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Development and evaluation of mucoadhesive microspheres of an antiviral drug

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ABSTRACT

The objective of this study was to prepare, characterize and evaluate mucoadhesive microspheres of acyclovir employing sodium alginate as coat along with synthetic/natural mucoadhesive polymers. The orifice ionic gelation method was adapted for preparation mucoadhesive microspheres of acyclovir. The six sets AF 1-3, AF 4-6, were prepared using sodium alginate as coating polymer and carbopol 934, chitosan, urad dal, fenugreek and Lady's finger as mucoadhesive polymer. The formulations AF-10, 14, 18 were prepared using combination of chitosan and urad dal, fenugreek and Lady's finger. The prepared microspheres were evaluated for particle size, particle shape, SEM, FTIR study, encapsulation efficiency, swelling ratio, in vitro wash off test and in vitro drug release. The release rates were studied by using dissolution software PCPDissoV3. Mucoadhesive microspheres were found to be spherical, discrete, free flowing. The microspheres appear with rough surface and encapsulation efficiency found to be in range of 95.21% to 99.12%. All the microspheres showed good mucoadhesive property and swelling index. The drug release was found to be in range of 70.89% to 97.95% over the period of 12 hours. In vitro release reveals drug follows Higuchi matrix and Peppas equation with **n** value less than 0.5 indicating release mechanisms to be Fickian diffusion.

Keywords: Mucoadhesive microspheres, acyclovir, sodium alginate, urad dal, fenugreek, lady's finger.

INTRODUCTION

Mucoadhesive drug delivery systems are one of the novel drug delivery system, which utilize the property of bio adhesion of polymers that become adhesive on hydration[1]. These drug delivery systems can be used for targeting a drug to a particular region of the body for extended period of time.[2] Bio adhesion is an interfacial phenomenon in which two materials, at least one of which is biological, are held together by means of interfacial forces[3]. The attachment could be between an artificial material and biological substrate, such as adhesion between a polymer and biological membrane. In case of polymer attached to the mucin layer of mucosal tissue, the term mucoadhesion is used**.** Mucosal adhesive materials have been investigated and identified[4]. These is generally hydrophilic macromolecules that contain numerous hydrogen bonds forming groups (e.g., hydroxyl and carboxyl groups) and will hydrate and swell when placed in contact with water. In most cases the materials require wetting to become adhesive however, over hydration may result in the formation of slippery mucilage and a loss of the adhesive properties.[5] The adhesive bond between a bioadhesive system and mucous gel can be investigated in terms of the contribution of three regions.

(i) The surface of the bioadhesive polymer
(ii) The interfacial layer between the bioadhesive material and mucosa;
(iii) The mucosa surface

Mechanism and theories of muco-adhesion

Mucoadhesion is believed to be interfacial phenomenon which is influenced by surface energies and involves formation of covalent bond between glycoprotein of mucus membrane and polymer. The first stage involves an intimate contact between a mucoadhesive and a membrane, either from a good wetting of the mucoadhesive surface, or from the swelling of the mucoadhesive. In the second stage, after the contact is established, penetration of the mucoadhesive into the crevices of the tissue surface or interpenetration of the chains of the mucoadhesive with those of the mucus takes

place. Several theories have been proposed to explain the fundamental mechanism of adhesion figure 2. The more important theories of mucoadhesion are Electronictheory, Adsorptiontheory, and Diffusiontheory

Polymers used for mucoadhesive microspheres

Carboxy methyl cellulose, Carbopol, Tragacanth, Poly acrylic acid, Sodium alginate, Hydroxy ethyl cellulose, Hydroxy propyl methyl cellulose, Gum karaya, Gelatin, Guar gum, Thermally modified starch, Pectin, Acacia, Polyethylene glycol, Hydroxyl propyl cellulose, Chitosan

Preparation of mucoadhesive microspheres

Solvent evaporation

It is the most extensively used method of micro-encapsulation, first described by Ogawa et al[6]. A buffered or plain aqueous solution of the drug (may contain viscosity building or stabilizing agent) is added to an organic phase consisting of the polymer solution in solvents like dichloromethane (or ethyl acetate or chloroform) with vigorous stirring to form the primary water in oil emulsion. This emulsion is then added to a large volume of water containing an emulsifier like PVA or PVP to form the multiple emulsions (w/o/w). The double emulsion, so formed is then subjected to stirring until most of the organic solvent evaporates, leaving solid microspheres. The microspheres can then be washed, centrifuged and lyophilise to obtain the free flowing and dried microspheres.

Hot melt microencapsulation

This method was first used by Mathiowitz and Langer[7] to prepare microspheres of poly anhydrides copolymer of poly [bis (p- carboxy phenoxy) propane anhydride] with sebacic acid. In this method, the polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50µm. The mixture is suspended in a non-miscible solvent (like silicon oil), continuously stirred, and heated to 5° C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, e.g. poly anhydrides. Microspheres with diameter of 1-1000µm can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disadvantage of this method is moderate temperature to which the drug is exposed.

Solvent removal

It is a non aqueous method of microencapsulation particularly suitable for water labile polymers such as the poly anhydrides. In this method, drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride[8].This mixture is then suspended in silicone oil containing span 85 and methylene chloride. After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting microspheres can then be dried in vacuum.

Hydrogel microspheres

Microspheres made of gel type polymers, such as alginates, are produced by dissolving the polymer in an aqueous solution, suspending the active ingredient in the mixture and extruding through a precision device, producing micro droplets which fall into a hardening bath that is slowly stirred. The hardening bath usually contains calcium chloride solution, whereby the divalent calcium ions cross linking the polymer formed gelled microspheres. The method involves an all aqueous system, which eliminates residual solvents in microspheres. Lim and Moss developed this method for encapsulation of live cells, as it does not involve harsh conditions, which could kill cells.

The surface of these microspheres can be further modified by coating them polycationic polymers, like poly lysine after fabrication. The particle size of microspheres can be controlled by using various size extruders or by varying the polymer solution flow rates. Addition of plasticizers,e.g. citric acid, which promote polymer coalescence on the drug particles and hence promote the formation of spherical and smooth surfaced microspheres. The size of microspheres can be controlled by the rate of spraying, the feed rate of polymer drug solution, nozzle size, and the drying temperature[9].

Phase inversion microencapsulation

The process involves addition of drug to a dilute solution of the polymer (usually 1-5%, w/v in methylene chloride). The mixture is poured into

Drug Profile: Acyclovir

Pharmaceutical applications

- **Micro capsules**: Used as a coat material for the preparation of micro capsules for sustained release of several drugs.
- **Gels:** Chitosan gel microspheres were used for the delivery of anticancer agents.
- Preparation of films for controlling drug release.
- **Bio adhesion:** Positive charges on the surface of chitosan could give rise to a bioadhesive strong electro static bon don a negatively charged mucosal surface and hence used as a bio adhesive polymer.
- It is an a absorption enhancer for nasal or oral drug delivery.
- For site-specific drug delivery of peptides and other drugs.(Stomach or colon).
- Absorption enhancer, wetting agents and improvement of poorly soluble drug substances.

URADI

Synonym: Vignamungo, Azukiamungo, Phase olusviridissimus.

Biological Source: These seeds are obtained from plant Vignamungo, family Fabaceae.

Color: Pale-yellow- yellowish brown or a ambercolor; **Odour:** Faint terbinthinate. **Taste:** Sweets. **Moisture:** Not more then 14 percent by weight **Occurrence:** In occurs in the form of angular, translucent masses of various sizes.

Bio chemical constituents of black gram: Block gram is a rich source of protein.It also contains albumin and globulin. Block gram is arch source of methionine, tryptophanandlysine. Black gram contains 5560% soluble

sugars, fibers, starch, soluble solids, non-nutritive factors such as trypsin inhibitors, phyticacid, and α-galacto sides and unavailable carbohydrates. It contains minerals like calcium, magnesium, zinc, iron potassium and phosphorus. 80% phosphate is present as phytate phosphate, complexed with protein.

Chemical constituents: Saponin, polyphenol, cysteine proteinase inhibitors (CPI), and hexasaccharideajuose, ascorbic acid, methionine, iron, phenolic compounds, soluble solids, ethylene.

Uses: Urd is an excellent source of late which improves sperm quality by reducingchromosomalabnormalities (Aneuploidy) in sperm. It has been traditionally used ayurveda to treat impotence.

Storage: Stored in well closed containers away from light.

METHI/ FENUGREEK

Synonym: Fenugreek, Helba, Basbasa.

Biological source: Methi is obtained from the seeds of Trigonella foenum-graceum L. family Fabaceae (Legguminosae).

Melting point: 75-85&C; **Color:** yellow wish brown/ brown; **Odour:** peculiar dour; **Acid value:** 1-2; **Iodine value:**115: **Saponification value:** 178-183; **Ash value:** 3.92 **Specific gravity:** 0.91gm/cm, **Taste:** Bitter, **Solubility:** It is in soluble in water but soluble in alcohol, 10% ethanol extractive.

Chemical constituents: Fenugreek seeds contains carbohydrates, mainly mucilaginous fiber (galactomannans), proteins, fixed oils), alkaloids, mainly trig onelline, gentianine, flavonoids, apigenin, luteollin, orient in quercetin, vitexin &isovitexin. Free amino acids as 4 - OH- is oleucine.Vitamins A,B & C, saponin and steroids.

Uses: Me the seeds are used as cardio tonic, hemolytic and diuretic. It also anticancer activity. Fenugreek seeds have been widely studied for the irrupted ant diabetic, hypocholesterolacmic, antifertility and hypolipidemic effects. Properties of fenugreek that have been reported but which have received less at tension include anti cancer, antibacterial, antihelmintic, anticholinergic and anti-inflammatory effects.

Storage: Stored in well closed containers away from light. Well drained place protected from humidity, excessive heat, direct sunrays, in sects and rodents.

BHENDI/ LADYSFINGER

Synonym: Hibiscus uses culentusL.

Biological source: these are the fruits obtained from the plant Abelmosehusesculentus belonging to the family malvaceae.

Color: Light brown colored; **Odor:** Character is tic odor, **Acid value:** 1.0%

Ash value: 5.68%, **Solubility:** Slightly soluble in water, practically in soluble in alcohol, chloroform and acetone and form thick gel in water.

Chemical constituents: Polysaccharide's, polyphenolic compounds, flavonoids, coumarin, mucilage contains carbohydrates such has D- glucose,Glucuronic acid, methyl pentose, fructose, sucrose, are binose, galactose

Storage: Stored in well closed containers away from light and well drained place. Protected from humidity, excessive heat, direct sunrays, in sects and rodents.

Uses: Used as suspending agent, binding agent.

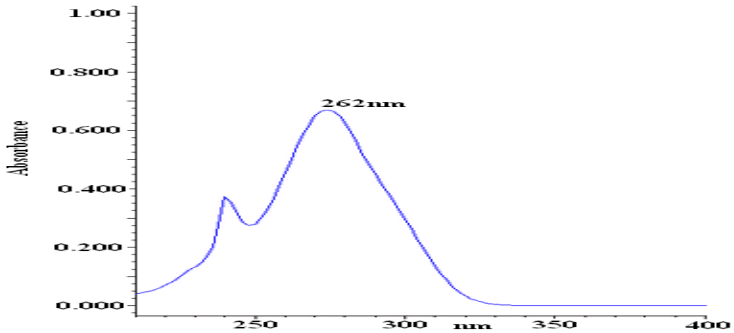

Fig 1: Absorption maxima of acyclovir in pH7.2 phosphate buffer solution.

Preparation of calibration curve

Pipette out 0.2, 0.4, 0.6, 0.8, 1.0 ml of II stock solution (100µg/ml) in to series of 10 ml volumetric flask and volume was adjusted to with pH 7.2 phosphate buffer solution to obtain 2,4,6,8, and 10µg/ml of solution. The absorbance of the resolutions was measured at 266nm keeping pH 7.2 phosphate buffer as blank, the optical density values are cored in table 3 with statistical data in table 4. Concentration versus optical density values are plotted and displayed in the figure 5 I the concentration range of 2-10µg/ml. the method obeyed Beer-Lamberts law and the solution was stable for 48h.

Table 1: Calibration curved at a for Acyclovir

Concentration (µg/ml)	Absorbance*±SD
2	0.069±0.006
4	0.136±0.003
6	0.204±0.005
8	0.275±0.004
10	0.346±0.005

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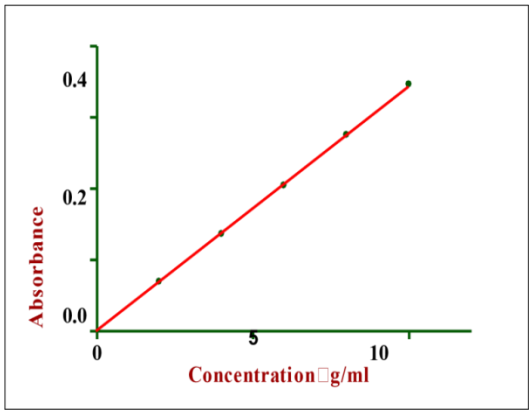

Fig 2: Calibration curve for acyclovir in ph 7.2 phosphate buffer

METHODS

Isolation of mucilage from Fenugreek by conventional procedure

Fenugreek was powdered for 5 mini name chemical blender and passed through the sieve no. 120 to get fine powder. It was then so a kedind is tilled water for 24 hours in a RB flask, it was boiled for 1 hour under reflux with occasional stirring and kept aside for 2 hours a for release of mucilage in to water. The material was filtered through muslin bag, hoted is tilled water added through theses of the mariachi and squeezed well in order to remove mucilage completely, equal volume of ethanol was add dried completely in a incubator at 370+.powdered, sieved and weigh had. It was subjected to chemical to firm identity.

Isolation of mucilage from Lady's finger by convention a procedure

The Lady "finger/ bhendi was procured from local market and was shade dried this was then powdered by crushing and. Lady's finger/ bhendi was dried in an oven at37&+ ordering and it was powdered for 5 minimum in a thick blender and passed through sieve no. 120 get fine powder. It was then so a kedging is tilled water 24 hours in a RB flask. It was boiled for 1 hour under reflux with occasional stirring and kept aside for 2 hours for release of mucilage in to water. The material was filtered through muslin bag, hoted it tilled water was added through the side of the mar cand squeezed well in order to remove mucilage completely. Equal volume of ethanol was added to the filtrate to precipitate the mucilage kept in side refrigerator for a day to effect settling. It was filtered and dried completely in a incubator at 37&c, powdered, sieved and weighed. It was subjected to chemical tests to confirm identity.

Isolation of mucilage from Black gram by convention a procedure

Black gram seeds were powdered for 5 mini name chemical blender and passed through sieve no. 120 to get fine powder. It was then so a ked in distilled water for 24 hours in a RB flask. It was boiled for 1 hour under reflux with occasional stirring and kept a side for 2 hours for release of mucilage in to water. The material was filtered through muslin bag, hoted is tilled water was added through the side of the marc and squeezed well in order to remove mucilage completely. Equal volume of ethanol was added to the filtrate to precipitate the mucilage and kept inside refrigerator for a day to effect setting. It was filtered and dried completely in an incubator at 37&c, powdered, sieved weighed, it was subjected to chemical tests to confirm its identity.1[0]

Preparation of mucoadhesive microspheres Orifice ionic elation method

Sodium alienates and muco adhesive polymer was dissolved in purified water (10) separately. Then both the solutions were mixed to from homogeneous polymer solution. The drug was added to the polymer solution and mixed thoroughly with help of pestle and mortar to form viscous person. There salting person was added drop wise in to 10% w/v calcium chloride solution (100mi) through a syringe with needle (size no 21) with continuo's stir ring at 500rpm. The added drop lets were retained in the calcium chloride solution for 15 minutes to produces spherical rigid microspheres. The microsphere was collected by centration, and the product thus separated was repeatedly with water and dried at 45&C for 12 hours and stored in desiccators. Similarly sodium alginate-fenugreek, sodium alginate- black gram, sodium alginate-okra microspheres prepared by dissolving required quantity of sodium alginate and muco adhesive polymer in water. Then drug is added to polymeric solution and mixed thoroughly with help of pestle and mort to form viscous dispersion. Then follow the procedure as mentioned above.

Table 2: Formula afford different sodium alginatemucoadhesive microspheres of a acyclovir Batch size:1G

Batches	Core: Coat	Acyclovir	Sodium alginate	Carbopol	Chitosan
AF1	1:1	500	375	125	--------
AF2	1:2	500	750	250	--------
AF3	1:3	500	1125	375	--------
AF4	1:1	500	375	--------	125
AF5	1:2	500	750	--------	250
AF6	1:3	500	1125	--------	375

Evaluation of mucoadhesive microspheres

Product on yield

The dried microspheres of each batch are weigh desperately and percentage yield is calculated by using following equation

Estimation of drug content

50 mg of mucoadhesive microspheres were weighing and powdered. This was dissolved or extracted in methanol in 100ml volumetric flask and made up to volume. The solution was shaken occasionally for 1 hand filtered. From this 1ml of solution was diluted up to 100ml with pH 7.2 buffer solution in 100ml volume metric flask. The drug content was analyzed by measuring absorb ancient UV spectra photometer at 266n musing pH7.2phosphate buffer as blank. The studies were carried out in triplicate.

FTIR spectral studies

The compatibility between pure drug and polymers were detected by IR spectra obtained on perk in Elmer 1600 series, (USA). The pellets were prepared on KBrpress. To prepare the pellets, a few mg of the micro spheres were ground to gather in a mortar with about 100 times quantity of KBr. The finally ground powder was in traduced in to a stain less steel die. The powder was then pressed in the die between polished stain less steel an will set a pressure of about 10 t/ in 2. The spectra as were recorded over the wave number range of 4000 to 500-/

Encapsulation efficien

100mg of mucoadhesive microspheres were accurately weighed. They were powdered and extracted with 100ml of methanol. Further it was serially diluted with pH7.2 phosphate buffer solution. There salting solution was analyzed for acyclovir drug content by measuring a absorb anceina UV-spectra. photometer at 266n musing pH7.2 phosphate buffer as blank. The studied were carried out in triplicate. Encapsulation efficiency (%) was calculated using the formula.

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Size analysis of microspheres: Different sizes in a batch are separated by sieving using a range of standard sieves 16/20,20/40 and the amountsretained on different sieves were weighed. Studies were carried out in triplicate
The sizes of the micro spheres were calculated by using the equation

$$D_{Avg} = \frac{\sum Xifi}{fi}$$

Where,
□$_2$- is the means size of the range, □$_2$- is the percent material retained on the small erosive in the size range.
Scanning electron micro scopy**:** The particle size, shape and surface morphology of microspheres were examined by scanning electron microscopy (SEM). Microspheres were fixed on aluminum studs and coated with gold using as gas putter coater SC 502, under vacuum [0.1mmhg]. he micro spheres were then analyzed by scanning electron microscopy (SEM) [Model JSM-840A, Joel. Japan]
Dissolution studies□: the release of a acyclovir form micro spheres was in vest gated in pH7.2 phosphate buffer solution as a dissolution medium (900ml) using USP type apparatus. As sample of microspheres equivalent to 50mg of acyclovir was taken in the basket. A speed of 59rpm and temperature of 37±0.5$_{\&}$c was maintained throughout the experiment. At fixed intervals, aliquot (5ml) was with draw and replaced with fresh dissolution media. The concentration of drug released at different time intervals was then determined by measuring the absorbance using Hitachi U-2000 spectra photometer at 266nm against blank. The stud dies were carried out in triplicate. The invitrodissolution data of formula muco adhesive microspheres were tabulated calculated by using dissolution of ware viz,. **PCPDISSOV3.0**.

RESULTS

Table 3: Production yield of AF-1,AF-2,AF-3,AF-4,AF-5 and AF-6 formulations.

Batches	Production yield*±SD
AF-1	93.29±0.87
AF-2	91.9±0.46
AF-3	89.46±0.78
AF-4	94.18±0.83
AF-5	95.56±0.31
AF-6	92.69±0.26

Table 4: Percent drug content ofAF-1,AF-2,AF-3,AF-4,AF-5 and AF-6 formulations

Batches	Theoretical drug content(mg)	Practical drug content (mg)	% Drug content ±SD	Coefficient of variation
AF-1	25	24.54	98.16±0.45	0.458
AF-2	25	24.20	96.81±0.35	0.361

AF-3	25	24.23	96.92±0.25	0.257
AF-4	25	24.53	98.12±0.54	0.550
AF-5	25	24.61	98.43±0.46	0.467
AF-6	25	24.66	98.65±0.31	0.314

Table 5: Micro encapsulation efficiency of AF-1,AF-2,AF-3,AF-4,AF-5andAF-6 formulations

Batches	Microencapsulation efficiency* ±SD
AF-1	98.16±0.45
AF-2	96.81±0.35
AF-3	96.92±0.25
AF-4	98.12±0.54
AF-5	98.43±0.46
AF-6	98.65±0.31

Table 6: Size analysis of AF-1,AF-2, and AF-3 formulations

Sizerange		Arithmetic meansize(μm)(Xi)	AF-4		AF-5		AF-6	
Iesh	μm		Percent* retained (F_i)	Weight Size(X_i F_i)	Percent* retained (F_i)	Weight size(X_i F_i)	Percent* retained (F_i)	Weigh Size(X_i F_i)
20	1190-840	1015.0	77.65	78814.75	78.14	79312.1	78.46	79636.
40	840-420	630.0	22.35	14080.5	21.86	13771.8	21.54	13570.
			D_{av}=928.95		D_{av}= 930.839		D_{av}= 932.071	

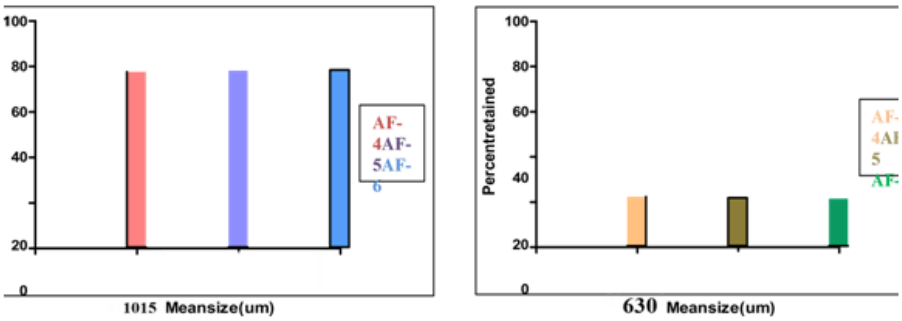

Fig 3: Size distribution of AF-1,AF-2andAF-3 formulations

Table 7: Size analysis of mucoadhesive microspheres of acyclovir of AF-4, AF-5, and AF-6 formulations

Size range		Arithmeticmean size (μm)(Xi)	AF-4		AF-5		AF-6	
			Percent* retained(F_i)	Weight Size (X_iF_i)	Percent* retained(F_i)	Weightsize (X_iF_i)	Percent* retained(F_i)	Weight Size(X_iF_i)
16/20	1190-840	1015.0 630.0	77.65 22.35	78814.75 14080.5	78.14 21.86	79312.1 13771.8	78.46 21.54	79636.9 13570.2
20/40	840-420							
			D_{av}=928.95		D_{av}= 930.839		D_{av}= 932.071	

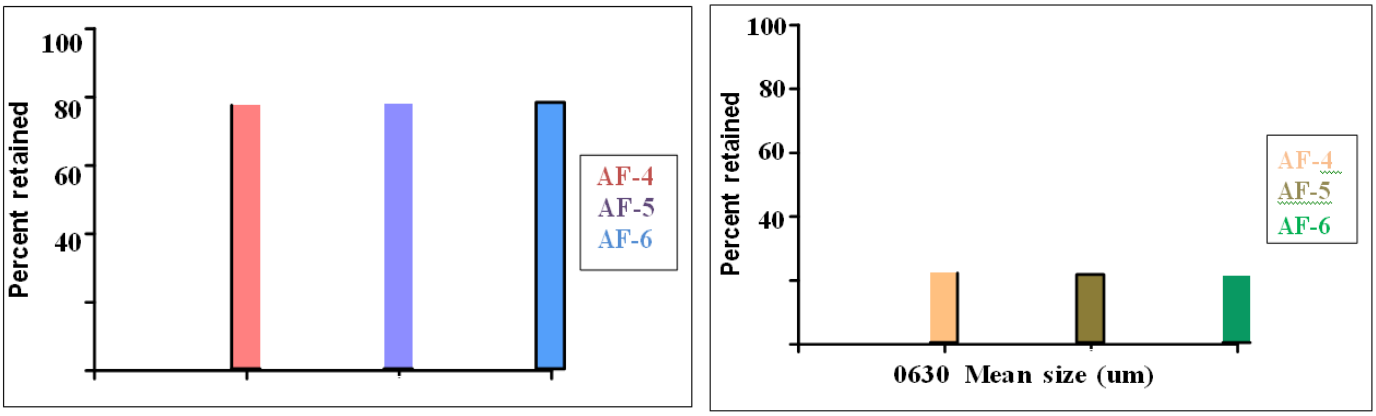

Fig 4 : Size distribution of AF-4,AF-5 and AF-6 formulation

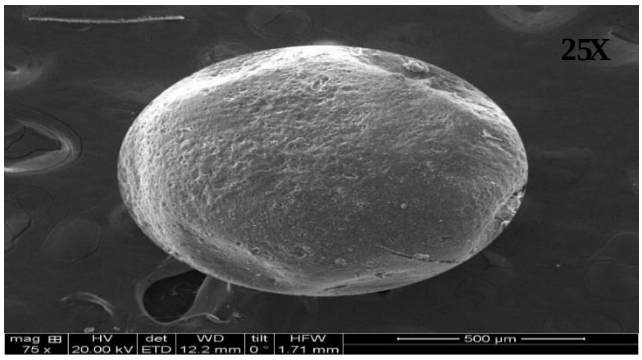

Fig 5: Scanning electron micrographs of AF-3 formulation

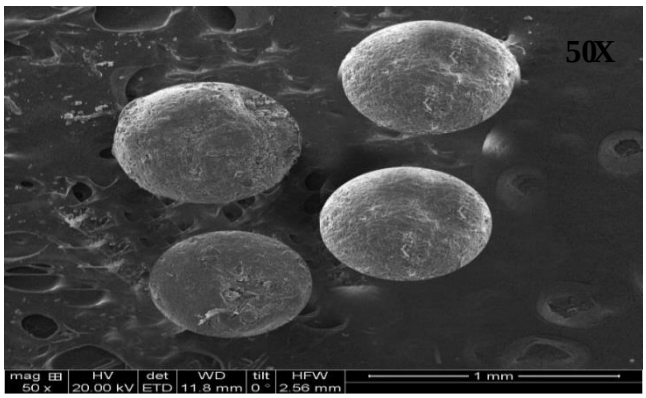

Fig 6: Scanning electron micrographs of AF-6 formulation

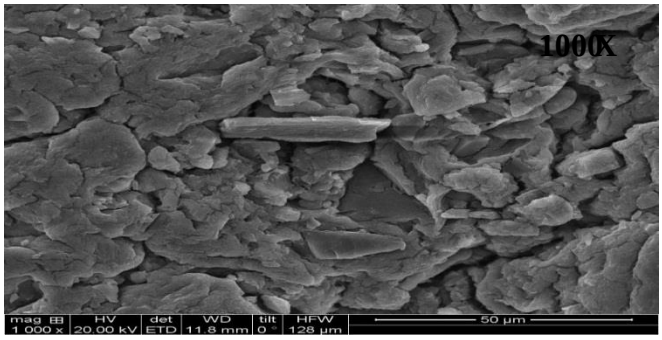

Fig 7: Scanning electron micrographs of AF-6 formulation

8: Swelling ratio of AF-1, AF-2, AF-3, AF-4, AF-5 and AF-6 formulations

Time (h)	AF-1		AF-2		AF-3		AF-4		AF-5		AF-6	
	Weight of MC after swelling	Relative swelling	Weightof MC after swelling	Relativeswelling	Weight of MC after swelling	Relative swelling	Weight of MC after swelling	Relative swelling	Weight of MC after swelling	Relative swelling	Weight of MC after swelling	Relative swelling
0	50	0	50	0	50	0	50	0	50	0	50	0
0.5	82	0.64	84	0.68	83	0.66	73	0.46	75	0.5	74	0.48
1	91	0.82	93	0.86	94	0.88	79	0.58	81	0.62	80	0.6
2	109	1.18	110	1.2	112	1.24	99	0.98	98	0.96	101	1.02
3	112	1.24	115	1.3	119	1.38	103	1.06	106	1.12	108	1.16
4	116	1.32	118	1.36	121	1.42	104	1.08	108	1.16	112	1.24
5	119	1.38	121	1.42	124	1.48	108	1.16	110	1.2	116	1.32
6	119	1.38	121	1.42	124	1.48	108	1.16	110	1.2	116	1.32

Table 9: Invitro wash off test of AF-1, AF-2, AF-2, AF-3, AF-4, AF-5, AF-6 formulations

Batches	Percentageofmicrospheresadheringtotissueatdifferenttimeinterval(h)					
	0	0.5	2.0	3.0	4.0	6.0
AF-1	50	88	80	64	60	60
AF-2	50	86	74	58	55	55
AF-3	50	96	85	77	65	65
AF-4	50	94	83	74	71	71
AF-5	50	96	88	79	68	68
AF-6	50	93	82	75	67	67

Table 10: Model fitting values for AF-1,AF-2,AF-3,AF-4,AF-5 and AF-6 formulations

	AF-1	AF-2	AF-3	AF-4	AF-5	AF-6
Zero order	0.6871	0.7221	0.7073	0.8258	0.8587	0.8478
1storder	0.964	0.9523	0.9484	0.9759	0.9732	0.9506
Matrix	0.9836	0.9877	0.9870	0.9959	0.9917	0.9936
Peppas	0.9886	0.9905	0.9932	0.9829	0.9710	0.9809
Hix.Crow	0.9284	0.9595	0.9598	0.9739	0.9796	0.9703

DISCUSSION

The mucoadhesive microspheres of Acyclovir were prepared by orifice ionic gelation method using sodium alginate-mucoadhesive polymers (synthetic/natural) and mucilage isolated from the natural sources. The mucoadhesive microspheres were prepared in 1:1,1:2 and 1:3 core: coat (Coat composition was rate controlling polymer: mucoadhesive polymerat1:1weightration). The polymer sodium alginate was selected to control the release rate and Carbopol as synthetic and chitosan, mucilage's isolated from uraddal, fenugreek and lady's finger were selected as natural mucoadhesive polymer. In first set three formulations viz., AF-1,AF-2 and AF-were formulated with Carbopol and three formulations viz., AF-4,AF-5 and AF-6 formulated with chitosan.

Production yield: The results of production yields are shown in tables 6-10. The percentage yield of set-1 formulations were in the range o f89.46±0.78 to 95.56±0.31,set-2 formulations in the range of 85.31±0.35 to 89.57±0.26 and set-3 formulations in the range of 84.51±0.38 to 86.51±0.37. The production yield was manageable with little loss of drug during the formulation stage.

Drug content: The results of drug content are shown in tables 11-15. The percentage drug content of set-1 formulations were in the range of 96.81±0.35 to 98.65±0.31,set-2 formulations in the range of 95.21±0.45to98.21±0.45 and set-3 formulations in the range of 98.42±0.82 to 99.12±0.45.The low SD and CV value indicates uniform distribution of drug with in the various batches of microspheres prepared. The drug content results suggest a negligible loss of drug during he formulation stage.

FTIR studies: The FTIR spectrum of pure acyclovir, carbopol, Chitosan, uraddal, fenugreek, lady's finger physical mixture and prepared mucoadhesive microspheres are shown in figures 6-14. The FTIR characteristic acyclovir bands are OH

stretching 3426cm^{-1},-NH stretching at 3169cm^{-1},Ar-CH=C stretching at 3043cm^{-1},CH2 and CH3 stretching at 2882cm^{-1} and 2821cm^{-1},C=O stretching at
1691cm^{-1} and NH-bending at 1264cm^{-1}. The FTIR spectra's of mucilage obtained from natural sources were complex in nature and hence, changes in the absorption bands of acyclovir were considered. FTIR spectra's of selected mucoadhesive microspheres showed all the characteristic absorption bands of acyclovir with little shifting toward lower / higher wavelength especially Ar-CH=C stretching at 3043cm^{-1} and C=O stretching at 1691cm^{-1} indicating minor interaction or no interaction.

Encapsulation efficiency: Encapsulation efficiency of all the formulations is presented in the tables 16-20. The percentage encapsulation efficiency of set-1 formulations were in the range of 96.81±0.35 to 98.65±0.31 forset-2 formulations in the range of 95.21±0.45 to 98.21±0.45 and set-3 in the range of 98.42±0.82 to 99.12±0.45. The results suggest encapsulation efficiency depend upon concentration of sodium alginate used in the formulation. The encapsulation efficiency is increased progressively with increase in the concentration of sodium alginate. This could beat tributed due to formation of larger microspheres with increasing concentration of sodium alginate, thus entrapping more amount of drug.

Size analysis: The size analysis is carried out by sieve analysis method and data is shown in tables 21-26. The sieve analysis of set-1 formulations found microspheres in the range of 928.95µm to 938.54µm [77.65 to 80.14 microspheres were distributed in the range of 840-1190 m(16/20mesh)],set-2 formulations found microspheres in the range of 914.28µm to 925.42µm [73.84 to 76.75 microspheres were distributed in the range of 840-1190 m(16/20mesh)] and set-3 formulations found microspheres in the range of 916.32µm to 930.83µm [74.37 to 78.14 microspheres were distributed in the range of 841-1190 m (16/20mesh)]. The size of microspheres is depending upon concentration of sodium alginate used in the formulation. The increase in size of microspheres was observed with increase in concentration of sodium alginate. This could be due to increase in viscosity of the polymeric dispersion, which eventually lead to formation of bigger particle during ionic gelation. [11]

Scanning electron microscopy: Scanning electron microscopy was used to know surface morphology of microspheres. The SEM photographs of AF-3,AF-6, AF-14 and AF-18 batches revealed that microspheres were spherical, discrete (Figure21-28). The outer surface of microspheres was coarse rough texture, with few pores, mild cracks and completely covered with coat materials.

Swelling studies by weight method: The swelling depends upon the polymer concentration, ionic strength as well presence of water. The relative swelling of mucoadhesive microspheres of set1 formulations were found in the range of 1.38,1.42,1.48 and 1.16,1.2,1.32 for AF-1,AF-2,AF-3 and AF-4,AF-5,AF-6 respectively and set-2 formulations were found in the range of 0.9,0.98,1.06;0.94,0.98,1.02; 0.94,1.06,1.08 for In all set of formulations as the concentration of sodium alginate increases the relative swelling increases which further depend on the type of mucoadhesive polymers. The results clearly suggested swelling ratio depends upon concentration of polymer and type of mucoadhesive polymer used in the formulation. Swelling ratio shows direct relationship with sodium alginate concentration and increased with increasing concentration of sodium alginate. In all three set, formulations having carbopol mucoadhesive polymers exhibited good swelling property compared toot her mucoadhesive polymers.

Invitro wash-off test: The mucoadhesion is a phenomenon in which two materials, at least one of which is biological are held together by means of inter facial force. The tables32-36 shows invitro mucoadhesion data of mucoadhesive microspheres carried out with averted at intestinal mucosa in presence of phosphate buffer pH7.2. The percentage of microspheres retained oneverted intestinal mucosa after 6hin set1 formulations were found in the range of 60,55,65;71,68,67 for AF-1,AF-2,AF-3 and AF-4,AF5,AF-6 respectively The overall results suggest that concentration and type of mucoadhesive polymer doesn't show much more difference in the mucoadhesive property.

Dissolution studies: The dissolution rate of mucoadhesive microspheres were studied by using USP type I apparatus
(Basket Method) and the dissolution at a was computed by using dissolution software
PCPDISSOV3.0. The dissolution profiles with various model fitted values are given in tables3741 and figures 29a-51. The percentage release of acyclovir from AF-1,AF-2, and AF-3formulations prepared with Sodium alginate : Carbopol and AF-4,AF-5 and AF-6 formulations prepared with Sodium alginate : Chitosan were 96.30±0.65, 97.38±0.25, 97.95±0.39 and 94.57±0.22, 95.50±0.28, 96.60±0.45 respectively over the period f1 2hours.

In mucoadhesive microspheres prepared with mucilage isolated from natural sources the release rate was maximum at low concentration of coating material and as the concentration increased the release rate was decreases. It is mainly attributed to the influence of swelling property on the release of the drug from the microspheres. The mucoadhesive microspheres prepared with mucilage isolated from natural sources in combination with Chitosan exhibit the release rate with respect to the type of mucilage. Microspheres prepared mucilage isolated from Bhendi shows maximum drug release when compared to microspheres prepared mucilage isolated from Methi and Urdi. The over all drug release was maximum at higher concentrations of coating polymer, where as incase of mucoadhesive microspheres prepared with mucilage isolated from natural sources it was found to be less compared to Carbopol and chitosan microspheres .It is mainly due to low swelling and less mucoadhesive property.

Further dissolution data were subjected for model fitting by using dissolution software DISSOV3. The release of the drug from AF-1,AF-2, and AF-3 formulations followed peppas equation with,, n‟‟ value of 0.3715,0.3834,0.3822 ,and the release from formulations AF-4t o AF-6 higuchi matrix with,, n‟‟ value of 0.4472,0.4291,0.4470.In all formulations the release exponent was found less than 0.5 indicating the release was fickian mechanism indicating the release rate was to be diffusion controlled. [12-14.]

CONCLUSION

The mucoadhesive microspheres of Acyclovir were conveniently prepared by orifice ionic gelation method using sodium alginate-mucoadhesive polymers (synthetic/natural) and mucilage isolated from the natural sources. The production yields were in the range of

84.51±0.38to95.56±0.31 and the percentage drug content were in the range of 95.21±0.45to99.12±0.45 with low SD and CV value indicating uniform distribution of drug within the various batches of microspheres prepared with negligible loss during the formulation stage. FTIR spectra's of selected mucoadhesive microspheres shows all the characteristic absorption bands of acyclovir with little shifting toward lower/higher wavelength especially Ar-CH=C stretching at 3043cm^{-1} and C=O stretching at1691cm^{-1} indicates minor interaction or no interaction. The percentage encapsulation efficiency was in the range of 95.21±0.45 to 99.12±0.45 and increased progressively with increase in the concentration of sodium alginate. This could be attributed due to formation of larger microspheres with increasing concentration of sodium alginate, thus entrapping more amount of drug. The microspheres were distributed in the range of 914.28µm to 938.54µm. The size of microspheres depends upon concentration of sodium alginate used in the formulation. The increase in size of microspheres was observed with increase in concentration of sodium alginate. This could be due to increase in viscosity of the polymeric dispersion, which eventually led formation of bigger particle during ionic gelation. The scanning electron microscopy reveals that the microspheres were spherical, discrete with rough texture. The swelling ratio depends upon concentration of polymer and type of mucoadhesive polymer used in the formulation. Swelling ratio shows direct relationship with sodium alginate concentration and increased with increasing concentration of sodium alginate. In all three set, formulations having carbopol as mucoadhesive polymers exhibited good swelling property compared to other mucoadhesive polymers. The invitro wash-off test results suggest that concentration and type of mucoadhesive polymer doesn't show much more difference in the mucoadhesive property. The overall drug release was maximum at higher concentrations of coating polymer, while it was less in mucoadhesive microspheres prepared with mucilages isolated from natural sources when compared to carbopol and chitosan microspheres. It is mainly due to low swelling and less mucoadhesive property. In mucoadhesive microspheres prepared with mucilage isolated from natural sources the release rate was maximum at low concentration of coating material and as the concentration increased the release rate was decreased. It is mainly attributed to the influence of swelling property on the release of the drug from the microspheres. The mucoadhesive microspheres prepared with mucilage isolated from natural sources in combination with chitosan exhibit the release rate with respect to the type of mucilage. Microspheres prepared mucilage isolated from lady's finger shows maximum drug release when compared to microspheres prepared mucilage isolated from fenugreek and Urad dal. The dissolution data were subjected for model fitting using dissolution software PCP DISSO V.3. The release from the mucoadhesive polymers follows higuchi matrix and peppas model. In all formulations the release exponent „n" was found to be less than 0.5 indicating the release was fickian mechanism indicating the release rate was diffusion controlled.

SUMMARY

Drug delivery systems [DDS] that can precisely control the release rate or target drugs to a specific body site had an enormous impact on the health care system. Microspheres constitute an important part of these particulate DDs by virtue of their small size and efficient carrier characteristics. However, the success of these novel novel DDS is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the DDS with absorbing membranes. It can be achieved by coupling mucoadhesion characteristics to microspheres and developing novel delivery systems as mucoadhesive microspheres.

Acyclovir, a nucleoside analogue of thymidine used in the treatment of HIV. Acyclovir has short half life of 2.3 hours thereby requiring twice daily in large number of patients which leads to no patient compliance. Thus the development of mucoadhesive microspheres for controlled release would be advantageous.

Chapter1 summarizes the detailed information regarding mucoadhesive drug delivery systems, polymers used, method of preparation and various applications of mucoadhesive drug delivery systems. Further this chapter gives information about various attempts made to prepare mucoadhesive drug delivery systems of various drugs under review of literature heading and profiles of drug and polymer are given in the later stages of this chapter.

Chapter2 describes the methodology which includes experimental methods. Experimental part gives information about various coating and copolymer used in the study and also method adapted for the preparation of mucoadhesive microspheres.

Further in this chapter enlisted in for various invitro characterization for the study of
mucoadhesive microspheres.

Chapter3 summarizes invitro characterization results along with the observations which are presented in the form of tables and graphs. The results reveal that the mucoadhesive microspheres of acyclovir can be conveniently prepared by adapted method. Further this chapter discusses the invitro release of drug from the microspheres and study of various dissolution parameters, model fitting data and release rate mechanism through dissolution software PCP disso V.3. The best fit model is korse-meyer peppas equation with exponential slope value **n<0.5** indicating that dissolution to be controlled and follows fickian diffusion mechanism.

Chapter4 describes brief summary of the present study with supported conclusions.

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