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Effect of Natural Mucilages on Mucoadhesion Property of Didanosine Loaded Sodium Alginate Microspheres

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Keywords: Didanosine, gastric residence time, sodium alginate, fenugreek mucilage, carbopol 934, inotropic gelation method.

ABSTRACT

The present work aims to prolong the gastric residence time of Didanosine by formulating into mucoadhesive control release microspheres. The mucoadhesive microspheres were prepared by ionotropic gelation method of polyelectrolyte complexation technique using sodium alginate as control release polymer and fenugreek mucilage, carbopol 934 as mucoadhesive agents in different proportions and calcium chloride as an agent of multivalent cations. The prepared microspheres were characterized by micrometric properties, mean particle size, invitro wash-off test, in vitro drug release and compatibility studies. The data obtained in this study suggests that a microparticulate mucoadhesive dosage form of Didanosine can be successfully formulated to give prolonged residence time using fenugreek mucilage.

INTRODUCTION:[1.2]

Microspheres are defined as solid colloidal approximately spherical shape particles size ranging from 1µm to 1000 µm in diameter. However, the success of these microspheres is limited due to their short residence time at the site of absorption. To overcome this limitation the microspheres are coupled with mucoadhesion character by incorporating mucoadhesive polymers into their formulation to develop mucoadhesive microspheres. Mucoadhesion is the attachment of the drug along with a suitable carrier to the mucosal layer. Mucoadhesion is a complex phenomenon which involves wetting, adsorption, and interpenetration of polymer chains.

The mucoadhesive microspheres are formulated by using mucoadhesive polymers, these mucoadhesive polymers have the property of adhering to mucin epithelial surface. A shortlist of Mucoadhesive polymers is given in Table no. 1.

MATERIALS AND METHODS:

MATERIALS:[3]

Table No. 1: List of materials

Sr. No.	Ingredients	Manufacturer/supplier
1	Didanosine	Hetero labs
2	Sodium alginate	Himedia laboratories
3	Carbopol 934	Himedia laboratories
4	Calcium chloride	Himedia laboratories

METHODS:[1,2,3,4,5]

Standard graph method of Didanosine

Preparation of sample solution:

Didanosine solution was prepared in 0.01M potassium phosphate buffer, pH7, with the final concentration of 0.2mg/ml. This dilution was used for the preparation of the calibration curve. The standard stock solution was prepared in triplicate for the calibration curve. The Quality control(QC) sample was prepared at a concentration of 75, 1500, and 3500ng/ml,

Preliminary test was carried out using a concentration range until the minimum and maximum values were obtained. To prepare the calibration curve,

Isolation of TFGSMP from *Trigonella foenum-graecum L*. seeds:

Trigonella foenum-graecum L. seeds (200g) were soaked in 1.5l of distilled water for 24h and boiled using water bath until slurry is obtained. The slurry was allowed to cool down and kept in the refrigerator overnight to settle out undissolved materials. The upper clear solution was gradually poured and concentrated at 60°C using a water bath to one-third of its original volume. The solution was allowed to cool down and transferred into three times the volume of acetone with continuous stirring. The precipitate was washed repeatedly with acetone and dried at room temperature for 24h. The isolated material was passed through sieve number 80 and stored in desiccators until further use.

Figure No. 1: *Trigonella foenum-graecum* L. Seeds Mucilage Powder

Evaluation of TFGSM:

- **Solubility:** Solubility of TFGSM was performed by dissolving in distilled water and organic solvents such as chloroform and methanol.

- **Swelling index:** TFGSM 1 g was transferred into a 25ml glass stoppered measuring cylinder and 25ml of distilled water was added and shaken vigorously every 10min for 1h and will be allowed to stand for 24h. The volume occupied by the TFGSM was measured. The process was repeated three times and the swelling index was calculated from the mean of three readings.

- **Loss on drying:** Loss on drying was determined by weighing 1g of TFGSM and heated at 105°C until a constant weight is achieved using a hot air oven. The percentage of moisture loss on drying was calculated using the following formula:

$$\textit{Loss on drying}\ (\%) = \frac{\textit{Weight of water in sample}}{\textit{Weight of dry sample}} * 100$$

- **pH:** TFGSMP 5g with 20ml of distilled water and stirred for 5min. a pH of the resulting TFGSM mixture was determined using a calibrated digital pH meter.

- **Bulk density:** Bulk density was determined by adding pre-weighed amounts of TFGSM into a graduated cylinder and the volume was recorded. The powders were introduced to tapping in a bulk density apparatus until a constant volume is achieved. The tapped density was determined as the ratio of the sample weight to the final sample volume.

- **Percentage yield:** The yield of the TFGSM was calculated using the formula below:

$$\textit{Yield}\ (\%) = \frac{\textit{Amount of TFGSM achieved}\ (g)}{\textit{Amount of TFG seed}\ (g)} * 100$$

PROCEDURE: Didanosine mucoadhesive microspheres were prepared by ionotropic gelation method using polyelectrolyte complexation technique. In this method, the required amount of Didanosine, sodium alginate, mucoadhesive agent and calcium chloride were weighed and passed through sieve no ≠ 60. Sodium alginate and mucoadhesive agent were dissolved in water to form a homogenous polymer solution by continuous stirring to which Didanosine was added. The resultant drug dispersion was loaded into the dry disposable syringe with needle size no 20 and added dropwise into 2% w/v solution of calcium chloride under constant stirring. The added droplets were retained in the calcium chloride solution for 15 min to complete the curing reaction and to produce the spherical rigid microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water and dried at room temperature for 24 hours. Total 6 formulations were prepared by using different drug and polymer ratios.

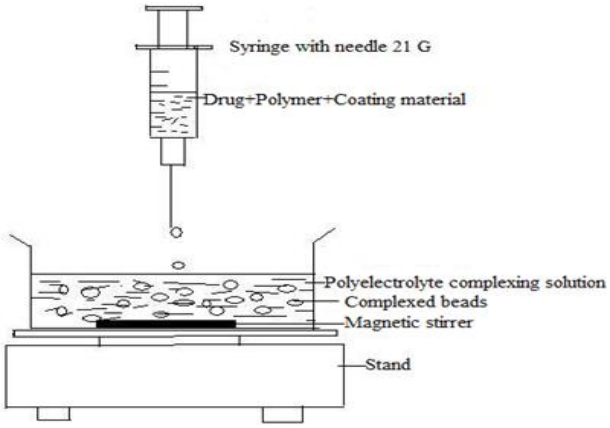

Figure No. 2: Polyelectrolyte Complexation Technique

Table No. 2: composition of different formulations

Sr. No.	FORMULATION CODE	DRUG: POLYMERS	RATIO
1.	F1	Didanosine : Sodium alginate	1 : 1
2.	F2	Didanosine : Sodium alginate : cabopol 934	1 : 1 : 0.5
3.	F3	Didanosine : Sodium alginate : cabopol 934	1 : 1 : 1
4.	F4	Didanosine : Sodium alginate : TFGSMP	1 : 1 : 0.5
5.	F5	Didanosine : Sodium alginate : TFGSMP	1 : 1 : 1

EVALUATION OF MUCOADHESIVE MICROSPHERES:

Micromeritic properties: The mucoadhesive microspheres were characterized by their micrometric properties such as particle size, bulk density, tapped density, compressibility index Hausner's ratio and angle of repose.

A) Tapped density: The prepared mucoadhesive were transferred to a measuring cylinder and tapped for 100 times. After tapping, the volume of microspheres was visually examined. The ratio of microspheres to the volume of microspheres after tapping gives tapped density.

Tapped density= Mass of microspheres in grams / Volume of microspheres after tapping

B) Bulk density: The prepared mucoadhesive Were transform to a measuring cylinder and the volume occupied by the microspheres was noted. This volume is bulk and it includes the true volume of the power and the void space among the microspheres.

Bulk density = Weight of microspheres in grams/ Bulk volume of microspheres in cm^3

C) Carr's compressibility index: The compressibility index is a measure of the flow of a powder to be compressed. It was determined from the bulk and tapped densities.

Carr's compressibility index = Tapped density- Bulk density x100/Tapped density.

D) Hausner's ratio: Tapped density and bulk density were measured and the Hausner's ratio was calculated using the following formula:

Hausner's Ratio = Tapped density/Bulk density

The values less than 1.25 indicate good flow whereas greater than 1.25 indicates poor flow.

E) The angle of repose: Angle of repose is defined as the maximum angle possible between the surface of the pile and the horizontal plane.

The fixed funnel method was employed to measure the angle of repose. A funnel was secured with its tip at a given height (h) above a graph paper that is placed on a flat horizontal surface.

Accurately weighed microspheres were carefully poured through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius(r) of the base of the conical pile was measured. The angle of repose (θ) was calculated following formula:

Following formula:

$$\theta = \text{Tan}^{-1}h/r$$

Where, θ → Angle of repose

h →height in cm

r →radius in cm

F) Particle size: The particle size was measured by microscopic technique with the help of ocular and stage micrometer. A drop of the suspension was mounted on a slide and observed under the optical microscope about 100 particles were measured and their average size was determined.

***In-vitro* wash-off test:** The mucoadhesive properties of the microspheres were evaluated by the invitro wash off test. A2 X 3 cm piece of goat intestine mucosa was tied on to a glass slide using thread. Microspheres were spread (~50) onto the wet, rinsed, tissue specimen and the prepared slide was hung onto to one of the grooves of the USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the beaker containing the simulated gastric fluid USP pH 1.2 and pH 6.8 buffer. At the end of 30 minutes, 1 hr and at hourly intervals up to 8 hrs the number of microspheres still adhering onto the tissue was counted. The results of invitro wash off the test of batches B1 to B7 were shown in table no 10 and 11.

Entrapment Efficiency: The capture efficiency of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyse. The lysate is then subjected to the determination of active constituents as per monograph requirement. The percent encapsulation efficiency of all formulations was shown in table no 9 and is calculated using the following equation:

% Entrapment = Actual content/Theoretical content x 100.

***In-vitro* drug release:** To carry out *In-vitro* drug release, accurately weighed 50 mg of loaded microspheres were dispersed in dissolution fluid in a beaker and maintained at 37±2°C under continuous stirring at 100 rpm. At selected time intervals 5 ml samples were withdrawn through a hypodermic syringe fitted with a 0.4 µm Millipore filter and replaced with the same volume of pre-warmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples were analyzed spectrophotometrically. The released drug content was determined from the standard calibration curve of the given drug.

Drug polymer interaction (FTIR) study:

IR spectroscopy can be performed by Fourier transformed infrared spectrophotometer. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wavenumber range of 4000-600 cm^{-1}. FTIR study was carried on a pure drug, physical mixture, formulations, and empty microspheres.

RESULTS AND DISCUSSION

Table No. 3: Flow properties of all formulations

BATCH CODE	ANGLE OF REPOSE (θ)	BULK DENSITY (g/cm2)	TAPPED DENSITY (g/cm2)	HAUSNERS RATIO	CARRS INDEX
F1	16±0.577	0.71±0.005	0.81±0.020	1.14±0.036	12.37
F2	12±1.00	0.79±0.005	0.85±0.005	1.07±0.010	7.05
F3	14±0.577	0.68±0.011	0.81±0.020	1.19±0.037	16.04
F4	11±0.577	0.80±0.057	0.87±0.040	1.08±0.050	8.04
F5	13±1.154	0.70±0.057	0.76±0.015	1.08±0.130	7.89

***For each sample n=3

Table No. 4: Mean particle size and entrapment efficiency of all formulations

BATCH CODE	PARTICLE SIZE µm	ENTRAPMENT EFFICIENCY (%)
F1	275±25	72±1.00
F2	285±25	79±0.577
F3	270±25	82±0.577
F4	280±25	85±1.154
F5	200±25	96±1.00

***For each sample n=3

Table No. 5: Percent mucoadhesive property of all formulations in pH 1.2 buffer

Time (hr)	F1	F2	F3	F4	F5
0.5	34±0.577	70±1.00	76±1.732	78±0.577	86±0.577
1	20±1.00	66±0.577	72±0.577	78±1.00	86±1.00
2	02±0.577	58±1.732	70±1.732	78±1.732	86±1.00
3	---	42±0.577	70±0.577	72±0.577	86±0.577
4	---	36±1.00	70±1.00	68±1.732	84±1.732
5	---	28±0.577	68±0.577	64±1.732	84±0.577
6	---	18±1.00	66±1.00	62±0.577	84±0.577
7	---	08±0.577	60±0.577	62±1.732	82±1.00
8	---	---	54±1.732	62±0.577	80±0.577

NOTE: The above values indicate percentage mucoadhesion at regular time intervals & ***For each sample n=3

Table No. 6: Percent mucoadhesive property of all formulations in pH 6.8 buffer

Time (hr)	F1	F2	F3	F4	F5
0.5	34±1.00	66±0.577	78±1.154	78±0.577	88±1.154
1	22±0.577	58±1.00	70±1.732	78±1.154	88±0.577
2	04±1.00	52±0.577	70±1.00	76±1.00	86±1.154
3	---	46±1.732	68±0.577	72±1.732	86±1.732
4	---	40±0.577	68±1.732	68±1.00	84±0.577
5	---	38±1.00	68±1.154	68±0.577	84±1.154
6	---	34±1.154	60±1.00	62±1.154	82±0.577
7	---	20±0.577	58±1.154	62±0.577	82±1.154
8	---	06±1.00	54±1.732	60±1.154	80±1.00

NOTE: The above values indicate percentage mucoadhesion at regular time intervals & *****For each sample n=3

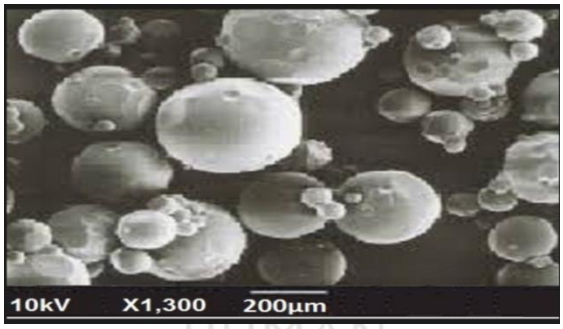

Figure No. 3: SEM of optimized formula F5

Table No. 7: *In-vitro* drug release results of all formulations in pH 6.8 buffer

TIME (H)	F1	F2	F3	F4	F5
0.5	2.050±0.110	2.505±0.112	3.058±0.118	2.524±0.129	3.806±0.123
01hr	05.029±0.140	05.241±0.124	08.746±0.105	04.269±0.124	06.039±0.145
02hr	10.219±0.125	11.816±0.145	14.328±0.122	11.351±0.123	18.156±0.145
03hr	13.112±0.145	15.006±0.156	19.159±0.012	16.896±0.123	29.842±0.123
04hr	24.158±0.163	25.103±0.169	25.753±0.022	20.846±0.145	34.147±0.145
05hr	30.312±0.114	33.517±0.132	32.456±0.115	29.258±0.123	45.354±0.124
06hr	33.359±0.154	39.841±0.122	37.258±0.145	35.371±0.164	53.318±0.114
07hr	39.105±0.126	45.732±0.123	44.862±0.164	40.394±0.124	60.164±0.164
08hr	42.801±0.129	52.352±0.124	50.248±0.169	47.682±0.114	67.489±0.114
09hr	49.248±0.189	59.621±0.114	56.624±0.193	55.317±0.145	74.148±0.124
10hr	54.359±0.174	63.195±0.160	63.486±0.156	66.753±0.114	80.254±0.123
11hr	61.215±0.165	69.168±0.131	72.426±0.165	74.691±0.124	86.889±0.164
12hr	68.368±0.149	74.915±0.135	78.482±0.159	83.186±0.123	95.599±0.124

NOTE: The above values indicate percentage release at regular time intervals& ***For each sample n=3

Table No. 8: *In-vitro* drug release results of all formulations in pH 1.2 buffer

TIME	F1	F2	F3	F4	F5
0.5	2.005±0.131	3.057±0.025	4.032±0.169	4.447±0.410	6.901±0.145
01hr	06.224±0.169	07.134±0.189	09.894±0.189	09.698±0.131	10.356±0.189
02hr	15.665±0.159	14.451±0.114	18.879±0.865	15.415±0.114	19.189±0.189
03hr	18.894±0.189	18.159±0.189	21.486±0.701	19.315±0.159	28.456±0.120
04hr	26.329±0.005	56.225±0191	26.558±0.174	23.618±0.022	37.183±0.250
05hr	32.025±0.189	35.154±0.37	34.693±0.131	29.987±0.065	48.297±0.169
06hr	37.684±0.025	38.154±0.159	39.841±0.145	38.167±0.009	58.628±0.125
07hr	39.881±0.114	47.126±0.058	48.678±0.189	42.189±0.169	69.428±0.131
08hr	44.369±0.145	55.119±0.114	55.964±0.189	49.684±0.018	76.462±0.174
09hr	48.786±0.174	58.589±0.131	59.497±0.201	58.691±0.291	80.391±0.174
10hr	53.220±0.131	65.564±0.169	65.537±0.145	67.351±0.501	85.971±0.021
11hr	60.001±0.189	68.756±0.845	72.918±0.306	76.369±0.080	89.713±0.006
12hr	65.091±0.069	78.345±0.174	78.008±0.333	89.321±0.114	97.139±0.601

NOTE: The above values indicate percentage release at regular time intervals & ***For each sample n=3

Table No. 9: Drug/ excipients Compatibility study

Sr. No.	Composition Details	D:E ratio	Observation at various storage conditions and Durations						
			Initial	40/75% RH		60±2°C		2±8°C	
				2W	4W	1W	2W	2W	3W
1	Didanosine (D)		White	NC	NC	NC	NC	NC	NC
2	D + Sodium Alginate	1:1	Off White	NC	NC	NC	NC	NC	NC
3	D + Carbopol	1:1	Off White	NC	NC	NC	NC	NC	NC
4	D + TFGSMP	1:1	Off White	NC	NC	NC	NC	NC	NC
5	F2	1:10	Off White	NC	NC	NC	NC	NC	NC
6	F3	1:0.5	Off White	NC	NC	NC	NC	NC	NC
7	F4	1:5	Off White	NC	NC	NC	NC	NC	NC
8	F5	1:10	Off White	NC	NC	NC	NC	NC	NC

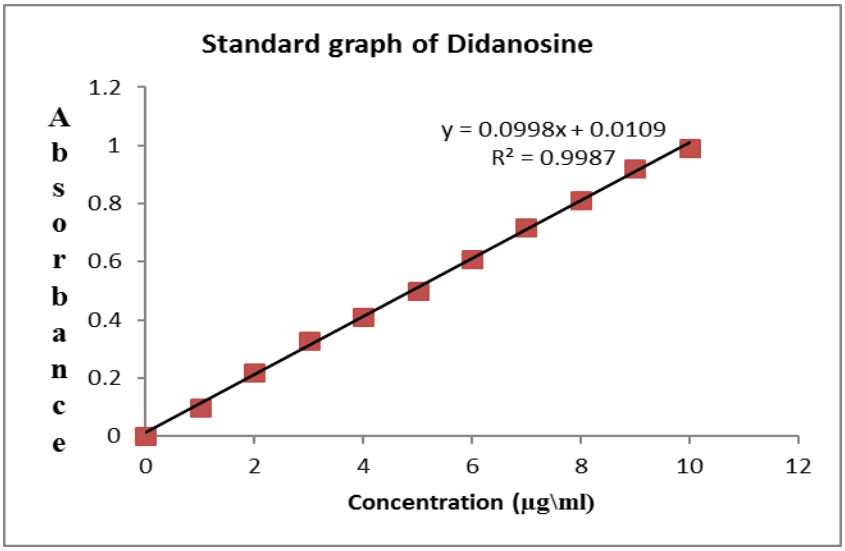

Figure No. 4: Standard Graph of Didanosine

Table No. 10: Physicochemical characteristics of TFGSM powder

TESTS	**RESULTS**
Organoleptic Characteristics	
Colour	Light brown powder
Odor	Odorless
Solubility	
Distilled water	Swells in contact with water and forms tacky mass
Methanol	Insoluble
Chloroform	Insoluble
Swelling index	
Method I	30ml
Method II	735
Loss on drying	0.46g
pH	6.23
Bulk density	0.60g/ml
Tap density	0.69g/ml
Yield	3.17%

CONCLUSION

The mucoadhesive microspheres of Didanosine using mucoadhesive agents (carbopol 934 and TFGSMP) and sodium alginate in different ratios were successfully formulated by ionotropic gelation method. All the formulations have shown good flow properties and good particle size. The *In-vitro* wash off comparison test, *In-vitro* drug release, were carried out in 1.2 pH and 6.8pH buffers and F5 formulation has shown good mucoadhesion property and *In-vitro* release amongst all the 5 formulations. Formulation F5 has shown good drug and excipient compatibility. TFGSMP microspheres have shown good results when compared to Carbopol 934 microspheres.

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