IMPROVING THE SOLUBILITY AND BIOAVAILABILITY OF DULOXETINE HCL: A NOVEL FORMULATION APPROACH

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ABSTRACT:

The objective of this study was to formulate and evaluate enteric-coated mucoadhesive microspheres of Duloxetine Hydrochloride to improve its solubility, gastrointestinal stability, enhance patient compliance and provide sustained drug release. Duloxetine HCl microspheres were prepared using emulsification phase seperation technique with varying ratios of polymer matrices. The formulations incorporated mucoadhesive polymers (such as Carbopol) and enteric polymers to achieve the desired release profile. The microspheres were characterized for their particle size, morphology, drug loading and release kinetics. The microspheres exhibited a uniform size distribution and spherical morphology. Drug loading and encapsulation efficiency were found to be optimal in formulations with higher polymerisation. The formulation of enteric-coated mucoadhesive microspheres of Duloxetine HCl successfully achieved targeted delivery and controlled release. This approach can potentially enhance the therapeutic efficacy of Duloxetine HCl by reducing gastrointestinal side effects and improving patient adherence.

Key words: Duloxetine HCl, Solubility Enhancement, Microspheres, Emulsification phase separation, Controlled release.

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INTRODUCTION:

Duloxetine Hydrochloride (HCl) is a widely prescribed serotonin-norepinephrine reuptake inhibitor (SNRI) used in the treatment of major depressive disorder, generalized anxiety disorder, and neuropathic pain. Despite its efficacy, Duloxetine HCl is often associated with gastrointestinal side effects, such as nausea and vomiting, which can impact patient adherence to the treatment regimen. Moreover, its therapeutic efficacy can be compromised by the drug's limited bioavailability due to its rapid release and degradation in the acidic environment of the stomach[1]. Duloxetine is newer and preferable antidepressant because of its favourable pharmacodynamic features viz. dual inhibition, tolerability, safety, faster recovery, fewer side effects and low affinity for other neuronal receptors[2].

Enteric Coating is a well-established method to enhance drug stability and release profile by protecting the drug from acidic gastric conditions and releasing it in the more neutral pH environment of the intestines. This technique can potentially mitigate the gastrointestinal side effects associated with Duloxetine HCl and improve its overall therapeutic effectiveness.

Mucoadhesion refers to the ability of a formulation to adhere to the mucosal surfaces of the gastrointestinal tract[3]. Incorporating mucoadhesive polymers into drug delivery systems can enhance the residence time of the drug in the gastrointestinal tract, leading to improved absorption and sustained drug release[4]. This property is particularly advantageous for drugs requiring prolonged action or localized delivery.

By combining enteric coating with mucoadhesive properties, microspheres can offer a dual advantage: protection from gastric degradation and enhanced adhesion for sustained release in the intestines. By combining enteric coating with mucoadhesive properties, microspheres can offer a dual advantage: protection from gastric degradation and enhanced adhesion for sustained release in the intestine. This approach has the potential to improve therapeutic outcomes by increasing drug bioavailability, reducing gastrointestinal side effects, and enhancing patient compliance.

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MATERIALS

Duloxetine was generously provided by Sainor Labs Ltd, (Hyderabad, India)

Carbopol was received as a gift from Hetero Labs Ltd.

All excipients and solvents used were of analytical grade

METHOD

Microsphere formulations utilizing Carbopol as a mucoadhesive and carrier polymer were created using a simple emulsification phase separation technique, as described previously[1]. In brief, a Carbopol solution (1% w/v) was prepared in glacial acetic acid (1% v/v). A 5 ml methanolic solution of the drug (1% w/v) was added to the desired volume of polymer solution as detailed in Table 1. This mixture was added to 50 ml of light liquid paraffin containing 0.1 ml of Span 80 as a surfactant, under constant stirring (2,000 rpm) using a three-blade propeller stirrer to form a w/o emulsion[1]. This was followed by the dropwise addition of 0.5 ml of glutaraldehyde, a cross-linking agent (25% v/v), with stirring at the same speed. Stirring continued for the next 5 minutes, after which the speed was adjusted according to the factorial design [Table 1]. Glutaraldehyde (0.25 ml) was further added twice, once after 1 hour and again after 2 hours, with continuous stirring. Stirring was stopped 1 hour after the final addition of glutaraldehyde. The obtained microspheres were separated by centrifugation and washed several times with petroleum ether to remove liquid paraffin. The microspheres were then suspended in a 5% w/v sodium bisulfite solution and stirred at the same speed for 15 minutes to remove residual glutaraldehyde. Finally, the microspheres were washed with distilled water and dried[1].

For enteric coating, 100 mg of microspheres were dispersed in 5 ml of an organic solvent (acetone: ethanol; 2:1) containing Eudragit L-100 (500 mg). This organic phase was poured into 70 ml of liquid paraffin containing Span 80[1]. The system was stirred at 1000 rpm at room temperature for 3 hours to allow the evaporation of the organic solvent[3]. The enteric-coated microspheres were then collected, rinsed with n-hexane, and air-dried. The same procedure was used to enteric coat all batches[5].

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Table 1: Composition of Duloxetine HCl microspheres

Optimisation of enteric coated microspheres using different parameters

Formulation code	Duloxetine HCl(mg)	Carbopol(mg)	Liquid paraffin(ml)	Glutaraldehyde(ml)	Methanol(ml)
F1	50	50	50	1	5
F2	50	50	50	1	5
F3	50	50	50	1	5
F4	50	100	50	1	10
F5	50	100	50	1	10
F6	50	100	50	1	10
F7	50	200	50	1	20
F8	50	200	50	1	20
F9	50	200	50	1	20

Particle size of microspheres:

The particle size of the enteric-coated microspheres was measured through optical microscopy. Around 100 microspheres were examined for particle size using a calibrated optical microscope**.**

Drug Entrapment Efficiency:

Microspheres (50 mg) were crushed using a glass mortar and pestle, and the resulting powder was suspended in 10 ml of phosphate buffer (pH 7.4). After 24 hours, the solution was filtered, and the filtrate was analysed for drug content[4]. The drug entrapment efficiency was calculated with the following formula:

$$\text{Entrapment Efficiency} = \frac{\text{Practical Drug Content}}{\text{Theoretical drug content}} \times 100$$

Swelling Index:

The equilibrium swelling studies were conducted as previously described[4]. A known weight (100 mg) of microspheres containing the drug was placed in 500 ml of phosphate buffer solution (pH 6.8) and allowed to swell for the required period at 37 ± 0.5°C using the United States Pharmacopoeia (USP) dissolution apparatus with a dissolution basket assembly at 50 rpm. To ensure complete equilibration, the samples were allowed to swell for 24 hours. Excess surface liquid drops were removed by blotting with soft tissue papers, and the swollen

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microspheres were weighed to an accuracy of 0.01 mg using an electronic microbalance. The microspheres were then dried in an oven at 60°C for 5 hours until there was no change in their dried mass[6]. The swelling index (SI) was calculated using the formula:

$$SI=\frac{Wc-Wo}{Wo}$$

where (Wo) is the initial weight of the dry microparticles and (We) is the weight of the swollen microparticles at equilibrium in the media. Each experiment was repeated three times, and the average value and standard deviation (SD) were taken as the SI value.

Mucoadhesion time:

Within an hour of the animal's death, a 5 cm length of recently cut pig intestine was procured from a nearby abattoir and cleansed with an isotonic saline solution. The mucosal surface of the microspheres was accurately weighed and then placed on a polyethylene plate that was mounted at a 40° angle to the horizontal plane. Over the tissue, phosphate buffer (pH 6.8) was applied at a rate of 5 ml/min after being warmed to 37 ± 1°C. Through visual inspection, the amount of time needed to remove every microsphere from the pig intestinal mucosa was noted.

In vitro drug release studies:

The in vitro drug release profile of the drug from enteric-coated microspheres was determined using the USP 23 method for modified release formulations (method A)[7]. Dissolution studies were performed using USP apparatus type-II (paddle type) at 50 rpm and 37 ± 0.5°C. Initial studies were conducted in 325 ml of 0.1 N HCl (pH 1.2) for 2 hours. The pH of the dissolution media was then adjusted to 6.8 by adding 125 ml of 0.2 M trisodium orthophosphate, with further adjustments made using 2 N HCl or 2 N NaOH. Dissolution continued in phosphate buffer for up to 24 hours. Samples were withdrawn at predetermined intervals, replaced with fresh media, filtered, and analysed using a UV-visible spectrophotometer at 288 nm[1].

Surface morphology:

The surface morphology of the microspheres was analysed using scanning electron microscopy (SEM). The microspheres were affixed to metal stubs with double-sided tape and coated with a 150 Å layer of gold under vacuum. The stubs were then examined under SEM.

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Stability analysis of microspheres:

In this study, the stability of the optimized formulation (loaded with Duloxetine HCl) was assessed by storing the microspheres at 4 ± 1°C and 25 ± 2°C with 60 ± 5% relative humidity for 30 days in screw-capped amber-coloured glass bottles. Every 15 days, the formulations were evaluated for the percentage of drug remaining and any physical changes, with the initial drug content taken as 100%[1].

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR spectrum of Duloxetine HCl is depicted in Figure 1. FTIR spectrum of optimised formulation is depicted in Figure 2. FTIR spectrum of DLX showed peaks at 1230.03 cm^{-1} (C-N stretching vibrations), 1061.08 cm^{-1} (C-O stretching vibrations), 1578.12 cm^{-1} (Aromatic C=C stretching vibrations) and 2768.26 cm^{-1} (Aliphatic C-H stretching vibrations). The IR spectra of Duloxetine HCl microspheres depicted all the characteristic peaks of DLX and Carbopol signifying zero interaction between them while preparation of the microspheres.

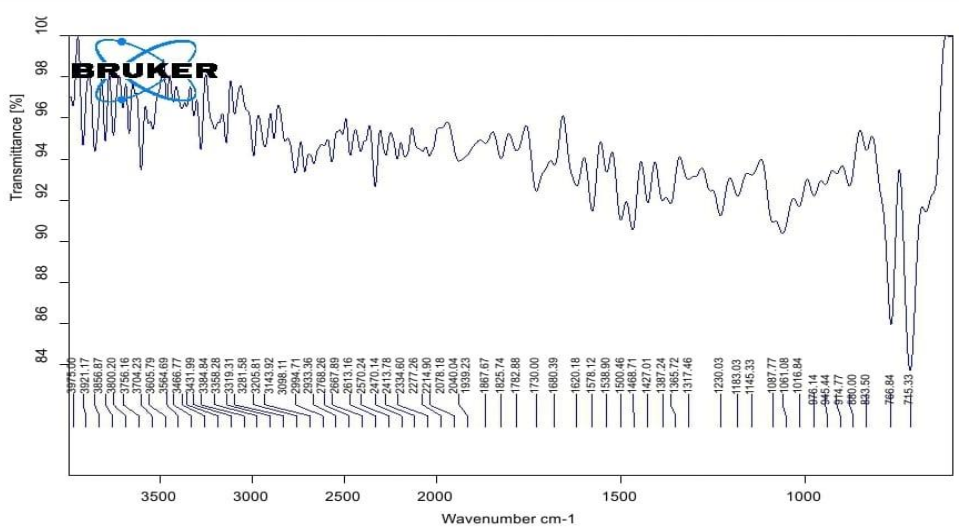

Figure 1: FTIR spectrum of Duloxetine HCl pure drug

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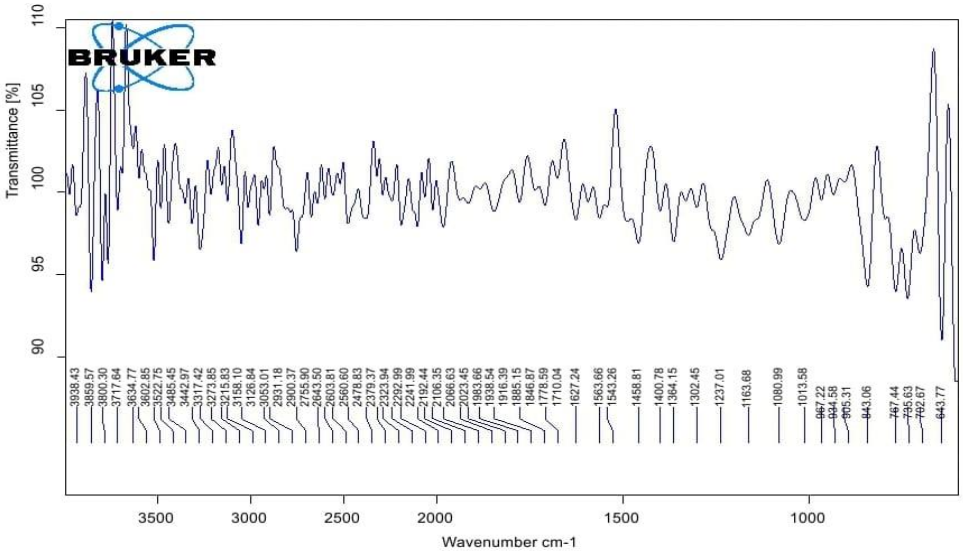

Figure 2: FTIR spectrum of optimised formulation

Characteristic properties of microspheres:

Table 2: Characteristic properties

Formulation code	Particle size (µm)	Drug entrapment efficiency (%)	Percentage swelling (%)	Mucoadhesion Time (h)	Percentage drug release in 24 hours (%)
F1	27.98±0.58	78.98±0.45	156.89±0.34	3.00±0.15	90.54±0.35
F2	25.78±0.67	80.65±0.55	220.65±0.45	2.58±0.30	91.65±0.46
F3	20.05±0.54	74.87±0.85	308.92±0.23	4.26±0.24	86.88±0.48
F4	40.54±0.65	84.76±0.64	200.76±0.25	2.56±0.12	93.23±0.45
F5	36.65±0.51	90.65±0.35	240.43±0.22	4.50±0.20	96.65±0.36
F6	32.76±0.78	72.59±0.63	330.56±0.33	3.67±0.32	88.86±0.56
F7	50.56±0.87	72.65±0.57	216.39±0.46	4.32±0.22	84.56±0.39
F8	46.34±0.74	74.06±0.46	256.89±0.56	2.54±0.21	89.65±0.53
F9	43.43±0.57	71.09±0.78	344.43±0.25	3.98±0.34	85.87±0.36

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In-vitro drug release

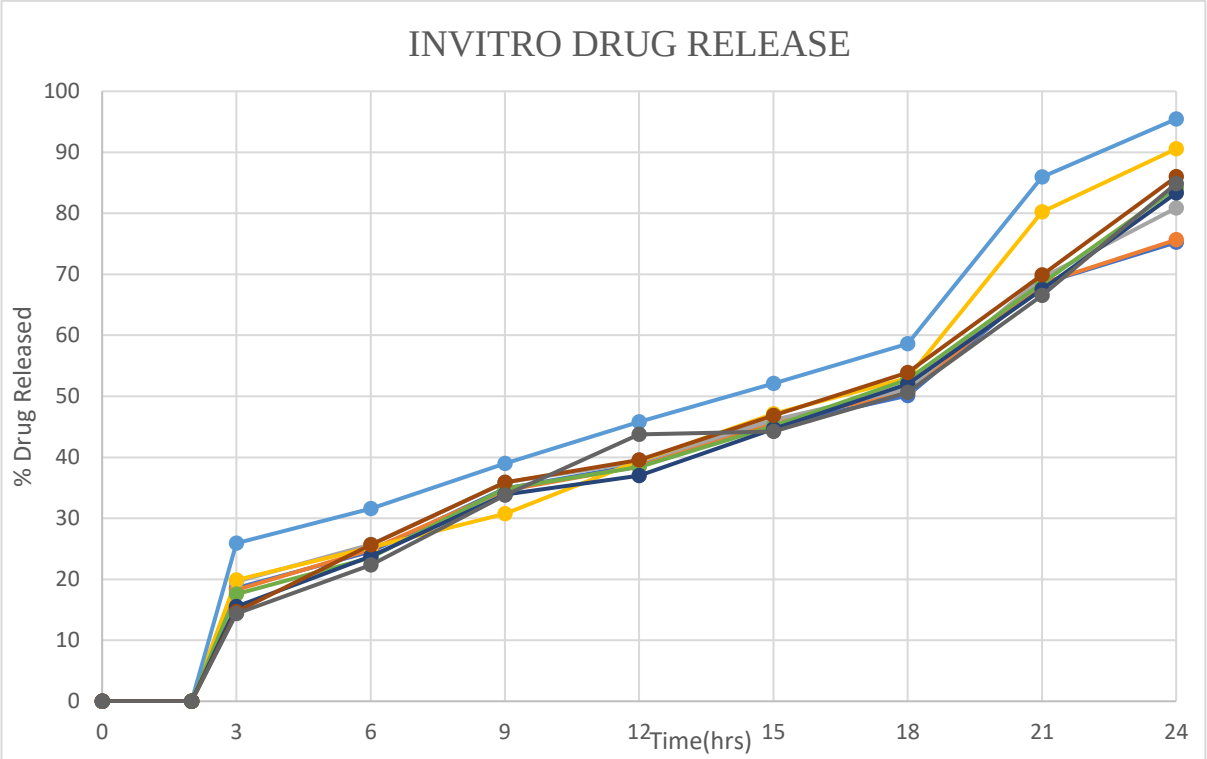

Figure 4: In-vitro drug release profiles of Duloxetine HCl microspheres

In-vitro release profiles of all the formulations were performed first in 0.1 N HCl for two hours and then in phosphate buffer (pH = 6.8). Because of the enteric coating on the microspheres, there was no drug release during the first two hours of the dissolution experiments. It was discovered that drug release increased as the drug polymer ratio increased, but drug release decreased as stirring speed increased. This was consistent with the maximum entrapment efficiency for the F5 Formulation, which also produced the maximum drug release in a 24-hour period**.**

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Drug release kinetics

Table 3: Kinetics of drug release of all formulations

Formulation code	Zero order release	First order release	Higuchi's release	Korsmeyer-Peppas release		Best fit model
				Slope(n)	R^2	
F1	0.824	0.954	0.924	0.710	0.813	First order
F2	0.855	0.998	0.967	0.686	0.772	First order
F3	0.845	0.983	0.948	0.642	0.817	First order
F4	0.920	0.995	0.975	0.694	0.720	Higuchi release
F5	0.915	0.954	0.965	0.737	0.685	Higuchi release
F6	0.927	0.974	0.999	0.620	0.783	Higuchi release
F7	0.884	0.986	0.969	0.520	0.838	First order
F8	0.945	0.998	0.975	0.600	0.698	Higuchi release
F9	0.957	0.998	0.964	0.634	0.787	First order

The Higuchi model drug release mechanism was followed by the release kinetics of the F4, F5, F6 and F8 batches, whereas the first order release mechanism was followed by the other batches. As with water soluble pharmaceuticals, the Higuchi model can be applied to a system where the medication is distributed across a consistent, swellable polymer matrix. Because it adheres to a controlled and continuous release process, the Higuchi model is also known as diffusion release.

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Surface morphology of microspheres

Surface morphology of the optimised formulation was studied by the SEM analysis. It revealed that the microspheres were spherical in shape and had a smooth surface. The homogeneity of the coating is indicated by how smooth the surface is.

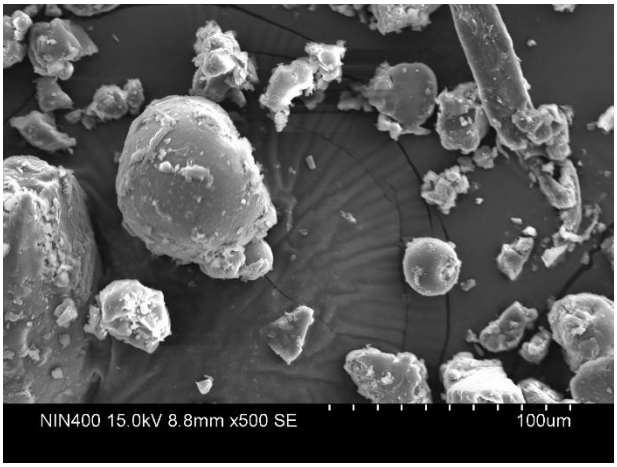

Figure 5: Scanning electron microscope photograph of Duloxetine HCl loaded microspheres

Stability studies of microspheres

Testing of the characteristics of the drug product that are prone to alter during storage and that could affect quality, safety, or efficacy should be a part of stability studies. The percentage of medication left and the physical (size and colour) variations of the microspheres were both observed to be affected by storage temperature. The original drug content was measured at 100% and contrasted with the formulations after a period of 15 days. The stability study's findings demonstrated that after 60 days, no changes in the size or colour of any of the microsphere formulations were noticed.

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Table 4: Stability study of optimised formulation(F5) at different temperatures

Time (days)	% Drug remained		Physical changes (size and colour)	
	4±1ºC	25±2ºC	4±1ºC	25±2ºC
0	100.00±0.3	100.00±0.2	-	-
15	99.96 ±0.2	99.87±0.3	No change	No change
30	99.75±0.3	99.65±0.2	No change	No change
60	99.53±0.2	99.45±0.2	No change	No change

CONCLUSION

The optimal batch of F5 microspheres displayed impressive characteristics, with improved solubility and highest percentage of drug released, including a 84.0% drug entrapment efficiency, and a substantial swelling index (SI) of 253.63%.

The stability study showed that there was no change observed in physical characteristic (size and colour) of final prototype formulation F5 at 15 days, 30 days, 45 days and 60 days. In vitro release studies indicated sustained drug release of Duloxetine from these mucoadhesive microspheres for almost 24 hours.

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