Development and characterization of Tulsion® (pH dependent) microspheres using quasi emulsion spherical crystallization technique

A. Jain[1], P. Kanaujia[1] and S. P. Vyas[1]*
[1] Department of Pharmaceutical Sciences, Dr. Hari Singh Gour University (Formerly University of Saugor), Sagar, India (ashishsemail@rediffmail.com)

1. Introduction

Spherical crystallization is a novel agglomeration technique, developed for use with the polymers, in which the precipitated crystals were designed to form functional drug devices such as microspheres (Kawashima et al., 1989), microballoons (Kawashima et al., 1991), biodegradable nanospheres (Niwa et al., 1993) and microcapsules (Niwa et al., 1994). *TULSION® Thermcoat L 30 D -55* is an aqueous dispersion of a solid polymer/ substance described as "Methacrylic acid copolymer dispersion" type C in USP/NF. Polymer exhibits a pH dependent solubility profile and reportedly has been used to prepare enteric dosage form for intestinal and colon delivery.

2. Material and methods

Clarithromycin was a gift sample obtained from Bennet Pharmaceuticals, Baroda (India). Thermocoat L 30 D55 polymer was obtained as a gift sample from Thermax ltd, Pune (India). All other chemicals used were of analytical grade unless mentioned.

The polymer was lyophilized to renovate it in to solid powder. The tulsion® polymer (500mg) and Clarithromycin (50mg) together were dissolved in 2.0 ml ethanol, subsequently 4.0 ml dichloromethane and 1.0 ml distilled water was added. The solution was added to 0.01N HCl drop wise under steady stirring at 1500 RPM for 30 min. The resultant visible suspension was centrifuged at 1000 RPM for 5 min. followed by drying at room temperature.

2.1 Characterization of prepared microspheres

Microspheres were observed directly under the optical microscope at 100x magnification to examine their shape. In order to examine the surface morphology, the formulations were examined under scanning electron microscope (SEM). Size distribution of dilute microspheres suspension was determined by laser diffraction particle size analyzer (CILAS 1064 L, France). The amount of Clarithromycin associated with the microspheres was analyzed in terms of surface adsorbed and entrapped drug. Percent yield was calculated by weight of microspheres formed with respect to weight of non-volatile substances (drug & polymer) used for preparation. *In-vitro* drug release at different pH was analyzed by dialysis membrane. The 100 mg microspheres were taken in treated dialysis bag which was dipped in 100 ml dissolution medium. The releasing media was magnetically stirred at 37±1°C and the pH of dissolution media was changed with respect to time. The release studies were performed in 0.1M HCl for first two hours, following this 340mg of KH_2PO_4 and 450mg of $Na_2PO_4.2H_2O$ were added, adjusting the pH to the desired value with 1.2 M NaOH. For all the formulation, the pH was adjusting to 5.5 for 1hr, 6.8 for 2hr and 7.5 until the end of the test. The final volume in all the cases was 100 ml (Rodriguez et al., 1998). The amount of drug in solution was analyzed spectrophotometrically using folin ciocaltue and sodium carbonate at 760 nm by UV-visible spectrophotometry.

3. Results and Discussion

Solid polymer was used for each formulation because liquid dispersion of thermocoat L 30 D 55 was not supported the formation of microspheres. The water of the initial stage interferes in the preparation mechanism.

When the drug- polymer solution was poured into poor solvent (0.01N HCl) under agitation at room temperature dispersed gel was formed. Due to affinity of the polymer to the organic solvent, good solvent (ethanol) and bridging liquid (dichloromethane) in the droplets however could not diffuse into 0.01N HCl instantaneously. Because ethyl alcohol, which is discretionarily miscible with 0.01N HCl, tended to diffuse out from quasi emulsion droplets under agitation thus drug and polymer in the droplets were supersaturated and as a result precipitated and consolidated into microspheres through the action of dichloromethane.

Water was used because in absence of water, droplets were adhered together which turned into bigger lumps instantaneously. The added dichloromethane (DCM) came in contact with HCl under agitation. Yield of microspheres was increased quantitatively on addition of water. However, beyond optimum limits the solubility of polymer was recorded to decrease in the solution, causing precipitation and subsequent agglomeration of the polymer. The water was in an appropriate range to assure not only the formation of quasi emulsion droplets at the initial stage of preparation but to affect the size of the microspheres. With increasing the amount of ethanol, the system moved towards the miscible region, rending the DCM in capable of bridging the drug and the polymer which cause dispersion of drug and polymer powder.

The good solvent, bridging liquid, dispersing agent and selected poor solvent of the system also influenced the average particle size. As the amount of ethanol (good solvent) increases the particle size tends to decrease. The decrease in particle size may probably due to the decrease in the viscosity of polymer solution with increasing amount of good affinity solvent. The particle size was also recorded to decrease on addition of dichloromethane, which may be due viscosity variation, but high quantity of DCM reduces the solubility of polymer and clump were formed hence 4 ml was considered to be optimum for the preparation of microspheres. Water was recorded to affect the particle size. At the low quantity of water the solution was not dispersed in the poor solvent properly resulting in to the higher particle size of the microspheres. As the normality of HCl increases the particle size were also increases. Polymer was insoluble in the acidic medium so the solubility rapidly decreases when polymer comes under contact with the solvent with low pH (poor solvent) hence precipitated and forming microspheres, thus higher normality causes spontaneous precipitation yielding the microspheres of higher particle size, therefore low normality was selected for the controlled precipitation of polymer with formation of microspheres. The effect of the different solvents on the particle size is shown in the table 1.

Agitation is an important factor, which affects the particle size of the microspheres. The increase in mechanical shear force produced by the agitation speed, divides the drug and polymer solution into small droplets rapidly, with decreased mean diameter of the microspheres. Agitation time also influenced the particle size up to a limit. As the agitation time period increased the particle size tended to decrease, but after 20 min. there was no detectable change recorded in the average particle size. The results show that after 20 min. all droplets were converted to microspheres. Effect of the agitation time and speed on the size is shown in the table 2.

Amount of Ethanol (ml)	Bridging liquid (ml)	Water (ml)	Normality of HCl (N)	Average Diameter (μm)	% Yield of microspheres
1	4	1	0.01	56.4 ± 0.58	48.10 ± 4.1
2	4	1	0.01	52.0 ± 0.46	57.27 ± 3.3
3	4	1	0.01	49.3 ± 0.24	52.72 ± 1.6
2	2	1	0.01	72.3 ± 0.86	34.54 ± 0.9
2	3	1	0.01	58.6 ± 0.38	44.54 ± 1.0
2	5	1	0.01	-	-
2	4	0.5	0.01	65.4 ± 1.06	39.09 ± 0.8
2	4	1.5	0.01	-	-
2	4	1	0. 1	95.2 ± 1.12	46.36 ± 1.2

Table 1- Effect of solvents on particle size, and percent yield of microspheres prepared using constant stirring speed (1500 rpm) and stirring time 20 min. (n = 3, $\bar{x}$ ± S.D.)

Stirring speed (RPM)	Stirring time (Min)	Average Diameter (μm)	Total drug loading efficiency (%)	% Yield of microspheres
500	20	76.2 ± 0.62	62.5 ± 2.5	48.10 ± 1.1
1000	20	61.6 ± 0.86	69.8 ± 2.9	55.45 ± 2.1
1500	20	52.0 ± 0.46	71.0 ± 3.1	57.27 ± 3.3
2000	20	49.8 ± 0.69	70.4 ± 1.9	56.36 ± 1.9
1500	10	63.7 ± 0.56	63.9 ± 2.8	49.09 ± 1.0
1500	30	51.8 ± 1.03	70.8 ± 4.0	57.27 ± 3.3

Table 2- Effect of stirring speed and stirring time on particle size, total drug loading efficiency and percent yield of microspheres. (n = 3, $\bar{x}$ ± S.D.)

The percent recovery may increase due to the increasing dispersion of the quasi emulsion droplets in the poor solvent with an increase in the amount of ethanol. The recovery improves up to a limit beyond which the increase on concentration. Water played a very important role in the recovery of microspheres. Unless water was used as dispersion medium, larger lumps of polymers were formed. However as the weight fraction of water increases the percent recovery of microspheres was also increased, however addition of water in volume more than 1 ml caused the precipitation of the polymer. Poor solvent affects remarkably the recovery of microspheres. As the pH of the solvent decreased the recovery was decreased due to sudden precipitation of polymer at higher H_3O^+ concentration and lumps formation. Percent yield of microspheres is mostly affected by the ratio of solvents as shown in table 1.

Total drug loading efficiency was influenced by the drug: polymer ratio. As table 5 shows the drug content increases with the drug: polymer ratio, i.e. 1: 10 Clarithromycin: thermocoat L 30 D 55

ratio has maximum drug loading capacity which shows polymer coated the drug efficiently with the higher concentration of polymer.

In vitro drug release study of all microspheres was conducted in simulated intestinal fluid with various pH. At the initial pH (0.1 N HCl) (simulated to stomach environment) less amount of drug was released. At the intestinal pH (5.5) after 2 hrs the release was increased invariably from each formulation further the release rate recorded to increase with the time of dissolution and pH of dissolution medium. The maximum release was observed at colonic pH, however maximum leaching should be there. Thus the formulation demonstrates sustained release in gastro intestine at the targeted site of the intestinal and colonic region. The drug release from formulation of different drug polymer ratio shows that the release rate was low in the case of formulation with higher polymer ratio. 1: 10 drug: polymer ratio shows controlled release of drug from microspheres. Clarithromycin suspension was released very rapidly and on the other hand increasing the amount of tulsion polymer resulted in a marked decrease in the drug release. It was evident that thermocoat L 30 D 55 polymer was an efficient retarding agent to control the drug release rate (Fig 1).

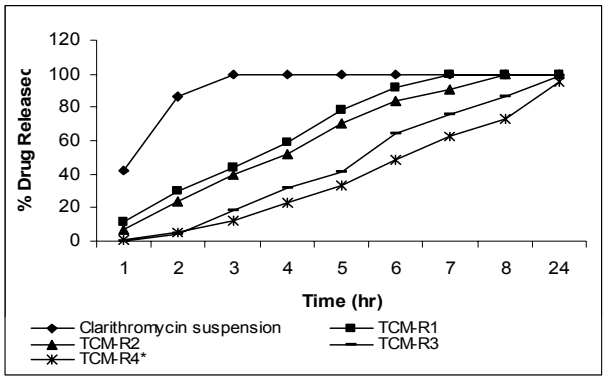

Fig 1- *In vitro* drug release from different formulation

4. Conclusion

Microspheres were prepared by the spherical crystallization technique, which is beneficial from conventional methods in various aspects like lack of time consumption, cheep and less use of costly and toxic solvents. The use of enteric polymers (Thermocoat L 30 D 55) as protective coating on the microspheres makes them to release the drug at particular pH of colonic fluid.

5. References

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