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DEVELOPMENT AND EVALUATION OF FLOATING MICROSPHERES OF ACYCLOVIR BY SOLVENT EVAPORATION TECHNIQUE

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ABSTRACT
The purpose of the current work is to formulate the floating microspheres of acyclovir, an antiviral drug, with an intention to prolong the residence time in the stomach and to achieve the prolonged release of the drug. A 3^2 full factorial design and solvent evaporation technique was utilized to formulate the floating microspheres. The formulated microspheres were evaluated for the various post formulation parameters including percentage encapsulation and *in vitro* drug release. The average particle size was found to be 175 µm with the percentage drug entrapment efficiency ranging from 64.92±0.763 % to 72.19±0.335% w/w. The total floating time of the microsphere was more than about 12 hours with the floating lag time ranging from 17.33±0.577 seconds to 39.66±2.081 secconds. The *in vitro* drug release showed that the acyclovir was released from the formulation at slow rate and sustained for more than about 12 hours. From the conducted study, it can be presumed that the fabrication of floating microspheres can successfully exert sustained release of the drug.

KEYWORDS: Acyclovir, Solvent evaporation, Floating microspheres, Sustained release, *in vitro* drug release.

INTRODUCTION
The drug carrier system plays a crucial role in the effectiveness of a drug. Amongst all other routes of drug administration, oral route is most preferred due to patient compliance, controlled delivery, and higher absorption. However, drug absorption in the gut is complex and challenging, with factors like uncertain gastric emptying and limited release affecting efficacy. Over the past two decades, various oral drug delivery systems have been developed to release active substances at a controlled rate over a defined time period. The main reason for this is the influence of gastrointestinal transit on drug effectiveness, with gastric residence time being a key factor. To address these concerns, floating gastric retentive drugs have been introduced, including tablets, beads, pellets, granules, and microspheres. These formulations are beneficial for drugs that need local action, have a narrow absorption window in the small intestine, and lower solubility at higher pH levels. [1-3] Floating drug delivery systems, also known as hydrodynamically balanced systems, have a lower bulk density than gastric fluids, allowing them to remain buoyant in the stomach for extended periods without affecting gastric emptying rates. This results in a longer gastric residence time and better control of plasma drug concentration.

Microspheres, also known as microparticles, are small spherical particles ranging from 1µm to 1000µm in diameter. They can be made from various natural and synthetic materials such as glass, polymer, and ceramic. Floating microspheres, also referred to as microballoons or floating microparticles, are empty particles without a core that have diameters in the micrometer range.[4 - 6] Acyclovir, a BCS class III/IV drug, is commonly used to treat herpes simplex and vericella zoster infections. It shows poor water solubility and have short biological half-life (2.5-3.3 h) and low bioavailability (10-20%) from the upper part of the gastrointestinal tract (GIT), making it a suitable candidate for floating drug delivery systems due to its acidic pH and limited absorption in the gastrointestinal tract.[7, 8]

The study aimed to create, design, and assess microspheres of Acyclovir to enhance its efficacy and stability in the stomach, thereby extending gastric residence time and increasing its bioavailability after oral administration.

MATERIALS AND METHODS
Acyclovir was obtained as a gift sample from Lomus Pharmaceuticals Pvt. Ltd and modified starch was purchased from Surya Chemicals Pvt. Ltd. All the other

excipients and reagents were of analytical grade. The study was conducted in the laboratory of Pharmaceutics located in the premises of National Academy for Medical Sciences, Kathmandu, Nepal.

Design of experiment: The experiments were designed using a $3^{[2]}$ full factorial, with polymer to drug ratio and stirring speed as independent variables and % entrapment efficiency, time for 80% drug release, and particle size as dependent variables. All the nine formulations (F1 – F9) were prepared using three different levels of polymer to drug ratio and stirring speed.[9]

Table 1: Formulation table of acyclovir floating microspheres.

Code	Acyclovir	HPMC K4M	Chitosan	Modified Starch	Ethyl Cellulose
M1	100	100	-	-	100
M2	100	100	-	-	150
M3	100	100	-	-	200
M4	100	-	100	-	100
M5	100	-	100	-	150
M6	100	-	100	-	200
M7	100	-	-	100	100
M8	100	-	-	100	150
M9	100	-	-	100	200

Calibration curve of acyclovir: 100 mg of the drug, was weighed in a 100ml volumetric flask and prepared using 0.1 N hydrochloric acid. A stock solution concentration of 100 µg/ml was prepared, and acyclovir aliquots were prepared from 2-10 µg/ml by transferring 0.2-1.0 ml of stock solution into a series of 10 ml volumetric flasks.[10,11]

Preparation of floating microspheres: Using a solvent diffusion-evaporation method with varying ratios of three different polymers and ethyl cellulose, floating microspheres containing acyclovir were developed as per the composition of formulations depicted in Table 1. A clear solution containing the drug and polymers dissolved in a mixture of 1:2 ratio of ethanol and dichloromethane was slowly poured into a 1% polyvinyl alcohol solution while continuously stirring for up to 5 hours at 27±2°C. The floating microspheres were collected while the non-floating microspheres were discarded. The microspheres were dried overnight and stored in a desiccator.[12 – 17]

Evaluation of microspheres[18 - 23]

Shape and Size: Optical microscopy method was used to evaluate the shape and size of the developed microspheres. The eye piece micrometer was calibrated with the help of a stage micrometer. The particle diameter of more than 50 microspheres was measured randomly. The average particle size was determined by using Edmondson's equation

$$D = \frac{\sum nd}{\sum n}$$

where, n = number of microspheres, and d = mean of the size range.

Drug entrapment efficiency: The drug content of different batches of floating microspheres was assessed by weighing and crushing microspheres equivalent to 50mg of Acyclovir. The resulting powder was dissolved in 5ml of ethanol and volumetrically made up to 100ml with 0.1N HCl. After filtering, the solution was diluted and analyzed spectrophotometrically at λ_{max}, using 0.1N HCl as blank. The drug entrapment efficiency was calculated using the following formula:

% drug entrapment efficiency = (particle drug content/Theoretical drug content) × 100

***In-vitro* drug release:** Accurately weighed microspheres equivalnet to 100 mg of the drug were placed in a gelatin capsule and added to a dissolution vessel. The dissolution study took place in 900 ml of 0.1N HCI (pH 1.2), maintained at 37 ± 0.5°C and stirred at 100 rpm. At specific intervals, 5 ml samples were withdrawn, filtered, and replaced with an equal volume of dissolution medium to maintain the sink condition. The concentration of the drug in the dissolution medium was analyzed spectrophotometrically at λ_{max}.

RESULTS AND DISCUSSION

Calibration curve

The UV Spectrum analysis of Acyclovir in the wavelength region of 200 – 400 nm showed maximum absorbance at 256 nm. Diluted solutions for calibration curve were analyzed for absorbance at 256 nm using a UV-VIS spectrophotometer, and a standard graph was plotted between concentration and absorbance which yield a coefficient of determination 0.9988.

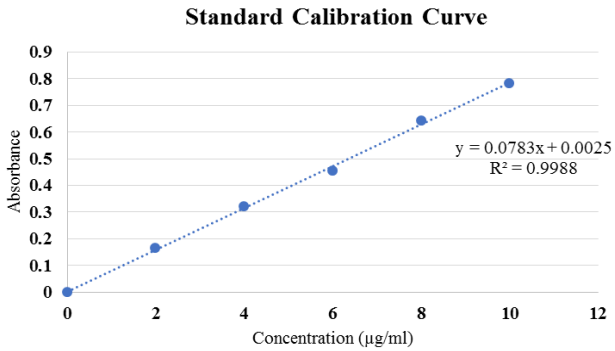

Fig. 1: Calibration curve of acyclovir.

Percentage yield

The dried microspheres were weighed and percentage yield was calculated for each developed batches. The percentage yield for the microspheres was found within the range of 66.81±1.626 to 74.20±0.711. Among all the formulations the highest yield was found to be of M3 All the results of obtained percentage yield is depicted in Table 2.

Angle of repose

The angle of repose was determined using the fixed funnel method and the obtained data for each formulation batch. The range of Angle of Repose varies from 24.66±0.514 to 31.87±0.965 which implies that the flow properties were excellent to passable as per USP data. The best flow properties was illustrated by microsphere M3. The overall results of angle of repose is tabulated in Table 2.

Particle size analysis

The particle diameter of more than 50 microspheres were studied using optical microscopy method and the average particle size was determined. The formulated microspheres average size ranged from 175 µm of formulation M3 to 204 µm of formulation M4. The data obtained by the optical microscopic studies is given in Table 2.

Drug entrapment efficiency

After the determination of the drug content, the microspheres were studied for the percentage drug entrapment efficiency as per the formula mentioned above in methodologies. Each batch of formulation were studied which showed a maximum percentage of 72.19±0.335 whereas, the least was shown by M4 with the efficiency of 64.92±0.763 %. The following results were obtained from the percentage drug entrapment efficiency.

Floating Lag Time: The time taken by the formulation to rise to surface is termed as floating lag time. The floating lag time of the prepared microspheres ranged from 17.33±0.577 seconds to 39.66±2.081 sec, which was shown by M3 and M4 respectively.

Table 2: Evaluation studies of prepared floating microspheres.

Formulation Code	Percentage yield (%)	Angle of Repose (°)	Average Particle Size (µm)	Drug Entrapment Efficiency (%)	Floating Lag Time (sec)
M1	70.92±0.704	30.93±0.169	183	68.25±0.658	19.66±1.154
M2	72.48±1.304	27.50±0.902	177	70.25±0.824	18.33±1.527
M3	74.20±0.711	24.66±0.514	175	72.19±0.335	17.33±0.577
M4	66.81±1.626	31.87±0.965	204	64.76±0.684	39.66±2.081
M5	67.02±0.588	30.58±0.141	198	64.92±0.763	32.33±1.527
M6	68.69±1.033	28.05±0.500	191	65.92±1.110	31.33±1.527
M7	69.97±0.106	30.01±0.606	194	67.10±1.214	24.66±1.154
M8	71.23±0.597	28.93±0.609	189	68.24±0.527	21.33±1.527
M9	71.56±0.245	26.01±0.593	180	69.14±0.514	20.66±0.577

***In-vitro* dissolution studies**

The *in vitro* dissolution studies was carried out in simulated gastric fluid of pH 1.2. The dissolution studies was carried out for 12 hours with an intention to study the percentage of drug release upto that duration. The study showed that at least 74.845% was released during the 12 hours interval. The formulation M3 conveyed a release of 99.16% at the end of 12 hours which can be said that almost full drug loaded in microspheres was released that signifies a sustained release of drug. The data obtained from the *in-vitro* dissolution study of all the nine formulations were revealed in a line graph as shown in figure 2.

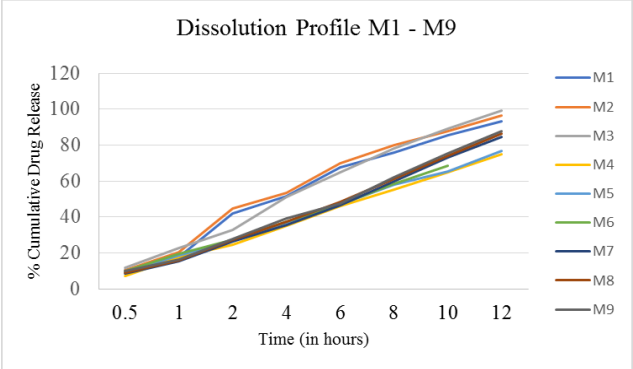

Fig. 2: Dissolution Profile of Floating Microspheres (M1 – M9).

CONCLUSION

An attempt was made to design, formulate and evaluate the floating microspheres containing acyclovir by solvent evaporation method. Among all the formulations, with reference to the evaluation parameters, M3 was considered to be the optimized formulation, as it was determined to have the best micrometric characteristics, percentage yield, percentage drug entrapment efficiency, floating lag time, and the highest *in vitro* drug release of 99.16% in a consistent and sustained manner over an extended period of time.

Thus the study successfully developed efficient floating acyclovir microspheres, concluding that the prepared floating microspheres of Acyclovir may be a safe and effective candidate for controlled drug delivery, reducing dosing frequency over an extended period.

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