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Research Article

BIOAVAILABILITY ENHANCEMENT OF ACYCLOVIR BY NANOSUSPENSION METHOD

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Abstract

The purpose of this study was to review about nanosuspension to enhance the bioavailability of Acylovir (ACL), a anti-viral agent with limited oral bioavailability. Size reduction to a nanoscale is a relatively new approach to overcome solubility and bioavailability issues of many drugs such as acyclovir (ACL). Acyclovir nanosuspensions were prepared by the precipitation-ultra sonication technique.The precipitation step was carried out by the addition of saturated drug solution in antisolvent solution (water and surfactant mixture) with stirring for pre-decided time interval subsequently the sample was subjected to probe sonication. The Acyclovir (200mg) and Eudragit RS100/RL100 were co-dissolved in 5 ml of dimethyl sulfoxide (DMSO) at 40°C to form uniform organic solution. *In vitro* diffusion studies of eight formulations of acyclovir nanoparticles were carried out by franz diffusion cell using pH 7.4 phosphate buffer. The cumulative percentage of the drug dissolved was 97.9 % at 120 min for selected nanosuspension (F4) while the cumulative percentage of the pure drug was 48 % at 120 min.

Keywords: Acylovir, Nanosuspension, *vitro* diffusion, precipitation-ultra sonication.

Introduction : Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. The main advantage of topical delivery system is to bypass first pass metabolism. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time are other advantage of topical preparations. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solution, and even medicated adhesive systems are in use. The topical drug delivery system is generally used where the others system of drug administration fails or it is mainly used in pain management, contraception, and urinary incontinence An ideal drug therapy achieves effective concentration of drug at the target for a specified period of time in order to minimize general and local side effects.

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An exciting challenge for developing suitable drug delivery systems targeted for ocular diseases is one of today's major focuses of pharmaceutical scientists.
Nanoparticles have become one of the most active areas of research in the field of drug delivery due to their ability to deliver drugs to the right place, at appropriate times, and in the right dosage. They have received considerable attention over the past 20 years due to their advantages compared to other drug delivery systems.[1-9]

Materials and Methods :

Procurement of drug and excipients:

The drug, excipients, chemicals/ reagents and equipments used for various experiments are enlisted as follows:
Acyclovir was gifted by ZCL chemicals Ltd. Mumbai, Maharashtra, India. SLS, Eudragit RS 100, Eudragit RL 100 and DMSO were purchased from Yarrow chemicals Mumbai, Maharashtra and are of AR grade.

Formulation of Polymeric Nanosuspension [10-16]

Acyclovir nanosuspensions were prepared by the precipitation-ultra sonication technique. The precipitation step was carried out by the addition of saturated drug solution in antisolvent solution (water and surfactant mixture) with stirring for pre-decided time interval subsequently the sample was subjected to probe sonication.
The Acyclovir (200mg) and Eudragit RS100/RL100 were co-dissolved in 5 ml of dimethyl sulfoxide (DMSO) at 40°C to form uniform organic solution. The solution was to be slowly injected with a syringe (at 0.5ml/mit rat) containing thin Teflon tube into 2% w/v, 40 ml water and alcohol mixture solution containing stabilizer SLS and it was maintained at low temperature in ice bath protected from sun light. During injection the mixture was stirred well by a high speed homogenizer at 8000 rpm speed. The solution immediately turned into pseudo emulsion of the drug and polymer solution in the external aqueous phase. Nanoparticles were spontaneously formed and turned the solution slightly turbid. Sonicate it with probe sonicator EI of 600 watt for 20 mint. Then, prepared nanosuspension was then stirred magnetically at 500 rpm at room temperature for 12 h to evaporate organic solvent.
The resulting particle suspension was filtered through 1.2 µm cellulose nitrate membrane filter in order to remove larger particle aggregates. Formulation were prepared with varying polymer & stabilizer ratio overall 8 formulation of drug Acyclovir were prepared with two

different polymer Eudragit RS100 & Eudragit RL100 with a stabilizer such as SLS .The prepared formulation were code as F1,F2,F3,F4,F5,F6,F7 and F8.

Evaluation of Nanosuspentions[12-20]

Particle size analysis:

Scanning electron microscopy (SEM) is a method for high resolution surface imaging. The SEM uses an electron beam for surface imaging. The advantages of SEM over light microscopy are greater magnification and much larger depth of field. Different elements and surface topographies emit different quantity of electrons, due to which the contrast in a SEM micrograph (picture) is representative of the surface topography and distribution of elemental composition on the surface.

From all the formulation F4 formulation was subjected to the particle size determination and the particle size was determined and recorded.

Percentage of drug entrapment in the polymeric nanosuspension:

Percentage Entrapment efficiency:

In order to determine the % entrapment around 2 ml of formulation were taken in the Nessler's cylinder tube (10 ml) the solution was centrifuge in the centrifuge machine at 2000-3000 rpm for 4 hrs. The supernatant layer was filter through whatmann filter paper number 41and diluted with phosphate buffer 7.4 pH up to 10 ml and the resultant solution were analyse at particular wavelength of drug in nm using UV Double beam Spectrophotometer These was carried out for three time and the result were calculated .

***In-vitro* drug Release studies**

The *in vitro* drug release of acyclovir nanoparticles were studied by using Franz diffusion apparatus. Freshly prepared pH 7.4 phosphate buffer was used as diffusion medium. Cellophane membrane previously soaked overnight in the distilled water was tied to one end of a specially designed glass cylinder (open at both ends). Accurately measured 1ml of nanosuspension was placed into this assembly. The cylinder was fixed to a stand and suspended above the receptor compartment containing 150 ml of diffusion medium maintained at 37± 0.5°C, so that the membrane just touched the receptor medium surface.

The diffusion medium was stirred at 50 rpm using magnetic stirrer. Aliquots, each of 1 ml volume was withdrawn at regular time intervals and replaced with equal volume of receptor medium. The aliquots were suitably diluted with receptor medium and analyzed by UV-Vis Spectrophotometer at 253 nm.

Stability studies

Stability studies were carried out at 2-8ºC and 30±2ºC/65±5% RH for optimized acyclovir nanoparticles (F4) for 60 days. The results of the stability study are shown in Table 7.5. The results showed no significant difference in the entrapment efficiency and cumulative drug release. There was no statistically significant difference between the initial results and the results obtained during stability studies.

Results and Discussion

Size analysis:

Particle size analysis/evaluation was carried out with Scanning electron microscopy (SEM) Make: JEOL Model JSM-6390lv Particle size in the nanosuspension is important because as the particle size reduce there is increase in the surface area which will result in the increase in the dissolution rate. As the particle goes in the nano more improving the dissolution of the poorly water soluble drug. The particle size of all the formulations were found in the range of 48.3 to 356.1nm.

Percentage drug unincorporated and entrapped for Acyclovir nanosuspension:

The percentage entrapment determination is equally important because during the formulation polymer form a polymeric coat and the drug is entrap inside it that will release slowly depend upon the polymer used Eudragit RS100/Eudragit RL100 release the drug slowly and sustained from the polymeric nanosuspension these was determined by appropriate method given in material and method. The entrapment efficiency of all the formulations was found in the range of 14.28 ± 0.25to 79.82 ± 0.01%. The results were shown in Table 2.

***In-vitro* drug Release studies**

In vitro diffusion studies of eight formulations of acyclovir nanoparticles were carried out by franz diffusion cell using pH 7.4 phosphate buffer. The observations of in vitro drug release were shown Fig 1. The most important feature of nanoparticles is the increase of the dissolution rate not only because of increase in surface area but also because the use of hydrophilic surfactant. The *In vitro* dissolution of acyclovir was carried out for all of the prepared nanosuspensions formulations and then compared to that of the pure drug powder. The cumulative percentage of the drug dissolved was 97.9 % at 120 min for selected nanosuspension (F4) while the cumulative percentage of the pure drug was 48 % at 120 min. The difference was significance at $p<0.05$ when t-test for unpaired data was applied, and the release kinetics was found to obey first-order kinetics with $R^2>0.98$ (table 3).

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Stability Studies

Stability studies were carried out at 2-8ºC and 30±2ºC/65±5% RH for optimized acyclovir nanoparticles (F4) for 60 days. The results of the stability study are shown in Table 4. The results showed no significant difference in the entrapment efficiency and cumulative drug release. There was no statistically significant difference between the initial results and the results obtained during stability studies.

Conclusions

The purpose of this study was to review about nanosuspension to enhance the bioavailability of Acylovir (ACL), a anti-viral agent with limited oral bioavailability. Size reduction to a nanoscale is a relatively new approach to overcome solubility and bioavailability issues of many drugs such as acyclovir (ACL). However, nanoparticles and specifically nanosuspensions can overcome many unstable conditions arises during the formulation of liquid formulations like aggregation and sedimentation. On the basis of this preliminary study we will try to enhance the biovailability of acyclovir by nanosuspension methods and its evaluations in the next semester.

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Table 1 : Formulation of Acyclovir polymeric Nanosuspension contents

Batch	Drug (mg)	Polymer (mg)		Surfactant		Distilled water: Ethanol (mL)
		Eudragit RS100	Eudragit RL100	SLS(%)	Poloxamer 407 (%)	
F1	200	40	-	1.0	0.5	10 : 20
F2	200	80	-	1.0	0.5	10 : 20
F3	200	40	-	1.0	1.0	10 : 20
F4	200	80		1.0	1.0	10 : 20
F5	200	-	40	1.0	0.5	10 : 20
F6	200	-	80	1.0	0.5	10 : 20
F7	200	-	40	1.0	1.0	10 : 20
F8	200	-	80	1.0	1.0	10 : 20

Table 2: Percentage drug unincorporated and entrapped for nanosuspension

Formulation Code	% Drug Unincorporated	Entrapment* efficiency (%)
F1	19.80	80.20
F2	16.31	83.69
F3	14.92	85.08
F4	**8.78**	**91.22**
F5	25.88	74.12
F6	26.12	73.88
F7	31.76	68.24
F8	32.11	67.89

**All the values are expressed as mean ±Standard deviation; n=3*

Table 3 : Cumulative Percentage drug release of acyclovir nanosuspension formulated with Eudragit RS100.

S.No	Time (Min.)	F1	F2	F3	F4
1	5	19.2	15	22.1	21.3
2	15	33	29.3	37.5	42
3	30	41.5	38.7	45	58.3
4	45	53.7	47	57.1	71.6
5	60	56	52.2	63.6	82.4
6	90	67.2	61.5	75.8	89.6
7	120	78	72.2	81	97.9

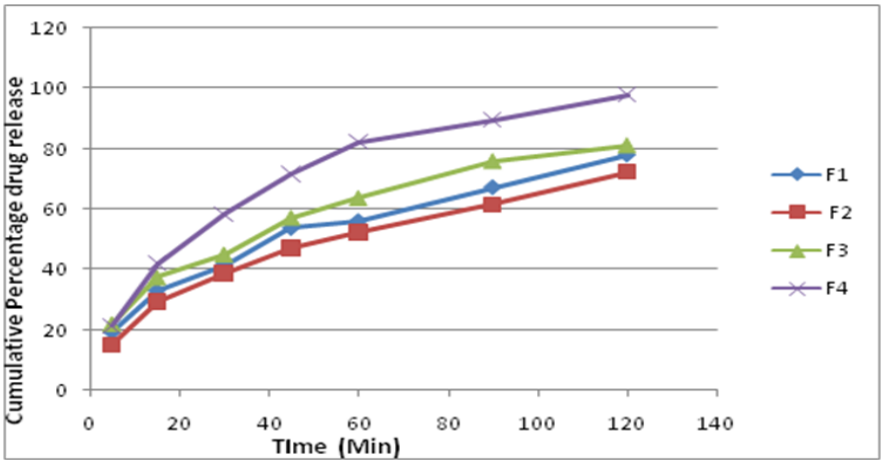

Fig. 1: *In vitro* release profile of Acyclovir F1, F2, F3 and F4 in phosphate buffer pH 7.

Table 4: Stability studies of acyclovir nanoparticles (F4)

Formulation Code	30 ± 2°C & 65 ± 5% RH for 60 days		2 - 8°C for 60 days.	
	In vitro drug release * (%)	Drug entrapment efficiency * (%)	*In vitro* drug release * (%)	Drug entrapment efficiency * (%)
F4	96.21	78.86	96.16	79.23

**All the values are expressed as mean ±Standard deviation; n=3*