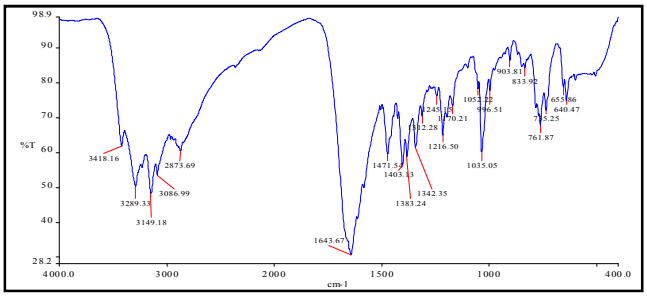

Figure 1 FTIR spectrum of Abacavir HCl

Table 3 Interpretation of FTIR spectrum of Abacavir HCl

Peak observed (cm^{-1})	Interpretation
3290.30	NH stretch
3148.09	OH stretch
1645.39	C=C Stretch Cyclopentene Ring
1471.10	CH Bend
2874.66	CH stretch of alkene

Physical Compatibility Test

For physical compatibility test by FTIR, drug and excipients were mixed and kept for 15 days. The spectrum was scanned over a frequency range 4000-400 cm^{-1}. FTIR spectra of drug-excipients mixtures retained the characteristic functional peaks of the drug as shown in figures 2 and 3.

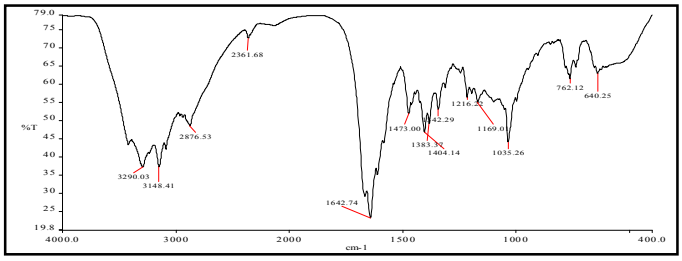

Figure2 IR spectrum of Abacavir HCl + Sodium alginate physical mixture

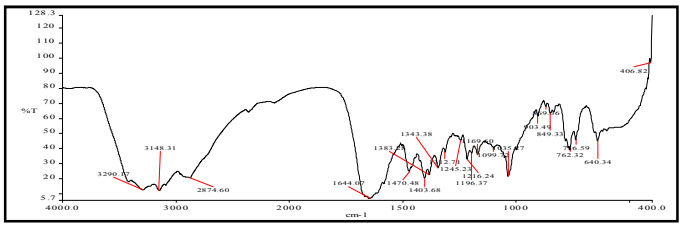

Figure 3 IR spectrum of Abacavir HCl + Gellan gum physical mixture

Table 4 Interpretation of FTIR Spectrum of Abacavir HCL and sodium alginate / gellan gum physical mixture

FTIR spectrum of Abacavir HCl + sodium alginate physical mixture		FTIR spectrum of Abacavir HCl + gellan gum physical Mixture	
Peak observed (cm-1)	**Interpretation**	**Peak observed (cm-1)**	**Interpretation**
3290.03	NH strecth	3290.17	NH stretch
3148.41	OH strech	3148.31	OH stretch
1642.74	C=C Strech Cyclopentene Ring	1644.07	C=C Strech Cyclopentene Ring
1473.00	CH Bend	1470.48	CH Bend
2876.53	CH strech of alkene	2874.60	CH strech of alkene

Differential Scanning Calorimetric (DSC) studies

Differential Scanning Calorimetry studies indicated a sharp endothermic peak at 135°C corresponding to melting of pure Abacavir HCl as shown in figure 4.

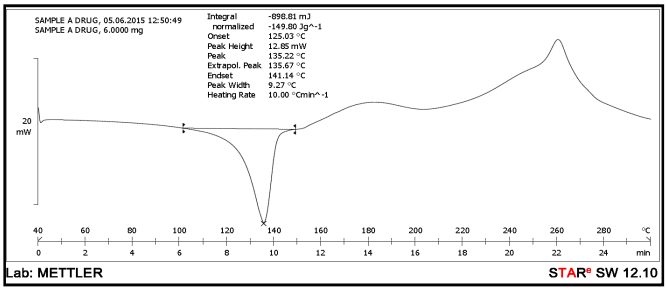

Figure 4 DSC thermogram of Abacavir HCl

The DSC thermogram of pure drug and physical mixtures is shown in the figure 4. Figure 5 which depicts the DSC thermogram of Abacavir HCl (a), Sodium alginate physical mixture, (b) Gellan gum physical mixture (c). The DSC thermogram of pure Abacavir HCl indicated a sharp endothermic peak at 135°C and exothermic peak at 260°C corresponding to melting of pure Abacavir HCl. The peaks broadening were observed in case of samples b and c, also the relative intensities were changed due to dilution of drug in the samples b and c. It can be concluded that the polymers and drug do not interact with each other. Also the drug didn't form a complex with the excipients as the endothermic peaks remained unchanged in position.

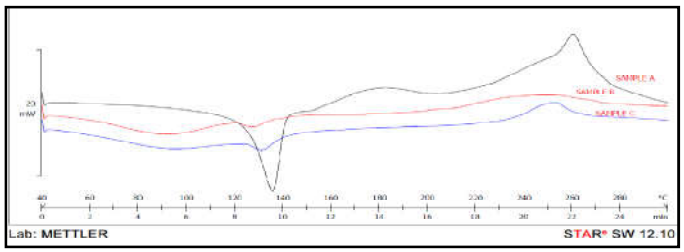

Figure 5 DSC thermogram of (a) Abacavir HCl (b) sodium alginate physical mixture (c) gellan gum physical mixture

From the above observation of FTIR and DSC study, it was concluded that the polymer and drug did not interact with each other and are compatible.

HPLC method of Abacavir HCl

The criteria employed for selection of particular solvent system for the analysis was cost, time required for analysis, sensitivity of the assay and solvent noise for the analysis of Abacavir HCl formulation. Literature review does reveal the specific mobile phase for Abacavir HCl estimation. It was clearly found that wavelength of Abacavir HCl is 218nm. At this wavelength chromatogram of pure drug was obtained as follows

Chromatogram of pure drug

The chromatogram of pure drug at concentration 100 µg/mL is shown in figure 6. The retention time was observed as 2.821 min.

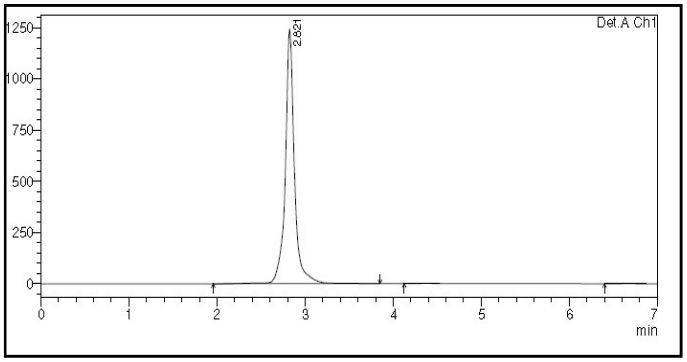

Figure 6 Chromatogram of Abacavir HCl

Evalution of microbeads

Percentage practical Yield

Percentage practical yield was increased with increase in polymer concentration. Batch with 1% gellan gum and 0.8% chitosan formulation showed highest percentage yield with spherical shape and no agglomeration of microbeads.

Table 5 Percentage yield of different formulation

Batch code	Sodium alginate solution (%)	Chitosan solution (%)	Percentage practical yield (%)
F1	1	0.3%	72.00
F2	1	0.5%	75.04
F3	1	0.7%	85.10
F4	1	0.8%	90.11
Batch code	**Gellan gum solution (%)**	**Chitosan solution (%)**	**Percentage practical yield (%)**
F13	1	0.3	72.10
F14	1	0.5	74.90
F15	1	0.7	87.63
F16	1	0.8	93.57

Micrometric properties of the beads

The mean particle size of drug loaded beads was determined by optical microscopy, and was found to be 510-620 µm as shown in Table no 6 and fig 7 and 8.

Figure 7 Sodium alginate formulation Paticle size (580-630µm)

Figure 8 Gellan gum formulation Paticle size (510-580µm)

The size of the microbeads increased as the concentration of chitosan increased. It may be due to formation of a thick chitosan layer with the increase of concentration of chitosan in the gelation medium.

Table 6 Evaluation parameters of Microbeads

Formulations	Angle of repose (0)	Carr's compressibility index (%)	Hausner's ratio	Bulk density		Particle size (µm)
				Loose (g/cm^3)	Tapped (g/cm^3)	
Sodium Alginate						
F1	43.28	21.05	1.26	0.414	0.525	580
F2	42.64	24.32	1.32	0.425	0.565	610
F3	41.82	23.94	1.31	0.443	0.583	620
F4	42.27	22.14	1.28	0.450	0.578	630
Gellan Gum						
F13	44.72	21.17	1.27	0.414	0.529	510
F14	42.25	24.65	1.32	0.431	0.572	550
F15	43.32	24.30	1.32	0.437	0.578	570
F16	42.12	22.85	1.29	0.450	0.583	580

Swelling studies

It was found that all beads swelled slowly in the presence of phosphate buffer pH7.2. It can be observed that maximum swelling occurred with F4 and F16 formulation and that gellan gum based microbead had the highest swelling.

Table 7 Swelling studies of different Batches

Formulation code	Initial volume	Final volume (after24hrs)	Swelling factor (%)
Sodium alginate			
F1	10	11	10
F2	10	12	20
F3	10	12	20
F4	10	13	30
Gellan gum			
F13	10	12	20
F14	10	12	20
F15	10	13	30
F16	10	14	40

Drug entrapment efficiency

The drug entrapment and loading increased as the concentration of chitosan increased. The drug entrapment efficiency of all formulations was found to be in the range of 55.07% to 91.30 % respectively as shown in table 8.

Table 8 Drug entrapment efficiency

Formulation code	Drug loading mg/100mg beads	Entrapment efficiencies (%)
Sodium alginate		
F1	6.35	55.07
F2	7.16	66.85
F3	7.19	71.90
F4	7.30	75.90
Gellan gum		
F13	6.93	60.10
F14	7.64	71.33
F15	8.74	87.47
F16	8.82	91.30

In vitro Release Profile of Drug loaded beads

Dissolution studies on all the eight formulations of Abacavir HCl microbeads were carried out using USP dissolution apparatus Type II. Phosphate buffer pH7.2 was used as the dissolution medium. The cumulative percent drug release at the end of 24 hr was found to be 54.760, 62.126, 68.740, and 72.934% for the formulations F1, F2 ,F3 and F4 respectively, whereas cumulative percent drug release at the end of 24 hr was 68.395, 70.661,81.098 and 89.378% for formulations F13, F14, F15 and F16 respectively.

Table 9 Drug release studies of sodium alginate formulations

Time (hr)	% Drug Release Formulation-1	% Drug Release Formulation-2	% Drug Release Formulation-3	% Drug Release Formulation-4
1	5.789	9.417	11.007	11.748
2	12.000	15.633	16.851	22.013
4	16.021	22.742	25.674	29.226
6	22.790	29.977	30.837	36.235
8	30.869	35.157	37.165	39.230
10	35.897	39.945	41.022	43.045
12	37.965	42.642	45.726	45.933
24	54.760	62.126	68.740	**72.934**

Table 10 Drug release studies of Gellan gum formulations

Time (hr)	% Drug Release Formulation-13	% Drug Release Formulation-14	% Drug Release Formulation-15	% Drug Release Formulation-16
1	9.513	9.880	11.560	11.774
2	13.610	16.309	16.825	22.776
4	17.357	25.916	26.068	31.021
6	22.272	29.393	30.951	36.566
8	30.529	36.418	38.089	40.508
10	35.649	40.325	43.578	44.170
12	40.440	43.568	46.187	46.847
24	68.395	70.661	81.098	**89.378**

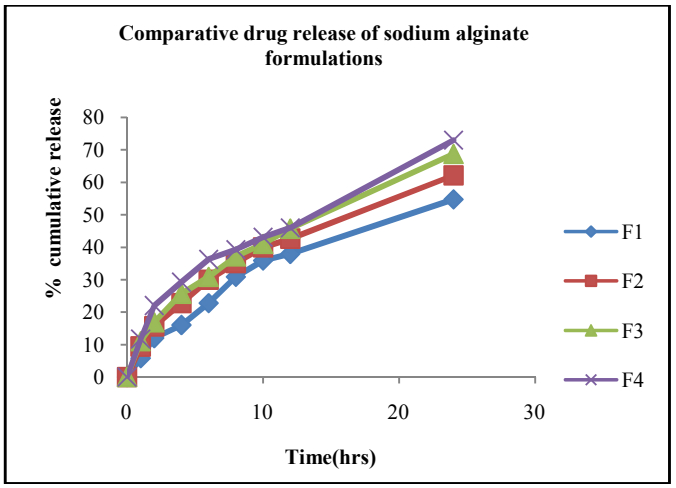

Figure 9 Dissolution profiles of formulations F1-F4

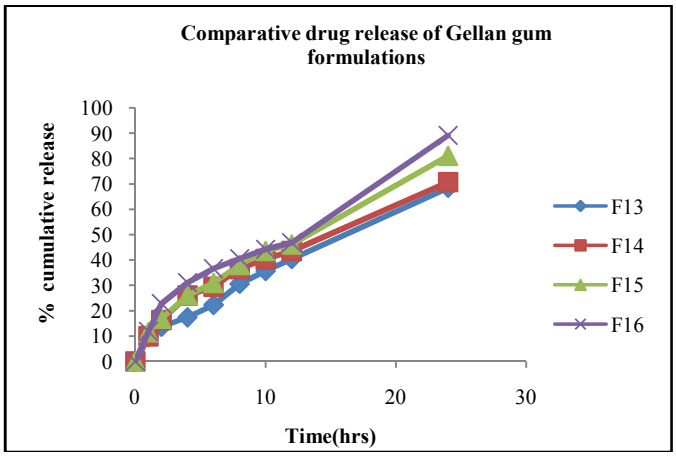

Figure 10 Dissolution profiles of formulations F13-F16

From the tables 9 and 10 it can be observed that maximum drug release occurred from F4 formulation. It was thus observed that the batches which had maximum swelling also brought about maximum release of Abacavir HCl. Based on their results F4 and F816 formulation were considered for further studies.

In Vitro Release Kinetics study of Abacavir HCl Microbeads

The optimized sodium alginate and Gellan gum formulations i.e. F 4 and F16 were subjected to kinetic treatment study to understand the drug release behaviour.

From table 11 it was found that the Korsmeyer Peppas model best fitted for the optimised formulations F4 and F16 as the regression value (R^2) was maximum for Korsmeyer-Peppas model. The 'n' value in Korsmeyer-Peppas model describes the drug release mechanism. It is known that, when $n \leq 0.5$ Fickian diffusion is observed and the release rate is independent of t, while $0.5 < n < 1.0$ indicate anomalous (non - fickian) transport and when n=1, the release is zero order. Here n value was found to be 0.5284 and 0.5656 respectively which signified that release pattern of optimized formulations followed non Fickian diffusion.

Table 11 Dissolution kinetics

Sr. No.	Model	Correlation Coefficient (r^2)	
		Sodium alginate (F4)	Gellan gum (F16)
1	Zero order	0.8384	0.9862
2	First order	0.9738	0.9494
3	Matrix	0.9882	0.9436
4	**Korsmeyer Peppas**	**0.9944 n = 0.5284**	**0.9919 n = 0.5656**
5	Hixson Crowell	0.9470	0.9803

Evalution of Abacavir HCl microbeads Flavoured Gel Organoleptic properties

Abacavir HCl microbeads Flavoured Gel found Orange in colure with pleasant odour and Smoth texture.

Determination of pH

The pH of the gel was determined using a calibrated pH meter. Average pH was found to be 6.1.

Rheological properties

All the formulations of the gel were found to be having good gelling capacity. In comparison to AG5, formulations AG2, AG3, AG6 had very high viscosity, while AG1 AG6 showed moderate viscosity. AG5 was found to have good gelling capacity (more than 24hr) and the required viscosity as compared to any other formulation.

Spreadability

From table 15 AG5 showed good spreadability as compared with any other formulation. The results shown reveal that gels of the formulation AG5 exhibited acceptable consistency and viscosity.

Table 12 Characteristics of gels using Gellan gum

Formulation	pH	Viscosity (cps)	Spreadability gms/sec
Chitosan: Sodium alginate Microbeads (0.8:1) F 4			
AG1	6.0	2152±40 (cPs)	15
AG2	6.1	11136±60 (cPs)	16
AG3	6.1	14965±50 (cPs)	11
Chitosan: Gellan gum Microbeads (0.8:1) F 16			
AG4	6.1	2546 ±45 (cPs)	16
AG5	6.0	8945 ±60 (cPs)	18
AG6	6.1	13448 ±55 (cPs)	13

Looking at the results of swelling behaviour, entrapment efficiency, in vitro release after 24hrs and paricle size for both F4 (sodium alginate based microbeads) and F16 (gellan gum based microbeads), it was decided to go ahead with F8 batch as it had superior properties in all respects over F4 batch. Besides this, the gel in which these beads were supposed to be finally added was to comprise of gellan gum as a gelling agent and it was thought that using microbeads based on gellan gum would be advantageous as compared to sodium alginate based beads as there would be no incompatibility issues.

In vitro Release of Abacavir from gel (AG5)

Dissolution study on gel formulation of Abacavir HCl was carried out using a USP dissolution apparatus Type II. Phosphate buffer pH7.2 was used as the dissolution medium. The cumulative percent drug release at the end of 24 hr was found to be 59.284, 66.326, 72.936, and 70.261%, 88.871, 80.254 for the formulations AG1, AG2, AG3, AG4, AG5, AG6 respectively.

Table 13 Drug release studies of gel formulations

Time (hr)	% Drug Release AG1	% Drug Release AG2	% Drug Release AG3	% Drug Release AG4	% Drug Release AG5	% Drug Release AG6
1	8.544	10.945	11.073	9.88	11.772	11.561
2	14.613	15.234	21.014	22.013	22.776	16.554
4	19.205	25.632	29.226	29.226	26.068	31.021
6	26.361	29.364	36.235	36.235	30.951	36.684
8	31.226	34.328	39.231	39.230	38.089	40.508
10	39.425	40.479	42.214	43.045	44.141	43.125
12	41.361	42.325	43.324	45.933	46.187	44.235
24	59.284	66.324	72.936	72.934	**88.871**	80.254

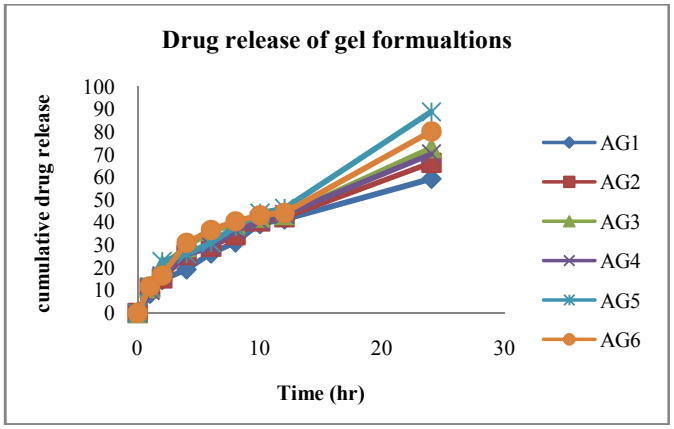

Figure 11 Dissolution profile study of gel formulations

From table 13 and fig 11, it can be observed that maximum drug release occurred from AG5 formulation.

All the batches of gels were transparent in appearance. The gel of formulation AG1 and AG4 exhibited fluid like consistency while the gel of formulation AG2, AG3 and AG6 were very thick in consistency. Drug release and Viscosity are important parameters which provide vital information during the optimization of the gel. The viscosity of the formulation AG1 and AG4 were low because of its fluid like consistency while the viscosity of the formulation AG2, AG3 and AG6 were high. The viscosity of formulation AG5 was acceptable. formulation AG5 consisting of 0.3% GG and 0.3% sodium citrate was considered as an optimum batch considering drug release, viscosity and appearance. Therefore it can be concluded that formulation AG5 is the optimised formulation for further studies.

Diffusion study of drug from beads

The content of Abacavir HCl was observed from 0-16 days.

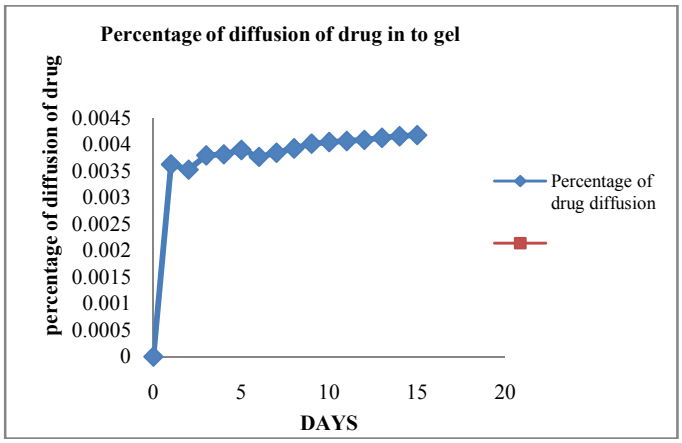

Figure 12 Percentage diffusion of drug into gel

It was observed that there was negligible diffusion of drug of from beads in to gel; this confirms the taste masking capacity of beads.

Homogeneity of drug containing pellets (microbeads) in the prepared gel

For uniform dispersion of beads in the gel and hence for content uniformity, test for homogeneity was carried out. It was observed that:

For 300mg of drug (≅ two doses of Abacavir HCl) gives 41.5 gm of beads (wet) are formed.

It is not feasible to add this large quantity in 10 gm gel (≅2 tea spoons) for administration to paediatric patients. Hence this was dried and then added to 10gm gel after drying to constant weight in vacuum oven at 45^0C for 24hrs. The resultant beads weighed 3 gm. which were easily dispensed in 10gm of gel. From this gel, 6 samples of gel having weight 1.5 gm was weighed and subjected to drug content analysis, the results of which are shown below

Table 14 Homogeneity of drug containing pellets (Microbeads) in the prepared gel

Sample no	Amount of drug in 1.5gm gel (mg)	% content
1	14.95	99.7
2	14.92	99.5
3	15.15	101
4	15.04	100.3
5	14.76	98.4
6	15.22	101.5

From table 14, percent drug content for Microbeads in gel showed significant homogeneity of drug in all samples.

Filling of Microbeads in Metered dose container/ Sachet

The formulation of dried Microbeads in gel was filled in a sachet equivalent to 5gm weight. This is a suitable and easiest way to deliver a sachet than a metered dose container.

A novel method of administration of Microbeads containing gel was thought in the present study. There is no literature available related to metered dose gels for taste masked gels to be taken orally. Oral liquids like syrup and suspension have not proved to be good carrier for bitter drugs due to their contact with the taste buds before they are digested and so it was decided to deliver Abacavir HCl in the form of taste masked Microbeads using flavoured gel as a carrier. Metering of it would ensure uniformity and accuracy of dose. As well as it can be added on biscuits, yoghurts, breads, etc. So the gel containing beads was filled in metered dose container.

Calculation for Metered dose delivery

Table 15 Evaluation of per actuation content for Abacavir HCL Metered dose by UV-spectophotometric method

Actuation times	Weight of per pump (g)	Abacavir HCL for per pump (mg)
1	0.7281	21.41
2	0.9125	24.30
3	0.6125	19.42
4	Blockages	--

Figure 13 Problems of blockages in metered dose container

From table 15 it can be observed due to blockages (figure 13) weight variation per pump/actuation occurred. Therefore, it was decided to fill in sachet which would also ensure accuracy and ease of dispensing, it can be also added on biscuits, yoghurts, breads, etc.

Taste evaluation

Six healthy, adult human volunteers participated in taste evaluation of Abacavir HCl gel (AG5). Abacavir HCl gel (1g) was given to every volunteer and they were told to keep the gel in mouth for 5 sec. The volunteers were instructed not to swallow the gel. The volunteers were asked to comment on the bitterness, aftertaste, sweetness and flavor of the gel. Mouth feel in terms of grittiness was also checked. The results of taste evaluation of Abacavir HCl gel are shown in table 16.

Table 16 Taste evaluation (formulation AG5)

Parameters	1	2	3	4	5	6
Bitterness	NB	NB	NB	NB	NB	NB
Aftertaste	NB	NB	NB	NB	BT	NB
Sweetness	VS	SW	VS	VS	VS	VS
Flavor	GD	GD	GD	GD	GD	GD
Mouth feel	GD	GD	GD	GD	MD	GD

NB Non bitter, BT bitter, SW sweet, VS very sweet, GD good, MD moderate.This indicated that the volunteers were not able to decipher the bitter taste of the drug and that the taste was masked.

Stability studies of Gel

The samples were kept at different temperatures (08°, 25±5°, 45±2°) for four weeks. The samples were observed for pH, viscosity, drug release and appearance at the intervals of 2 week**.**

Appearance

Formulations AG5 kept for stability studies were examined. The colour of the formulations was similar before and after stability studies. The results of the stability studies are shown in table 17.

Table 17 Stability studies of Abacavir HCl gel (Formulation AG5)

Temperature	0°C		Room Temperature	
Weeks	2	4	2	4
pH	6.1	6	6.05	6.1
Viscosity(cPs)	8950	8952	8961	8967

Table 17 indicate no considerable changes in pH, viscosity and appearance of the formulations. Precipitation of Abacavir HCl in the gel was also not observed.

In vitro drug release studies

Dissolution profiles of formulations AG5 after stability study are shown in figures 14.

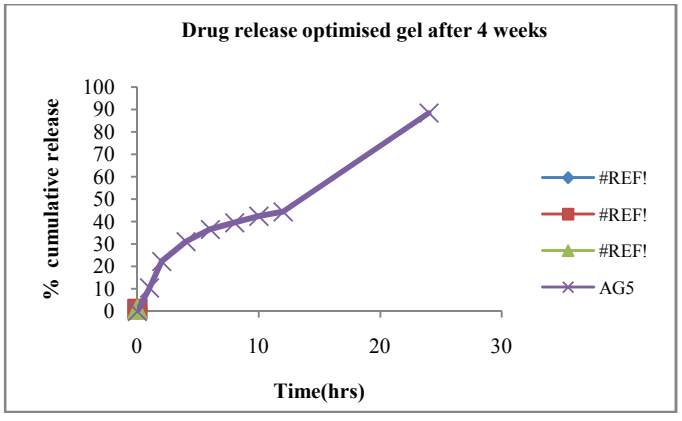

Figure 14 Dissolution profile study of optimised gel formulation (AG5) after 4 week

From figure 14 it can be observed that formulation AG5 showed 88.43% release at the end of 24hrs after 28 days. It was concluded that there was no significant change in release profiles of formulation. So the formulations were found to be stable.

Drug content

Drug content was determined for gel formulations of AG5 after 4 weeks. The drug content was determined on the basis of HPLC study.

Table 18 Drug content for Gel formulation (AG5)

Formulation	Days	AUC	Drug Content
AG5	0	8930280	99.21
	30	8872942	97.40

From Table 18 the drug content was found to be within acceptable limits of I. P. Thus the formulations AG5 was found to be stable.

Stability study by HPLC

The gel formulation of AG5 was subjected to HPLC study after 1 month and was observed for any changes in retention time.

From the chromatograms it can be concluded that there is no significant change in the retention time for gel formulations of AG5. So the gel formulation was found to be stable after 1 month (4 week).

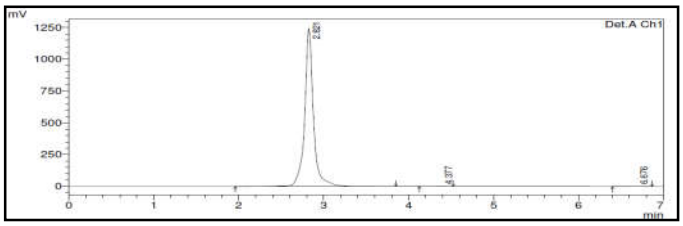

Figure 15 Chromatogram of Gel formulation of AG5 before stability study

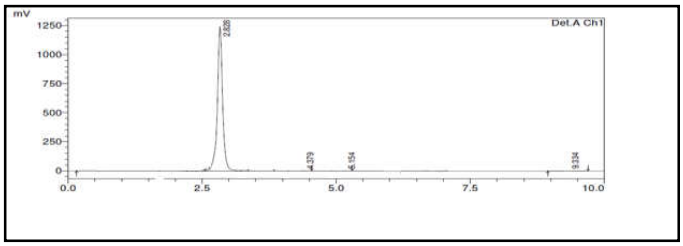

Figure 16 Chromatogram of Gel formulation of AG5 after stability Study

CONCLUSION

Bitter undesirable taste of most drugs is important factor to affect the patient compliance. In case of paediatric patients, taste of drug product is one of the important criteria that should be kept in mind while designing it. Taste masking of the bitter drugs is required in order to improve the patient's acceptance and adherence to the particular drug therapy. Abacavir HCL is widely used as an antiviral drug but it is very bitter and so it was thought of solving this problem by forming Microbeads and then dispensing it in the form of an oral gel.

Fourier transforms infrared spectroscopic (FTIR) studies and DSC revealed that there was no interaction between the drug, polymers and excipients. Microbeads prepared with drug: polymer: polymer ratio of 1:1:0.8 (Abacavir HCl: gellan gum:

chitosan) showed excellent practical yield (93.57%), entrapment efficiency (91.30%), drug loading (8.80mg/100mg beads), particle size (580µm) as compared to Abacavir HCl: sodium alginate: chitosan (1:1:0.8) ratio. The best fit model for prepared formulations F4 (72.934% release) and F16 (89.378% release) followed Korsmeyer-Peppas model (R^2=0.9944 and 0.9919) respectively and n value was found to be 0.5284 and 0.5656 which signified that release pattern of optimized formulations followed non fickian diffusion. The release was sustained for 24hrs from both the formulations. Therefore, one can assume that the Gellan Gum and Sodium alginate are promising natural biopolymers which can provide sustained release drug delivery as well as can mask the taste.
For forming the base of oral gel which could act as a carrier for the microbeads, Formulation AG5 oral gel (gellan gum based microbeads) consisting of 0.3% gellan gum and 0.3% sodium citrate was considered as an optimum batch considering drug release, viscosity and appearance. The pH of the maximum stability of Abacavir HCl in aqueous phase is in between 1.5 to 7. Therefore, the pH of the formulated gels was adjusted and maintained in between 5 to 7 with help of buffering agents; citric acid and sodium citrate. Sucrose may crystallize in presence of citric acid on standing. Therefore, the amount of citric acid was kept minimum, just to adjust to the required pH. Sodium citrate was selected as a salt to contribute as a cation because it also act as sequestrant, buffering agent and also helps in maintaining mechanical property of the gel. Test for homogeneity revealed that drug content uniformity is maintained throughout the handling and filling process.

Though metered dose container was used to fill the final product, blockage occurred per pump actuation resulting in weight variation. Therefore, it was decided to fill the product in a sachet which would not only ensure accuracy and ease of dispensing but can be also added on biscuits, yoghurts, breads, etc easily in case of paediatric patients as well it can be easily adopted for large scale filling. All the six volunteers perceived the gel as nonbitter. Addition of flavors and sweeteners is the foremost and simplest approach for taste masking especially in the case of pediatric formulations. Sucrose (20%) was not able to mask the bitter taste completely because sugar molecules might have been trapped into the gel network. Orange flavor was selected because to certain extent it helps in masking the bitter taste of drug and also improves patient acceptance.

The results of stability studies indicated no considerable changes in pH, viscosity and appearance of the optimised AG5 formulation. Precipitation of Abacavir HCl in the gels was not observed. There were no changes observed in the drug content, dissolution profile and HPLC chromatogram which showed that formulation AG5 is stable at 40ºC/75% RH for one month. Conclusively, the current study attained the successful design, preparation and evaluation of novel taste masked Abacavir HCl using chitosan microbeads in oral flavored gel which sustained the drug release for 24hrs and thus can be used for paediatric and geriatric patients.

Acknowledgment

Authors are thankful to Sinhgad College of pharmacy, Vadgaon, Pune for providing all important facilities and guidance to carry out the research work.

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How to cite this article:

Vikas Thorat *et al* (2019) ' Formulation, Development and Evaluation of Taste Mask Abacavir Hcl Using Chitosan Microbeads in Flavoured Gel', *International Journal of Current Advanced Research,* 08(12), pp. 20767-20777. DOI: http://dx.doi.org/10.24327/ijcar.2019. 20777.4066

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