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Formulation and Evaluation of Sustained Release Vildagliptin Microspheres

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HUMAN

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ABSTRACT

The objective of the present study is to develop biodegradable polymeric microspheres for controlled release of anti- diabetic drug Vildagliptin loaded microspheres. Encapsulated slow release spheres can be prepared by the process of microencapsulation. Microspheres of Vildagliptin were prepared by ionotropic gelation technique. In the present work four sets Microspheres were prepared by using sodium alginate alone in different concentrations and with different concentrations of coating polymers like Sodium CMC, Pectin and Calcium chloride as counter ion. The prepared batches of vildagliptin loaded microspheres can be evaluated for measurement of micromeritic properties of microsphere like granulometric study, angle of repose, bulk and tapped densities, particle size analysis, surface morphological details, swelling properties, drug entrapment efficiency and *In-Vitro* dissolution Studies. By increase in the percentage of polymer concentrations, has significantly affected on the size of Spears and the optimum release of vildagliptin from the Microspheres which showed formulation F9 is 98.89 of drug released respectively within 12 hrs. The formulation (sodium alginate, Sodium CMC and Calcium chloride) F9 is compared to other formulations the F9 is the best formulation of the released the percentage drug of microspheres.

INTRODUCTION

Sustained release systems include any drug delivery system that achieves slow release of drug over an extended period of time. More precisely, sustained drug delivery can be defined as "Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects[1].

In sustained release dosage forms, a sufficient amount of drug is initially made available to the body to cause a desired pharmacological response. The remaining fraction is released periodically and is required to maintain the maximum initial pharmacological activity for some desirable period of time in excess of time expected from usual single dose. A sustained release is facilitated through the consistent. Rejuvenation of drug molecules2.

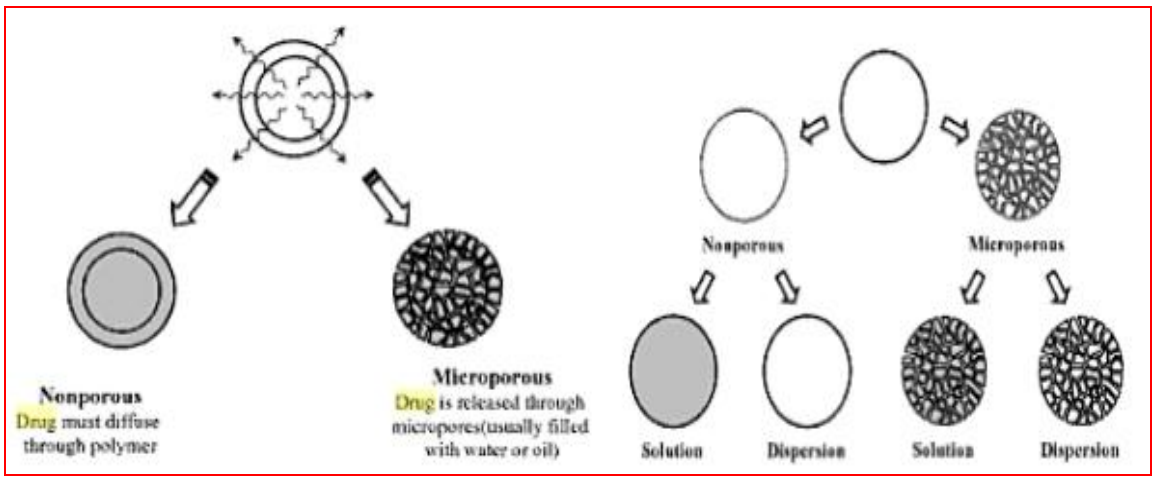
Potential advantages and disadvantages of Sustained release drug delivery systems[1, 2]

1. Avoid patient compliance problems.
2. Employ less total drug.
3. Minimize or eliminate local side effects.
4. Minimize or eliminate systemic side effects.
5. Obtain less potentiating or reduction in drug activity with chronic use.
6. Minimize drug accumulation with chronic dosing.
7. Improve efficiency in treatment.
8. Cure or control condition more promptly.
9. Improve control of condition, i.e., reduce fluctuation in drug level.
10. Improve bioavailability of some drugs.
11. Make use of special effects like sustained release aspirin for morning relief of arthritis by dosing before bedtime.
12. Economy

Disadvantages

1. High cost
2. Unpredictable or poor *in vitro – in vivo* correlation
3. Dose dumping
4. Reduced potential for dosage adjustment

5. Increased first pass clearance
6. Poor systemic availability in general



(a) (b)

Fig 1 Sustained release mechanisms (a) Diffusion sustained reservoir systems. (b) Diffusion sustained monolithic systems.

MATERIALS AND METHODS

Vildagliptin, Sodium CMC was obtained as a gift sample from spectrum lab Hyderabad. Sodium alginate, Pectin and Calcium Chloride from SD Fine Chem. Ltd., Mumbai. All other chemicals used were of pharmaceutical grade.

Preparation of Microspheres[3, 4]

Microspheres of Vildagliptin were prepared by Ionotropic gelation technique. In the present work four sets Microspheres were prepared by using sodium alginate alone in different concentrations and with different concentrations of coating polymers like Sodium CMC, Pectin and Calcium chloride as counter ion.

Table No.1 Formulation Design of Microspheres

	Formula-tion	Vildagliptin (mg)	Sodium Alginate (w/v)	Calcium Chloride (w/v)	Pectin (w/v)	SCMC (w/v)
I	F1 F2 F3	500 500 500	0.5% 1% 1.5%	3% 5% 7%		
II	F4 F5 F6	500 500 500	0.25% 0.5% 0.75%	5% 5%	0.25% 0.5% 0.75%	
III	F7 F8 F9	500 500 500	0.25% 0.5% 0.75%	5% 5%		0.25% 0.5% 0.75%

Preparation of Sodium alginate Microspheres:-[5]

In the first set, three batches of drug-loaded Microspheres were prepared (F1, F2, and F3). A solution of sodium alginate (2-4% w/v) was prepared in 100ml of deionized water. In 50ml of sodium alginate solution, weighed quantity (100mg) of Vildagliptin sodium was dispersed uniformly. Bubble free dispersion was dropped through a syringe into 100ml aqueous calcium chloride solution and stirred at 100rpm. After stirring for 10minutes, the gelled spheres were separated by filtration, washed with distilled water, air dried and finally dried at 60^0C for 6 hrs in an oven.

Preparation of Alginate Pectin Microspheres:-[6]

In the second set, two batches of drug-loaded Microspheres were prepared (F4, F5, F6) using sodium alginate and Pectin as a coating polymer. To 50 ml of deionized water, pectin (0.5 – 1%w/v) were added and stirred with the electric stirrer to form mucilage. Then sodium alginate (3%w/v) was added to form uniform mucilage. Then finally weighed quantity (100mg) Vildagliptin was added and homogenized for 5min. The resulting dispersion was dropped through syringe into 100 ml of 5% w/v aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 10 minutes the formed spheres were separated,

washed with distilled water, air dried and finely dried 60^0C for 6 hrs.

Preparation of Alginate-Sodium CMC Microspheres:-[7]

In third set, two batches of Microspheres were prepared (F7, F8, F9) using sodium alginate and Sodium CMC as a coating polymer. To 50ml aqueous sodium alginate solution weighed quantity (100mg) Vildagliptin was dispersed uniformly. Bubble free dispersion was dropped through a syringe into 100ml of Sodium CMC solution containing 5% w/v calcium chloride (Sodium CMC dissolved in 10ml of 5% w/v acetic acid). Stirred at 100rpm. After stirring for 30 minutes, the coated spheres were separated by filtration, washed with distilled water, air dried and finally dried 60^0 C for 6 hrs.

Preparation of Alginate-Calcium chloride Microspheres:-[8]

In fourth sets, two batches of Microspheres were prepared (F7, F8, F9) using sodium alginate and Calcium chloride as a polymer. These Microspheres were prepared as described above same as alginate-CMC Microspheres, with a slight modification. In this pectin was mixed along with weighed quantity (100mg) Vildagliptin and dispersed in 50ml sodium alginate solution.

EVALUATION PARAMETERS OF MICROSPHERES:

1. Measurement of Micrometric Properties of Microspheres[9, 10]

Granulometric Study:-

The particle size has very significant effect on the release profile of Microspheres. Granulometric study was conducted to determine the particle size distribution pattern. For this study sieve analysis was carried out on mechanical sieve shaker, using different meshes (#12, #16, #22, #30) of American Society of Testing Materials (ASTM).

Flow Property:-[11]

The flow properties were investigated by measuring the angle of repose of drug-loaded Microspheres using fixed-base cone method to assess the flowability. The fixed-base cone method, a funnel was secured with its tip at a 1cm height (H) above the graph paper that was placed on a flat horizontal surface. Microspheres were carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. Measure the height of

the pile (h) and the radius of the base (r) with ruler. The angle of repose was determined by using the equation

$$\tan\theta = \frac{H}{R} \quad \text{or} \quad \theta = \tan^{-1}\frac{H}{R}$$

Where θ = angle of repose, R = radius of the base of pile

H = height of pile.

Bulk and Tapped Density:-[12]

The bulk and tapped densities were measured in a 10ml graduated measuring cylinder to measure packability of the Microspheres. The sample contained in the measuring cylinder was tapped mechanically by means of constant velocity rotating cam with a change in its initial bulk density to a final tapped density when it has attained its most stable form. Each experiment was carried out in triplicate. The bulk and tapped density can be determined.

Particle Size Analysis:-[13]

Particle size analysis of drug-loaded Microspheres was performed by optical microscopy (Olympus Model Szx-12). A small amount of microspheres was suspended in purified water (10ml). Mount the sample on a clean glass slide and placed it on mechanical stage of the microscope. The eyepiece of microscope fitted with a micrometer by which the size of the spheres could be determined.

2. Surface Study: [14]

The surface morphological details of the Microspheres were determined by using a scanning electron microscope (SEM) model JSM, 35CF JEOL, Japan. The samples were dried thoroughly in vacuum desiccator before mounting on brass specimen studies. The samples were mounted on a specimen studies using double-sided adhesive tape, and gold-palladium alloy of 120Å K-ness was coated on the sample using spatter coating unit (Model E5100 Polaron, UK) in an argon ambient of 8-10 pascal with plasma voltage about 2Kv and discharge current about 20mA. The sputtering was done for nearly 3minutes to obtain

uniform coating on the samples to enable good quality SEM images. The SEM operated at low accelerating voltage of about 15Kv with load current of about 80mA.The condenser lens position was maintained between 4.4 – 5.1. The objective lens aperture has a diameter of 240 microns and the working distance WD = 39mm.

3. Loose-Surface Crystal Study: [12]

In this study accurately weighed 25mg of Microspheres (#16) was suspended in the phosphate buffer pH 7.4 and was shaken vigorously for 5min. The drug leached out from the surface of the micro pellets was analyzed at 210 nm wavelength spectrophotometrically.

4. Swelling Properties: [13]

The swelling properties of prepared Microspheres were determined in acidic buffer pH 1.2. Thirty dried spheres were placed in a beaker to which 200ml of buffer solution and then stirred with a magnetic stirrer at a speed 50 rpm. After 1hr interval, the equilibrium swollen spheres were observed and measured under optical microscope. The magnitude of swelling was presented by the ratio of the mean diameter of swollen spheres to the mean diameter of the dried spheres before the test.

5. Drug Entrapment Efficiency (DEE) [14]

Drug entrapment efficiency of Microspheres was performed by accurately weighed 50mg of Microspheres were suspended 100ml of phosphate buffer pH 7.4±0.1. The resulting solution was kept for 24 hours. Next day it was stirred for 15 min and subjected to filtration. After suitable dilution, Vildagliptin content in the filtrate was analyzed spectrophotometrically at 210nm using Shimadzu 1201 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 we get the percentage of actual drug encapsulated in Microspheres. The drug entrapment efficiency was determined using following relationship:-

$$\%DEE = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

6. In-Vitro Dissolution Studies[14]

The physicochemical property of most drugs that has greatest influence on their absorption characteristics from the GIT is dissolution rate. "The drug is expected to release from the solid dosage forms (granules, tablets, capsules etc.) and immediately go into molecular solution. This process is called as dissolution".

Drug Release Studies:-

Apparatus: **-** USP XIII dissolution rate test apparatus employing the round bottom dissolution vessel and rotating basket assembly.

Acid Stage: **-** 900ml of simulated gastric fluid TS (acid buffer pH 1.2±0.05 without enzymes.

Buffer stage: **-** 900ml of pH 6.8 (duodenal fluid) and simulated intestinal fluid TS (phosphate buffer pH 7.4±0.05 without enzymes.

Table No. 2: Dissolution Conditions:-

Volume of Dissolution Media	Dilution factor	pH Condition	Time (h)	Simulated GI region
900 ml	10	1.2	2	Stomach
900 ml	10	6.8	1	Duodenum
900 ml	10	7.4	7	Lower small intestine

Procedure:-

Hard gelatin capsules were filled with microspheres equivalent to 100 mg of vildagliptin and were evaluated for in-vitro dissolution studies. The study was carried out in a USP XIII rotating basket apparatus. Dissolution fluid consists of 900ml of simulated gastrointestinal fluids of increasing pH namely pH 1.2 (-2 hrs), pH 6.8 (1hr) and pH 7.4 up to 10hrs) maintained temperature at 37ºC ±0.5ºC and the basket was rotated at a constant speed of 50rpm. Aliquots of samples were withdrawn after predetermined periods of time and the same volume of fresh medium was added immediately to the test medium. The

withdrawal samples were filtered through a 0.45µm membrane filter. The drug content was determined in the filtrate after appropriate dilution and analyzed at 210 nm spectrophotometrically using Shimadzu 1201 UV-visible spectrophotometer. Corresponding concentrations in the samples were calculated from standard plot and calculate cumulative percentage of drug release from each formulation.

RESULTS AND DISCUSSION

EVALUATION OF MICROSPHERES:

In the present work vildagliptin, Microspheres were prepared by ionotropic gelation technique using sodium alginate and also with three different coating polymers. Total nine batches of Microspheres (F1-F9) were prepared and investigate the physicochemical properties like granulometric study, flow properties, particle size, drug entrapment efficiency, swelling properties, scanning electron microscopy, loose-surface crystal study and in-vitro drug release behaviors.

a) Granulometric Study:

In the granulometric study, it was observed from the table. The size distribution of the microspheres in different sieves was observed, that about 42.46% to 79.50% of microspheres were retained in #20 sieve, which proves the uniformity size of microspheres. It was observed that by decreasing the concentration of sodium alginate and $CaCl_2$ solution were not formed spherical spears. However, increase in the concentration of sodium alginate and calcium chloride solution tends to make the particles more spherical and obtaining the uniform size spheres. On other hand with increase in the concentration of coating polymers in the formulated microspheres of batch F1, F2, F3 (Sodium alginate) F4, F5, F6 (Pectin), F7, F8, F9 (Sodium CMC) and observed that the distribution of the particle size slightly shifts to the lower pore size due to increase in the physical behaviours of the microspheres.

b) Flow Property:

The flow property of the prepared formulations was determined by measuring the angle of repose, using fixed-base cone method. All the formulations showed an acceptable range of angle of repose. The determined range of the formulations as reported in table no. 5. The batches prepared with coating polymers such as Pectin (F4, F5, F6), and Sodium CMC

(F7, F8, F9) showed good flowability compared with batches F1, F2 and F3. It indicates that the presence of the coating polymers will also affect the flow property of Microspheres. Bulk and tapped density of the Microspheres also determined in a 10 ml measuring cylinder. All the formulations show good acceptable range and found to have higher packability. The determined values of bulk and tapped densities as reported in table.

c) Particle Size:

Particle size of drug-loaded Microspheres was performed by optical microscopy. The size of the spears was obtained in the range 1 to 1000 μm. The mean diameter of the particles was found to decrease by increasing the concentration of calcium chloride solution and also increasing the concentration of sodium alginate by increase in the diameter of the particles. The mean diameter of the Microspheres was reported in table. It has been stated that when a drop of alginate solution comes in contact with calcium ions, gelation occurs instantaneously. As Ca^{+2} ions, penetrates into interior of droplets, water is squeezed out of the interior of droplets resulting in contraction of the spears. Thus increase in concentration of calcium chloride solution will significantly affect the contraction of the spears leading to decrease in diameter On other hand at fixed concentration of sodium alginate and calcium chloride in the formulation batches F4, F5, F6, F7, F8 and F9 (3% sodium alginate and 5% calcium chloride) and increases in the coating polymer concentration leading to increase in the diameter of formed Microspheres.

d) Scanning Electron Microscopy:

The physical parameters like shape and particle size were analyzed by scanning electron microscopes, which were presented for determining the surface and size, in the photographs 1 to 4. The SEM's of the formulation F2, F5, F7 and F9 showed that the spears are having the size range within the standard limits. The SEM of the Microspheres prepared from sodium alginate alone (F2) are spherical in shape, exhibits uniformity and rough surface has a sandy appearance, however in case drug loaded sodium alginate Microspheres containing coating polymers like Pectin (F5), Sodium CMC (F9) appeared to be spherical, although the surface was not smooth as sodium alginate Microspheres. This was due to coalescence and fusion to the colloidal aqueous polymer dispersions in the alginate matrix. The average diameter of the particles increases and decreases the porosity accounts for slow release of drug. From the photomicrographic observation, it can be stated that bridging and dense

nature of the formulation batches F5 and F7 were significantly prolonged the From the photomicrographic observation it can be stated that bridging and dense nature of the formulation batches F5 and F7 were significantly prolonged the drug release compared with other formulation batches.

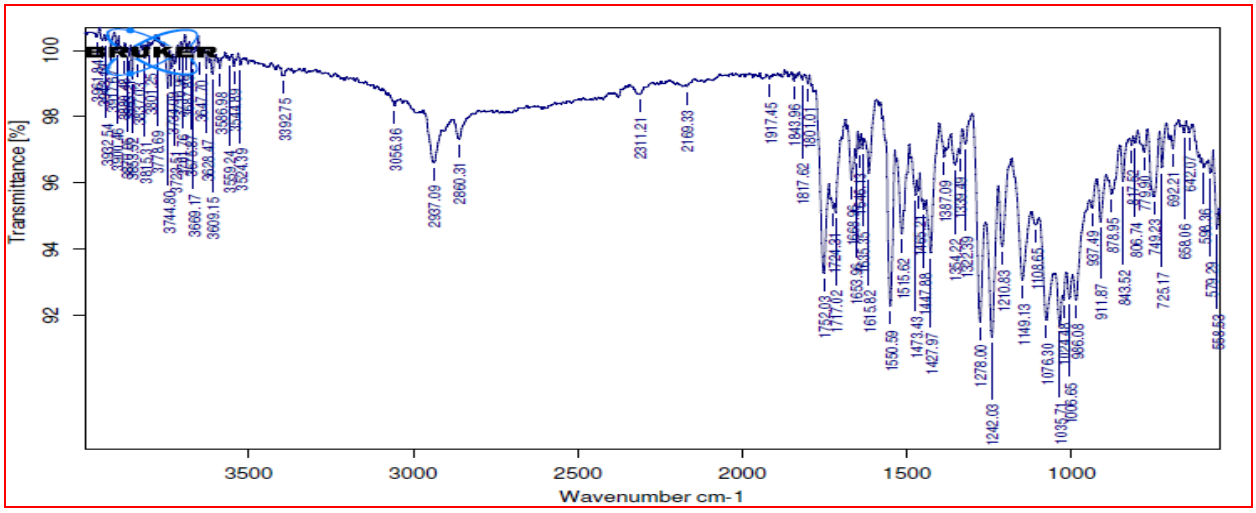


Figure 2: IR Spectra of Vildagliptin Optimised Formulation

SEM Photograph Vildagliptin

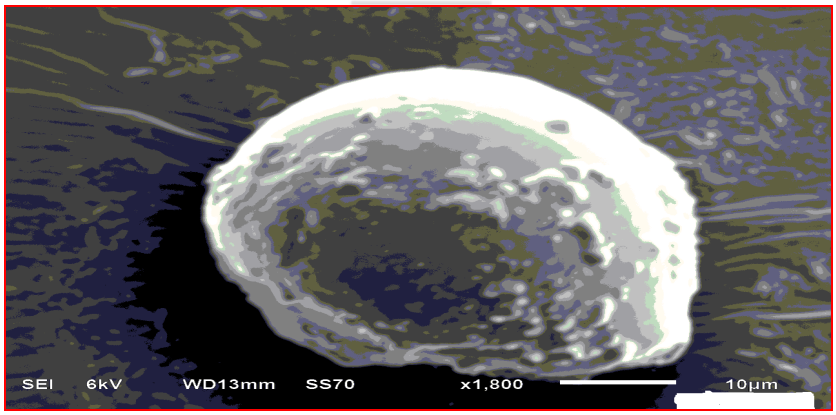


Figure 3: Vildagliptin microspheres

II. Preformulation Studies:

Calibration development for vildagliptin adopting spectrophotometric technology. The λmax of Vildagliptin at 210 nm were identified using UV-Visible Spectrophotometry, A standard curve from the stock solution was obtained in the range of 2-12 µg/ml concentrations using pH 1.2 (acid buffer), pH 6.8 (phosphate buffer) and pH 7.4 (phosphate buffer) by measuring absorbance at 210nm.

Table No 3: Standard Calibration Curve of Vildagliptin

Sl. No.	Concentration (µg/ml)	Absorbance at 210nm		
		pH 1.2	pH 6.8	pH 7.4
1	2	0.025	0.031	0.052
2	4	0.047	0.061	0.113
3	6	0.074	0.090	0.163
4	8	0.097	0.117	0.220
5	10	0.121	0.147	0.284
6	12	0.143	0.177	0.335

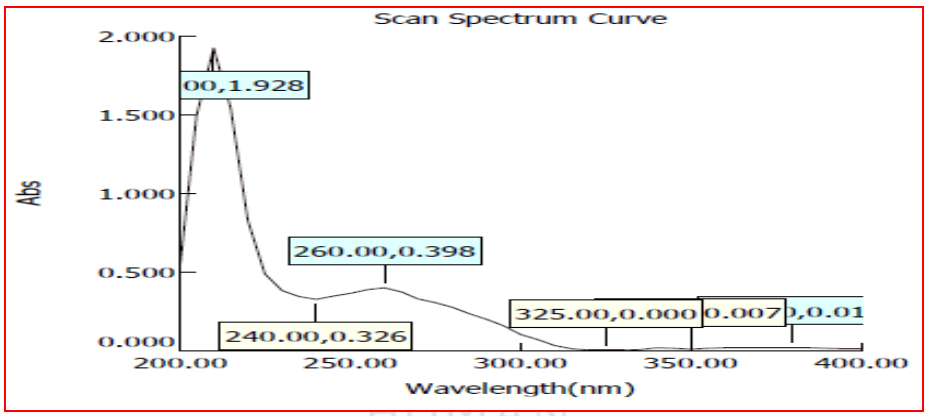


Figure 4: Spectrum curve of Vildagliptin 210nm

UV Spectrum of Vildagliptin 210nm

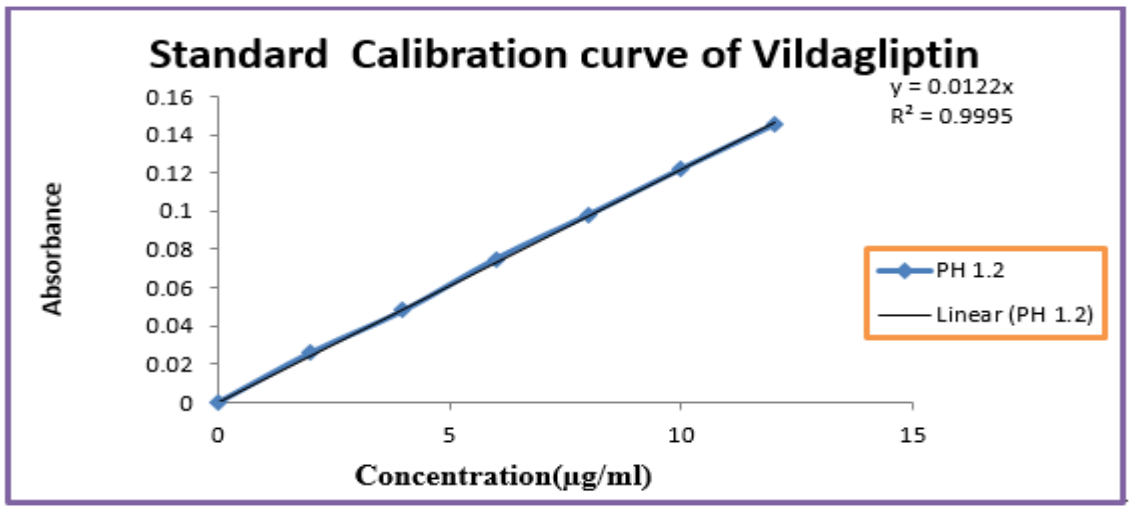


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Figure 5: standard calibration curve of Absorbance, pH 1.2 of Vildagliptin

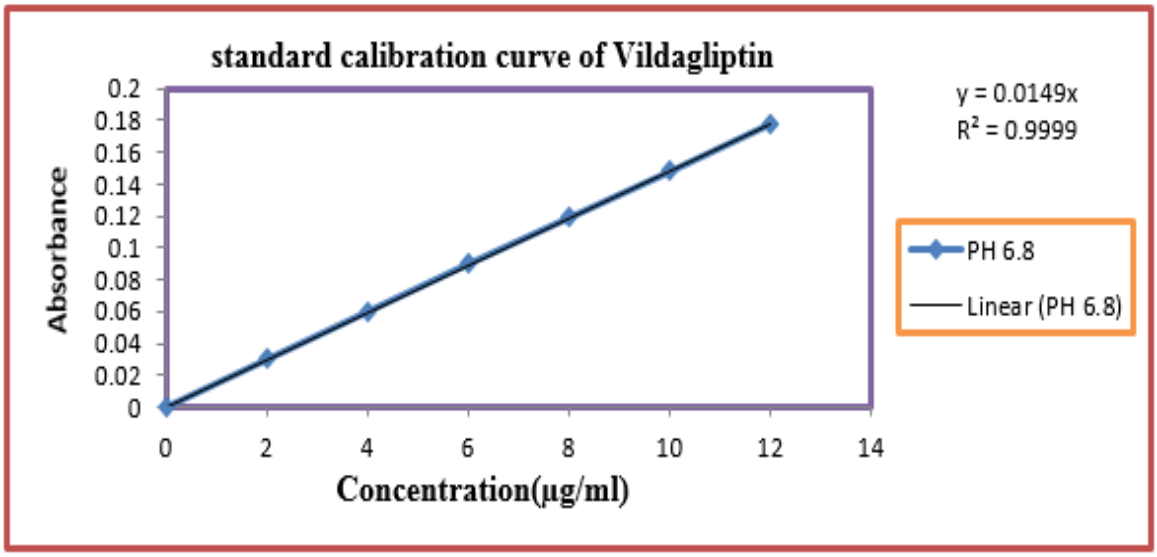

Figure 6: standard calibration curve of Absorbance, pH 6.8 of Vildagliptin

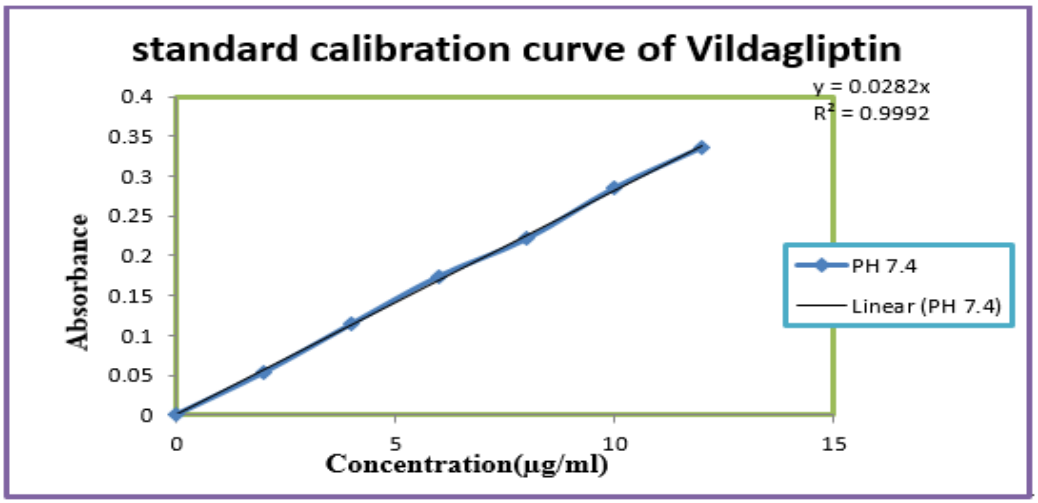

Figure 7: standard calibration curve of Absorbance, pH 7.4 of Vildagliptin

Table No 4: Percentage of Weight Remained on Various Sieve Size

Batch No.	#12 (1.68mm) 1190-1680µm	#16 (1.19mm) 840-1190µm	#20 (0.84mm) 590-840µm	#30 (0.59mm) 297-590µm
F1	8.42	7.54	79.54	4.62
F2	5.17	22.85	68.40	3.64
F3	4.79	27.15	63.64	4.49
F4	5.98	34.37	57.84	1.88
F5	8.68	38.94	51.42	1.03
F6	5.87	49.98	42.48	1.74
F7	7.98	45.65	44.83	1.62
F8	8.32	38.32	50.22	3.22
F9	9.41	40.71	47.82	2.13

Table No 5: Micromeritic Properties of Drug-loaded Microspheres

Batch No.	Angle of Repose (°)	Bulk Density (g/ml)	Tapped Density (g/ml)	Mean Diameter (µm)
F1	31°.25	0.487	0.696	823.16
F2	30°.15	0.698	0.757	795.26
F3	29°.20'	0.788	0.866	779.50
F4	27°.20'	0.739	0.808	863.17
F5	26°.30'	0.767	0.813	892.58
F6	26°.40'	0.728	0.833	970.60
F7	25°.60'	0.747	0.841	985.19
F8	28°.15'	0.788	0.855	908.60
F9	27°.80'	0.748	0.864	923.18

g) Drug Entrapment Efficiency:

The drug entrapment efficiency of all the batches is shown in table no.6. The batches F1, F2

and F3 shows the drug entrapment efficiency values of 71.4%, 76.2% and 84.3% respectively. Drug entrapment efficiency of Microspheres increases with increasing the percentage of sodium alginate as well as by concentration of coating polymers like Sodium CMC and pectin. The observations are still further depicts the drug entrapment efficiency of batch F4 and F5, were 91.9% and 93.7% similarly for batch F6, F7, F8 and F9 were 88.9%, 94.2%, 86.4% and 88.0% respectively. These may be due to drug adhering property of the polymers and also reduced the loss of drug in the curing medium and formation of dense matrix structure shows increase in the drug entrapment efficiency of the Microspheres. The amount of calcium chloride has probably no significant effect on the drug entrapment efficiency.

Table No 6: Drug Entrapment Efficiency of Microspheres

Sl. No.	Theoretical Drug Content (%)	Actual Drug Content (%)	Drug Entrapment Efficiency (%)
F1	81.53	57	71.405
F2	80.52	62	76.256
F3	77.76	66	84.336
F4	73.74	66	91.916
F5	71.87	67	93.713
F6	80.69	72	88.934
F7	76.89	71	94.276
F8	74.59	63	86.459
F9	78.76	69	88.091

f) Swelling Properties:

The Swelling-dissolution-erosion process is highly complex. In systems based on sodium alginate cross-linked with calcium chloride, the osmotic pressure gradient that exists between

the alginate gel and the environment comprises an important factor in the swelling process. Under acidic conditions swelling of calcium alginate spears scarcely occurs. The swelling behavior of the prepared Microspheres was studied in pH 1.2 up to 2 hrs. Increasing concentration of calcium chloride in the counterion solution produces spears with higher levels of Ca2+ ions will reduce the swelling of the spears, consequently increasing the concentration cross-linking polymers also decreasing of the swelling properties of the spears in acidic medium. Due to increased dissolution behavior of the Microspheres in the pH 6.8, and pH 7.4 the swelling behavior study was not carried out in those phosphate buffers.

Table No 7: Swelling Properties of Drug-loaded Microspheres in Different Time Periods at pH 1.2

Formulation Code	Mean Diameters of Microspears		
	0 hr (μm)	*1 hr (μm)*	*2 hr (μm)*
F1	524.19	640.70	664.35
F2	496.30	608.23	625.44
F3	480.46	589.45	604.25
F4	562.20	686.09	612.45
F5	592.61	607.72	633.35
F6	672.57	698.47	731.15
F7	684.20	705.27	728.19
F8	610.71	634.52	660.75
F9	622.56	642.32	664.76

h) *In-vitro* Dissolution Studies:

The dissolution studies were conducted by using three different dissolution mediums simultaneously at pH 1.2 for 2hrs, then the spears shifted at pH 6.8 determined for 1 hour and

finally transferred to pH 7.4 and studied up to 12hrs. The results of the *in-vitro* dissolution studies of formulations F1 to F9 as shown in table. The formulations F1, F2 and F3 containing 2%, 3% and 4% sodium alginate showed the drug release of 48.30%, 44.02%, 30.32% after 2 hrs respectively. The similar observations were determined in batch F4, F5 and F6 the percentage of drug release about 45.97% and 28.36% w/w after 2 hrs, when using 0.5% and 1% w/v of Sodium CMC used as coating polymer. The results were depicted in batch F7 and F9 shows 18.58% and 11.36% w/w when using 0.5% and 1% w/v of Pectin used as coating polymer. Further, the drug release pattern resembles the similarly way were seen in batch F7 and F8. The percentage cumulative drug releases were 18.58% and 10.26% w/w when using 1% and 2% w/v pectin used as coating polymer. The percentage cumulative drug release for batch F1 to F9 were 8.98, 11.36, 14.63, 21.26, 33.42, 48.91, 52.83, 64.56, 76.30, 84.13, 90.97 and 98.93 respectively. The observed values show that the prepared Microspheres showed increase in drug release at pH 6.8 than in pH 1.2.This shows the Microspheres formulated with pectin slightly increases the release in acidic condition compared to Microspheres formulated only with sodium alginate. The resulting alginate-pectin spears showed release behavior dependent on pH. The pectin polymer is poorly soluble in water. In acidic medium, protonation of amine groups improves solubility. The interpolymeric complex between alginate and pectin exists in a gel at low pH. At neutral pH the viscous complex will swell and gel formed will slowly disintegrate, release the drug. The release rate is a function of the degree of cross-linking between both polymers and moreover pectin also suppresses the gel matrix erosion of alginate spears, leads to retard the drug release.

The sodium alginate and pectin forms rigid gel with calcium ions relatively easily would be expected to resist erosion in higher pH and prolong drug release when compared to formulations with only sodium alginate, but somewhat faster release is observed when compared with formulations of Sodium CMC Microspheres.

The drug release behavior was increased in pH 7.4 phosphate buffer were observed in almost all batches due to increasing swelling behavior as well as the drug entrapment efficiency. The dissolution profile shows the increased release of drug may due to the increased drug released characteristics in pH 7.4 when compared to pH 1.2 and pH 6.8. But due to the presence of various coating polymers in the formulation namely batch F4 to F9, the drug release pattern will be retarded and shows the sustained release property, these drug

formulation batches when compared to the batches F1 to F3 where there is no polymer available for getting the sustained effect. The *in-vitro* drug release observations were continued for 12 hrs to estimates the sustained release property of the prepared formulations. It proves that these formulation batches shows that having sustained release behaviors were observed. The in-vitro dissolution study was compared simultaneously in 3 different buffers to analyze drug release behavior in respected reasons. *In-vitro* release kinetic data proves that very low release in acidic medium (pH 1.2) and the release behavior slowly increase by increasing the buffer concentrations. The *in-vitro* release data shows the % cumulative drug release at 12^{th} hour for batch F7 to F9 in range 93% to 98% w/w. Whereas batch F4, F5 and F6 were 81 to88% w/w, and even the same drug release were observed F1, F2 and F3batches. The formulated Microspheres showed increase in drug at pH 6 .8 and trend is continued in pH 7.4 because due to cross-linking takes place only between carboxylate residue of GG-blocks and Ca^{+2} ions forms a tight gel network structure. The toughness of the network structure and subsequent disintegration and dissolution of alginate particles taking place through ion-exchange between the bound Ca^{+2} ions and Na^{+} ions present in dissolution medium leading to extended release some extent. Thus the release of vildagliptin from the microspheres appears to be significantly with increase in initial alginate concentration but not with calcium ions. Based on these values the maximum drug release was obtained in the batches F7 to F9 about 98% due to the alginate spears having fast disintegration in simulated intestinal fluid and high porosity which results in rapid drug release. The estimated physicochemical and *in-vitro* release data proves that the formulated vildagliptin microspheres having the characteristics that required for the formulation of sustained release dosage forms. However, it can be proved the released drug may be retarded and produce proper – sustained effect in the formulation batches F4, F5, F6, F7 and F9 as the percentage of Sodium CMC, Pectin was showing better-sustained release pattern when compared to the other batches.

Table 8: In-Vitro Release Data of Drug-loaded MicrospheresF1-F9

TIME (Hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	24.53	27.59	19.6	28.39	29.48	23.66	10.56	8.56	9.87
2	49.13	45.31	31.56	46.89	35.48	35.82	19.67	11.35	10.48
3	64.48	57.68	39.84	57.85	53.59	48.79	32.41	20.69	15.78
4	75.68	66.7	50.84	66.68	64.06	58.84	38.15	28.56	22.38
5	88.89	77.3	63.74	73.59	75.59	69.58	55.68	46.89	34.56
6	99.13	89.06	74.85	86.58	89.23	74.48	74.48	57.89	49.86
7		99.69	88.62	97.89	89.23	88.09	85.25	68.59	53.94
8			99.78			98.59	98.79	74.89	65.68
9								83.58	77.56
10								99.82	85.17
11									91.85
12									98.89

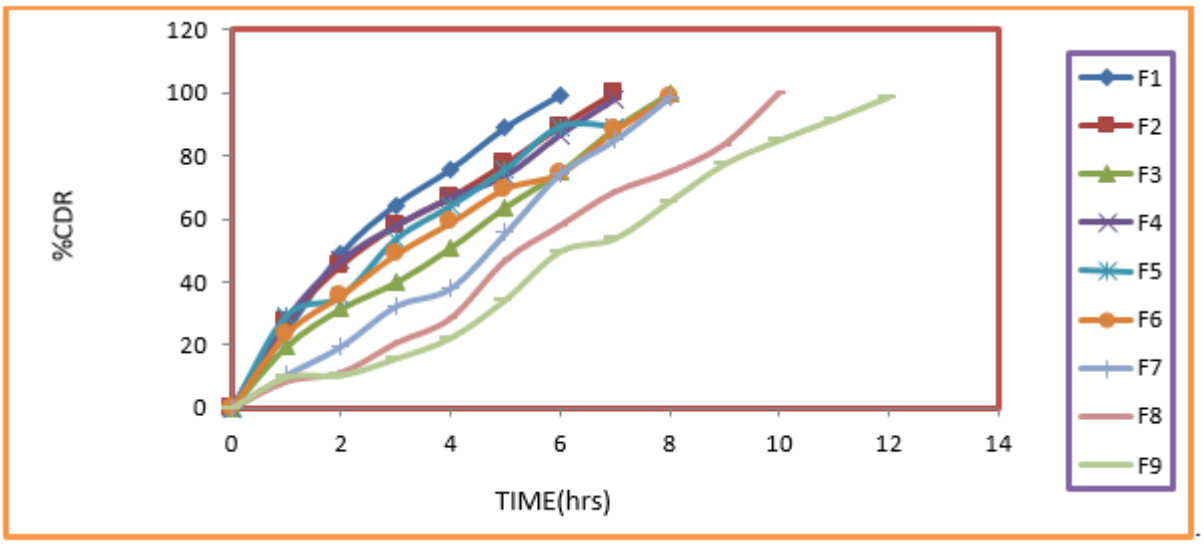

Figure: 8 % Cumulative Drug Release of Formulation F1-F9

CONCLUSION

By studying all the experimental results of the prepared vildagliptin microspheres, the results suggest that microspheres containing antihyperglycemic agent Vildagliptin were successfully formulated by an ionotropic gelation technique by using sodium alginate, Sodium CMC and pectin as polymers and calcium chloride as cross linking agent to produce sustained release

delivery systems.

The prepared microspheres were strong spherical with narrow size distributions could be prepared with high yields and good entrapment efficiencies. By increase in the percentage of polymer concentrations, has significantly affected on the size of Spears and the optimum release of Vildagliptin from the microspheres which showed formulation F9 is 98.89 of drug released respectively within 12 hrs. The formulation (sodium alginate, Sodium CMC and Calcium chloride) F9 is compared to other formulations the F9 is the best formulation of the released the percentage drug of microspheres. Remaining formulation is drug releasing percentage showing respectively of microspheres of vildagliptin.Our study has suggested that microencapsulation by ionotropic gelation technique is inexpensive compared with other techniques and also advantages to prevent the drug related adverse effects of conventional dosage forms and maintain the sustained drug release over an extended period of time.

REFERENCES

1. Remington. The science and practice of pharmacy 21st edition, vol. 1, 939.
2. Eroglu H, Suheyla KASH, Oner L, Sargon M, Hincal AA. Preparation of bovine serum albumin microspheres containing Dexamethasone Sodium Phosphate and the *in vitro* evaluation. Turk J Med Sci 2000; 30:125–8.
3. Hong W, Kinam P. Oral controlled formulation design and drug delivery: Theory practice 1st edition, 6.
4. Venkatesan.P, Manavalan.R, Valliappan.K. Microencapsulation: a vital technique in novel drug delivery system. J Pharm Sci & Res 2009; 1(4):26-35.
5. Gunja C, Prakash. B, Saha.RN. Microspheres technology and its applications. (PDF). http://docstoc.com/docs/38166355/review-on-microspheres, Nov 2010.
6. Jain NK. Biodegradable polymers Controlled and Novel Drug Delivery 1st edition, 81-239.
7. Murali Mohan Babu GV, Himasankar K, Churuvu PS Narayan, Ramana Murthy KV. Controlled release of diclofenac sodium by gum karaya-chitosan complex coacervate: *In vivo* evaluation. Ind J Pharm Sci 2001; 63(5):408-12.
8. Sinha VR, Singla AK, Wadhawan S, Kaushik R, Kumria R, Bansal K, Dhawan S.Chitosan microspheres as a potential carrier for drugs. Int J Pharm Sci 2004; 274:133.
9. Murata, Y., Toniwa, S., Miyamoto, E., Kawashima, S., 1999. Preparation of alginate gel beads containing chitosan nicotinic acid salt and the functions. Eur. J. Pharm. Biopharm.48, 49–52.
10. Shailendra Shukla, Deepak Jain, Kavita Verma, Sofiya Verma. Formulation and *in vitro* characterization of alginate microspheres loaded with diloxanide furoate for *colon*- specific drug delivery.2010; 4(4):199-204.
11. Yueling Zhang,Wei Wei, Piping Lv, Lianyan Wang, Guanghui Maa. Preparation and evaluation of alginate–chitosan microspheres for oral delivery of insulin. Eur. J.Pharm.2011; 77, 11-19.
12. Torrado JJ, Illum L, Davis SS. Particle size and size distribution of albumin microspheres produced by heat and chemical stabilization. I J Pharm 1989; 51(1): 85-93
13. Sahoo SK, Mohapatra S, Dhal SK, Behera BC, Barik BB. Formulation of floating microspheres of Ciprofloxacin Hydrochloride by crosslinking agent. Ind pharm 2007: 6(58): 65-8
14. Rajamanickam D, Rajappan M, Varadharajan M, Srinivasan B. Formulation and evaluation of albumin microspheres containing aceclofenac; IJPSRR 2010; 4(1): 112-7.