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Formulation and Evaluation of Spironolactone Microspheres using natural and synthetic polymers as a Carrier

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Abstract

The present study reports a novel attempt to prepare microspheres of the diuretic drug spironolactone by using natural ,semisynthetic and synthetic polymers as carrier. Microspheres were prepared by solvent evaporation method using an acetone / liquid paraffin system and Phase separation coacervation method using petroleum ether and coconut oil as dispersion and continuous phase systems. n-hexane was added to harden the microspheres. The prepared microspheres were evaluated for their micromeritic properties, drug content and encapsulation efficiency and characterized by Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM). The in vitro release studies was performed by buffer change method to mimic Gas Intestine Tract(GIT) environment in pH 1.2, carbonate buffer (acidic) and pH 7.4, phosphate buffer (Alkaline). The prepared microspheres were yellow, free flowing and spherical in shape. The mean particle size of the microspheres was found in the range of 150 to 408µm.. The drug-loaded microspheres showed 82-91% of entrapment and release was extended up to 6 to 12 h releasing 77% of the total drug from the microspheres. The infrared spectra showed stable character of Spironolactone in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. Scanning electron microscopy study revealed that the microspheres were spherical and porous in nature. The best-fit release kinetics was achieved with Koresmeyer-Peppas plot followed by zero order and First order. The release of Spironolactone was influenced by the drug to polymer ratio and particle size & was found to be both diffusion and dissolution controlled.

Key-Words: Spironolactone, Egg albumin, Eudragit, HPMC, Ethyl cellulose, Solvent Evaporation technique, Phase Separation technique, Microspheres

Introduction

The drug delivery system employed plays a vital role in controlling the pharmacological effect of the drug as it can influence the pharmacokinetic profile of the drug, the rate of drug release, the site and duration of drug action and subsequently the side-effect profile. An optimal drug delivery system ensures that the active drug is available at the site of action for the correct time and duration. However, it is necessary to develop suitable dosage forms or drug delivery systems to allow the effective, safe and reliable application of these bioactive compounds to the patient.

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It is important to realise that the active ingredient (regardless of whether this is a small-molecular weight 'classical' drug or a modern 'biopharmaceutical' drug like a therapeutic peptide, protein or antigen) is just one part of the medicine administered to the patient and it is the formulation of the drug into a dosage form or drug delivery system that translates drug discovery and pharmacological research into clinical practice.

The efficacy of a drug in a specific application requires the maintenance of appropriate drug blood level concentration during a prolonged period of time. However the conventional administration of drugs, gives a poor control of the concentration of these substances in plasma because of variations in the concentration of the bioactive product, once a specific dose has been administered [1]. The conventional dosage systems can give rise to alternative periods of inefficacy or toxicity. These difficulties have been called for the development of new administration techniques for bioactive compounds, directed towards

attaining the steady state plasma concentration [2]. Microspheres have played a vital role in the development of controlled/sustained release drug delivery systems. After the employment in the body, biodegradable polymers of natural and synthetic origin like Eudragit, ethyl cellulose and egg albumin have a unique advantage that after performing their function they degrade into non toxic monomers. Administration of drugs in the form of microspheres, usually improves the treatment by providing the localization of the active substance at the site of action and by prolonging release of drug. Pharmaceutically acceptable techniques using hydrophobic biodegradable polymers as matrix materials include Emulsion-solvent evaporation, Phase separation (non solvent and solvent partitioning), Interfacial polymerization and Spray drying. Spironolactone was selected as a model drug which is a synthetic, steroidal antimineralocorticoid and antiandrogen that also has some weak progestogen properties, as well as some indirect estrogen and glucocorticoid effects. It is used primarily as a diuretic andantihypertensive, but may also be employed for the purpose of reducing elevated or unwanted androgen activity in the body. It acts predominantly as an antagonist of the mineralocorticoid (or aldosterone) and androgen receptors, and in relation to its antimineralocorticoid effects, belongs to a class of pharmaceutical drugs known as potassium-sparing diuretics.

Material and Methods

Spironolactone (Jubliant Life Sciences Ltd), Eudragit RS 100 and RL 100 (Jubliant Life Sciences Ltd), Egg albumin (SD fine chemicals), HPMC (SD fine chemicals), Ethyl cellulose (SD fine chemicals), Petroleum ether (Reachem laboratories limited), N-hexane (Finar Reagents), Liquid paraffin (Qualigens Fine chemicals), Acetone (SD fine-chemicals limited) and all other solvents and reagents used were of analytical grade.

Experiment

Spironolactone microspheres were formulated using two techniques i.e. phase separation and solvent evaporation method depending upon the type of polymer employed for the formulation of microspheres. Biodegradable polymers employed were Eudragit RS 100 and RL 100 (synthetic), ethyl cellulose (semi synthetic), HPMC and egg albumin (natural). In the formulations concentration of polymers were varied from 1-2-3-4% w/v respectively to study their effects on the drug release. The objective of varying the concentration of the polymers was to achieve optimized formulations, which would give a sustained release of the drug over a period of 8 hours.

Preparation of Polymeric-Spironolactone microspheres

Phase separation emulsion polymerization method was employed for the preparation of microspheres. Polymeric drug solution (Spironolactone with HPMC, Ethyl Cellulose, Egg Albumin, Eudragit RS 100 and RL 100) were prepared by adding required amount of drug to egg albumin solution and stirred continuously until uniform dispersion. In a separate beaker, to 86ml of coconut oil 1ml of 0.5% sodium lauryl sulphate was added to give organic phase. Polymeric drug solution was added drop-wise using 22 gauge needles into the organic phase and stirred continuously to form uniform dispersion. The temperature of the solution was gradually increased to 80°C and stirred at this temperature for 2 hours. The solution was cooled to room temperature with continuous stirring using mechanical stirrer. After the room temperature was attained, 1ml of formaldehyde and 20ml of n- Hexane was added to separate the microspheres from the organic phase which were filtered and separated. The obtained microspheres were washed thrice with n-Hexane then followed by distilled water, dried and stored in air tight containers until further analysis.

Characterization

The prepared microspheres were characterized by Fourier Transformed Infrared Spectroscopic analysis, The FT-IR spectral measurements were taken at ambient temperature using a Shimadzu, Model 8300 (USA) using KBr pellet method by applying 6000 kg/cm2 pressure to study the polymer-drug interactions. The SEM analysis of the microspheres was carried out by using Jeol JSM T-330A Japan, to determine size, shape and surface morphology of the prepared microspheres.

Evaluation

Drug-Excipients Compatibility Studies

Excipients are integral components of almost all pharmaceutical dosage forms thus it is mandatory to detect any possible physical or chemical interaction of the drug with the excipients since the excipient can affect the bioavailability and stability of the drug. The drug and the excipients must be compatible with one another to produce a product that is stable, efficacious, attractive, easy to administer and safe. If the excipients are new and have not been used in formulations containing the active substance, the compatibility studies have a considerable importance. FTIR technique was commonly used to investigate the compatibility between the drug and the various excipients used in the formulation.

Sample preparation and analysis by FTIR

FTIR spectra data was taken on a Shimadzu (FTIR-8300) instrument to find out the chemical stability of the drug with excipients. FTIR spectra of drug, polymer and composition of final formulation were obtained by mixing with potassium bromide and converted into pellets by pressing at 1 ton/unit. Spectral scanning was done in the range of 4000 – 400^{-1} cm. FTIR study was carried on spironolactone, physical mixture of spironolactone and polymer, spironolactone microspheres and blank microspheres.

Surface morphology (SEM)

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry spironolactone microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of spironolactmicrospheres were taken by random one scanning of the stub.

Percentage yield

These studies involve determination of the amount of microsphere obtained at the end of preparation and polymer and drug that are consumed in its preparation. The yield of microspheres was determined by comparing the whole weight of microspheres formed against the combined weight of the copolymer and drug. The yield of microspheres was determined by the formula:

$$\% \text{ Yield} = \frac{\text{Total Weight of Microspheres}}{\text{Total Weight of Raw Material}} \times 100$$

The percentage yield of each formulation was determined according to the total recoverable final weight of microsphere and the total original weight of spironolactone and polymer**.**

Bulk density

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it was handled. In the present study, we had taken the weighed quantity of air-dried and presieved (#40/120) microspheres. Carefully added them to a cylinder with the aid of a funnel without any losses. The initial volume was noted, it gives the bulk volume.

Bulk density= weight/ bulk volume

Tapped Density

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume or mass readings are taken until little further volume or mass change is observed. We had taken the weighed quantity of air-dried and presieved (#40/120) microspheres. Carefully added them to a cylinder with the aid of a funnel without any losses.The sample was then tapped until no further reduction in volume was noted. After tapping the volume reduced, giving the value of tapped volume

Tapped density= weight/tapped volume

Carr's Index

Carr's index has been used as an indirect method of quantifying powder flowability from bulk density; this method was developed by Carr. The percentage compressibility of a powder is a direct measure of the potential powder arch or bridge strength and stability, and is calculated according to following equation. It can be measured of potential strength that powder could build up in its arc in hopper and also the case with an arch could be broken.

Carr's index= Tapped density- Bulk density/ Tapped density

Hausner's Ratio

It is an indirect index of case of measuring the powder flow. It is calculated by the following formula:

Hausner's ratio = Tapped density/Bulk density

Angle of repose

Angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles. The angle of repose of microsphere and commercial crystals was measured by fixed funnel method. A funnel with the end of the stem cut perpendicular to the axis of symmetry is secured with its tip at a given height (1 cm), H, above graph paper placed on a flat horizontal surface. The microspheres were carefully poured through the funnel until the apex of the conical pile so formed just reached the tip of the funnel. Thus, the r being the radius of the base of the microspheres conical pile.

$$\theta = \text{Tan-1}(h/r)$$

Where, θ = Angle of Repose, h= height of the heap

Particle size analysis

Particle size characterization is an important study of ensure that the particle size of the formulation lies in the optimal range. Determination of average particle size of spironolactone microspheres was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of spironolactone microspheres was spread on a clean glass slide and average size of 100 spironolactone microspheres was determined in each batch. In order to be able to define a size distribution or compare the characteristics of particles with many different diameters, the size distribution can be broken down into different size ranges, which can be presented in the form of a histogram. Histogram presents an interpretation of the particles size distribution and enables the percentage of particles having a given equivalent diameter to be determined The average particle size was determined using Edmondson's equation.

$$D = \sum nd / \sum n$$

Where, n =number of microspheres, d= mean of the size range, D = average particle size (in μm).

Drug loading

The prepared microspheres were powdered and passed through sieve no (85/120). The powder retained on the sieve 120 was taken for content uniformity studies. A weight of powder containing 100 mg of the drug was taken in a 100ml standard volumetric flask. To this of ph 7.4 buffer solution was added and made upto the mark and kept overnight. The final solution was filtered using what man filter paper. From this 10 ml was pipetted out into a 100 ml standard volumetric flask and made up to the volume with phosphate buffer solution and estimated spectrophotometrically for drug content.

% Drug Loading = Amount of drug present in microspheres/weight of microspheres X 100

Drug entrapment efficiency of microspheres

It is determined by calculating the amount of drug that is entrapped in the microsphere and the drug which is adsorbed on the surface or interior of the polymer. The amount of free, adsorbed and entrapped drug should be capable of being determined separately and this determination indicated the efficacy of the microsphere produced in terms of its active ingredients. For the drug entrapment efficiency of microspheres, 50 mg of microspheres were accurately weighed. Then dissolved in 50 ml of methanol in a volumetric flask to get a solution containing one mg drug per ml. The resulting solution was filtered through whatman filter paper and then suitably diluted to check for the absorbance on the UV spectrophotometer.

% Drug entrapment efficiency=Amount of drug actually present / Theoretical weight of the drug X 100

***In vitro* dissolution studies of microspheres**

These studies aid in understanding the behavior of these system in terms of drug release and their efficacy. Since microsphere is heterogeneous system, the drug release form the polymer taken place through a diffusion process, in an in vitro environment. As a result, the drug and polymer matrix are phase separated and form a biphasic system. The release of the drug is determined by the extent of degradation of polymeric microsphere. In the dissolution test the micro particles were firstly subjected to a pH 1.2 buffer for 2 hours and then to a pH 7.4 phosphate buffer for next 10 hrs. The volume of the dissolution media was maintained at 900 ml with constant stirring (100 rpm) and the temperature was maintained at 37±0.50C. After a time interval of 1 hr. sample were withdrawn and replaced with fresh media immediately after sampling. The samples withdrawn were analyzed for the drug content by scanning the sample at 238 nm using UV spectrophotometer. Dissolution studies were carried out for all the batches of the prepared formulations.

Results and Discussion

FT-IR analysis

Spironolactone and one of the formulations of each polymer were subjected to FTIR spectroscopic analysis, to ascertain whether there is any interaction between the drugs and the polymers used. The FT-IR spectra obtained is given in **Fig. (1,2,3,4,5,6).** The characteristic peaks of the pure drug were compared with peaks obtained from their respective formulations and are given in the tables and for Spironolactone and formulations respectively. From the data obtained it was observed that characteristic peaks appears with identical or with minor differences, at frequencies and formulations, 1777, 1700, 1677 and 1619 cm-1 corresponding to –C=O stretching of lactone ring, –C=O stretching of thioacetyl group, –C=O stretching of α, β-unsaturated ring and –C=C stretching of α, β-unsaturated ring.from the peaks it was evident that there was no chemical interaction between the drug and polymers.

Scanning electron microscopy

Scanning electron microscopy (SEM) is one of the most commonly used method for characterizing drug delivery systems, owing in large part of simplicity of sample preparation and ease of operation. Scanning electron microscopy was carried out in order to characterize surface morphology of the microspheres. In this study the morphological observations were carried out to study the surface morphology of

microspheres. SEM micrographs and typical surface morphology of the microspheres. It was observed that microspheres were spherical in nature, the microspheres ranged in size 150 to 408µm.

Conclusion

Microspheres as controlled release preparations were formulated and evaluated containing highly water-insoluble drug, spironolactone, using natural polymer, semi synthetic polymer and Synthetic polymer as the retardant materials by phase separation coacervation method and emulsion solvent evaporation method where the polymer concentration was varied keeping the drug concentration constant. The FTIR Spectra revealed that there was no interaction between polymers and spironolactone. All the polymers used were compatible with the spironolactone. The microspheres are spherical and Surface smoothness of the spironolactone microspheres was increased by increasing the polymer concentration in case of each polymer, which was confirmed by SEM. % yield of microspheres increases with increasing polymer concentration and egg albumin microspheres gives highest % yield. The micromeritics properties show that all the formulations are free flowing. As the drug to polymer ratio was increased, the mean particle size of spironolactone microspheres was also increased. Egg albumin microspheres have minimum particle size. Entrapment efficiency increase with increase in the polymer concentration. Egg albumin and Combination of ethyl cellulose and Eudragit RS-100 microspheres shows maximum drug entrapment. The study also indicated that the amount of drug release decreases with an increase in the polymer concentration. Combination of Eudragit RS-100 and RL-100 shows best sustained release action upto 12 hrs. The *in-vitro* performance of spironolactone microspheres showed prolonged and sustained release of drug

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References

1. Bhaskar Mazumder., Sanjib Bhattacharya., Bibhash Mohanta., Sanjay Dey., Anindya Maity. (2009) *Int J PharmTech Research*, 1(3), 905-913.
2. Sunit Kumar Sahoo., Abdul Arif Mallick., Barik B.B., Prakash Ch Senapati. (2005) *Tropical journal of Pharmaceutical Research*, 4 (1), 369-375.
3. Cheu S.J, Chen R.I, Chen P.F, Lin W.J, In vitro modified release of acyclovir from ethyl cellulose microspheres, (2001), Journal of Microencapsulation, 18(5), 559-565.
4. Tamer Guneri, Mesut Arici, Gokhan Ertan, Preparation and Diffusional Evaluation of Sustained -Release Suppositories Containing Ibuprofen Microspheres (2004) , Fabad j. pharm. Sci, 29, 177- 184. [5] Radi Hejazi, Mansoor Amiji. (2002), Int J Pharmaceutics, 235, 87-94.
5. Ibrahim M. El-Bagory, Ehab A. Hosny, Saleh A. Al-Suwayeh, Gamal M. Mahrous, Fahad I. Al-Jenoobi. (2007), Saudi pharm Journal, 15(3-4), 213-217.
6. Kotadiya R, Patel V, Patel H, Koradiya H, Effect of cross-linking on physicochemical properties of chitosan mucoadhesive microspheres: A factorial approach (2009) Int J Green Pharmacy, 14, 58-62.
7. Waree Tiyaboonchai, Garnpimol C. Ritthidej, Develoment of indomethacin sustain release microcapsule using chitosan as polymer, (2003) , Songklanakarin J. Sci. Technol, 25(2), 245-254.
8. Pachuau L., Sarkar S, Mazumder B, Formulation and evaluation of matrix microspheres for simultaneous delivery of salbutamol sulphate and theophylline, (2008), Tropical Journal of Pharmaceutical Research, 7 (2), 995-1002.

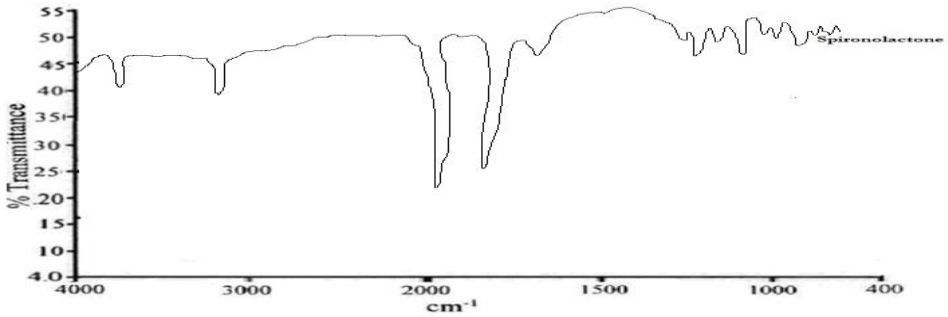

Fig. 1: FTIR of Spirnolactone

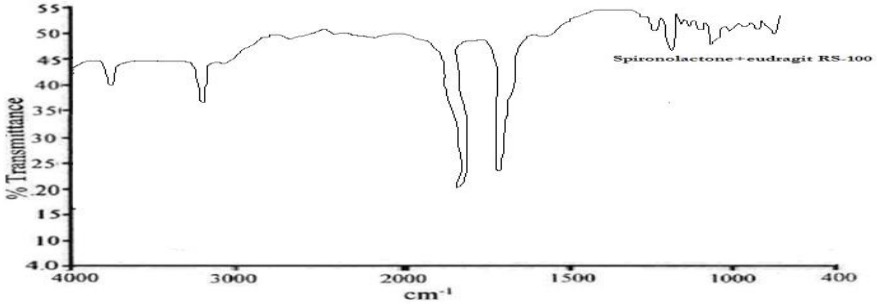

Fig. 2: FTIR of Spirnolactone + Eudragit RS 100

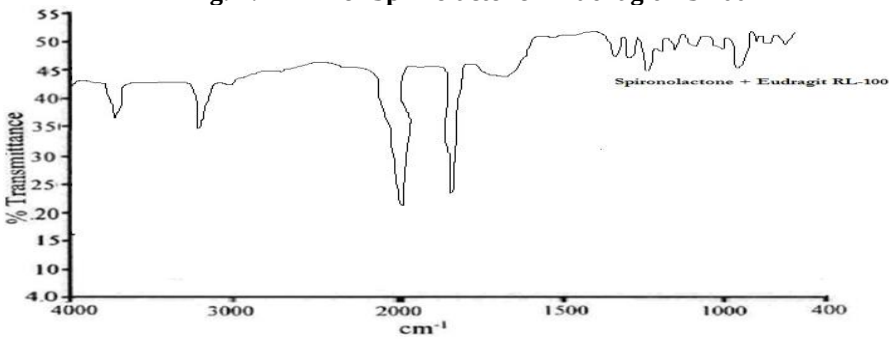

Fig. 3: FTIR of Spirnolactone + Eudragit RL 100

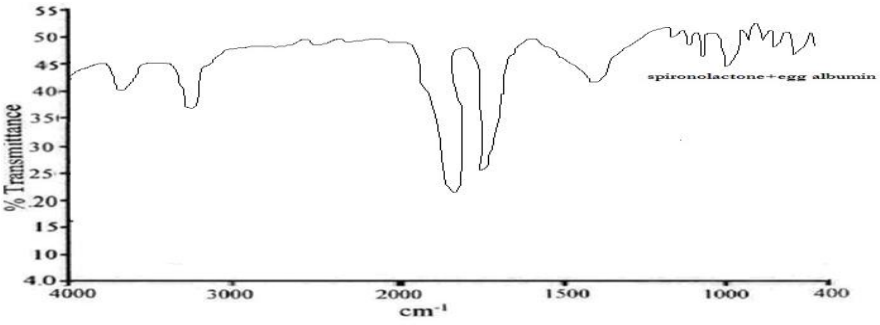

Fig. 4: FTIR of Spirnolactone + Egg Albumin

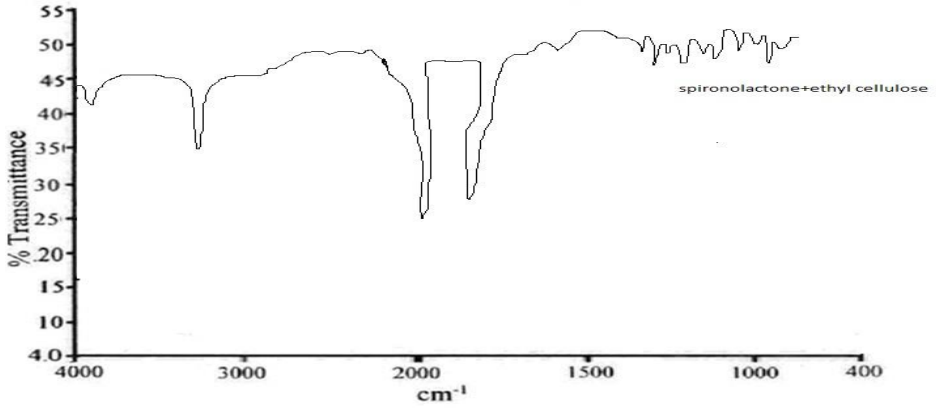

Fig. 5: FTIR of Spirnolactone + Ethyl Cellulose

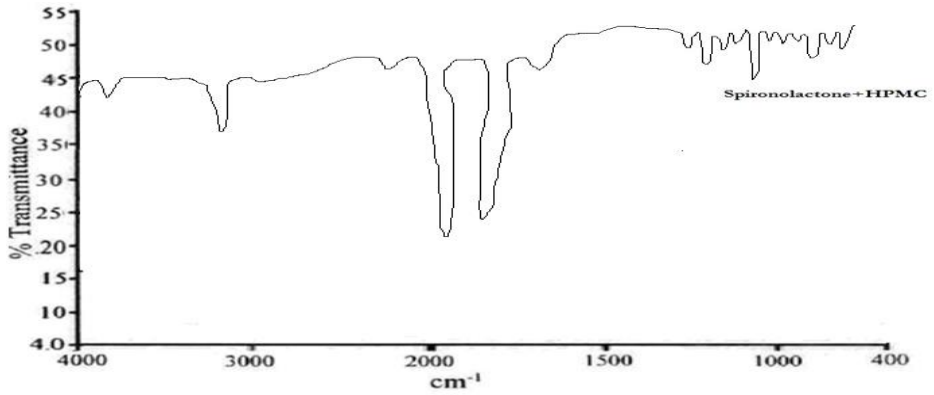

Fig. 6: FTIR of Spirnolactone + HPMC

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