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Design, Evaluation and Statistical Optimisation of a Controlled Release Multiparticulate Acyclovir Delivery System

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SUMMARY. This work aims at designing and evaluating a multiparticulate controlled release dosage form of Acyclovir using Ethyl Cellulose as the matrix-forming polymer employing the solvent evaporation technique of microencapsulation. The product was characterized by physicochemical parameters such as yield (51%-86%), drug entrapment efficiency (88% - 96%), particle size (mainly #30 mesh ASTM), surface topography, drug-excipient compatibility and *in vitro* release (11% - 81% after 8 h). The controlled release profile was optimized using a simplex lattice mixture design for achieving the correct blend of microparticles (proportion of particles of drug-polymer ratio 1:1 to 1:2 = 68%: 32% by weight) that closely matches the target release profile. The error between the target (% release at 2nd, 6th and 8th h of 40, 60 and 75 respectively) and optimum blend of the microparticles was less than 10%. The study illustrates the utility and advantage of designed experimentation in controlled drug delivery research.

RESUMEN. "Diseño, Evaluación y Optimización Estadística de un Sistema Multiparticulado de Acyclovir de Liberación Controlada". El presente trabajo tiene por objeto el diseño y la evaluación de una forma de liberación controlada de Acyclovir utilizando etilcelulosa como polímero formador de la matriz y empleando la técnica de evaporación del solvente para la microencapsulación. El producto fue caracterizado por parámetros fisicoquímicos tales como el rendimiento (51%-86%), la eficiencia de retención de la droga (88% - 96%), el tamaño de partícula (principalmente tamiz de malla 30 ASTM), la topografía superficial, la compatibilidad droga-excipiente y la liberación *in vitro* (11% - 81% después de 8 h). El perfil de liberación controlada fue optimizado utilizando una mezcla de látex sencilla diseñada para lograr la combinación más adecuada de las micropartículas (proporción de partículas de relación droga-polímero 1:1 a 1:2 = 68%:32% de peso), que es el más apropiado para el perfil de liberación. El error entre el blanco perseguido (% de liberación a las 2 h, 6 h y 8 h de 40, 60 y 75, respectivamente) y la combinación óptima de las micropartículas fue menor que el 10%. El estudio demuestra la utilidad y ventajas del diseño experimental en la investigación de la liberación controlada de drogas.

INTRODUCTION

Acyclovir is an acycloguanosine analogue effective against Herpes Simplex Virus (HSV-1), Herpes Zoster and certain strains of Varicella Zoster virus (VZV). It is seen that, even after the symptoms recede, the HSV-1 may remain undetected and dormant in the body at the nerve trunks. Acyclovir has often been reported to be less toxic and more effective than many other antivirals of its class. However, it has a low half-life (2-3 hs) and requires at least 5 dosage/day for effective therapy, which causes patient incompliance and therefore failure of therapy [1], not to mention the high cost of therapy that often is a reason for discontinuation of the regimen. In such cases for complete eradication and treatment of both acute and chronic phases of Herpes, a Sustained Release Drug Delivery System would be beneficial which can maintain the required plasma level. The virus in most cases becomes chonic and maintains a latent phase in the biosystem [2] and this warrants prolonged low dose Acyclovir therapy. Various multiparticulate Drug Delivery Systems of Acyclovir have been designed for this purpose utilizing such varied polymers as poly (DL-lactide-co-glycolide, PLGA) [3], polybutylcyanoacrylate [4] and chitosan [5] with the aim of achieving greater control over

KEY WORDS: Acyclovir; Controlled release; Microparticle; Mixture design; Optimization.
PALABRAS CLAVE: Acyclovir, Diseño de mezclado, Liberación controlada, Micropartícula, Optimización.

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release rate in the biosystem and enhancing the bioavailability. The controlled release multiparticulate formulations could be designed to release small amounts of the antiviral drug slowly but effectively over a long period of time and prevent the latent phase of the viral residues and thereby prevent the relapse by passage into either the respiratory or peripheral reproductive and urinary systems. Acyclovir-loaded microparticles have shown to be promising carriers for the effective delivery of acyclovir in the treatment of HSV-1 infections in cell cultures so they can have a potential use *in vivo* [6]. This type of suppressive therapy has shown to be highly effective in randomized controlled trials [7]. Therefore, an attempt has been made in this investigation to develop an economically viable microparticulate acyclovir delivery system for better control of chronic recurrent herpes utilizing the industrially widely used polymer ethyl cellulose and employing emulsification solvent evaporation technique of microencapsulation. The release rate optimization has been achieved using simplex mixture design.

MATERIALS AND METHODS

Materials Used

Acyclovir was generously supplied by Torrent Pharmaceuticals Ltd., India; Ethyl Cellulose, degree of substitution 2.43-2.53, Viscosity ≈14 cp (measured for a 5% w/w solution in 80:20 toluene:ethanol by volume at 25 °C), Central Drug House(P) Ltd., India; Acetone ExcelaR, Glaxo (India) Ltd.; Light and Heavy Liquid Paraffin I.P, Caxton Pharmaceuticals, India; Petroleum Ether (40°-60 °C) ExcelaR, Glaxo (India) Ltd.; 'SQ' Hexane (Fraction from Petroleum) 65-70ºC (95%), Glaxo (India) Ltd.

Preparation of the Microcapsules

Microcapsules of Acyclovir were prepared by solvent evaporation technique of microencapsulation [8]. The microcapsules were washed with petroleum ether (40°-60 °C) or hexane for thee to four times and were stored overnight in a desiccator over anhydrous Calcium chloride. By varying the amount of polymer, different batches of microcapsules were prepared.

Physicochemical Studies of the microcapsules

Yield of the microcapsules was calculated by weighing the product after overnight desiccation using the formula: Yield % = (Wt. of microcapsules obtained / Total weight of drug + polymer) x 100.

Particle size analysis was carried out by standard sieving method using a nest of ASTM sieves placed on a mechanical shaker.

Drug entrapment efficiency. The microparticles were crushed and acyclovir was extracted using distilled water by stirring the crushed powder in distilled water for 2 h on a magnetic stirrer around 100 rpm.

Surface characteristics study was done by Scanning Electron Microscope, using -Hitachi - 415A Scanning Electron Microscope. The particles were coated with a thin gold film using sputter coater. Different accelerating voltages were used and the electron beam was focussed on the gold-coated particles perpendicularly.

Drug-excipient interaction was studied using Infrared spectroscopy utilizing JASCO 500 IR Spectroscope.

Dissolution Studies [9]. *In vitro* dissolution studies for all the preparations were carried out in USP Apparatus type I [DISSO 2000, Labindia, Chennai, India] at 100 rpm at a temperature of 37° ± 0.5 °C. The release medium was 900 ml of 0.1 N hydrochloric acid. Aliquots of 5 ml were withdrawn at predetermined intervals and replenished with 5 ml of 0.1 N HCl to maintain sink conditions. Aliquots, after suitable dilution, were assayed spectrophotometrically against suitable blank at 255 nm with reference to a calibration curve prepared previously.

RESULTS AND DISCUSSION

The microparticles prepared by solvent evaporation technique were found to be spherical, white to off-white in colour and free flowing in nature with occasional small aggregates. The results of the various physicochemical studies on the developed microparticles are presented below.

Yield

It was found that the yield of the products varied from 51 to 86% (Table 1). The yield varied inversely with the polymer loading, presumably due to polymer loss at high viscosity of the slurry during preparation.

Particle size analysis

It was observed that the size distribution of the microparticles varied from #12 to #60 mesh ASTM. But in each case maximum weight was retained on sieve #30 and the mean size (μ) ± sd is reported (Table 2) which appears to be optimum for the solvent evaporation method at low speed.

Product code	Drug:Polymer	% yield	% Drug Entrapment efficacy	% Release after 8 h
F1	1:1	86	89.93	81.72
F2	1:2	75	96.67	31.26
F3	1:3	62	88.32	20.11
F4	1:4	51	89.00	11.11

Table 1. Characteristics of the microcapsules.

Formulation (Drug: Polymer)	% Weight retained on ASTM Sieve Nos.								Weight average Mean Size (μ)	SD (μ)
	#12	#16	#20	#30	#40	#60	#80	#140		
F1 (1:1)	0	0	12.76	53.75	18.71	10.75	4.03	0	544.61	57.48
F2 (1:2)	6.23	4.97	5.90	26.18	14.05	10.04	13.58	19.05	501.24	148.35
F3 (1:3)	4.64	1.76	5.17	20.5	17.05	15.57	4.47	0	563.45	159.72
F4 (1:4)	0.374	0.629	8.195	80.29	8.89	1.62	0	0	607.03	138.73

Table 2. Particle size analysis data.

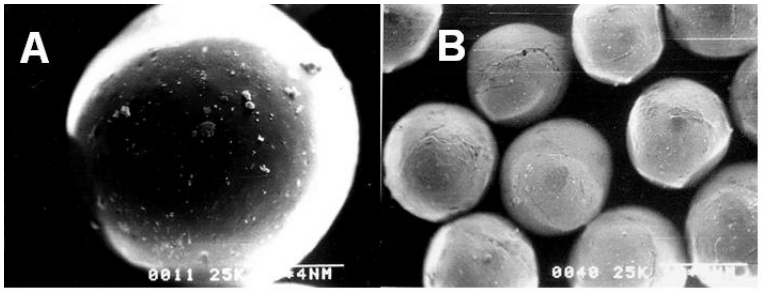

Figure 1. SEM Photographs of Acyclovir microcapsules before and after dissolution. **A**: SEM Photograph of microcapsules (before dissolution). **B**: SEM Photograph of microcapsules (after dissolution).

Drug entrapment analysis

The microparticles showed high drug entrapment efficiency ranging from 88% to 96% (Table 1).

Surface characteristics study

SEM study revealed that microcapsules were spherical in shape, with minute pores and occasional aggregates. Their size did not change after dissolution, though the number and size of the pores increased after drug release commensurate with the insoluble nature of ethyl cellulose (Fig. 1).

Drug-Excipient interaction study

The IR spectroscopy revealed that there was no interaction between drug and polymers and they are mutually compatible (data not shown). The IR spectra of acyclovir and drug containing microparticles revealed broad bands between 3500-2900 cm^{-1} corresponding to stretching vibrations of N–H, O–H, and Ar C–H, all superimposed on each other. However, the broadened peaks did not disappear on dilution of the samples indicating that the broadening is due to intramolecular H-bonding between –C=O and –NH groups present in the acyclovir molecule which is reported to exist in keto form in solid state and in polar solvents [10]. On the other hand, the polymer functional group peaks did not alter significantly. It appeared, therefore, that H-bonding centers in acyclovir becomes preoccupied in intramolecular bridging, and hence, are unavailable for intermolecular interactions with lone pair of electrons available on oxygen atom in the side chains of ethyl cellulose backbone. Therefore, it may be safely inferred that there is no potential interaction existing between acyclovir and ethyl cellulose. Hence, they are mutually compatible.

Dissolution study

The *in vitro* drug dissolution study as per USP indicated that the microparticles released drug in accordance to their drug-to-polymer ratio. With increasing polymer load, drug release slowed down as expected. The release varied widely from about 10% for drug-polymer ratio

Formulation Drug: Polymer)	R^0	R^1	R^{HC}	R^{HG}	Model of Best Fit
F1 (1:1)	0.9509	0.9948	0.7542	0.9978	Higuchi
F2 (1:2)	0.9432	0.9986	0.7346	0.9958	Higuchi
F3 (1:3)	0.9652	0.9860	0.7776	0.9982	Higuchi
F4 (1:4)	0.9761	0.9792	0.7902	0.9971	Higuchi

Table 3. Correlation Coefficients Of Four Models Of Release Kinetics. [R^0 = Correlation Coefficient for Zero Order, R^1 = Correlation Coefficient for First Order, R^{HC} = Correlation Coefficient for Hixon-crowell, R^{HG} = Correlation Coefficient for Higuchi.]

Formulation (Drug: Polymer)	K^0	K^1	K^{HC}	KHG
F1 (1:1)	9.206	-0.086	-0.334	29.209
F2 (1:2)	3.373	-0.018	-0.233	10.799
F3 (1:3)	2.322	-0.011	-0.216	7.289
F4 (1:4)	1.291	-0.006	-0.178	3.999

Table 4. Release Rate Constants of The Four Models Of Release Kinetics [K^0 = Release Rate Constant for Zero Order, K^1 = Release Rate Constant for First Order, K^{HC} = Release Rate Constant for Hixon-crowell, K^{HG} = Release Rate Constant for Higuchi.]

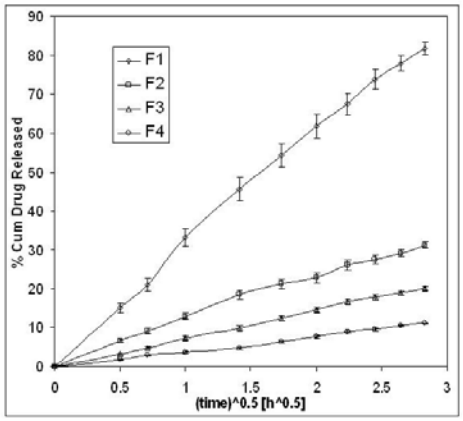

Figure 2. Comparative Release profiles of Acyclovir loaded microcapsules.

1:4 to approximately 80% for drug-polymer ratio 1:1 after 8 h of dissolution (Table 1, Fig. 2). After performing the *in vitro* dissolution study, release data were subjected to linear regression analysis to investigate the release kinetics of best fit. The release kinetics models investigated statistically were compared for best fit with respect to their correlation coefficients.

Tables 3 and 4 give the values of R (correlation coefficient) and K (release rate constant/regression coefficient) for different release kinetics models investigated. In all cases, it was observed that the regression coefficient was highest for the Higuchi model. So the release pattern of microcapsules followed Higuchi Release Kinetics. Since microcapsules are actually monolithic or matrix systems, the release of drug from multiparticulate dosage forms should be expected to follow diffusion controlled model in accordance to Higuchi [11].

The plot (Fig. 2) of the amount of Acyclovir released from ethyl cellulose microcapsules vs. Square root of time indicated the amount of acyclovir released from micropellets increased linearly with square root of time ($Q = Kt^{1/2}$). Further analysis of the release data by subjecting to Ritger-Peppas model yielded the 'n' and 'k' parameters, which showed that the n values for all the formulations were within the theoretical limit $0.43 < n < 1.0$ (non-swellable matrix) for anomalous release, though the values were close to the theoretical 'n' (= 0.43) for diffusion dependent release [12] (Table 5 and Figure 3). This proves that the nature of drug release is predominantly controlled by pore diffusion commensurate with Higuchian release [13] which is characteristic of the non-swellable matrices.

Further it was found during dissolution studies, that the particles remained buoyant (data not shown) in the dissolution fluid for more than 8 hours indicating their potential as gastroretentive systems.

Statistical optimization of the drug release

The release profile of the developed microparticles with varying drug-polymer ratio indicated a release proportional to the drug load. Hence, the next step taken was to optimize this

Formulation	n	k
F1 (1:1)	0.498	3.941
F2 (1:2)	0.451	1.958
F3 (1:3)	0.538	0.750
F4 (1:4)	0.534	0.407

Table 5. Ritger-Peppas parameters [n = Release exponent, K = Release rate constant].

release profile to fit a suitable controlled release profile [1]. A simplex lattice mixture design was adopted to obtain 20 different blends of microparticles and their release profile was obtained (Table 6). The % by weight of microcapsule of each drug-polymer ratio was taken as the independent variables and the % cumulative drug release at 2nd, 6th and 8th h were taken as the response variables for the optimization (Table 6). Polynomial models were generated for each of the response variables via multiple least square regression analysis and the models were validated though ANOVA as well as other diagnostic statistics (Table 7).

The model equations indicate a complex non-linear relationship between the independent variables and the response except for CR8 which showed linearity with the proportion of each formulation in the blend. This was followed by numerical optimization of the model

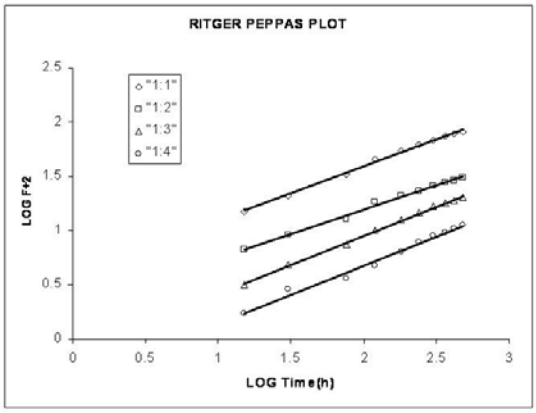

Figure 3. Ritger-Peppas Plot for the formulations.

equations (Equations 1 -3) with set target of %CR2 = 40, %CR6 = 60 and %CR8 = 75. The predicted blend of microparticles obtained was F1:F2:F3:F4 = 0.68 : 0.32 : 0 : 0 with high desirability statistics nearing 1. The blend was then actually formulated and subjected to dissolution study. The % error of the optimized release profile in comparison to the target controlled release profile was minimal (Table 8 and Figure 4). Hence, the product could be optimized by blending microparticles with drug polymer ratio of 1:1 and 1:2 in a ratio of 68% to 32% to achieve the desired controlled release profile.

Code	Ethyl Cellulose Formulations				Responses Modelled		
	F1	F2	F3	F4	CR2	CR6	CR8
S1	1	0	0	0	45.63	73.81	81.72
S2	0.5	0.5	0	0	32.06	50.68	56.48
S3	0.5	0	0.5	0	27.76	45.87	50.92
S4	0.5	0	0	0.5	25.18	41.71	46.42
S5	0	1	0	0	18.49	27.55	31.26
S6	0	0.5	0.5	0	14.19	22.74	25.68
S7	0	0.5	0	0.5	11.61	18.58	21.18
S8	0	0	1	0	9.89	17.93	20.11
S9	0	0	0.5	0.5	7.31	13.77	15.61
S10	0	0	0	1	4.73	9.61	11.11
S11	0.625	0.125	0.125	0.125	32.66	53.02	58.88
S12	0.125	0.625	0.125	0.125	19.09	29.89	33.65
S13	0.125	0.125	0.625	0.125	10.32	25.08	28.07
S14	0.125	0.125	0.125	0.625	12.21	20.92	23.58
S15	0.25	0.25	0.25	0.25	19.68	32.22	36.05
S16	0	1	0	0	18.49	27.55	31.26
S17	0	0	1	0	9.89	17.93	20.11
S18	1	0	0	0	45.63	73.81	81.72
S19	0	0	0	1	4.73	9.61	11.11
S20	0	0.5	0.5	0	14.19	22.74	25.68

Table 6. Blended formulations as per Simplex Lattice mixture design [CRx = % cumulative drug release at x th hour].

Response Parameter	Model obtained [n = 20]	F_0	p < F	Std. Dev.	$R^2_{adj.}$	Press	No
%CR2 =	Logit(CR2) = Ln[(CR2 - 4.28)/ (47.76 - CR2)] = +3.53769 * F1 -0.66713 * F2 -1.81897 * F3 -3.54151 * F4 -3.13434 * F1 * F2 -3.36745 * F1 * F3 +0.15794 * F1 * F4 +0.73999 * F2 * F3 +3.54132 * F2 * F4 +2.31497 * F3 * F4	109.05	< 0.0001	0.26	0.9808	3.53	1
%CR6 =	Sqrt(CR6) = +8.60770 * F1 +5.36411 * F2 +4.35541 * F3 +3.32796 * F4 +0.74643 * F1 * F2 +4.15647 * F1 * F3 +1.72514 * F1 * F4 +0.16751 * F2 * F3 +0.30971 * F2 * F4 +4.67839E-003 * F3 * F4	791.86	< 0.0001	0.083	0.9973	0.77	2
%CR8 =	CR8 = +82.66131 * F1 +32.72964 * F2 +21.93873 * F3 +12.22313 * F4	8489.79	< 0.0001	0.57	0.9993	8.58	3

Table 7. Model equations and diagnostic statistics.

	Predicted microparticle Blends (Total = 1)				Response Parameters			Desirability Statistics
Formulation	**F1**	**F2**	**F3**	**F4**	**%CR2**	**%CR6**	**%CR8**	
Predicted Optimum Blend	0.68	0.32	0.00	0.00	40	59.9296	66.8384	0.939
Experimental Blend	0.68	0.32	0.00	0.00	37.61	57.87	68.18	
				Target release % =	40	60	75	
				% Error of prediction w.r.t target data =	5.98	3.44	2.01	
				% Error of experimental w.r.t target data =	5.98	3.55	9.09	

Table 8. Predicted and optimised Microparticle Blends and Release Parameters.

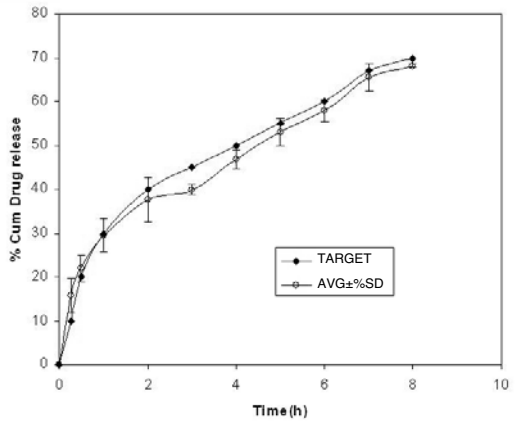

Figure 4. Comparison of Target and Optimised release profile of the Acyclovir microcapsules.

CONCLUSION

It is expected that the developed formulation would be a better candidate with ease of industrial production and robust enough to ensure a sustained plasma drug level of acyclovir for better therapy of Herpes and other conditions. This type of delivery systems ensures slow release of drugs in the stomach while being buoyant for long time. This ensures that the dosage form would not be emptied easily from the stomach and hence would not quickly overshoot the major absorption zone of the drug. Further, slow release in stomach would ensure that the drug arrives and stays at the absorption site for a prolonged period, thereby improving its bioavailability. This can lead to reduction of the therapeutic dose and hence an affordable therapy. The experimental design supported formulation development is also more predictable and economic through which a drug delivery system could be developed in a well planned manner and in much less time than the trial and error methodology.

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