Available online at http://jddtonline.info

RESEARCH ARTICLE

DEVELOPMENT AND CHARACTERIZATION OF SRM MICROSPHERES OF REPAGLINIDE

*Gupta AK[1], Garg SK[2], Pal SK[1], Saxena M[1], Sharma A[1]

[1]Micro Labs ltd, Baddi (H.P.), INDIA
[2]National Institute of Pharmaceutical Education and Research (NIPER), Hazipur, INDIA
*Corresponding Author's E-mail: anishpharma02@yahoo.co.in

Received 26 Sep 2011; Revised 19 Oct 2011; Accepted 21 Oct 2011, Available online 26 Oct 2011

ABSTRACT

The aim of current work to develop and evaluate sustained release mucoadhesive (SRM) microspheres of Repaglinide using emulsification solvent evaporation technique. Effects of formulation variables i.e. polymer concentration and phase volume ratio on particle size, % mucoadhesion and drug release were investigated in this study. Scanning electron microscopy of microspheres with maximum drug content (Formulation CH1:8) demonstrated smooth surface spherical particles with mean diameter of 64.78 ± 3.26 µm. The mean Particle size, % drug loading and mucoadhesion were found to vary by changing the formulation variables. Microspheres size was significantly increased as increasing the polymer concentration in the aqueous phase while size of microspheres decrease as increase in volume of continuous phase. Decrease in size of microspheres leads to decrease in mucoadhesion time, % drug loading and faster the drug release. It can be concluded that the present mucoadhesive microspheres can be an ideal system to deliver the Repaglinide in sustained release manner for management of Type II Diabetes Mellitus.

Key Words: Sustained release microspheres, Repaglinide, formulation variables, mucoadhesion, drug loading.

INTRODUCTION

Substantial efforts have recently been focused upon placing a drug or drug delivery system in a particular region of the body for extended period of time[1]. From a technological point of view, an ideal Sustained Release Mucoadhesive (SRM) dosage form must have three properties. It must maintain its position in the mouth for a few hours, release the drug in a controlled fashion and provide the drug release in a unidirectional way towards the mucosa[1]. Microspheres form an important part of such novel drug delivery systems. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres.[2-7] Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site.[8, 9] Mucoadhesive microspheres that are retained in the stomach would increase the drug absorption and decrease dosing frequency which provides better patient compliance as compared to conventional dosage forms.

Repaglinide is an oral hypoglycaemic agent which acts by stimulating the release of insulin from pancreatic beta-cells by inhibition of potassium efflux resulting in closure of ATP regulates K+ channels[10].

The bioavailability of the oral formulation was found to be 63%[11]. The effective control of diabetes type-II requires administration of Repaglinide 0.5 – 4 mg three times daily. Owing to its short biological half life (1 hours) and low bioavailability (63%)[12]; it's necessary to develop a sustained release mucoadhesive dosage form of Repaglinide which adhere to the mucosa and release the drug in sustained release manner.

These microspheres would prolonged, relatively constant effective level of Repaglinide and improve patient compliance. Thus SRM microspheres of Repaglinide are suitable candidate for effective control of diabetes type-II.

Literature survey revealed that Carbopol (CP) [13-15] and hydroxy propyl methyl cellulose (HPMC) [16, 17] are the polymer which shows good mucoadhesive properties, high drug entrapment efficiency and release the drug in sustained release manner. Therefore in the present study Repaglinide is selected as a model drug and CP and HPMC are chosen as a mucoadhesive polymer for design and evaluation SRM Microspheres for treatment of diabetes type-II.

MATERIALS:

Repaglinide was obtained as gift sample from Sun Pharmaceuticals Ltd., Mumbai, INDIA. CP was gifted from Colorcon Asia Pvt. Ltd, Goa INDIA. HPMC was received as gift sample from Zydus-Cadila Healthcare Ltd, Ahmadabad, INDIA. n-Hexane and span 20 were procured from central drug house, New Delhi INDIA. Liquid paraffin was procured from Loba Chemie Pvt. Ltd., Mumbai INDIA. All the reagents were used of analytical grade.

METHODS:

Assay of Repaglinide:

Repaglinide was estimate using an UV spectrophotometer method. Different solutions of Repaglinide were prepared in simulated gastric fluid (pH 1.2) and absorbance was measured on Shimazdu UV spectrophotometer at 247 nm. The method was validated for linearity, accuracy, and precision. The regression coefficient was found to be 0.991.

Preparation of microspheres7, 18:

Mucoadhesive microspheres of Repaglinide were prepared by emulsification solvent evaporation method using various ratios of CP and HPMC. For this, aqueous solution of drug and polymer is prepared. Then drug and polymer solution was added drop wise to the liquid paraffin containing 0.5 % span 20 as an emulsifying agent with constant stirring. The constant stirring was carried out using magnetic stirrer. The beaker and its content were heated at 800C with constant stirring for 4 hrs until the aqueous phase was completely removed by evaporation. The liquid paraffin was decanted and collected microsphere were washed 5 times with n-hexane, filtered through whattman's filter paper and dried in hot air oven at 50°C for 2 hours. Table 1 shows composition of various formulations of microspheres.

Surface morphology[19, 20]:

The surface morphology and structure were visualized by scanning electron microscopy (SEM). 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

Table-1 Composition *of drug loaded microspheres*

Formulation code	Drug	Stirring Speed	Variables		
			Polymer conc.		Phase volume ratio (D/C)
			Carbopol 934	HPMC	
C1:8	10 mg	500 rpm	1.0%	-	1:8
C1:12	10 mg	500 rpm	1.0%	-	1:12
C1:16	10 mg	500 rpm	1.0%	-	1:16
H1:8	10 mg	500 rpm	-	1.0%	1:8
H1:12	10 mg	500 rpm	-	1.0%	1:12
H1:16	10 mg	500 rpm	-	1.0%	1:16
CH1:8	10 mg	500 rpm	1.0%	1.0%	1:8
CH1:12	10 mg	500 rpm	1.0%	1.0%	1:12
CH1:16	10 mg	500 rpm	1.0%	1.0%	1:16

Particle Size[21, 22]:

Particle size analysis of drug-loaded microspheres was performed by optical microscopy using a compound microscope (Erma, Tokyo, Japan). A small amount of dry microspheres was suspended in n-hexane (10 mL). The suspension was ultra-sonicated for 5 seconds. A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and 300 particles were measured using a calibrated ocular micrometer. The average particle size was determined by using the Edmondson's equation $D_{mean} = \sum nd/\sum n$, where n= number of microspheres observed and d= mean size range. The process was repeated 3 times for each batch prepared.

Drug entrapment efficacy[21]:

50 mg of microsphere were taken and drug was extracted from microspheres by digesting for 24 hours with 10 ml of simulated gastric fluid (pH 1.2). During this period the suspension was agitated. After 24 hours, the solution was filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated using the following formula:

Entrapment efficiency = (Practical drug content/theoretical drug content) ×100

In-vitro mucoadhesivity[7, 8, 23]:

The mucoadhesive properties of the microspheres were evaluated by in vitro wash-off test as reported by Lehr et al. A 1-cm by 1-cm piece of rat stomach mucosa was tied onto a glass slide (3-inch by 1-inch) using thread. Microspheres were spread (∽50) onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid (pH 1.2). At hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue was counted. Percent mucoadhesion was given by the following formula.

% mucoadhesion = (no. of microspheres remains / no. of applied microspheres) ×100

The observations are expressed in figure 2-4.

In-vitro drug release[24, 25]:

In-vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using simulated gastric fluid (pH 1.2) as dissolution medium, volume of dissolution medium was 900 ml and bath temperature was maintained at (37±1) °C throughout the study. Paddle speed was adjusted to 50 rpm. An interval of 1 hour, 10 ml

of sample was withdrawn with replacement of 10 ml fresh medium and analyzed for drug content by UV-Visible spectrophotometer at 247 nm. All the experimental units were analyzed in triplicate (n=3). Cumulative percentage drug release was calculated using an equation obtained from a standard curve. The observations are expressed in figure 5 to 8 and table 3.

RESULTS AND DISCUSSION

Surface morphology:

Surface morphology of the mucoadhesive microspheres was examined by scanning electron microscopy (SEM). The SEM showed that the microspheres obtained from all the formulations are spherical with smooth surface. The SEM showed that CP produced spherical with smooth surface microspheres due to their high solubility in water[13, 14]. The SEM of microsphere of formulation C1:8 are shown in figures 1

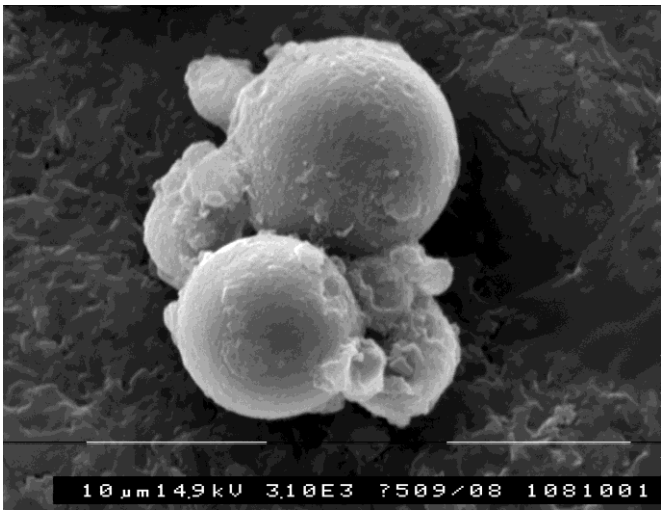

Figure- 1 SEM of formulation C1:8 showing population of microspheres

Particle size analysis:

Particle size analysis of different formulations was done by optical microscopy[20, 21]. The average particle size was found to be in the range of 28.43 to 64.78 µm. The mean particle size was significantly varied according to type of polymer used for the preparation of microspheres; this may be due to fact that difference in the viscosity of the polymer solution[13]. Since high viscosity of polymer solution requires high shearing energy for breaking of droplets of the emulsion. Microspheres containing HPMC are larger as compared to CP microspheres because HPMC solution has more viscosity at the same concentration. Particle size decreased with increase in volume of continuous phase due to the fact that increased in continuous phase, more efficiently utilized the energy produced by stirring, which leads to further decrease in droplets size of internal phase. increase in concentration of polymer in internal phase leads to increase in size of microspheres because at higher concentration polymer solution have more viscosity which requires more energy to breaking the droplets of dispersed phase. Results of particle size analysis are shown in table 2.

Drug entrapment efficiency:

Drug content in different formulations was estimated by U V Spectrophotometric method. Percent drug loading efficiency of microspheres was found in the range of 62.13 to 76.5 % (table- 2). Formulation CH1:8 containing blend of CP and HPMC showed maximum % drug loading about 76.5 % because these microspheres have larger size as compared to other formulations. Whereas formulation H1:16 containing HPMC showed minimum % drug loading about 63% because these microspheres are small in size which results more loss of drug from surface during washing of microspheres. Increase in polymer concentration of internal phase also increase in drug entrapment of microspheres. Rank order of % drug loading of various formulations was found to be as follows:

CH1:8>C1:8>C1:12>CH1:12>C1:16>CH1:16>H1:8>H1:12>H1:16

In-vitro mucoadhesivity test:

To assess the mucoadhesive property of microspheres, In-vitro wash-off test was performed for all the formulations. In the mucoadhesion process, it is necessary for swelling and expansion of the polymer chain since interpenetration and entanglement of the polymers and the mucous networks are considered to be responsible for adhesion[13]. Therefore, bioadhesives should swell and expand rapidly when they come in contact with water. Adhesion of polymer with the mucus membrane is mediate by hydration in the case of hydrophilic polymer. Upon hydration these polymers becomes sticky and adhere to mucus membrane. A high percentage of adhesion indicates that microspheres have excellent mucoadhesion to mucosal tissue. Carbopols are interacts with the mucin, resulting in adhesion of the polymer to the mucin. Formulation H1:8 containing HPMC showed the highest mucoadhesivity. Formulation C1:16 containing CP showed the shortest mucoadhesion time due to the small size of microsphere which takes short time for solubilization. The results of % mucoadhesivity test of all the formulations are expressed in figure 2, 4 and 5.

Drug release study:

Drug release form these microspheres were slow, extended and dependent on the type of polymer and concentration of polymer used. The rate of release of drug from the bioadhesive microspheres was slow and found to further decrease with increase in drug to polymer ratio. Formulation H1:16 containing HPMC showed the fast drug release due to rapid swelling property in dissolution environment (0.1 N HCl). Dissolution medium permeation in to the microspheres is facilitated due to high swelling action of the HPMC which leads to more medium for the transport of the drug is available. While HPMC microspheres showed the least drug release. A drug release form microsphere is significantly affected by the size of microspheres. Increase in polymer concentration leads to increase in size of microspheres thus drug release from microspheres having low drug to polymer ratio found to significantly decrease. FormulationC1:16 shown fastest drug release among all the formulation due to fact that these microspheres are small in size. Results of drug release study are expressed in figure 6 to 9.

Table 2: % yield, % drug entrapment and Particle size of microspheres

Formulation code	% yield	Particle size (µm)	% Drug entrapment
C1:8	78.46±2.45	44.23	75.23
C1:12	75.65±2.55	35.88	71.31
C1:16	72.26±2.80	28.43	69.50
H1:8	73.22±2.40	59.44	66.45
H1:12	70.83±2.64	48.94	64.86
H1:16	66.85±1.90	41.25	62.13
CH1:8	80.50±2.12	64.78	76.50
CH1:12	76.40±2.35	50.34	70.20
CH1:16	74.36±2.30	43.68	68.84

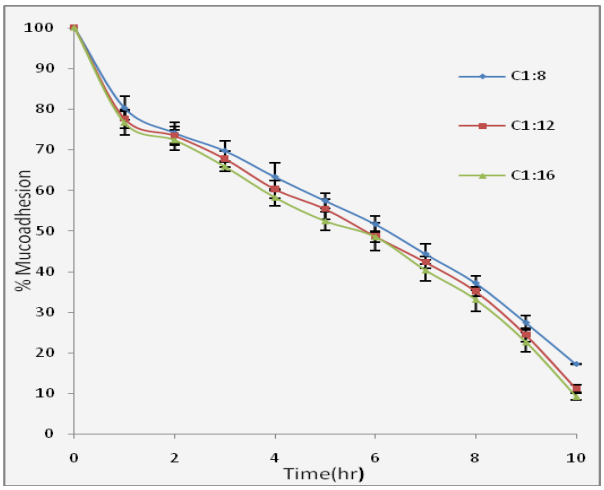

Figure 2: Comparative % mucoadhesion of formulations C1:8, C1:12 & C1:16

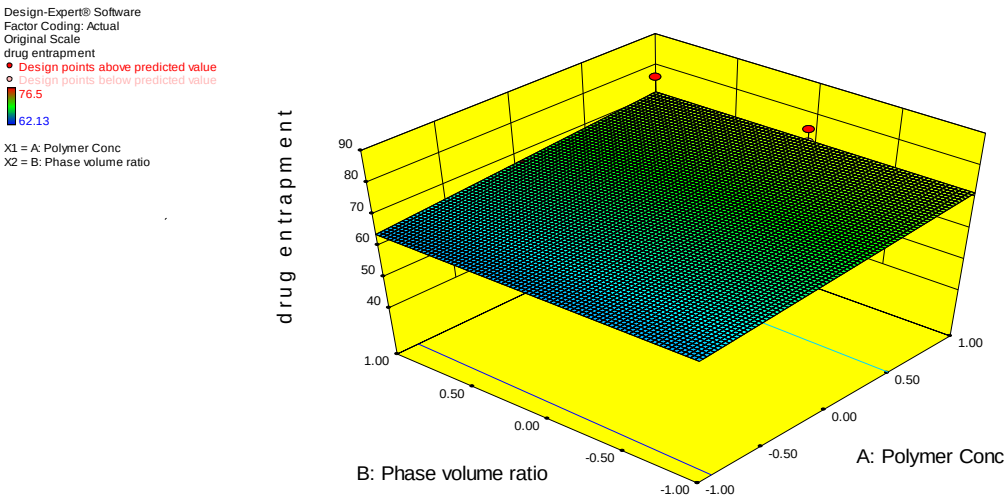

Figure 3: Surface Response Curve shows effect of polymer conc and phase volume ratio on drug entrapment

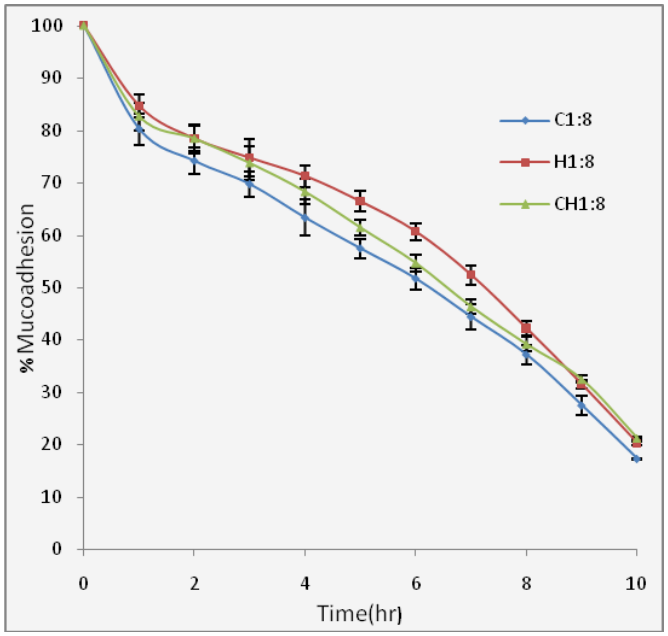

Figure 4: Comparative % mucoadhesion of formulations C1:8, H1:8 & CH1:8

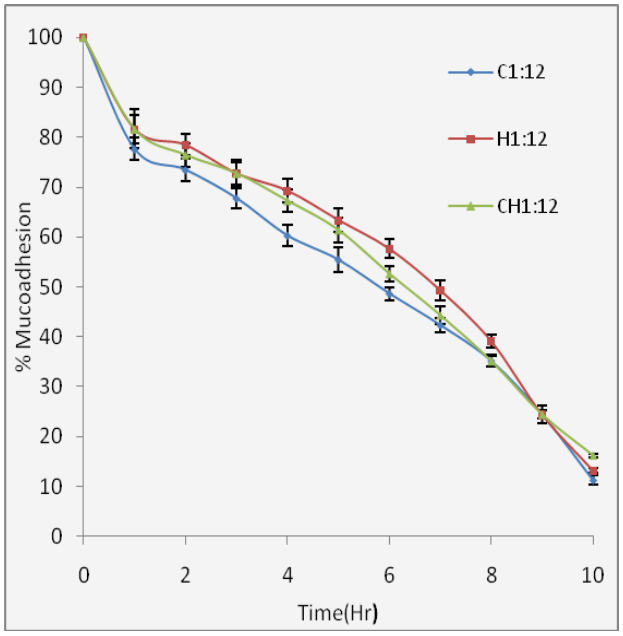

Figure 5: Comparative % mucoadhesion of formulations C1:12, H1:12 & CH1:12

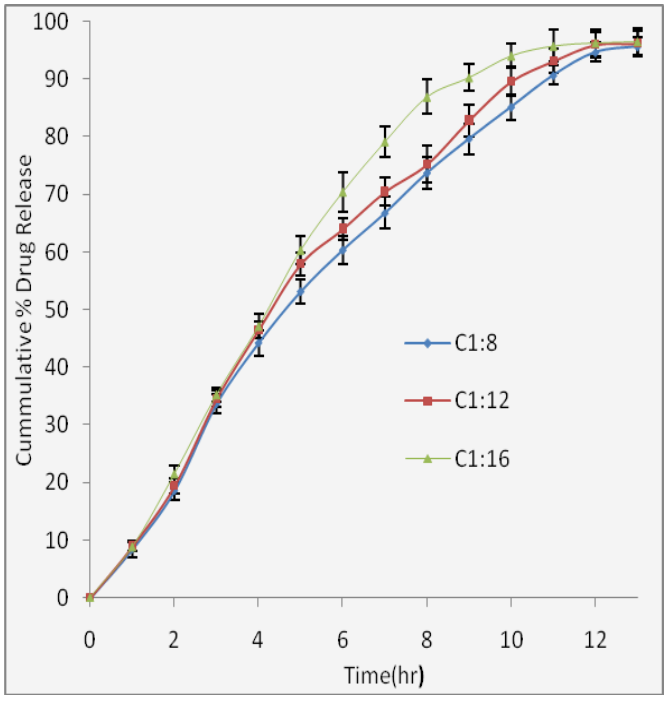

Figure 6: Cumulative % drug release from formulation C1:8, C1:12 & C1:16

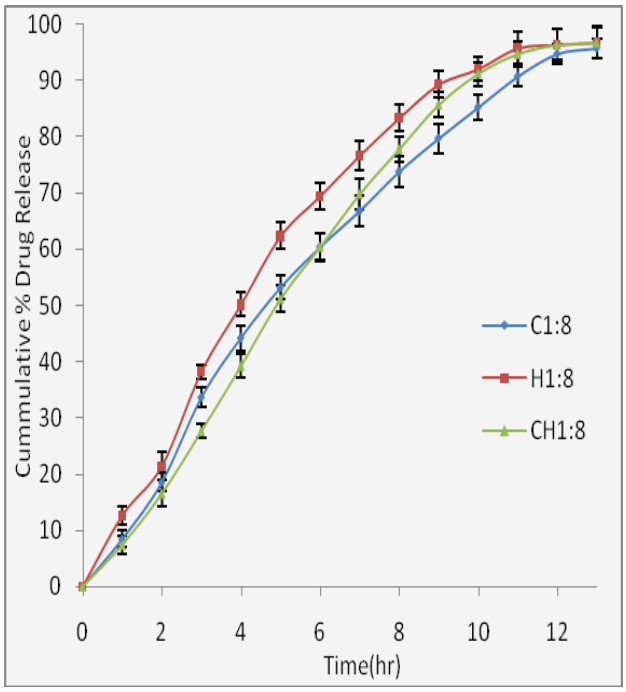

Figure 7: Cumulative % drug release from formulation C1:8, H1:8 & CH1:8

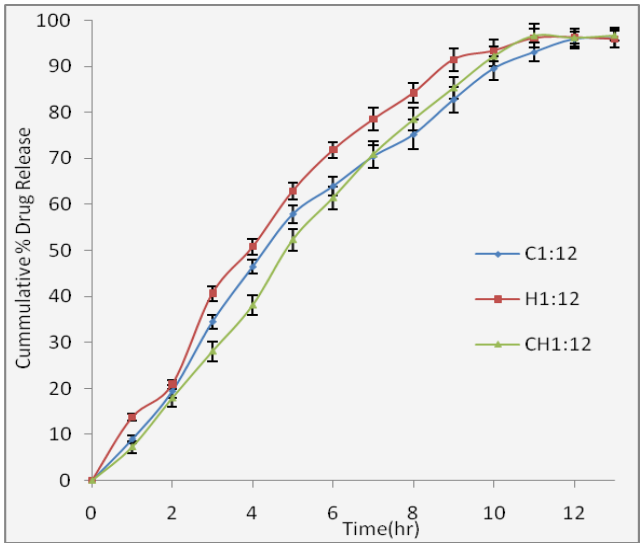

Figure 8: Cumulative % drug release from formulation C1:12, H1:12 & CH1:12

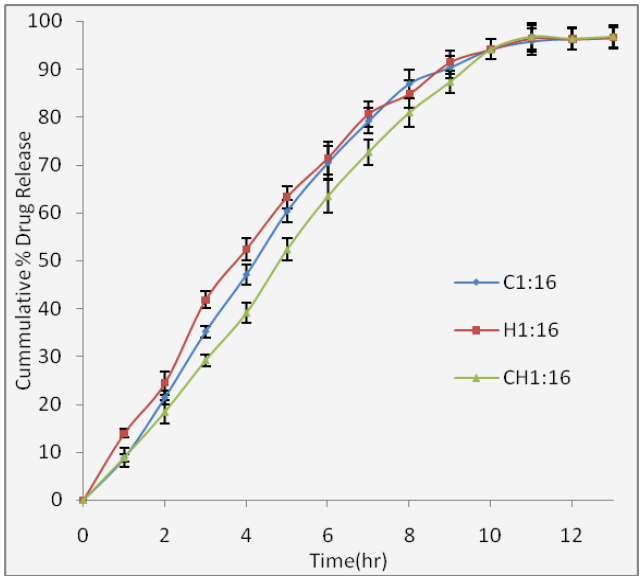

Figure 9: Cumulative % drug release from formulation C1:16, H1:16 & CH1:16

Table 3: % Mucoadhesion, t_{50} and t_{80} of Repaglinide Release from microspheres

Formulation	% Mucoadhesion after 1 Hour	T_{50} of drug release(Min)	T_{80} of drug release(Min)
C1:8	80.25±2.95	280	544
C1:12	77.62±2.23	263	521
C1:16	76.60±2.91	254	420
H1:8	84.65±2.23	238	448
H1:12	81.62±3.86	235	431
H1:16	82.82±2.94	223	415
CH1:8	82.65±2.63	292	498
CH1:12	81.42±2.86	264	497
CH1:16	79.82±2.74	298	470

Table 4: Application of kinetic models to access drug release behavior

Formulation Code	Kinetic Models		
	Zero Order	First Order	Second Order
C 1:8	y = 7.5766x + 8.2311 R^2 = 0.964	y = 37.159ln(x) - 2.5625 R^2 = 0.9718	y = -0.3971x^2 + 12.739x - 2.0931 R^2 = 0.9979
C 1:12	y = 7.6829x + 9.6586 R^2 = 0.9501	y = 37.83ln(x) - 1.4454 R^2 = 0.976	y = -0.4753x^2 + 13.862x - 2.7 R^2 = 0.9966
C 1:16	y = 7.9215x + 11.553 R^2 = 0.9161	y = 39.522ln(x) - 0.67 R^2 = 0.9715	y = -0.6512x^2 + 16.387x - 5.3775 R^2 = 0.9953
H 1:8	y = 7.6754x + 13.309 R^2 = 0.9225	y = 37.664ln(x) + 2.7216 R^2 = 0.9752	y = -0.6119x^2 + 15.631x - 2.6018 R^2 = 0.9975
H 1:12	y = 7.6357x + 14.402 R^2 = 0.9096	y = 37.473ln(x) + 3.9524 R^2 = 0.9711	y = -0.6591x^2 + 16.204x - 2.7343 R^2 = 0.9964
H 1:16	y = 7.5676x + 15.634 R^2 = 0.9072	y = 36.971ln(x) + 5.6727 R^2 = 0.977	y = -0.6712x^2 + 16.294x - 1.8179 R^2 = 0.9985
CH 1:8	y = 8.1384x + 5.2654 R^2 = 0.9621	y = 40.36ln(x) - 7.3768 R^2 = 0.9529	y = -0.4121x^2 + 13.495x - 5.4481 R^2 = 0.9936
CH 1:12	y = 8.1681x + 5.5983 R^2 = 0.9594	y = 40.509ln(x) - 7.0688 R^2 = 0.9526	y = -0.4263x^2 + 13.71x - 5.4849 R^2 = 0.9928
CH 1:16	y = 8.1675x + 6.6591 R^2 = 0.9525	y = 40.407ln(x) - 5.7532 R^2 = 0.9496	y = -0.4635x^2 + 14.193x - 5.3925 R^2 = 0.9918

FUTURE PROSPECTS:

While the control of drug release profiles has been a major aim of pharmaceutical research and development in the past two decades, the control of GI transit profiles could be the focus of the next two decades and might result in the availability of new products with better therapeutic possibilities and substantial benefits for patients. Mucoadhesive microspheres would become the promising candidate for delivery various drugs in sustained release manner. Dosing frequency and loss of drug also reduced by use of such type of formulations. Thus SRM microspheres of Repaglinide would become a promising candidate for therapy of diabetes type-II in the future.

ACKNOWLEDGEMENT

Author is highly thankful to Dept of pharmacy, IEC Group of Institution, Greater Noida, INDIA for providing him best lab facilities & atmosphere for this research.

REFERENCES:

1. Dhakar RC, Maurya SD, Aggarawal S, Kumar G, Tilak VK, Design and evaluation of SRM microspheres of metformin hydrochloride, Pharmacie Globale(IJCP), 2010, 1(6), 1-5.
2. Rajput G, Majumdar F, Patel J, Thakor R & Rajgor NB. Stomach Specific mucoadhesive microspheres as a controlled drug delivery system. Sys Rev Pharm, 2010, 1(1), 70-78.
3. Capan Y, Jiang G, Giovagnoli S & DeLuca PP. Preparation and characterization of poly (D,L-lactide-co-glycolide) microsphere for controlled release of human growth hormone. AAPS PharmSciTech. 2003. 4:E28.
4. Gohel MC & Amin AF. Formulation optimization of controlled release diclofenac sodium microspheres using factorial design. J Control Release. 1998. 51:115-122.
5. Nagai T, Nishimoto Y, Nambu N, Suzuki Y and Sekine K. Powder dosage form of insulin for nasal administration. J Control Release. 1984.1:15-22.
6. Ilium L, Farraj NF, Critchley H and Davis SS. Nasal administration of gentamicin using a novel microsphere delivery system. Int J Pharm. 1988. 46:261-265.
7. Wang J, Tabata Y and Bi D Morimoto K. *J. Control Release*. 2001. 73, 223-231.
8. Lehr CM, Bouwstra JA, Schacht EH and Junginger HE In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int J Pharm. 1992.78:43-48.
9. Chowdary KPR and Rao. YS. Design and in vitro and in vivo evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: a technical note. AAPS PharmSciTech. 2003, 4:E39.
10. Gromada J, Dissing S, et al. Effects of the hypoglycaemic drugs repaglinide and glibencamide on ATP-sensitive potassium-channels and cytosolic calcium levels in beta TC3 cells and rat beta pancreatic cells. Diabetologia 1995; 38:1025-32.
11. Hatorp V, Nielsen K, et al. Bioavailability of repaglinide after administration as either 2mg tablet or 2mg I.V. infusion. J Clin Pharmacol 1997; 37: 874.
12. European Agency for the Evaluation of Medicinal Products. European Public Assessment Report Novonorm CPMP/866/98.
13. Ponchel G, Jeanne M, Dembri M, Durrer C, Duchene D. Mucoadhesion of colloidal particulate systems in the gastrointestinal tract. *European. J. Pharm Biopharm.* 1997. 44: 25-31.
14. Semalty A and Semalty M, Preparation and characterization of mucoadhesive microspheres of ciprofloxacin hydrochloride. Indian drugs. 2007.44(5), 92-113.
15. Thakur RS, Ray S and Shiva SK, Formulation and evaluation of mucoadhesive dosage form containing Rosiglitazone maleate. Pak. J. Pharm. Sci. 2006. 19(3):208-213.
16. Venkateswaramurthy N, Sambathkumar R, Vijayabaskaran M and Perumal P, Formulation and *in vitro* evaluation of furazolidone mucoadhesive microspheres, Int J Pharm and Pharm, Sci, 2010, (2)3.
17. Prajapati S.K, Tripathi P, Ubaidulla U and Anand V. Design and Development of Gliclazide Mucoadhesive Microcapsules: In Vitro and In Vivo Evaluation AAPS PharmSciTech, 2008, 9(1).
18. Bomati-Miguel O, Morales MP, Tartaj P, Ruiz-Cabello J., Bonville P, Santos M, Zhao X and Veintemillas-Verdaguer S. Fe-based nanoparticulate metallic alloys as contrast agents for magnetic resonance imaging. Biomater. 2005; 26:5695–5703.
19. Miyazaki S, Kawasaki N, Kubo W, Endo K and Attwood D. Comparison of insitu gellig formulations for the oral delivery of cimetidine. Int. J Pharm. 2001; 200:161-168.
20. Kubik H, Muller BW. Rheological properties of polymer solutions as carrier for nasal drug delivery systems. Eur. J. Pharm and Biopharm. 1993; 39:192-196.
21. Dashora K, and Saraf S. Effect of processing variables on micro particulate system of aceclofenac. Pak J Pharm Sci. 2006; 19:6-10.
22. Martin A, Swarbrick J and Cammratha A. Physical pharmacy: Physical and chemical principles in the pharmaceutical sciences. Indian Edition by Verghese Publishing House, Bombay: 3rd ed. 1991; 494-495
23. Singh C, Jain KA, Kumar C and Agrawal KA. Design and in vitro evaluation of mucoadhesive microspheres of pioglitazone. J. Young Pharm. 2009; 1(3):195-198.
24. Jian-Hwa Guo, PhD. Carbopol polymers for pharmaceutical drug delivery applications. Excipient Updates. Drug Delivery Technology. September 2003; 3 (6)
25. Gohel MC, Parik RK, Amin AF and Surati AK. Preparation and formulation optimization of sugar cross linking gelatin microspheres of diclofenac sodium. Indian J. Pharm Sci. 2005; 67(8):575-581.