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Formulation Development and Evaluation of Duloxetine Hydrochloride Microspheres By Ionotropic Gelation Technique

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A B S T R A C T

The present study was envisaged to reduce the dosing frequency and improve patient compliance by designing and evaluating sustained release microspheres of Duloxetine for effective control of major depressive disorder, generalized anxiety disorder, fibromyalgia, and neuropathic pain. Microspheres were prepared by Ionotropic gelation technique using Chitosan and sodium alginate as sustained release agents. The prepared microspheres were evaluated for particle size, drug content, surface morphology, drug entrapment efficiency, flow properties, in vitro drug release studies. The drug excipients compatibility was determined by FTIR studies. The surface morphology of prepared microspheres was measured by SEM and the particle size distribution was determined using an optical microscope. The particles were found to be discrete and spherical with the average particle size in the range of 147±0.05 µm to 193±0.05 µm. The formed Duloxetine microspheres showed high drug entrapment efficiency of 73.15 to 98.52 %. In vitro results showed that the formulation D7 containing 1:3 ratio of Sodium alginate released maximum amount of drug i.e. 18.81% (pH 1.2) and 99.35% (pH 7.4) and D7 formulation was considered as optimised formulation. The release kinetics was also studied and it was shown that the release profiles of D7 formulations showed good correlation with the Zero order release kinetics.

Keywords: Duloxetine, Chitosan, Sodium Alginate, Ionotropic Gelation Technique And Microspheres

1. Introduction

The term microsphere is defined as a spherical particle with size varying with diameters in the micrometer range (typically 1µm to 1000µm), containing a core substance. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 micrometer.[1] Depression is a severe illness with a lifetime prevalence of between 10 and 20% according to WHO. Mood disorders are among the most prevalent forms of mental illness.Major depressive disorder (MDD) is the most common of all psychiatric disorders.[2] DuloxetineHCl is a potent dual acting serotonin and norepinephrine reuptake inhibitor (SNRI) being investigated for the treatment of major depressive disorder, general anxiety disorder, urinary incontinence and diabetic peripheral neuropathic pain.SNRI antidepressants work by preventing serotonin and noradrenaline from being reabsorbed (inhibit the reuptake) back into the nerve cells in the brain. This helps prolong the "mood lightening" effect of any released serotonin and noradrenaline. In this way SNRIs help to relieve depression.The recommended therapeutic range of oral duloxetine is 40 to 60 mg/day. Duloxetine HCl can be administered either in a twice daily dose or thrice daily. It is currently supplied in 20,30,and 60mg delayed release capsules and tablets.[3, 4]

The oral bioavailability of duloxetine HCl is 50%. Duloxetine is approximately 96% bound to human plasma proteins. Duloxetine binds to both albumin and alpha1-acid glycoprotein. Protein binding is not affected by renal or hepatic impairment.[5, 6] .In the present study, efforts were made to incorporate duloxetine HClin ethyl cellulose polymer by applying the solvent evaporation technique.

2. Materials and Methods

Duloxetine HCl received from MaanPharma Ltd.,Mehsana, ethyl cellulosereceived from BiomedicarePvt.Ltd., Ahmedabad, Polyvinyl alcohol& other material are received from Loba chemicals Pvt.Ltd., Mumbai.

3. Preparation of Microspheres

The microspheres were prepared by the Ionotropic gelation technique. The Chitosan and sodium alginate solution was prepared by dispersing the Chitosan and sodium alginate in de-ionized water under continuous stirring for 30 minutes. The weighed amount of the drug was thoroughly mixed with Chitosan and sodium alginate dispersion. By following the same procedure the of different ratios of drug: polymers were prepared. The resulted homogeneous dispersion was extruded in to the calcium chloride solution through hypodermic syringe with flat tip needle (20G) and stirred for 15 minutes at 100rpm using magnetic stirrer. The formed micro beads were allowed to cure for 30 minutes in the calcium chloride solution to complete the gelation reaction. The microspheres were then filtered and dried in hot air oven at 60ºC for 3 hr.

Table 1.Formulation of Microspheres

INGREDIENTS	FORMULATIONS							
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**
Duloxetine	20	20	20	20	20	20	20	20
Chitosan (w/v)	20	40	50	60	-	-	-	-
Sodium alginate (w/v)	-	-	-	-	20	40	50	60
Calcium Chloride (w/v)	60	60	60	60	60	60	60	60

4. Evaluation of Microspheres

4.1 Percentage yield

Prepared microspheres were weighed after drying, and percentyield was calculated by help of this formula. The yield was calculated as theweight of the microspheres recoveredfrom each batch divided by total weightof drug and polymer used to prepare thatbatch multiplied by 100.8

$$\% \text{ yield} = Yp / Yt \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

Where, Yp =Practical yield,

Yt = Theoratical yield.

4.2 Particle size analysis

The microsphere size distribution was determined by the optical microscopy method using a calibrated stage micrometer (μm). The size of microspherewas calculated by using this equation.9

$$X = 10 \times [(ni \times \log Xi) / N] \quad \ldots\ldots\ldots (2)$$

Where, X =particle's mean diameter,

ni =number of particle in range,

Xi = the midpoint of range and

N = total number of particles

4.3 Drug entrapment efficiency

The amount of drug entrapped was calculated from the difference between the total amount of drug added and the amount of drug found in the filtered solution. About 100 mg of microspheres were completely dissolved in 500 ml of phosphate buffer solutions (pH 7.4), and stirred for 1h. Then, 2 ml of solution was filtered and the concentration of drug was determined spectrophotometrically by UV.10, 11 Efficiency of drug entrapment was calculated in

terms of percentage drug entrapment (PDE) as per the following formula:

PDE = (Pdl/Tdl) ×100 …… (3)

Where,Pdl =Practical drug loading, Tdl =Theoretical drugloading.

4.4 The In-Vitro dissolution study

These studies were performed by using USP type II dissolution test apparatus.Dissolution medium used was phosphate buffer (pH 7.4), each 900 ml, temperature was maintained at 37 ± 2°C and 100 rpm stirring was provided for each dissolution study. Duloxetine HCl microspheres equivalent to 100 mg of pure drug were used for each dissolution study. Samples were collected periodically and replaced with a fresh dissolution medium. After filtration through whatman no. 1 filter paper, concentration of duloxetine HCl was determined spectrophotometrically at 288.8 nm.12

Release kinetic study

Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of drug release from microspheres. The kinetic models usedwere zero order, first order, Higuchi and Hixson-Crowell model.12, 13

For zero order release kinetics, the equation is,

$Q = K0\ t$ …… (4)

Where, Q = Amount of drug release per unit surface area, K0 = Zero order release rate constant andt = Time.

For first order release kinetics, the equation is,

$\ln q0/q = -K1t$ …… (5)

Where, q = Amount of drug released per unit surface area, K1 = First order release rate constant, q0 = Initial amount,Cs= Saturation solubility,Ct= Concentration and t = time.

For Hixson-Crowell release kinetics, the equation is,

$W01/3 - W1/3 = KHC\ t$ … (6)

Where, W0 = Initial weight of the particles,W= Weight of the particles,KHC= Hixson Crowell release rateconstant and t = time.

For Higuchi release kinetics, the equation is,

$Q = KHG\ t\ 0.5$ …… (7)

Where, Q = Amountof drug released per unit surface area of the dosage form,KHG= Higuchi release rate constant, andt = time.

Scanning electron microscopy (SEM) study SEM of the duloxetine HCl microsphere was performed by scanning electron microscope. The samples were prepared by lightly sprinkling the microspheres powder on a double side adhesive tape which already shucked to on aluminum stubs. The stubs were then placed into fine coat ion sputter for gold coating. After gold coating samples were randomly scanned for particle size and surface morphology.14, 15

4.5 Zeta potential

The zeta potential of duloxetine HCl microspheres was determined by malvernzetasizer. Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the ethanol and rinsed using the sample to be measured before each experiment. The zeta potential is an indication of the stability of the colloidal system and indicates the charge present on the colloidal system.16

5. Results and Discussion

5.1 Micrometric Properties

The mean size increased with increasing polymer concentration which is due to a significant optimum in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres containing Chitosan as a polymer had a size range of 130±0.02µm to 196±0.02 µm. Microspheres containing Sodium alginate as polymer exhibited a size range between 147±0.05 µm to 193±0.05 µm.

The particle size data is presented in Tables 8.3 and displayed in Figures. The effect of drug to polymer ratio on particle size is displayed in Figure. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentration.The bulk density of formulation D1 to D8 containing Chitosan and Sodium alginate formulation was in the range of 0.4136 to 0.4394 gm/cm3 (as shown in table 8.3), tapped density 0.5126 to 0.5428 and hausners ratio 1.21 to 1.26.

The Carr's index of formulation D1 to D8 containing different grades of Chitosan and Sodium alginate 17.29 to 20.60 respectively. The angle of repose of formulation D1 to D8 containing Chitosan and Sodium alginate formulation was in the range <26.37 respectively (as shown in table 8.3) the values of car's index and angle of repose indicate good flow properties.

Table 2. Micromeritic property of microspheres of Duloxetine

Formulati on code	Mean particle size	Bulk density (gm./cm^3)	Tapped density (gm./cm^3)	Hauseners ratio	Carr's index	Angle of repose
D1	130±0.02	0.4394	0.5428	1.24	19.05	25.30±0.062
D2	158±0.01	0.4225	0.5203	1.23	18.80	21.73±0.040
D3	183±0.03	0.4172	0.5164	1.24	19.21	25.49±0.010
D4	196±0.02	0.4275	0.5274	1.23	18.94	23.93±0.069
D5	147±0.05	0.4247	0.5135	1.21	17.29	22.74±0.026
D6	162±0.01	0.4136	0.5164	1.25	19.91	26.37±0.021
D7	178±0.03	0.4156	0.5126	1.23	18.92	23.52±0.013
D8	193±0.05	0.4244	0.5345	1.26	20.60	21.59±0.012

In preformulation studies we concluded that UV analytical method was found to shown good linearity. The melting point of duloxetine HCl was found within range. From the above studies of DSC and FT-IR, it was concluded that the excipients and drug did not interact with each other and are compatible.

Table 3.Percentage yield and percentage drug entrapment efficiency of theprepared microspheres

Formulation code	%yield	Drug content (mg)	% Drug entrapment efficiency
D1	89.35	90.59	73.15
D2	90.19	93.76	86.91
D3	95.78	95.20	90.33
D4	96.14	98.42	96.75
D5	83.99	86.47	85.42
D6	87.38	91.50	94.21
D7	91.25	97.28	98.52
D8	98.57	98.83	98.10

5.2 Swelling studies

The swelling ratio is expressed as the percentage of water in the hydrogel at any instant during swelling. Swell ability is an important characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems. Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux. Swellability data revealed that amount of polymer plays an important role in solvent transfer. It can be

concluded from the data shown in Table 8.5 that with an increase in polymer concentration, the percentage of swelling also increases. Thus we can say that amount of polymer directly affects the swelling ratio.Asthepolymertodrugratioincreased,thepercentageofswellingincreasedfrom59.37to 90.68 % for microspheres containing Chitosan as polymer, 76.49 to 92.68 % for microspheres containing sodium alginate as polymer. The percentage of swelling of the prepared microspheres is displayed in Figures.

Table 4: Swelling studies

S.NO.	FORMULATIO N CODE	INITIAL (Wt)	FINAL (Wt)	PERCENTAGE SWELLING
1	D1	18	21.15	85.10
2	D2	18	20.36	88.40
3	D3	18	20.14	59.37
4	D4	18	19.85	90.68
5	D5	18	23.53	76.49
6	D6	18	21.74	82.79
7	D7	18	20.69	86.99
8	D8	18	19.42	92.68

***5.3 In Vitro* Mucoadhesion Test**

As the polymer to drug ratio increased, microspheres containing Chitosan exhibited % mucoadhesion ranging from 62.23 to 92.41 %, microspheres containing sodium alginate exhibited % mucoadhesion ranging from 60.92 to 96.17 %. The results of *in-vitro* mucoadhesion test are compiled in Table 8.6.

Table 5: *In Vitro* Mucoadhesion Test Of All Formulations

S.NO.	FORMULATION CODE	No. OF MICROSPHERES		PERCENTAGE MUCOADHESION
		INITIAL	FINAL	
1	D1	16	13.48	83.15
2	D2	16	10.14	62.23
3	D3	16	14.95	92.41
4	D4	16	13.83	84.78
5	D5	16	10.21	60.92
6	D6	16	12.74	78.39
7	D7	16	13.91	87.52
8	D8	16	15.13	96.17

5.4 In-vitro drug release studies

Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2. The results of the *in-vitro* dissolution studies of formulations D1 to D8 are shown in below table. The plots of Cumulative percentage drug release Vs Time. Figure shows the comparison of % CDR for formulations D1 to D4, figure for formulations D5 to D8.

The formulations D1, D2, D3 and D4 containing Chitosan showed a maximum release of 95.01% at 8 hours, 96.63% after 10 hours, 97.19% 11 hours and 98.17% after 12 hours respectively.The formulations D5, D6, D7, and D8 containing Sodium alginate polymer showed a maximum release of 95.86% 10 hours, 98.84% after 11 hours, 99.35% after 12 hours and 96.35 % 12 hours respectively.

Table 6: *In-Vitro* drug release data of Duloxetine microspheres containing Chitosan

TIME (h)	CUMULATIVE PERCENT OF DRUG RELEASED			
	D1	**D2**	**D3**	**D4**
0	0	0	0	0
1	15.98	20.47	25.89	10.15
2	28.63	26.35	31.93	16.29
3	42.24	31.12	42.52	23.31
4	53.15	38.86	48.17	30.55
5	67.76	46.90	53.75	36.90
6	72.83	52.63	59.53	41.21
7	88.25	60.52	64.98	49.18
8	95.01	75.71	70.16	58.63
9		85.18	75.24	61.71
10		96.63	83.13	76.82
11			97.19	90.49
12				98.17

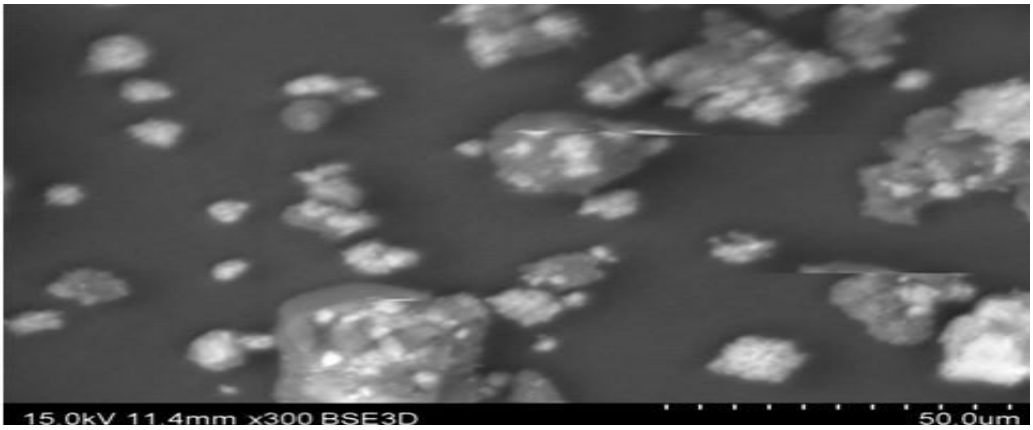

Figure 1:SEM of Optimised formulation

TABLE 8: Release kinetics studies of the optimized formulation (D7)

CUMULATIV E (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAI N	RELEASE RATE (CUMULATIV E % RELEASE / t)	1/CUM% RELEAS E	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3 - Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
14.24	1	1.000	1.154	0.000	1.933	14.240	0.0702	-0.846	85.76	4.642	4.410	0.232
18.81	2	1.414	1.274	0.301	1.910	9.405	0.0532	-0.726	81.19	4.642	4.330	0.311
23.92	3	1.732	1.379	0.477	1.881	7.973	0.0418	-0.621	76.08	4.642	4.237	0.404
30.23	4	2.000	1.480	0.602	1.844	7.558	0.0331	-0.520	69.77	4.642	4.117	0.525
36.56	5	2.236	1.563	0.699	1.802	7.312	0.0274	-0.437	63.44	4.642	3.988	0.653
45.6	6	2.449	1.659	0.778	1.736	7.600	0.0219	-0.341	54.4	4.642	3.789	0.853
50.98	7	2.646	1.707	0.845	1.690	7.283	0.0196	-0.293	49.02	4.642	3.660	0.982
57.71	8	2.828	1.761	0.903	1.626	7.214	0.0173	-0.239	42.29	4.642	3.484	1.158
62.25	9	3.000	1.794	0.954	1.577	6.917	0.0161	-0.206	37.75	4.642	3.355	1.287
74.16	10	3.162	1.870	1.000	1.412	7.416	0.0135	-0.130	25.84	4.642	2.956	1.685
82.53	11	3.317	1.917	1.041	1.242	7.503	0.0121	-0.083	17.47	4.642	2.595	2.047
99.35	12	3.464	1.997	1.079	-0.187	8.279	0.0101	-0.003	0.65	4.642	0.866	3.775

6. Conclusion

7. In the present study ight formulations are formulated by using Chitosan and Sodium alginate in various proportions. All the formulations are subjected for evaluation. Results of preformulation studies, IR, Swelling index, particle size, % yield, *in vitro* dissolution and, Flow property had shown satisfactory results. Preformulation studies like UV analysis of Duloxetine are complied with IP standards. The IR Spectra's revealed that, there is no interaction between polymer and Duloxetine. The polymer used is compatible with the Duloxetine. As the drug to polymer ratio is increased, the mean particle size of Duloxetine microspheres was also increased. On the basis of release data and graphical analysis formulation D7 showed a good sustained release profile with maximum entrapment efficiency because of high polymer concentration. Hence, from all the above obtained data it can be summarized that it is possible to formulate a promising sustained release mucoadhecive microspheres of Duloxetine by Ionotropic gelation technique using an ideal polymer like Chitosan and Sodiumalginate.

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