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FORMULATION AND EVALUATION OF MICROSPHERES OF NATURAL GUM CONTAINING CIDOFOVIR, AN ANTIVIRAL DRUG

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Abstract: In the present work, microsphere formulation to enhance transdermal permeation of was prepared and evaluated. Colloidal suspensions of microsphere were prepared by reported method. Microsphere system was found to be easy to prepare and composed mainly of phospholipids and ethanol, compounds commonly found in pharmaceutical preparations. The average vesicle size of optimized formulations determined by Malvern Zeta master was 1.112±0.053 µm. The smooth surface of vesicles was confirmed by SEM. The entrapment efficiency of microsphere was determined for all formulations. Effect of ethanol concentration was observed on percent drug entrapment of microsphere. The maximum entrapment efficiency was found to be 90.06±0.79% for formulation ETE3 and minimum 52.36±0.82% for formulation ETE5, respectively. The drug content after treatment with triton X100 and % residue of cidofovir was calculated. Only 2±1% degradation was observed at 37±2°, at room temperature only 3% degradation was observed however all formulations were almost stable at 8±2° and 4±2° and only 1.57±0.2% degradation of Cidofovir was observed showed that developed system is stable at low temperature. These flexible vesicles may become a promising carrier for the transdermal delivery of Cidofovir. It helps in reducing the dose of drug applied topically.

Keywords: Cidofovir, Microsphere, FT-IR

Introduction: Microspheres are polymeric micron range particles with sizes from 1 to 1000 µm .These microspheres are used for drug delivery, wherein the drug can be encapsulated or entrapped form. Based on the polymeric composition of microspheres, they can be classified into two types: natural and synthetic. Natural polymers include carbohydrates (e.g., chitosan, agarose, starch, alginate) and proteins (e.g., albumin, gelatin), while synthetic polymers include non-biodegradable (e.g., polymethyl methacrylate, epoxy polymers) and biodegradable (polylactic acid/polyglycolic lactic acid). Microspheres in drug delivery are used for targeted as well as prolonged drug release in the diseased area. It also protects the unstable or pH-sensitive drugs before and after the administration. There are two types of microspheres: Microcapsules and Micromatrices. Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall. And Micromatrices are those which entrapped substance is dispersed throughout the matrix. Microspheres are sometimes referred to as microparticles.

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Ideal characteristics of microspheres:

•The ability to incorporate reasonably high concentrations of the drug. Stability of the preparation after synthesis with a clinically acceptable shelf life. • Controlled particle size and dispersability in aqueous vehicles for injection. •Release of active reagent with a good control over a wide time scale. •Biocompatibility with a controllable biodegradability. •Susceptibility to chemical modification.

Methods of Preparations

1. Spray Drying: In Spray Drying technique, the polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution with high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100µm.

2. Solvent Evaporation: This process is carried out in a liquid manufacturing vehicle phase. The microcapsule coating is dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microcapsule.

3. **Single emulsion technique:** The micro particulate carriers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium like oil. In the next step, the cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using the chemical cross linkers. The chemical cross linking agents used are glutaraldehyde, formaldehyde, acid chloride etc.

4. Double Emulsion Technique: Double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited for water soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase.

5. Phase separation coacervation technique: This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer.

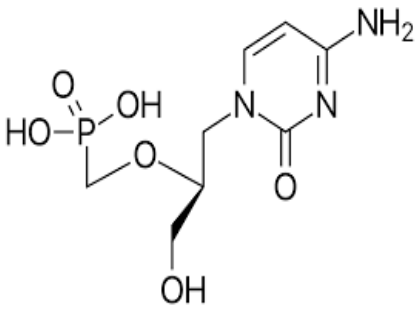
6. Spray drying and spray congealing: These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization.

7. Solvent extraction: Solvent evaporation method is used for manufacturing of microparticles, involves removal of the organic phase by extraction of or non aqueous solvent. This method involves water miscible organic solvents as isopropanol. Organic phase can be removed by extraction with water. This process decreases the hardening time for the microspheres.

8. Quassi emulsion solvent diffusion: A novel quasi-emulsion solvent diffusion method to manufacture the controlled release microspheres of drugs with acrylic polymers has been reported in the literature. Microsponges can be manufactured by a quasi emulsion solvent diffusion method using an external phase containing distilled water and polyvinyl alcohol. The internal phase consists of drug; ethanol and polymer. The concentration of polymer is in order to enhance plasticity. At first, the internal phase is manufactured at 60ºC and then added to the external phase at room temperature. After emulsification process, the mixture is continuously stirred for 2 hours.

Materials and Method: Cidofovir was obtained as a gift sample from Alembic Ltd., Vadodara, India. Soya lecithin purchased by Acros Organics, New Delhi, India, Triton-X 100 was purchased by Himedia Laboratories Pvt. Ltd. Mumbai, India and Methanol, Propylene Glycol, Ethanol was purchased by Merck India Ltd. Mumbai, India.

Structure of Cidofovir



Chemical Formula : $C_8H_{14}N_3O_6P$

Synonyms: Cidofovir, Cidofovirum

IUPAC Name: ({[(2S)-1-(4-amino-2-oxo-1,2-dihydropyrimidin-1-yl)-3-hydroxypropan-2-yl]oxy}methyl)phosphonic acid

Description:

Cidofovir is an injectable antiviral medication employed in the treatment of cytomegalovirus (CMV) retinitis in patients diagnosed with AIDS. It suppresses CMV replication through selective inhibition of viral DNA synthesis. It was manufactured by *Gilead* and initially approved by the FDA in 1996, but has since been discontinued.[1]

Pharmacodynamics:

Cidofovir is a new anti-viral drug. It is classified as a nucleotide analogue and is active against herpes cytomegalovirus (CMV) retinitis infection. Most adults are infected with CMV. Cidofovir suppresses cytomegalovirus (CMV) replication by selective inhibition of viral DNA synthesis.

Mechanism of action:

Cidofovir acts through the selective inhibition of viral DNA polymerase.Biochemical data support selective inhibition of CMV DNA polymerase by cidofovir diphosphate, the active intracellular metabolite of cidofovir. Cidofovir diphosphate inhibits herpesvirus polymerases at concentrations that are 8- to 600-fold lower than those needed to inhibit human cellular DNA polymerase alpha, beta, and gamma(1,2,3). Incorporation of cidofovir into the growing viral DNA chain results in reductions in the rate of viral DNA synthesis.

Half-life: 2.4 to 3.2 hours

Toxicity: Kidney damage, fall in the number of white blood cells, decreased platelets

Pre-formulation Studies: Pre-formulation may be described as a phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of new drug substances, in order to develop stable, safe and effective dosage forms. These studies are designed to determine the compatibility of initial excipient with the active substance for a biopharmaceutical, physicochemical and analytical investigation in support of promising experimental formulations.

Organoleptic evaluation: It is the initial evaluation during Pre-formulation studies which assess the colour, odor and taste and appearance of the substance.

Solubility: Solubility is an important physicochemical property of drug substance, which determines its systemic absorption and in turns its therapeutic efficacy. Solubility of drug was determined in different solvents.

Melting Point: Melting point of drug was determined by Open capillary method.

Loss on drying: Weigh accurately a dry empty glass Petri dish. Put the sample (about 0.5 to 5 gm as par requirement, it mean if sample having less wet take 5 g weight. If sample having more weight than take 0.5 g weight in dish and weigh. Note down the reading. Distribute the sample in Petri dish by gentle shaking. Place the loaded dish (without cover) in the drying chamber for two hours. Maintain the oven temperature 105 +/- 5^0C.After drying is completed, open the drying chamber, cover the dish and allow it to cool at room temp. Keep it in the desiccator for

15 minutes. Weigh it and calculate the loss on drying percentage as follow.

Calculation

$$\text{Loss on drying \%} = \frac{(W1 - W2) \times 100}{M}$$

Weight of Petri dish =W

Weightof Petri dish + sample = W1

Weight of sample= M

After drying wt of Petri dish + sample = W2

Partition co-efficient: 50 mg of drug was taken in three separating funnels. The separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically. The partition coefficient of the drug in phases was calculated by using formula:

$$KPC = \frac{\text{Concentration of Drug in Oil Phase}}{\text{Concentration of Drug in Water Phase}}$$

Determination of λ_{max}: A solution of drug containing the concentration 10 µg/ ml was prepared in ph 7.4 phosphate buffer. The solution was scanned in the range of 279 nm UV spectrum using Systolic double beam spectrophotometer.

Preparation of standard curve: Cidofovir standard stock solution (1000µg/ml), 1ml solution was diluted to 10 ml using phosphate buffer (pH 7.4) to get concentrations of 100 µg/ml. from this solution, aliquots of, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml from standard drug solution were diluted to 10 ml with phosphate buffer (pH 7.4) and the absorbance of these solutions was measured spectrophotometrically using phosphate buffer (pH 7.4) as a blank at 279 nm. A standard curve was plotted using concentration on X-axis and the absorbance obtained on Y-axis.

Drug excipient compatibility: Drug excipient compatibility was determining by the FTIR or DSC method.

FTIR Study: Fourier Infrared spectroscopy is one of the powerful analytical techniques which offer the possibility of chemical identification. The technique is based on the simple fact that chemical substance shows selective absorption in infrared region. After absorption of IR radiations, the molecules vibrate, giving rise to absorption spectrum. It is an excellent method for the qualitative analysis because except optical isomers.

Preparation of Microspheres: Microspheres were prepared by emulsification solvent evaporation technique. Briefly, Cidofovir and polymers were mixed in 50ml distilled water. A different polymer ratio 1:1, 1:2, 1:3, and 1:4, used to prepare the different formulations. Polymeric aqueous solution was made in which the drug was dispersed and then the solution was added drop wise into 200 ml of light liquid paraffin containing 0.5% span-80 as an emulsifying agent. The aqueous phase was emulsified in oily phase by stirring the system in a 500ml beaker, Constant stirring at 500 rpm was carried out using magnetic stirrer at 80ºC, stirring and heating were maintained for 4hrs, Until The aqueous phase was evaporated. The microspheres were washed 5 times with n-hexane, filtered through whatman's filter paper and dried in hot air oven at 50°C for 2 hours.

Table 1 : Microsphere formulations with different concentration

Formulation code	Drug	HPMC K-100+ Carbopol	HPMC K-4+ Carbopol	Liquid paraffin (ml	Span-80 (0.5 %)
F1	100	1:1	-	200	0.5
F2	100	1:2	-	200	0.5
F3	100	1:3	-	200	0.5
F4	100	1:4	-	200	0.5
F5	100	-	1:1	200	0.5
F6	100	-	1:2	200	0.5
F7	100	-	1:3	200	0.5
F8	100	-	1:4	200	0.5

Results and discussion:

The physical characterization results of Cidofovir are as follows:

Sr. No.	Name Of Parameter	Result
1	Color	Fluffy white powder
2	Odor	Odorless
3	Taste	tasteless
4	Appearance	solid
5	Solubility in water	Soluble
6	Solubility in ethanol	Insoluble
7	Solubility in DMSO	Insoluble
8	Melting Point	480^0C
9	Loss on drying	40
10	Partition co-efficient in n-Octanol/Water (pH 7.4)	3.9

Determination of λ max

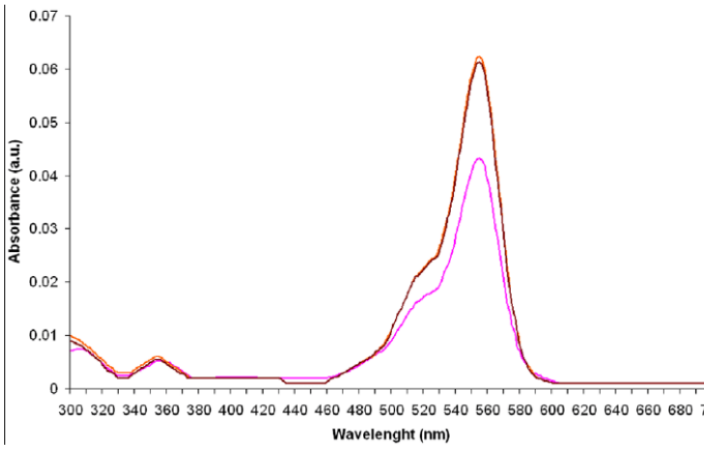


Figure 1: λ max of Cidofovir

Table 2: Preparation of standard curve

S. No.	Concentration (µg/ml)	Absorbance (279nm)
1	10	0.5
2	20	1.0
3	30	1.5
4	40	2.0
5	50	2.5

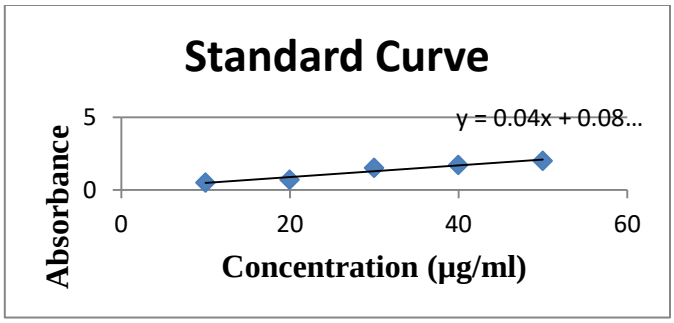


Figure 2: Standard Curve of Cidofovir

Drug excipient compatibility by FTIR: It was done by making pellets of the drug in KBr. FTIR spectra was taken at Thermo Instrument. The observed peaks were compared with reported spectra for groups.

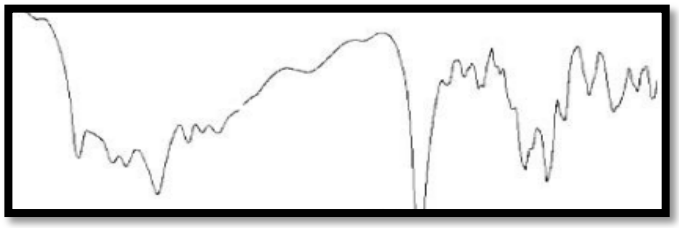


Figure 3: FTIR of Cidofovir

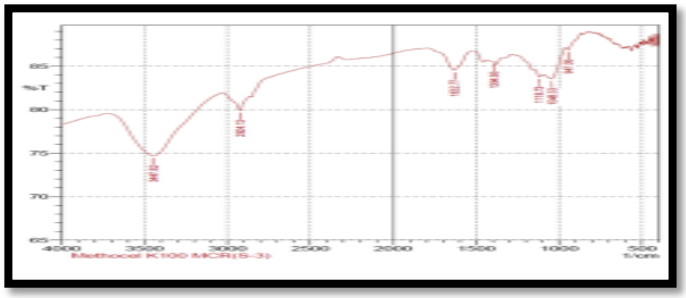


Figure 4: FTIR of Excipient

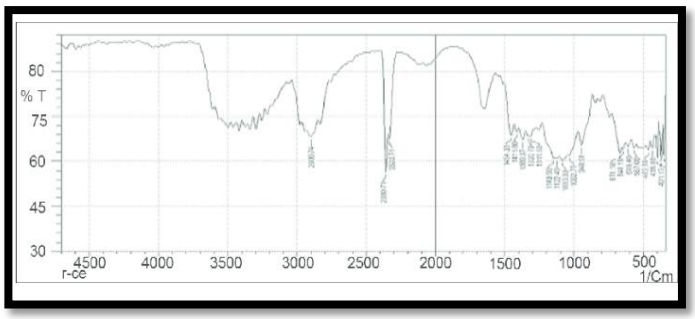


Figure 5: FTIR of Excipient

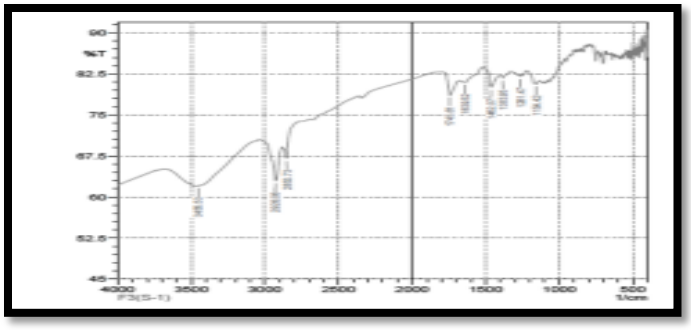


Figure 6: FTIR of Drug + Excipient

Preparation of Microsphere: After optimization of microsphere containing cidofovir was evaluated.

Characterization of Microsphere: Drug entrapment efficiency

Table 3: The result of entrapment efficiency

S.No	Formulation code	Entrapment efficiency	Mean particle size (µm	Shape	Bulk Density (gm/cm3)	Tapped Density (gm/cm3)
1	F1	71.14	323.12	Oval	0.277	0.322
2	F2	68.12	342.10	Oval	0.294	0.344
3	F3	64.28	427.14	Irregular	0.312	0.370
4	F4	58.14	453.46	Irregular	0.322	0.384
5	F5	80.07	253.26	Round	0.285	0.322
6	F6	69.21	363.86	Oval, Round	0.303	0.357
7	F7	66.42	437.78	Oval	0.344	0.416
8	F8	62.28	518.56	Irregular	0.357	0.434

Table 4: Carr's Index/ Compressibility, Hausner Ratio, Swelling Index and Mucoadhesion Index

S.No	Formulation code	Compressibility index (%)	Hausner ratio	Swelling Index after 6th hours (%)	Mucoadhesion after 6th hours (%)
1	F1	13.97	1.16	69%	50
2	F2	14.53	1.17	85%	54
3	F3	15.67	1.18	89%	58
4	F4	16.14	1.19	94%	62
5	F5	11.49	1.12	73%	74
6	F6	15.12	1.17	87%	74
7	F7	17.30	1.20	91%	77
8	F8	17.74	1.21	97%	79

Summary and Conclusion

In the present work, microsphere formulation to enhance transdermal permeation of was prepared and evaluated. Colloidal suspensions of microsphere were prepared by reported method. Microsphere system was found to be easy to prepare and composed mainly of phospholipids and ethanol, compounds commonly found in pharmaceutical preparations.

The average vesicle size of optimized formulations determined by Malvern Zetamaster was 1.112±0.053 µm. TEM photographs showed the surface morphology of the microsphere as well as existence of unilamellar vesicular structure. The smooth surface of vesicles was confirmed by SEM.

The entrapment efficiency of microsphere was determined for all formulations. Effect of ethanol concentration was observed on percent drug entrapment of microsphere. The maximum entrapment efficiency was found to be 90.06±0.79% for formulation ETE3 and minimum 52.36±0.82% for formulation ETE5, respectively. There was increase in percent drug entrapment was observed with an increase in ethanol concentration, but when ethanol concentration exceeded 30%, a decrease in percent drug entrapment was observed. Improvement in aqueous solubility of cidofovir was achieved with higher concentration of ethanol, which could be due to its co-solvent effect. Therefore, the more drug amount could be accommodated in the aqueous core of the vesicles however, as the concentration of ethanol increased above 30% resulting into leakage of drug from fluidized bilayer of vesicles.

The entrapment efficiency also increased with an increase in concentration of lecithin but, after 3 % of lecithin concentration there was no significant increase in percent entrapment was observed. The minimum percent entrapment observed for formulation ETL1 was 76.03±0.80% and maximum for formulation ETL4 was 90.21±0.80%, respectively. This may be due to the lipophilic nature of drug which could get more encapsulated in lipid bilayer of the formulation.

Increase in propylene glycol concentration decreases the entrapment of drug in formulations. Percent entrapment of ETP1 and ETP4 formulations was found to be 89.11±0.1% and 67.31±0.90%, respectively. The results revealed that as the concentration of permeation enhancer increased, the fluidity of bilayerd also increased causing the drug to leach out from vesicles.

The effect of ethanol, lecithin and propylene glycol on drug permeation rate of cidofovir was studied. On increasing the amount of ethanol from 10-0.30%, drug permeation was found to be increased whereas further increase in ethanol concentration the drug permeation was found to be decreased which could be due to the disruption of bilayer vesicles at higher concentration of ethanol. The maximum permeation was found to be 0.41±0.080 mg/cm2 at 30% ethanol concentration.

As the lecithin is the main component of microsphere system play important role in formulation. In present study the effect of lecithin concentration on drug permeation was also evaluated to optimize the formulation for sustained release properties on system. In prepared microsphere system, as the concentration of lecithin increased above 2%, permeation of drug decreased from 0.42±0.022 mg/ cm2 due to decrease in the bilayer deformability of vesicles.

Variation in propylene glycol concentration also influenced the amount of drug permeated. The cumulative amount of drug permeated was maximum (formulation ETP2) 0.49±0.032 mg/cm2 and minimum system play important role in formulation. In present study the effect of lecithin concentration on drug permeation was also evaluated to optimize the formulation for sustained release properties on system. In prepared microsphere system, as the concentration of lecithin increased above 2%, permeation of drug decreased from 0.42±0.022 mg/ cm2 due to decrease in the bilayer deformability of vesicles.

Variation in propylene glycol concentration also influenced the amount of drug permeated. The cumulative amount of drug permeated was maximum (formulation ETP2) 0.49±0.032 mg/cm2 and minimum formulations were stored in amber glass container at different temperature. The drug content after treatment with triton X100 and % residue of cidofovir was calculated. Only 2±1% degradation was observed at 37±2°, at room temperature only 3% degradation was observed however all formulations were almost stable at 8±2° and 4±2° and only 1.57±0.2% degradation of Cidofovir was observed showed that developed system is stable at low temperature.

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