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Formulation and evaluation of acyclovir microcapsules using bakers yeast

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ABSTRACT

Objective: To formulate and evaluate acyclovir microcapsules using bakers yeast. Methods: Acyclovir, pretreated yeast and deionized water were taken at a volumetric ratio of 1:2:4 respectively. This suspension was agitated in a magnetic stirrer at 25°C, 30°C, 35°C, and 40°C for 4 hours. The suspension was then centrifuged for 10 minutes at 2000 rpm. The supernatant solution was decanted and the cells were washed 3 times with deionized water. Then the suspended drug entrapped yeast cells were dried in a lyophilizer for 48 hours. The yield was noted. Results: The first four formulations were done with 200 mg of the drug, followed by 400 mg for the next four formulations and 800 mg for the last four formulations. SEM showed that the surface of the microcapsules was heterogeneous, with no limit characteristics. FTIR showed no interaction between acyclovir and the cell wall. USP showed that the peak was within the standard values. The mean particle size for all the samples was 8 μm in diameter. The dissolution studies were done for all the twelve samples and showed a Fickian model of diffusion. Conclusions: From the results it is inferred that the samples prepared at 40°C (FY-4, FY-8, FY-12) show better entrainment and release. So these samples are formulated in the form of a suspension and compared with marketed acyclovir suspension using HPLC technique. The formulated suspensions with FY-4, FY-8 and FY-12 shows drug content in accordance with the standards of the pharmacopoeial limits.

1. Introduction

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body in order to promptly achieve and thereby to maintain the desired concentration. Microencapsulation is a process in which tiny particles or droplets are surrounded by coating to give small beads with many useful properties. In its simplest form a microcapsule is a small sphere with a uniform wall around. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating or membrane. A range of materials is suited for use as the capsule material: lipids, wax, starch, modified starch, cellulose, phospholipids and other polymers. In general, microcapsules size ranges from 5-500 μm. They can be made below 1 μm and up to 5 000 μm in size. Micro-organisms offer certain advantages over the conventional process, as microcapsules are preferred.\textsuperscript{45} The technology is based on using yeast and other simple microorganisms as capsules to protect and deliver the active drug. Microorganisms were first used to encapsulate unidentifiable materials.\textsuperscript{67} Yeast contain very low level of fat (less than 10 percent) and used as micro capsules without lipid extending substances.\textsuperscript{87} The technology of microencapsulation using yeast cell is unique as it involves the use of preferred walls and membranes of microorganisms to provide the capsules.\textsuperscript{90,91} This method can improve the shelf life and bioavailability of active ingredients. Antiviral drugs are a class of medication used specifically for treating viral infections.\textsuperscript{101} Like antibiotics, specific antivirals are used for specific viruses.\textsuperscript{108} Most of the antiviral drugs available are designed to deal with HIV. Herpes virus, which is best known for causing cold sores, but actually covers a wide range of diseases, hepatitis B and hepatitis C viruses which can cause liver cancer.\textsuperscript{109,110} Acyclovir is an analogue of 2-deoxyguanosine that exerts its antiviral effects after being metabolized to acyclovir triphosphate. Acyclovir triphosphate is 30-50 times more potent inhibitor of herpes simplex type-1 DNA polymerase.\textsuperscript{114} Their major production of acyclovir triphosphate in uninfected cells and its specificity for viral DNA polymerase result in minimal
cellular toxic effects. Acyclovir has proved effective for the treatment of infections caused by herpes simplex virus type 1 and 2 and varicella-zoster virus and for suppression of some forms of cytomegalovirus disease. Acyclovir when orally administered, peak plasma concentration occurs after 1–2 hours. It has a high distribution rate, only 30 percent is protein bound in plasma. The elimination half-life of acyclovir is approximately three hours. It is renally excreted partly by glomerular filtration and partly by tubular secretion. It has not been shown to cause teratogenic or carcinogenic effects. Acyclovir is marketed as tablets (200 mg, 400 mg and 800 mg), topical cream (5%), intravenous injection (25 mg/ml), and ophthalmic ointment (3%).

2. Materials and methods

2.1. Pre-treatment of yeast cells

Pre-treatment of these cells was done prior to the encapsulation process to assess the importance of cell viability. A suspension of fresh yeast (100 ml of 50% solids) was treated overnight with sodium azide (2 g), a respiratory inhibitor used to prevent the cells from performing any energy-dependent process. Sterilization by autoclaving is a thermally destructive process demamining any carrier protein molecules likely to be involved in facilitated diffusion process which may be responsible for encapsulation process.

2.2. Preparation of acyclovir microcapsules using Baker's yeast

Acyclovir, yeast and distilled water were taken in the volumetric ratio of 1:2:4 respectively. This suspension was agitated in a magnetic stirrer for 4 hours. The suspension containing the cells were then centrifuged for 10 minutes at 2,000 rpm. The supernatant solution was decanted and the cells were washed 3 times with deionized water and dried in a lyophilizer for 48 hours.

The effect of temperature and the drug concentration on microcapsule formulations were studied. The above procedure was followed at 4 different temperatures (25°C, 30°C, 35°C and 40°C) for 3 different doses of drug (200 mg, 400 mg, 800 mg) (FY1–FY12).

3. Results

The microcapsules of acyclovir was prepared using the drug, Baker's yeast and water at specific ratio and various temperatures and lyophilized and the yield was obtained. The surface morphology shown by scanning electron microscopy (SEM) was intact and with no burst on the surface. The SEM of FY-3 was considered to be the best which was done at 10 μm and 500 μm (Figures 1 & 2).

The standard graph of the pure sample was made as per the procedure. The particle size analysis showed size of 0–10 μm in diameter was common in most groups except group FY-9, where most particles were found to be at 10–20 micrometer. Particle size analysis of sample FY-5 microcapsules by optical microscopic method was shown in (Figures 3, 4, 5).
The entrapment efficiency of the twelve samples was done for every 100 mg of the sample. FY-1 showed the least entrapment efficiency of 90.35% and FY-8 showed the most (97.61%) (Table 1). According to the amount of drug released from these samples (Table 1), it was shown that the amount released increases with temperature.

The release study of aceclovir was done for the first two hours at an acidic pH using hydrochloric acid (pH 1.2) and then it was continued at an alkaline pH using phosphate buffer (pH 6.8). This was carried out for all the twelve formulations. The cumulative release at the end of the 2nd hour in the acidic pH and at the end of the 8th hour in alkaline pH were 29.5 mg and 191 mg for FY-1, 30.3 mg and 195 mg for FY-2, 30.1 mg and 193 mg for FY-3, 31.5 mg and 197.5 mg for FY-4, 55 mg and 395 mg for FY-5, 59 mg and 392 mg for FY-6, 61 mg and 396 mg for FY-7, 113 mg and 398 mg for FY-8. From FY-9 to FY-12, the cumulative release at 6th hour was also calculated, at the end of 2nd, 6th and 8th hour, they were 118 mg, 156 mg and 776 mg for FY-9, 114 mg, 152 mg and 792 mg for FY-10, 124 mg, 136 mg and 798 mg for FY-11. For FY-12 it was 122 mg at the end of the 2nd hour in the acidic pH and 792 mg in the alkaline pH.

The FY-18 of the microcapsules FY-1 to FY-12 was done. The spectra shows a strong absorption band at 1716.78 cm⁻¹ for C=O group. An intense peak is observed at 1535.1 cm⁻¹ for C=C aromatic nuclei. Two strong absorption bands were seen at 1488.01 and 1541.68 cm⁻¹ for 8 C=C, 9 C=N stretching in ring. Absorption is seen at 1308.91 cm⁻¹ for C=N stretching for primary amino group. Absorption is seen at 902.01 cm⁻¹ for 5,6 alkene -CH=CH- group. Two strong absorption bands at 3441.87, 3480 cm⁻¹ and this confirms primary amino group. Absorption at 3551 cm⁻¹ indicates presence of O-H (primary alcohol). Absorption is seen at 3186.66 cm⁻¹ for C-H stretching at alkenes. It was concluded that there was no interaction with aceclovir and bakers yeast based on the

Table 1
Preparation of aceclovir loaded microcapsules using bakers yeast.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Sample No</th>
<th>Temperature</th>
<th>Drug (mg) in 5 ml buffer (pH5)</th>
<th>Yeast (g) in 10 ml water</th>
<th>Water (ml)</th>
<th>Volume taken for stirring (ml)</th>
<th>Stirring rpm</th>
<th>Yield %</th>
<th>Entrapment efficiency</th>
<th>Amount of C=O released (mg)</th>
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<tr>
<td>1</td>
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<td>25°C</td>
<td>200</td>
<td>0.4</td>
<td>20</td>
<td>35</td>
<td>2000</td>
<td>69</td>
<td>0.759</td>
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<td></td>
<td>FY-5</td>
<td>25°C</td>
<td>400</td>
<td>0.8</td>
<td>20</td>
<td>35</td>
<td>2000</td>
<td>76</td>
<td>0.770</td>
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<tr>
<td></td>
<td>FY-9</td>
<td>30°C</td>
<td>800</td>
<td>1.6</td>
<td>20</td>
<td>35</td>
<td>2000</td>
<td>94</td>
<td>0.773</td>
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<td>0.4</td>
<td>20</td>
<td>35</td>
<td>2000</td>
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<td>0.784</td>
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<td>2000</td>
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<td></td>
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<td>20</td>
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<td></td>
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<td>20</td>
<td>35</td>
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<td>92</td>
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Table 2

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<tr>
<th>Sl. No.</th>
<th>Sample taken</th>
<th>Peak area</th>
<th>Amount of acyclovir present in 400 mg of sample (mg)</th>
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<tr>
<td>1</td>
<td>Standard drug</td>
<td>4462551</td>
<td>436.0</td>
</tr>
<tr>
<td>2</td>
<td>Marketed sample</td>
<td>4151954</td>
<td>397.9</td>
</tr>
<tr>
<td>3</td>
<td>Sample 4</td>
<td>4389299</td>
<td>396.7</td>
</tr>
<tr>
<td>4</td>
<td>Sample 8</td>
<td>4394368</td>
<td>391.9</td>
</tr>
<tr>
<td>5</td>
<td>Sample 12</td>
<td>4326804</td>
<td>386.0</td>
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</tbody>
</table>

The High Performance Liquid Chromatography (HPLC) was done for the pure drug acyclovir, FY-4 (2400 mg) and the peak found was 4389299, FY-8 (400 mg) the peak was 4394368, and FY-12 (900 mg) the peak was 4326804 and the content uniformity was assayed (Table 2).

The Thermal analysis (DSC) was done for the standard (pure drug) acyclovir and sample FY-1, sample FY-3, sample FY-5 and sample FY-7. For the standard (pure drug-acyclovir) two peaks were obtained at 168.54 C and 278.99 C. For sample FY-1 a single peak was obtained at 263.70 C. Sample FY-3 showed a single peak at 116.72 C, sample FY-5 showed two peaks at 263.49 C and 260.77 C. Sample FY-7 gave one peak at 265.59 C. As per the standard (pure sample) the peak value is between 168.54 C and 278.99 C. Except sample FY-3 which showed a peak value of 116.72 C all the other samples are within the range of the peak values of the standard acyclovir (Figure 6).

4. Discussion

The microencapsules of acyclovir was prepared using the drug, baker's yeast and water at specific ratio, at various temperatures and pH to optimize the yield. The yield was maximum in the case of FY-9 (94%). The entrapment efficiency was found to be maximum in FY-8 (97.61%). The SEM analysis revealed there was no burst on the surface and it was intact. The SEM of FY-5 was considered to be the best which was done at 10 µm and 500 µm. The particle size analysis showed size of 0-10 µm in diameter was common in most groups except group FY-9, where most particles were found to be 10-20 µm.

Entrapment efficiency for every 100 mg of the samples was done for all the twelve samples, FY-3 showed maximum entrapment of 97.61 mg.

The FTIR of the twelve samples showed no interaction between acyclovir and baker's yeast. The thermal analysis of standard (pure drug acyclovir) showed two peaks at 168.54 C and 278.99 C, peaks of all samples were within the peak values of the standard one.

The dissolution study of the twelve samples showed the amount of release increased with temperature and sample FY-8 had a cumulative drug release of 398 mg.

HPLC study showed FY-4, FY-8 and FY-12 were prepared in the form of suspension. It was found that the amount of acyclovir present in 400 mg marketed sample was 397 mg, for FY-4, FY-8 and FY-12 was 390.7 mg, 391.9 mg and 386 mg, which are all above 95% and almost equal to the label claim of the marketed samples. So it has confirmed that sample FY-8 is the best microencapsulated sample using acyclovir, yeast and water for microencapsulation.

Conflict of interest statement

We declare that we have no conflict of interest.

References

Formulation and evaluation of acyclovir microcapsules using biodegradable and non-biodegradable polymers

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ABSTRACT

To formulate and evaluate Acyclovir microcapsules using biodegradable and non-biodegradable polymers namely egg albumin, guar gum and ethyl cellulose and its invitro evaluation. The Acyclovir microcapsules were prepared using different concentrations of egg albumin, guar gum and ethyl cellulose. The microcapsules were prepared using solvent diffusion method and heat coagulation method. The microcapsules were then studied for entrapment efficiency at two different stirring speeds, drug polymer compatibility and surface morphology. The invitro release study was also done. Further kinetic modeling was employed to find out the release mechanisms. Acyclovir loaded microcapsules formulated with guar gum showed an entrapment efficiency of 92.97%, with ethyl cellulose 91.96% and the entrapment efficiency with egg albumin was 90.61%. The SEM showed that the microcapsules were free flowing, non aggregated and spherical between 700-1000 µm in diameter. The surface was wavy in microcapsule formulated with guar gum, porous in microcapsules using ethyl cellulose and smooth in the case of microcapsules formulated with egg albumin. The FTIR spectrum showed that there is no interaction between the polymer and the drug. The invitro release study was found to be the best in the case of acyclovir microcapsules formulated with guar gum. The rate of drug release follows a time dependent process based on fickian diffusion and korsemeyer-peppas model that the drug release is by diffusion and by erosion. The acyclovir microcapsules using various polymers can be used as oral controlled delivery of the antiviral drug acyclovir.

Key words. Polymers, Acyclovir, Microcapsules
INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body in order to promptly achieve and thereby to maintain the desired concentration. Recently, several technical advancements have been made. They have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity, or targeting the delivery of drug to a tissue. These advancements have led to the development of several novel drug delivery systems that could revolutionize the method of medication and provide a number of therapeutic benefits and the ultimate aim of these systems are to achieve the extended duration of drug levels but the methods of achieving this and the clinical performance of the products can vary considerably. Prolonged release or sustained release dosage forms have many advantages in safety and efficacy over immediate release drug product in that the frequency of dosing can be reduced, drug efficacy can be prolonged and intensity of adverse effects can be decreased. Many techniques are capable of controlling the rate of the drug delivery. Sustaining the duration of therapeutic activity and / or targeting the delivery. Sustained release[1,2,3,4.] systems are designed to achieve slow release of drug over an extended period of time after administration of single dose. If the system can provide control whether this be of a temporal or spatial nature or both of drug release in the body it is considered as a controlled release system. Control delivery attempts to sustain drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body. Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small beads with many useful properties. In its simplest form a microcapsule is a small sphere with a uniform wall around it. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating or membrane. Most microcapsules have diameters between a few micrometers and a few millimeters. A range of materials are suited for use as the capsule material: lipids, wax, crystal starch, modified starch, cellulose, phospholipids and other polymers.[5,6] Many microcapsules however bear little resemblance to these simple spheres. The core may be a crystal a jagged adsorbent particle, an emulsion, a suspension of solids or a suspension of smaller microcapsules. The microcapsule even may have multiple walls. The uniqueness of microencapsulation is the smallness of the coated particles and their subsequent use and adaptation to a wide variety of dosage forms and product applications which otherwise might not have been technically feasible. Because of the smallness of the particles, drug moieties can be widely distributed throughout the gastrointestinal tract, thus potentially improving drug sorption. Converting liquids to solids, providing environmental protection, improved material handling properties, colloidal and surface properties can be altered, control the release characteristics and masking or protecting the core material as well as decreasing the volatility. Microencapsulation[7,8] holds great promise for increased product value and effectiveness, particularly within the pharmaceutical field. There are great benefits arising from the use of microencapsulation in Pharmaceutical products. For many treatments, microencapsulation will allow patients to take lower doses for the same pharmacological effect, lower the risk of side effects, allow patients to take fewer doses; e.g. one a day instead of five a day, enable taste – masking for children’s medicines.[9,10]. Microencapsulation will enable the production of taste masked chewable tablets, powders and suspensions, sustained or prolonged action medication, single layered tablets containing chemically incompatible ingredients, new formulation concepts for cream, ointments, dressing, aerosols, plasters, injectables and suppositories. Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antiviral are used for specific viruses. Antiviral drugs are one class of anti-microbial, a larger group which includes antibiotics, antifungal and anti-parasitic drugs. They are relatively harmless to the host and therefore can be used to treat
infections. Most of the antiviral now available are designed to help deal with HIV, herpes virus, which is best known for causing cold sores but actually covers a wide range of diseases, and the hepatitis B and C viruses, which can cause liver cancer. Researchers are now working to extend the range of antiviral to other families of pathogens.[11,12] The emergence of antiviral is the product of a greatly expanded knowledge of the genetic and molecular function of organisms, allowing biomedical researchers to understand the structure and function of viruses, major advances in the techniques for finding new drugs and the intense pressure placed on the medical profession to deal with the deadly virus. Eleven drugs approved by the Food and Drug Administration for the treatment of viral infections (other than those caused by human immunodeficiency virus). They are seven nucleoside analogues, two closely related 10-carbon ring amines, one pyrophosphate analogue, and a recombinant protein produced in bacteria. Acyclovir[13,14] is poorly water soluble and has poor oral bioavailability (10-20%) hence intravenous administration is necessary if high concentrations are required. When orally administered, peak plasma concentration occurs after 1-2 hours. Acyclovir has a high distribution rate; only 30% is protein-bound in plasma [15]. The elimination half life of acyclovir is approximately 3 hours. It is renally excreted partly by glomerular filtration and partly by tubular secretion. The USP describes guar gum as a gum obtained from the ground endosperms of Cyamopsis tetragonolobus. It consists chiefly of a high molecular polysaccharide, composed of galactan and mannan units combined through glycoside linkages, which may be described chemically as a galactomannan. Guar gum occurs as an odorless or nearly odorless, white to yellowish-white powder with a bland taste. Ethyl cellulose is a tasteless free flowing white to lighten colored powder. Egg albumin is obtained from the hen’s eggs. In the solid state, albumin appears as yellow, brownish amorphous lumps, scales or powder. Denaturation can be induced by heating to 56°C by vigorously shaking with various acids. Albumin is a complex protein consisting of a single polypeptides chain of about 400 residues, a maximum of two phosphate residues per mole, and an oligosaccharide side chain of composed of mannose & glucosamine residues. Acyclovir being an antiviral drug was microencapsulated using egg albumin, Guar gum [18,19] and ethyl cellulose. Egg albumin and Guar gum being natural biomaterials was selected for micro encapsulation. Guar gum[20,21] has a good binding property so has got adhesive property towards drugs in a better manner when compared to that of egg albumin and ethyl cellulose. The release of the drug was done in the acidic pH and then in the alkaline pH and evaluation was done as per the plan of work. The work is evaluated against the marketed preparation of Acyclovir

**MATERIALS AND METHODS**

The microcapsules using guar gum [22.23] was prepared by water-in-oil-oil(w/o/o) solvent diffusion method. A weighed amount of acyclovir (0.2 to 0.8gm) and guar gum were dissolved in 30ml of a mixture of acetonitrile and dichloromethane (1:1 v/v). The initial 50ml water-in-oil emulsion was formed by adding 2ml of deionized water to the drug polymer solution with constant stirring at 500rpm for 10 minutes. The w/o primary emulsion was then slowly added to 50ml light liquid paraffin containing span 80 (0.1 ml) as a surfactant with constant stirring for 2 hours. The glutaraldehyde (1 ml) was added and the stirring was further continued for 1 hour. The resulting microcapsules were separated by filtration, freed from liquid paraffin by washing with petroleum ether and finally air dried over a period of 24 hours in desiccators. Microcapsules using egg albumin were prepared by the water-in-oil-in-oil (w/o/o) double emulsion (heat coagulation) method. A weighed amount of acyclovir and the egg albumin were dissolved in 30ml of water. The initial w/o emulsion was formed by adding 0.1ml of span 80. Added albumin solution drop wise to liquid paraffin 50 ml and kept stirring for 20 minutes. 50 ml of liquid paraffin was heated in a heating mantle at a temp of 80°C and to this albumin emulsion was
added drop wise and stirred for one hour. Precipitation of albumin formed which was separated by centrifugal process. The upper layer was discarded. The lower layer was washed four times with petroleum ether to remove traces of reactants and dried in an oven at 40°C. The microcapsules using ethyl cellulose was prepared by water-in-oil-oil (w/o/o) solvent diffusion method. A weighed amount of acyclovir (0.2 to 0.8gm) and Ethyl cellulose were dissolved in 30ml of a mixture of acetonitrile and dichloromethane (1:1 v/v). The initial 50ml water-in-oil emulsion was formed by adding 2ml of deionised water to the drug polymer solution with constant stirring at 500rpm for 10 minutes. The w/o primary emulsion was then slowly added to 50ml light liquid paraffin containing span 80 (0.1 ml) as a surfactant with constant stirring for 2 hours. The glutaraldehyde (1 ml) was added and the stirring was further continued for 1 hour. The resulting microcapsules were separated by filtration, freed from liquid paraffin by washing with petroleum ether and finally air dried over a period of 24 hours in a desiccator.

Table No: 1  Formulation details of acyclovir microcapsules using Guar gum

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<th>FG-I</th>
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<td>Yield (%)</td>
<td>95.45</td>
<td>93.75</td>
<td>96.42</td>
<td>93.18</td>
<td>91.66</td>
<td>94.64</td>
</tr>
<tr>
<td>Entrapment Efficiency (%)</td>
<td>89.88</td>
<td>76.27</td>
<td>92.97</td>
<td>84.25</td>
<td>66.72</td>
<td>92.55</td>
</tr>
</tbody>
</table>
Table No: II Formulation details of acyclovir microcapsules using Egg albumin

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>FA-I</th>
<th>FA-II</th>
<th>FA-III</th>
<th>FA-IV</th>
<th>FA-V</th>
<th>FA-VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclovir (mg)</td>
<td>200</td>
<td>400</td>
<td>800</td>
<td>200</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Solvent (ml)</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Liquid paraffin (ml)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Span 80 (ml)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Glutarardehyde (ml)</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Speed (rpm)</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1500</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>86.36</td>
<td>87.50</td>
<td>89.28</td>
<td>88.63</td>
<td>89.58</td>
<td>92.85</td>
</tr>
<tr>
<td>Entrapment Efficiency (%)</td>
<td>76.40</td>
<td>49.75</td>
<td>90.61</td>
<td>70.23</td>
<td>48.37</td>
<td>89.28</td>
</tr>
</tbody>
</table>

Table No: III Formulation details of acyclovir microcapsules using Ethyl cellulose

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>FE-I</th>
<th>FE-II</th>
<th>FE-III</th>
<th>FE-IV</th>
<th>FE-V</th>
<th>FE-VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclovir (mg)</td>
<td>200</td>
<td>400</td>
<td>800</td>
<td>200</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Solvent (ml)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Liquid paraffin (ml)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Span 80 (ml)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Glutarardehyde (ml)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Speed (rpm)</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1500</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>90.90</td>
<td>95.83</td>
<td>94.64</td>
<td>93.18</td>
<td>95.83</td>
<td>96.42</td>
</tr>
<tr>
<td>Entrapment Efficiency (%)</td>
<td>82.32</td>
<td>79.72</td>
<td>91.76</td>
<td>90.03</td>
<td>71.05</td>
<td>90.21</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

1. Entrapment efficiency of Acyclovir microcapsules

**Determination of entrapment efficiency of acyclovir loaded microcapsules**

About 100 mg of the sample was taken and 60 ml of 0.1 M sodium hydroxide was added and dispersed well for 15 minutes. Then sufficient quantity of 0.1 M Sodium hydroxide was added to produce 100 ml. Mixed well and filtered. 15 ml of the filtrate was taken and 50 ml of water and 5.8 ml of 2 M Hydrochloric acid and sufficient water was added to produce 100 ml. To 5ml of this solution sufficient 0.1 M Hydrochloric acid was added to produce 50 ml and mixed well. The absorbance of the resulting solution was measured at 255nm, using 0.1 M hydrochloric acid in the reference cell. The same procedure was followed for all the samples.

The quantity of acyclovir present in 100 mg of the sample taken was calculated by the following formula:

\[
\text{Absorbance} \times \frac{100}{\text{A (1%, 1cm)}} \times \text{dilution factor} \times 1000 \times \text{Weight taken}
\]

\[\text{A (1%, 1cm) of Acyclovir} = 560\]

The entrapment efficiency of the 18 formulations was done and it was found that formulation with Guar gum the minimum % entrapment efficiency was 66.72% and maximum was 92.91%. Formulation with Ethyl cellulose the minimum to maximum % entrapment efficiency was 71.05% to 91.76 %. Formulation with Egg albumin % entrapment efficiency the minimum to maximum was 49.75% to 90.61%. The entrapment efficiency of the formulation with guar gum in FG-III at 1000 rpm was 92.97% and FG-VI at 1500 rpm was 92.55% consequently. The entrapment efficiency of the formulations with the ethyl cellulose in FE-III at 1000 rpm was 91.76% and FE-VI at 1500 rpm was 90.21% consequently. The entrapment efficiency of the formulations with Egg albumin FA-III at 1000 rpm was 90.61% and FA-VI at 1500 rpm was 89.28 % consequently.

2 Compatibility study using FT-IR:

FT-IR was done for pure drug and 18 microcapsules formulation for drug identification. It indicates no chemical reaction between drug and polymers and also confirmed the stability of the drug during micro encapsulation process. The characteristic peaks were due to pure Acyclovir at 615 cm\(^{-1}\), 1215 cm\(^{-1}\),1440 cm\(^{-1}\),1515 cm\(^{-1}\),1610 cm\(^{-1}\), 1627 cm\(^{-1}\) for Aromatic ring - NH\(_2\) Aromatic, ether, - OH binding secondary amine, primary amine, C=C ring aromatic stretching, C=O stretching have appeared in microcapsules spectra peaks, without any change in their position after successful encapsulation. The pure drug spectra peaks correlated with the microcapsules formulations peaks.

3. Particle size analysis of microcapsules:

The particle size analysis of formulations was carried out by optical microscopy and the size range of maximum particles in formulation was found. FG-I to FG-III (140 -160µm), FG-IV to FG-VI (80-100µm), FE-I to FE-III (160 -180µm), FE-IV to FE-VI (140 - 160µm), FA-I to FA-III (140 -160µm) and FA-IV to FA-VI (60 - 100µm). The size range of particles were assessed between 80µm and 320µm.
Fig. No. I Histogram of FG-III microcapsules

Fig. No. II Frequency distribution curve of FG-III microcapsules

Fig. No. III Histogram of FG-VI microcapsules
Fig. No : IV Frequency distribution curve of FG - VI microcapsules

![Frequency distribution curve](image)

4. Morphological evaluation of microcapsules:
The surface morphology of Acyclovir microcapsules was seen using Scanning Electron Microscope. The surface morphology was done with magnifications 50 x, and 2500x as shown.

**SEM of Acyclovir Microcapsules**

**Fig No: V, FG- III (50x, 500 µm)**

![SEM image 1](image)

**Fig No: VI, FG- III (2500x, 10 µm)**

![SEM image 2](image)
5. In vitro drug release study under simulated gastrointestinal conditions:
The in vitro dissolution apparatus used in the present study was specially designed. 100 mg of sample was weighed and placed in dialysis membrane. A 250 ml beaker with 187.5 ml of dissolution fluid was kept on a magnetic stirrer. A magnetic bead was placed and stirred at 100 rpm. The temperature of 250 ml beaker was maintained at 37±0.5°C. The dialysis membrane was tied at the bottom of two side open glass tube and it was immersed in the dissolution fluid so that it touches the surface of the dissolution fluid. Samples were withdrawn every half an hour for 2 hours in case of 0.1N HCl as dissolution medium and after two hours 62.5 ml of 0.2M tribasic sodium phosphate solution was added for change the pH 1.2 to 6.8 and samples were taken every 1 hour interval up to 6 hours. 5 ml of the sample was withdrawn and replaced with 5 ml of the dissolution medium. The samples were analyzed spectrophotometrically at 255 nm with suitable dilution. The dissolution as carried out for 18 samples using 0.1N HCl pH 1.2 and phosphate buffer pH 6.8 as dissolution medium. The release study was performed for eight hours in simulated GI fluid. For FG-III at the end of the 2nd hour the cumulative release was 38.76% and at the end of the 8th hour it was 84.14%. For FG-VI at the end of the 2nd hour the cumulative release was 40.11% and at the end of the 8th hour it was 85.12%. For FE-III at the end of the 2nd hour the cumulative release was 38.49% and at the end of the 8th hour it was 81.93%. For FE-VI at the end of the 2nd hour the cumulative release was 40% and at the end of the 8th hour it was 86.72%. For FA-III at the end of the 2nd hour the cumulative release was 38.76% and at the end of the 8th hour it was 86.88%. For FA-VI at the end of the 2nd hour the cumulative release was 39.70% and at the end of the 8th hour it was 87.69%.
Fig. No: IX, Comparative study of % Cumulative drug released vs Time FA – Formulations.

Comparative study of % Cumulative drug released vs Time

Fig. No: X, Comparative study of % Cumulative drug released vs Time FG – Formulations.

Comparative study of % Cumulative drug released vs Time

Fig. No. XI, Comparative study of % Cumulative drug released vs Time FE – Formulations

Comparative study of % Cumulative drug released vs Time
6. Mathematical modelling of Acyclovir microcapsules:

In order to investigate the mode of release of the microcapsules, the release data were analysed with the following mathematical models.

\[ Q_t = K_0 \cdot t \] (Zero Order kinetics)
\[ \log \left( \frac{Q_t}{Q_0} \right) = K_1 \cdot \frac{t}{2.303} \] (First Order Kinetics)
\[ Q_t = K_{KP} \cdot t^n \] (Korsmeyer and Peppas equation)
\[ Q_t = K_H \cdot t^{1/2} \] (Higuchi’s equation)

Where \( Q_t \) is the percent of drug released at time “\( t \)”, \( K_0 \), \( K_1 \), \( K_{KP} \), and \( K_H \) are the coefficient of Zero order, First order, Korsmeyer-Peppas and Higuchi’s equation.

CONCLUSION

From the entrapment efficiency done for the eighteen formulations it was seen that on increasing the rpm from 1000 to 1500 the entrapment efficiency decreased except in the case of the acyclovir microcapsules encapsulated with guar gum which was 92.97%. In the case of FT-IR after interpretation through the spectra it was confirmed that there were no major shifting of functional peaks between the spectra of the drug, polymer and the drug loaded microcapsules. It can be concluded from the IR spectroscopic studies that the drug acyclovir was entrapped in the polymer matrix and there was no chemical interaction because there was no shifting of the functional peaks From the SEM analysis it was seen that there was no burst in the encapsulated material. Surface was wavy in FG, porous in FE and smooth in FA formulations. The microcapsules were spherical, free flowing and non aggregated.

The rate of drug release follows a time dependent process based on fickian diffusion and korsmeyer-peppas model that the drug release is by diffusion and by erosion.

REFERENCES