Research Article

Formulation and Evaluation of Furosemide Microcapsules

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Present research work was focused to enhance bioavailability and reduce the short half life problem of Furosemide by preparation of sustained release microcapsule. Cellulose acetate microcapsules were prepared by coacervation phase separation technique and phase separation was induced using distilled water. Prepared microcapsules were evaluated for Particle Size Analysis, Flow properties i.e. Angle of Repose Carr's Index and Hauser's Ratio, Scanning Electron Microscopy, Coating Wall Thickness, Drug Content and Microencapsulation efficiency, Dissolution studies. All the studies were performed in triplicate and standard deviation was calculated.

Key Words: Furosemide, Microcapsules, coacervation phase separation technique, Mucoadhesion, coating wall thickness.

INTRODUCTION:

Present research work was focused to enhance bioavailability and reduce the short half life problem of Furosemide by preparation of sustained release microcapsule. Cellulose acetate microcapsules were prepared by coacervation phase separation technique and phase separation was induced using distilled water. Prepared microcapsules were evaluated for Particle Size Analysis, Flow properties i.e. Angle of Repose Carr's Index and Hauser's Ratio, Scanning Electron Microscopy, Coating Wall Thickness, Drug Content and Microencapsulation efficiency, Dissolution studies.

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MATERIALS AND METHOD

Furosemide was obtained as gift sample from Intas labs, Mumbai; Cellulose acetate was purchased from Intas Lab Pvt Ltd., Mumbai. All other chemicals and solvents used were of analytical grade and double distilled water was used during whole study.

Ethyl cellulose Microcapsules

Ethyl cellulose was dissolved in 50 ml of toluene to form homogeneous polymer solution. Core material was then added to the polymer solution and dispersed thoroughly with the aid of a mechanical stirrer (1000 rpm) for 10 min.

Coacervation was induced by addition of petroleum ether slowly over a period of 1 hour, while stirring at same speed. The system was then chilled for 20 min. with stirring to rigidise the coating of microcapsules. The encapsulated product was then collected by vacuum filtration and air dried to obtain discrete microcapsules[3].

Alginate microcapsules

Sodium alginate was dissolved in purified water to form a homogeneous polymer solution. The active substance, Furosemide was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added manually drop wise into calcium chloride (10% w/v) solution through a syringe with a needle of size no. 18. The added droplets were retaining in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce spherical rigid microcapsules. The microcapsules were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45oC for 12 hours. The alginate microcapsules were prepared in the same manner with and without the incorporation of Mucoadhesive polymers[4]. Table1 shows different formulation codes with Drug: Polymer ratios for the prepared microcapsules.

Table1: Formulation Code of Prepared Microcapsules

Formulation Code	Polymer	Drug:Polymer Ratio
F4	Ethyl Cellulose	1:2
F5	Ethyl Cellulose	1:3
F6	Ethyl Cellulose	1:4
F7	Sodium alginate	1:2
F8	Sodium alginate	1:3
F9	Sodium alginate	1:4

Evaluation of Prepared Microcapsules

Particle Size Analysis

The microcapsules distribution was determined averaging the values of diameter of 100 microcapsules using optical microscope (Olympus KH) [5].

Flow properties[6]

Angle of Repose

Angle of repose (θ) was determined by fixed funnel method and was calculated using formula:

$$\tan \theta = 2h/D$$

Where

h is cone height

D is diameter of heap

Carr's Index and Hausner's Ratio[7]

Tapped density was determined by placing a graduated cylinder containing a known mass of the prepared microcapsules on a mechanical tapping apparatus, which was

operated for a fixed number of taps until the bed volume reached to a minimum.

Scanning Electron Microscopy

The microcapsules were observed under a scanning electron microscope (LEO 430, Japan). They were mounted directly onto the SEM sample stub using double sided sticking tape and coated with gold film under reduced pressure (0.001mm of Hg) [8].

Coating Wall Thickness

Wall thickness of microcapsules was determined by the method of Luu et al. using the equation[9].

$$h = \frac{r\,(1-p)\,d_1}{3[p\text{-}d_2 + (1-p)\,d_1]}$$

Where

h = wall thickness of microcapsules

r = mean radius of the microcapsules

d_1 = density of the core material

d_2 = density of coating material

p = proportion of the medicament in the microcapsules.

Mucoadhesion Properties

The mucoadhesive property of the microcapsules was evaluated by an in vitro adhesion testing method known as the wash-off method. Freshly excised pieces of intestinal mucosa (2x2 cm) from goat were mounted onto glass slides (3x1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. About 50 microcapsules were spread onto each wet rinsed tissue specimen, and immediately thereafter the support was hung onto the arm of a USP tablet disintegrating test apparatus. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up-and-down movement in the test fluid at 37^0C C contained in a 1 L vessel of the machine. At hourly intervals up to 12 hours, the machine was stopped and the number of microcapsules still adhering to the tissue was counted. The test was performed at intestinal pH (phosphate buffer, pH 6.2)[10].

Drug Content and Microencapsulation efficiency

Microcapsules were triturated in a mortar-pestle; accurately weighed amount of powder (100mg) was dissolved in 5ml ethanol and diluted to 50 ml with phosphate buffer pH 7.4 in a volumetric flask and filtered. Furosemide content in the microcapsules after suitable dilutions was estimated by U.V. spectrophotometer at 237nm using phosphate buffer pH 7.4 as blank[11].

Microencapsulation efficiency was calculated using formula:

$$MEE = \frac{EPDC}{TPDC} \times 100$$

MEE-Microencapsulation efficiency

EPDC-Estimated percentage drug content

TPDC-Theoretical percentage drug content

Dissolution Studies

Release of Furosemide from the microcapsules was studied in phosphate buffer pH 7.4(900ml) using an USP XXll six station Dissolution Rate Test Apparatus with a rotation paddle stirrer at 50 rpm and 37 ± 1^{o}C. A Sample of microcapsule equivalent to 100mg of Furosemide was used in each test. Samples of dissolution fluid were withdrawn at different time

Results and Discussion

Physical characterization like %Yield, Particle Size, Flow properties, Coating wall thickness and Mucoadhesive properties are tabulated in table 2.

Table 2: Drug Content and Encapsulation efficiency of prepared Microcapsules

Formulation Code	Drug Content (mg/250mg)	Encapsulation Efficiency (%)
F4	164.40	65.76
F5	174.43	69.77
F6	159.78	63.91
F7	143.93	57.57
F8	137.78	55.11
F9	137.28	54.91

Table3: Physical characterization of prepared Microcapsules

Formulation Code	Yield (% w/w)	D mean (µm)	Angle Of Repose (θ)	Carr's Index (%)	Hausner's Ratio	Coating Wall Thickness (µm)	Mucoadhesion (%) 12 h
F4	3.982±0.03	67.50±0.02	18.01^{0}±0.03	8.29±0.01	1.68±0.01	20.11±0.01	-
F5	4.327±0.02	68.22±0.01	28.27^{0}±0.01	9.74±0.02	1.77±0.03	20.62±0.01	-
F6	4.821±0.01	70.57±0.02	19.91^{0}±0.06	7.04±0.02	1.51±0.02	23.35±0.01	-
F7	3.818±0.01	69.94±0.03	18.79^{0}±0.03	10.01±0.05	2.11±0.02	29.82±0.01	29±1
F8	4.417±0.02	70.29±0.04	19.29^{0}±0.01	12.04±0.02	2.71±0.02	30.14±0.02	32±3
F9	4.712±0.02	73.91±0.01	18.57^{0}±0.02	9.72±0.03	2.09±0.04	33.32±0.05	36±2

Dissolution studies of the prepared microcapsules were performed using six station dissolution rate apparatus U.S.P. XXll in phosphate buffer pH 7.4.

The amount of Furosemide released was determined at pre determined time intervals and the data were expressed graphically as:

Table 4: In-vitro Drug release data of Microcapsules of Furosemide for formulation code F1 to F6 (Zero order release kinetics)

Time	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0
1	4.07	3.98	3.71	4.12	3.76	3.29
2	8.85	8.23	7.68	8.71	8.09	7.21
3	13.25	12.84	12.01	13.68	12.71	11.89
4	18.15	17.64	16.82	18.45	17.02	16.12
5	24.05	23.73	22.11	23.91	22.14	21.09
6	30.09	29.06	26.06	29.63	27.92	27.92
7	35.68	33.22	32.21	33.25	31.02	30.28
8	39.67	37.48	35.73	38.27	36.08	35.69
9	43.58	41.66	38.96	42.99	41.03	40.07
10	48.29	47.01	42.42	47.19	45.09	43.98
11	51.65	50.61	46.25	52.92	51.01	50.84
12	55.77	54.53	51.61	57.69	56.19	54.28

Table 5: In-vitro Drug release data of Microcapsules of Furosemide for formulation code F4 to F9 (First Order release)

Time	F4	F5	F6	F7	F8	F9
0.000	2.000	2.000	2.000	2.000	2.000	2.000
1.000	1.982	1.982	1.984	1.982	1.982	1.984
2.000	1.960	1.963	1.965	1.960	1.963	1.965
3.000	1.938	1.940	1.944	1.938	1.940	1.944
4.000	1.913	1.916	1.920	1.913	1.916	1.920
5.000	1.881	1.882	1.891	1.881	1.882	1.891
6.000	1.845	1.851	1.869	1.845	1.851	1.869
7.000	1.808	1.825	1.831	1.808	1.825	1.831
8.000	1.781	1.796	1.808	1.781	1.796	1.808
9.000	1.751	1.766	1.786	1.751	1.766	1.786
10.000	1.714	1.724	1.760	1.714	1.724	1.760
11.000	1.684	1.694	1.730	1.684	1.694	1.730
12.000	1.646	1.658	1.685	1.646	1.658	1.685

Table 6: In-vitro Drug release plot of Microcapsules of Furosemide for formulation code F1 to F6 (Higuchi Model)

Sqrt.Time	F4	F5	F6	F7	F8	F9
1	4.07	3.98	3.71	4.12	3.76	3.29
1.414214	8.85	8.23	7.68	8.71	8.09	7.21
1.732051	13.25	12.84	12.01	13.68	12.71	11.89
2	18.15	17.64	16.82	18.45	17.02	16.12
2.236068	24.05	23.73	22.11	23.91	22.14	21.09
2.44949	30.09	29.06	26.06	29.63	27.92	27.92
2.645751	35.68	33.22	32.21	33.25	31.02	30.28
2.828427	39.67	37.48	35.73	38.27	36.08	35.69
3.000000	43.58	41.66	38.96	42.99	41.03	40.07
3.162278	48.29	47.01	42.42	47.19	45.09	43.98
3.316625	51.65	50.61	46.25	52.92	51.01	50.84
3.464102	55.77	54.53	51.61	57.69	56.19	54.28

Table 7 : Fit of Various Kinetic Models for microcapsules of Furosemide (F4 –F9)

Code	Zero order			First order			Higuchi Model		
	Intercept	R^2	K ($mg.hr^{-1}$)	Intercept	R^2	K (hr^{-1})	Intercept	R^2	K ($mg\ hr^{-1/2}$)
F4	0.1771	0.9963	4.8129	2.0198	0.9935	0.06955	22.855	0.9846	22.133
F5	0.366	0.9982	4.6763	2.0197	0.9922	0.06679	22.408	0.9824	21.501
F6	0.2338	0.9971	4.3412	2.0157	0.9926	0.05988	20.699	0.9825	19.953
F7	0.5191	0.9994	4.8405	2.0231	0.9854	0.07047	23.251	0.9780	22.217
F8	1.0509	0.9988	4.6887	2.0242	0.9806	0.06702	23.226	0.9714	21.566
F9	1.4453	0.9975	4.634	2.0248	0.9821	0.06541	23.645	0.9720	21.416

1. Cumulative % drug release Vs Time (Zero order release kinetics plot)

2. Log Cumulative % drug retained Vs Time (First order release kinetics plot)

3. Cumulative % drug release Vs Square root of Time (Higuchi Model)

The in vitro release data are shown in Table 4 to 6.

The in-vitro release data ere fitted to zero order kinetics, first order kinetics and Higuchi model.

The maximum drug release at the end of 12 hours was found to be 55.77 for cellulose acetate microcapsules (F_4) and 57.69 for alginate microcapsules (F_7). It is evident from the data that as the polymer concentration increased the rate of release decreased significantly. The release profile was found to vary with the nature of coating materials; as the coating wall thickness increased the release rate was found to decrease.

The polymer can be arranged in the order of release rate as : ethyl cellulose > sodium alginate. Change in the proportion of the polymer also affected the release profile and can alter the release characteristics.

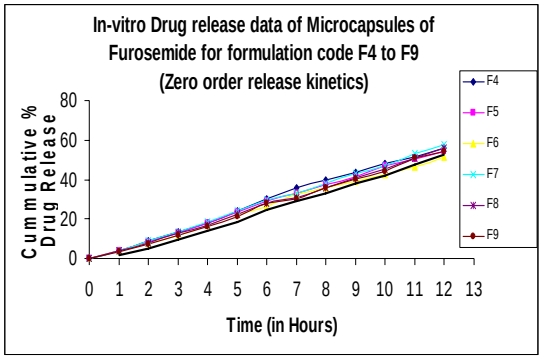

Fig. 1: In-vitro Drug release plot of Microcapsules of Furosemide for formulation code F4 to F6 (Zero order kinetics)

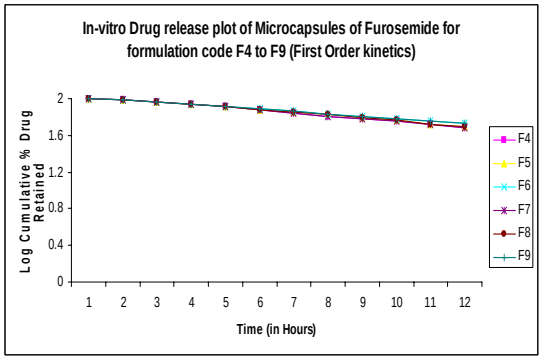

Figure 2: In-vitro Drug release plot of Microcapsules of Furosemide for formulation code F4 to F9 (First Order kinetics)

The drug release was found to follow zero kinetics as evident from the graphical representations (Figure 1 to 3) and kinetic parameters (Table 4 and 7).

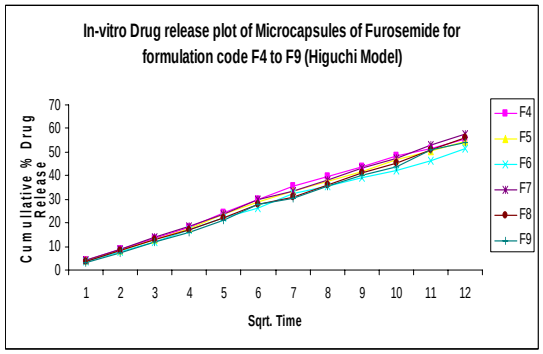

Figure 3: In-vitro Drug release plot of Microcapsules of Furosemide for formulation code F4 to F9 (Higuchi Model)

The release mechanism was to diffusion controlled as indicated by the Higuchi plots with r^2>0.97. Hence it can be concluded that the nature, proportion of polymer and the manufacturing process influenced the release characteristics of Furosemide from the microcapsules.

REFERENCE

1. Brunton LL, Chabner BA, Knollmann BC. In Goodman Gilman Edited. Goodman & Gillman's The Pharmacological Basis of Therapeutics. 12th Ed., New York: Mc Grow Hill; 2011:682–684

2. Mathiowitz E, Langer R. Polyanhydride Microcapsules as Drug Carriers. J Cont Rel 1987; 5: 13-22.

3. Arigita, C., Berg, J., Wensink, K., Steenbergen, M., Hennink, W. E., Crommelin, D.A.J., Kersten, G.F.A. and Jiskoot, W. Immunogenicity of meningococcal PorA formulations encapsulated in biodegradable microspheres, Eur. J. Pharm. Sci.,, 2004, 21(2-3), 131-141.

4. Jeong, Y., Song, J., Kang, S., Ryu, H., Lee, Y., Choi, C., Shin, B., Kim, K., Ahn, K. and Shin, J. Preparation of poly(DL-lactide-co-glycolide) microspheres encapsulating all-trans retinoic acid. Int. J. Pharm., 2003, 259 : 79-91.

5. S. Bozdag- Pehlivan , I. Vural, M. Cetin, Y. Capan , Design of Microencapsulated Alginate Microspheres for Colon Targeted Drug Delivery, AAPS Pharm Sci Tech, 2006, 7, 1-9.

6. Miyazaki, Y., Ogihara, K., Yakon, S., Nagai, T., Takayama, K. In vitro and in vivo evaluation of mucoadhesive microspheres consisting of dextran derivatives and cellulose acetate butyrate., Int. J. Pharm., 2003, 258: 21-29.

7. Martin, A., Swarbrick, J., Commarata, A., "Physical Pharmacy", Varghese Publishing House, Mumbai, 1986 : 104

8. Sahin, S., Selek, H., Hincal, A. A. and Kas, H. S. Preparation, characterization and in vivo distribution of terbutaline sulfate loaded albumin microspheres., J. Control. Rel., 2002, 82(2-3) : 345-58.

9. Roullin, V.G., Deverre, J.-R., Lemaire, L. and Benoit, J.P. Anti-cancer drug diffusion within living rat brain tissue: an

experimental study using [3H](6)-5-fluorouracil-loaded PLGA microspheres., Eur. J. Pharm. Biopharm., 2002, 53 : 293-99.

10. Sakagami, M., Sakon, K., Kinoshita, W., Makino, Y. Enhanced pulmonary absorption following aerosol administration of mucoadhesive powder microspheres, J. Control. Rel., 2001, 77(1-2): 117-29.

11. Shi, L., Caulfield, M. J., Chern, R.T., Wilson, R. A., Sanyal, G., Volkin, D. Pharmaceutical and immunological evaluation of a single-shot hepatitis B vaccine formulated with PLGA microspheres., J. Pharm. Sci., 2002, 91 (4) : 1019-35.

12. Hejazi, R. and Amiji, M. Stomach-specific anti-H. pylori therapy. I: Preparation and characterization of tetracyline-loaded chitosan microspheres., Int. J. Pharm., 2002, 235: 87-94.