Formulation and evaluation of Acyclovir microparticles for Ocular Delivery

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The objective of the present work was to formulate and evaluate microparticles of Acyclovir and produced sustained drug delivery for ocular delivery. In this 9 batches (A1-C3) of acyclovir microparticle was prepared with ethyl cellulose, PVA and other ingredients by solvent evaporation technique. The prepared microparticles were evaluated for different parameters i.e % Drug yield, % Drug entrapment, Surface morphology, Zeta potential and in-vitro drug release for 24hrs in phosphate buffer 7.4 and simulated tear fluid. The best batch was performed stability studies for 6 months. The research concluded that Acyclovir microparticles could be a alternative for conventional dosage formand other phytochemical in herbs.

Keywords:
Acyclovir, Microparticles, Ocular delivery

Introduction:

Microparticles are particles between 0.1 and 100 μm in size. Commercially available microparticles are available in a wide variety of materials, including ceramics, glass, polymers, and metals. Microparticles encountered in daily life include pollen, sand, dust, flour, and powdered sugar. Microparticles have a much larger surface-to-volume ratio than at the macroscale, and thus their behavior can be quite different. For example, metal microparticles can be explosive in air. Microspheres are spherical microparticles, and are used where consistent and predictable particle surface area is important. Microparticulate drug delivery system is one of the processes to provide the sustained & controlled delivery of drug to long periods of time. They are small particles of solids or small droplets of liquids surrounded by walls of natural & synthetic polymer films of varying thickness & degree of permeability acting as a release rate controlling substance. Microparticles are small [0.2-5um], loded microspheres of natural or synthetic polymers. Microparticles were initially developed as carriers for vaccines and anticancer drug. [1]

Material and Method

Acyclovir was acquired as a gift sample from Archerchem, Mumbai, India. All other ingredients were of laboratory grades (Ethyl cellulose, PVA).

Compatibility Studies

The compatibility studies were carried out, by adopting IR spectroscopy with reference to the pure drug alone and its combination with chosen ingredients.

FTIR analysis

The drug-polymer compatibility was studied by FTIR (Shimadzu IR Affinity-1) spectroscopy. The mixture of individual drug and potassium bromide was ground intoa finepowderusingmortarPestleandthencompressedintoaKBrdis csinahydraulic press at a pressure of 75Kg/cm². Each KBr disc was scanned 45 times at a resolution of 2 cm⁻¹. The characteristic peaks were recorded in the case of each drug individually.
Formulation design
Acyclovir was studied for physicochemical characteristics. Microparticles of Acyclovir were produced, by employing technology. Various ingredients were selected, for Acyclovir, formulation design of Microparticles as represented in the Table no 1.

Preparation of Drug loaded Microparticles
Acyclovir were micronized by adopting solvent evaporation technique. Drug loaded Microparticles of Acyclovir, were prepared individually by using single emulsion solvent evaporation method. Drug and polymer in different proportions were weighed and co-dissolved at room temperature in to a mixture of ethanol and dichloromethane (2:1%v/v) with magnetic stirring. This was slowly poured (drop wise) into the dispersion medium consisting of 20 ml of 1% (w/v) aqueous PVA and 1.5% (w/v) span 80,during sonication by means of probe sonicator for 2h on the ice bath. Afterwards, the system was put on magnetic stirrer over night for complete evaporation of organic solvents. The prepared suspension was centrifuged at 1,500 rpm for 1hourinthepresence of 5% mannitol (cryoprotectant). The supernatant was removed and these diment was freeze dried for 48h for further analysis. The obtained particles, with each drug, were kept in dehydrated conditions.[2]

Evaluation of Microparticles
The Microparticles produced with each drug i.e. Acyclovir, was evaluated for various parameters i.e. %yield, entrapment efficiency, determination of particle size & Zeta potential, surface morphology, in-vitro drug release, release kinetics and stability studies.

Percentage yield (%yield)
The yield values were calculated as the weight of the Microparticles recovered from each batch divided by total weight of drug and polymer used in the preparation of the particular batch[3].

\[
\% \text{ Yield} = \left( \frac{\text{Weight of microparticles obtained}}{\text{Weight of drug + polymer}} \right) \times 100
\]

Determination of drug entrapment efficiency
The formulations were dissolved in a minimum quantity of methanol individually and centrifuged at 1,500 rpm for 20 minutes. These dements were separated and upper layers were filtered, suitably diluted and analyzed spectrophotometrically at respective wavelengths. Each experiment was repeated in triplicate. Percentage drug entrapment, for each class of Microparticles, was determined by the following formula:

\[
\text{E. E.} = \left( \frac{\text{Amount of drug actually present in microparticles}}{\text{Amount of drug actually used}} \right) \times 100
\]

Particle size and zeta-potential
The mean particle size of drug-loaded Microparticles and zeta potential of Acyclovir was determined by a Malvern Zeta sizer nanozs (Malvern instrument ltd).

Surface morphology
Surface morphology was determined by scanning electron microscopy of Microparticles. It determined whether particles had a uniform shapes or not and whether they were uniformly/ununiformly distributed. It also confirmed the obtained particle size in each case.[4].

In-vitro drug release from Drug-loaded Microparticles
Drug-loaded Microparticles obtained with Acyclovir was suspended in pH 7.4 phosphate buffer in a glass vial which was placed in a mechanical shaking bath (100cycles/min) at the temperature adjusted to 37°C. At selected time intervals sample was removed and replaced with fresh buffer medium. Each withdrawn sample was then centrifuged at15000 rpm (Acyclovir microparticles) and supernatant was analyzed using UV spectrophotometry.[5]

Accelerated Stability studies
The selected (optimized) formulation, in each case, was packed in amber-colored bottles which were tightly plugged with cotton and capped. These were then stored at 400 ±20°C / 75% ±5% RH for 6 months and evaluated ,for its physical appearance & drug contents at specified intervals of time.[6-7]

Result and Discussion
Compatibly Studies

FTIR analysis
Fig. 1: FTIR spectra of Acyclovir (pure drug)

Fig. 2: FTIR spectra of Ethyl cellulose

Fig. 3: FTIR spectra of Acyclovir and Ethyl cellulose
**Formulation Design**

Table 1: Formulation design of Microparticles of Acyclovir

| Formulation Code | Drug (mg) | Polymer (Ethyl cellulose) (mg) | PVA (%) | Dichloromethane (ml) | Ethanol (ml) | Span80 (1.5 %µl) |
|------------------|-----------|--------------------------------|---------|----------------------|--------------|------------------|
| A1               | 100       | 100                            | 1       | 5                    | 10           | 100              |
| A2               | 100       | 200                            | 1       | 5                    | 15           | 100              |
| A3               | 100       | 300                            | 1       | 5                    | 20           | 100              |
| B1               | 100       | 100                            | 2       | 5                    | 10           | 100              |
| B2               | 100       | 200                            | 2       | 5                    | 15           | 100              |
| B3               | 100       | 300                            | 2       | 5                    | 20           | 100              |
| C1               | 100       | 100                            | 3       | 5                    | 10           | 100              |
| C2               | 100       | 200                            | 3       | 5                    | 15           | 100              |
| C3               | 100       | 300                            | 3       | 5                    | 20           | 100              |

**Percentage yield:** The maximum percentage yield was found to be 55.8% with batch C3 (Acyclovir), while minimum of 35.12% with batch A1 (Acyclovir).

Table 2: Percentage yield of Acyclovir Microparticles (batches A1- C3)

| Microparticulate Batches | Total amount of Ingredient (mg) | Practical yield (mg) | Percentage yield (%) |
|--------------------------|---------------------------------|----------------------|----------------------|
| A1                       | 200                             | 70.24                | 35.12                |
| A2                       | 300                             | 135.8                | 45.2                 |
| A3                       | 400                             | 216.3                | 54.0                 |
| B1                       | 200                             | 73.2                 | 36.6                 |
| B2                       | 300                             | 147.3                | 49.1                 |
| B3                       | 400                             | 219                  | 54.75                |
| C3                       | 200                             | 75.6                 | 37.8                 |
| C2                       | 300                             | 149.2                | 49.74                |
| C3                       | 400                             | 223.2                | 55.8                 |
Drug entrapment efficiency: The % drug entrapment of Acyclovir microparticles (batches A1-C3) was determined. It ranged between (53.23%-62.37%) respectively.

Table3: Percentage drug entrapment of Acyclovir Microparticles (batches A1- C3)

| Microparticulate Batches | % Drug content |
|-------------------------|---------------|
| A1                      | 53.23         |
| A2                      | 56.42         |
| A3                      | 60.19         |
| B1                      | 54.55         |
| B2                      | 57.31         |
| B3                      | 61.65         |
| C1                      | 55.21         |
| C2                      | 58.15         |
| C3                      | 62.37         |
Particle size analysis: The analysis was performed for all nine batches prepared with Acyclovir. The mean diameters of particles for all batches were found in the range of 164-198 nm.

Table 4: Particle size analysis of Acyclovir Microparticles (batches A1-C3)

| S. No. | Microparticulate Batches | Mean particle size (µm) |
|-------|--------------------------|-------------------------|
| 1     | A1                       | 166                     |
| 2     | A2                       | 174                     |
| 3     | A3                       | 195                     |
| 4     | B1                       | 164                     |
| 5     | B2                       | 172                     |
| 6     | B3                       | 191                     |
| 7     | C1                       | 170                     |
| 8     | C2                       | 178                     |
| 9     | C3                       | 198                     |
Zeta potential: The zeta potential of Acyclovir (batch A₁-C₃) was determined. It ranged between \((-7.45\text{ to } -20.80\text{ mV})\) respectively.

### Table 5: Zeta potential analyses of Acyclovir Microparticles (batches A₁-C₃)

| S. No | Microparticulate Batches | Zeta Potential Mean (mV) |
|-------|--------------------------|--------------------------|
| 1     | A₁                       | -7.45                    |
| 2     | A₂                       | -12.30                   |
| 3     | A₃                       | -14.66                   |
| 4     | B₁                       | -13.49                   |
| 5     | B₂                       | -15.85                   |
| 6     | B₃                       | -14.80                   |
| 7     | C₁                       | -19.80                   |
| 8     | C₂                       | -20.80                   |
| 9     | C₃                       | -16.89                   |
**In-vitro** dissolution studies

Table 6: *In–vitro* comparative release study of Acyclovir Microparticles (batches A₁-C₃) in pH 7.4 phosphate buffer

| Time (hr.) | A₁    | A₂    | A₃    | B₁    | B₂    | B₃    | C₁    | C₂    | C₃    |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0         | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| 2         | 25.369| 23.147| 21.756| 18.256| 17.925| 16.482| 15.254| 13.346| 11.321|
| 4         | 40.215| 38.542| 37.418| 35.627| 33.625| 32.246| 31.634| 29.674| 26.249|
| 6         | 56.254| 53.874| 53.691| 52.368| 50.045| 49.857| 48.659| 47.123| 45.371|
| 8         | 65.258| 64.254| 63.201| 62.526| 61.258| 60.324| 59.624| 58.321| 57.136|
| 10        | 74.658| 73.628| 72.364| 70.025| 69.357| 67.208| 66.358| 65.159| 64.268|
| 12        | 79.365| 77.218| 76.354| 75.42  | 73.201| 72.135| 71.25  | 70.243| 69.31  |
| 14        | 82.654| 81.625| 80.693| 78.651| 77.269| 75.249| 74.125 | 72.049| 71.428 |
| 16        | 85.125| 84.357| 83.651| 82.367| 81.561| 80.234| 78.687 | 76.254| 75.365 |
| 18        | 87.209| 86.951| 84.502| 83.259| 82.349| 81.136| 80.695 | 78.153| 77.016 |
| 20        | 89.561| 88.692| 86.36  | 85.208| 84.159| 83.902| 82.348 | 81.715| 80.242 |
| 22        | 92.657| 91.258| 90.321| 89.361| 88.795| 87.462| 86.168 | 85.134| 84.139 |
| 24        | 94.368| 93.625| 92.354| 91.935| 91.458| 90.845| 89.894 | 89.151| 88.654 |

Fig. 7: Comparative % drug release of Acyclovir microparticles (batches A₁- C₃) in pH 7.4 phosphate buffer.
Table 7: *In–vitro* comparative release study of Acyclovir Microparticles (batches A1-C3) in simulated tear fluid

| Time (hr.) | A1   | A2   | A3   | B1   | B2   | B3   | C1   | C2   | C3   |
|------------|------|------|------|------|------|------|------|------|------|
| 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| 2          | 24.264 | 22.954 | 20.444 | 17.138 | 16.065 | 15.055 | 13.523 | 12.302 | 11.575 |
| 4          | 39.624 | 37.856 | 36.295 | 34.852 | 32.631 | 31.249 | 29.326 | 27.255 | 25.366 |
| 6          | 56.357 | 54.217 | 52.361 | 51.741 | 49.413 | 47.368 | 46.652 | 44.042 | 43.333 |
| 8          | 64.128 | 63.163 | 62.049 | 61.652 | 60.523 | 59.387 | 58.915 | 57.384 | 56.626 |
| 10         | 73.026 | 72.825 | 71.387 | 69.963 | 68.748 | 67.808 | 65.348 | 64.971 | 62.236 |
| 12         | 78.354 | 77.209 | 76.629 | 74.987 | 73.965 | 72.634 | 71.267 | 70.263 | 69.864 |
| 14         | 81.247 | 80.145 | 79.123 | 77.348 | 76.142 | 74.562 | 73.625 | 72.485 | 71.961 |
| 16         | 84.569 | 83.276 | 82.349 | 81.921 | 80.146 | 79.125 | 77.325 | 75.106 | 74.527 |
| 18         | 86.327 | 85.329 | 84.278 | 82.654 | 81.795 | 80.121 | 78.967 | 77.685 | 76.354 |
| 20         | 88.315 | 86.32 | 85.147 | 84.557 | 83.624 | 82.209 | 81.324 | 80.369 | 78.051 |
| 22         | 91.052 | 90.021 | 89.304 | 88.344 | 87.657 | 86.666 | 85.743 | 84.694 | 83.256 |
| 24         | 93.154 | 92.192 | 91.682 | 90.322 | 89.202 | 88.303 | 87.691 | 86.208 | 85.382 |

Fig. 8: Comparative % drug release of Acyclovir Microparticles (batches A1-C3) in simulated tear fluid
Accelerated Stability studies

Table 8: Stability data of Acyclovir microparticle A1 batch in simulated tear fluid

| Time (hr) | 0 days | 60 days | 120 days | 180 days |
|-----------|--------|---------|----------|----------|
| 0         | 0      | 0       | 0        | 0        |
| 2         | 24.264 | 24.112  | 24.056   | 23.888   |
| 4         | 39.624 | 39.254  | 39.106   | 38.754   |
| 6         | 56.357 | 56.221  | 56.187   | 56.024   |
| 8         | 64.128 | 64.028  | 63.864   | 63.784   |
| 10        | 73.026 | 72.981  | 72.686   | 72.545   |
| 12        | 78.354 | 78.112  | 77.898   | 77.696   |
| 14        | 81.247 | 81.102  | 81.025   | 80.689   |
| 16        | 84.569 | 84.205  | 84.068   | 83.753   |
| 18        | 86.327 | 86.153  | 85.878   | 85.698   |
| 20        | 88.315 | 88.121  | 87.877   | 87.712   |
| 22        | 91.052 | 90.988  | 90.875   | 90.568   |
| 24        | 93.154 | 93.089  | 92.785   | 92.622   |

Fig. 9: Comparative release profile of Acyclovir Microparticles batch A1 on stability studies
Conclusion
In the Present study the Acyclovir microparticles were evaluated for different parameters i.e % Drug yield, % Drug entrapment, Surface morphology, Zeta potential and in-vitro drug release for 24hrs in phosphate buffer 7.4 and simulated tear fluid. The latter revealed that A1 batch from the nine formulations shows maximum sustained release (93.154%) in 24 hr. The A1 batch was performed for stability studies for 6 months. The research, reference characterized that acyclovir microparticles could be alternative than conventional dosage for sustained action in ocular delivery.

References

1. Takalea.A., Banerjee SK., Gadhavem.V., Gaikwadd.D, Microparticles In Drug Delivery System: A Review, International Journal of Institutional Pharmacy and Life sciences 2012;2(2):349-359.
2. Huang Yuan, XuXuefan, Xiang Qingyu, He Zhiyao, Liu Yuchua, Zhou Dan, “Crystalline drug aconitine-loaded poly (d,L-lactide-co glycolide) nanoparticles preparation and in vitro release”, The Pharmaceutical society of Japan, 2010;130: 409-418.
3. Patel PareshN., PatelL.J, and Patel J.K. “Development and testing of novel Temoxifen citrate loaded chitosan nanoparticles using ionic gelation method "Der Pharmacia Sinica", 2011;2(4):17-25.
4. Das swarmali, Sureshk. Preeti. “Drug delivery to eye: Special reference to nanoparticles” International Journal of Drug Delivery, 2010;2: 12-21.
5. Agnihotri M. Sagar, Vavia, R. Pradeep. “Diclofenac-loaded biopolymeric nano suspensions for ophthalmic application”. Nanomedicine: nanotechnology, biology, and medicine, 2009;5:90-95.
6. Bhambere Deepak S; Deshmukh NarendraV; DoijadRajendraC;SomapurCandGojeArjun;PatelKareeshmaS. “Colloidal drug delivery of biodegradable poly (lactide-coglycolide) (PLGA) injectable nanoparticles for anti cancer drug” Int. J. Drug Dev. & Res., 2010;2(4):681-689.
7. ICH Q1A (R) guidelines: “Stability testing of New Drug Substance and Product”, (2003),1-18.