WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Formulation and Evaluation of Microsphere Containing Metronidazole

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ABSTRACT :

This work has been done to develop microsphere of metronidazole, slow drug release for prolonged period reduce dose dumping and produces prolong therapeutic effect and develop modified release dosage forms for targeted or sustained release purpose with masking the taste of bitter or noxious drugs for their convenient handling. Microspheres were prepared using different speed at three different drug: polymer ratio, (1:1, 1:1.5, and 1:2) In case of micrometric properties all the formulation show angle of repose value in the range of 15.69±0.745 to 34.21±0.61. these value for angle of repose <40 indicated good flow properties of microspheres the values of bulk density were found to range from 0.124±0.032 to 0.961±0.03 .the values for tapped density were found to range from 0.205± 0.064 to 0.89 ±0.009 .the value of compressibility index found in range of 17.443±0.024 to 12.76±0.023 these values for compressibility index .indicated good flow properties the values for Hausner's ratio 1.085±0.0652 to 1.18±0.021.i.e. all the preparation show that they had good flow property the average particle size of microspheres is found to be within 44.73±0.57 to 132.452±0.37µm. The percent encapsulation efficiency is found to be in the range of 63.74±0.88 to 98.76±4.68. In-vitro drug release study it can be seen that the F1 batch, show in cumulative percentage drug release 84.77 % in the 12 hrs. From these result formulation F1 was selected as optimized formulation and evaluated for FTIR XRD,SEM .The IR data of metronidazole loaded ethyl cellulose microsphere batch F5 also show than bands as bands in IR data of pure drug ,these suggest that no chemical interaction between ethyl cellulose and metronidazole.

Keywords: Micro-particulate systems, Metronidazole, Microspheres.

Introduction :

The sustained release drug delivery includes the application of physical and polymer chemistry. These polymers slowly release the drug in bio-system and maintain drug blood level within therapeutic range for longer duration. Some of the products characterize the drug permeation through the appropriate biological membrane and ay first pass metabolic effect prior to the entry of drug into systemic circulation. The fact that the absorption and release rate of the drug from the dosage form, is one of the interesting and most recent development in pharmaceutical field. [(1).]Micro-particulate systems refer to the technology of re-arranging and processing of atom/molecules to fabricate material of micrometre specifications. The availability of the spheres of different size allows flexibility regarding specific in vivo applications and forms of administration. In general, these systems consist of polymeric materials in which the drug is dispersed, entrapped, dissolved or adsorbed. This relatively young branch of sciences draws expertise from chemical sciences, polymer science, physical sciences, biomedical sciences, pharmaceutical sciences, engineering and biotechnology, etc., in a truly inter-disciplinary manner.

Carrier is one of the most important entities essentially required for successful transportation of loaded drugs overcoming challenges posed in drug delivery. The function of drug carrier is not only confined as guiding devices but to protect and retain the bioactivity of drugs including enzymes, proteins, peptides, etc., from different physiological environments against inactivation or excretion, to prevent them from eliciting adverse immune reactions or to store them in a reservoir for their release in a gradual fashion [(2).]

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 µm3 [(3,4).]

Multiparticulate delivery system H.Steckel, F.Mindermann-Nogly have prepared chitosan pellets using the extrusion/spheronization technology. Microcrystalline cellulose was used as additive in concentrations range from 0-70 %. The powder mixture was extruded using water and dilutes acetic acid in different powder to liquid ratios. The study showed that chitosan pellets with a maximum of 50 % (m/m) could be produced with demineralized water as granulating fluid. The mass fraction of chitosan within in the pallets could be increased to 100% by using dilute acetic acid for the granulation step [(5,6).]The colon targeted drug delivery is beneficial for the localized treatment of several colonic diseases mainly inflammatory bowel diseases (IBD),

irritable bowel syndrome and colonic cancer. To achieve clinically relevant bioavailability of poorly absorbed drugs from the upper parts of the gastrointestinal tract because of their polar nature and/or vulnerability to chemical and enzymatic degradation in the small intestine specifically for proteins and peptides3. The colonic drug delivery provide more effective therapy of colon associated diseases such as irritable bowel syndrome, IBD including Crohn's disease and ulcerative colitis, and also has potential to deliver macromolecular drugs orally. Colon related pathologies range in seriousness from constipation and diarrhoea to the incapacitating inflammatory bowel diseases through to colon cancer, the third most widespread form of cancer in both women and men.

Drugs that are easily absorbed from the gastrointestinal tract and have a short half-life are eliminated quickly from the blood circulation, required frequent dosing. To avoid this problem, the oral sustained release formulations have been developed in an attempt to release the drug slowly into the GIT and maintain a constant drug concentration in the serum for longer period of time. For better absorption and enhanced bioavailability of some drug, prolongation of retention time of the dosage form in the stomach is essential. Some of the oral drug delivery devices have restriction due to gastric retention time (GRT) a physiological limitation. Have a restriction due to gastric retention time, a physiological limitation. Therefore, prolonged gastric retention is important in achieving control over the GRT because this helps to retain the sustained release system in the stomach for longer time in predictable manner [(7-8).]

Metronidazole is antibacterial, antiprotozoal, and antiamoebic agent is initially introduced for the treatment of vaginal infections caused by Trichomonas vaginalis but has since been shown to be effective for treatment of Amoebiasis, giardiasis, and anaerobic bacterial infections including clastidium difficulties.as the half-life of metronidazole is 8 hrs. And pka is 2.5 hence it is required to take thrice daily and it is decomposed at alkaline [(9)] Adverse effects seen on occasion with metronidazole are nausea, vomiting, upset stomach, loss of appetite, dry mouth, and metallic taste, due to this reason Microparticulates dosage is important to masking metallic taste of metronidazole The literature review revealed that metronidazole, ethyl cellulose yet not studied sustained release microsphere by crosslinking method hence present work was prepare.

Materials and Methods

Materials:

Metronidazole was received from Aarti Pvt. Ltd, Bhoisar as a gift sample. Ethyl cellulose, Glutaraldehyde, Acetic acid, Light paraffin, Heavy paraffin & Tween 80 were purchased from Merck & Loba Chemicals pvt. Ltd. Mumbai. All the other chemicals and reagents used in this study were of analytical grade.

Methods

Glutaraldehyde cross linking method

2.5% (w/v) ethyl cellulose and metronidazole solution in aqueous acetic acid was prepared. This dispersed phase was added to continuous phase (100mL) consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 0.5% (w/v) Span 85 to form a water in oil (w/o) emulsion. Stirring was continued at 2000 rpm using a 3-blade propeller stirrer. A drop-by-drop solution of a measured quantity (2.5 mL each) of aqueous glutaraldehyde (25% v/v) was added at 15, 30, 45, and 60 minutes. Stirring was continued for 2.5 hours and separated by filtration under vacuum and washed, first with petroleum ether (60°C-80°C) and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde [(10)]

Table no.1 Formulation of sustained release microparticles of metronidazole

Sr. no	Batch	Metronidazole	EC	Acetic acid	Continuous phase	Glutaraldehyde	Span 80
1	F1	1g	1g	10ml	100ml	4ml	0.025%
2	F2	1g	1.5g	10ml	100ml	4ml	0.025%
3	F3	1g	2g	10ml	100ml	4ml	0.025%
4	F4	1g	2.5g	10ml	100ml	4ml	0.025%
5	F5	1g	3g	10ml	100ml	4ml	0.025%

Physiochemical Characterization of metronidazole:

The observation of colour or colour change hassled to significant discoveries in the field of chemistry, simple colour test have often proved to be useful preliminary or confirmatory evidence for presence of a particular chemical species. The basic principle of most colorimetric measurements consists in comparing, under well define condition, the colour produced by substances is unknown amount with the same colour produced by known amount of material being determined [(11,12)] Also using visual method colour, appearance data of drug id determined. The visual method consisted of viewing approximately 50 mg of API placed on a white sheet paper under laboratory lighting. Odour of drug and taste is mention in official books like Indian

Pharmacopoeia, National Formulary.

Melting point determination:

The physical properties of compound, such as melting point and boiling point can provide useful information which can help in identication of sample or establish its purity. Melting point of metronidazole was determined by melting point apparatus using capillary method, (Thiele tube)

Solubility determination of metronidazole: Solubility of drug was determined by dissolving both dug and carrier in different solvent and common solvent dissolving was selected [13]

UV spectroscopic characterization of metronidazole:

Preparation of calibration curve of metronidazole:

Weigh accurately 100mg of pure metronidazole and dissolve in minimum quantity of methanol and dilute to 100.0ml with 0.1 N HCL. Pipette out 0.2, 0.4 ml, 0.6ml…of this solution and dilute to 10.0 ml in separate 10.0ml volumetric flask to make 2, 4, 6, 8 …20µg/ml concentration solutions. Measure the absorbance (in UV spectrophotometer) at λ max 277 nm.

Accurately weighed amount (100mg) of Metronidazole was dissolved in a small portion of 0.1 N HCl in a volumetric flask (100 ml) and the volume was made up to 100 ml with 0.1 N HCl. This was primary stock solution (solution A) containing 1000ug/ml of metronidazole. From solution A, accurate volume (10 ml) was pipetted out and transferred to volumetric flask (100ml) and made up the volume with 0.1 N HCl producing solution of concentration 100ug/ml (solution B) From solution B, various aliquots in the concentration range 2-30 ug/ml were prepared. The absorbances of these solutions were measured against the 0.1 N HCl. As blank at 277nm using UV-Visible double beam spectrophotometer and calibration curve was plotted taking concentration in ug/ml on x-axis and absorbance on Y-axis.

λmax determination of metronidazole with pH 6.8 phosphate buffer.

100 mg of metronidazole was accurately weighed and was dissolved in 100 ml phosphate buffer (pH 6.8). 1 ml of this solution was then diluted to 100 ml using phosphate buffer (pH 6.8) to get a final solution of conc. 10ug/ml. taking a concentration as per method done in 1N HCI the UV spectrum was recorded in the wavelength range 277nm

IR spectroscopic characterization of metronidazole:

Identification of metronidazole by FT-IR

FT-IR is analytical technique used to identify organic materials. This technique measure absorption of various infrared light wavelengths by the material of interest. These infrared absorption bands identify specific molecular components and structures. Absorption range of 4000-1500 wavenumbers are typically due t functional group.

IR absorption spectrum of metronidazole was determined by Fourier Transform Infrared Spectrophotometer using KBr dispersion method. The dry sample of metronidazole was mixed by triturating with dry potassium bromide (A. R. Grade) ad placed in sample cell. The IR spectrum of drug sample was recorded and spectral analysis was done using FT-IR (schimadzu co japan) [14].

Identification of metronidazole by XRD

X-ray crystallography is one of the most useful methods for exploring the nature of matter. X-ray diffractometry XRD is used to determine the phase content in many mierals and materials. It is used as an adjunct to chemical analysis in the identification of the constituents of mixtures of crystalline phase.

Microsphere were recorded with an X-ray diffractometer (XPERT-PRO, PAN analytical, The Netherlands) using the PRS measurement program using Ni-filtered, CuKa radiation with a voltage of 45 kV and a current of 40 mA. The instrument was operated at continuous scanning speed over 2*q* range of 5 to 49°.

Metronidazole and polymer interaction study by FTIR

FTIR Spectra help to confirm the identity of drug and to detect the .interaction of drug with carrier. The IR Spectroscopy of pure drug and physical mixture of drug with polymer was carried out to check the compatibility between drug and polymer. IR Spectra of drug with polymers were compared with standard IR Spectrum of the pure drug.

The drug polymer interaction was carried out using FT-IR (schimadzu co japan). Individual IR spectra of drug and polymer as well as in combination were taken and compared to see if interaction has occurred or not. The above stated procedure is followed to record IR spectra

The Fourier transform infra-red analysis was conducted for the analysis of drug polymer interaction and stability of drug during microspheresformulation process .spectrum of pure metronidazole, ethyl cellulose and sustained microsphere were recorded

The FT-IR spectrums of pure metronidazole and physical mixture of metronidazole and ethyl cellulose were analysed for compatibility study [15].

Metronidazole polymer interaction study by XRD:

XRD of Metronidazole drug and polymer EC were recorded on powder X-Ray diffractometer (Philips APD15). Samples were irradiated with monochromatized X-rays (Cu-kα) and XRD patterns were recorded with scanning rate 2° min-1 in the range of 4-40° of diffraction angle (2ɵ).

Preparation of microspheres from method Cross-Linking by Chemical Method

Generally, aldehydes like glutaraldehyde or tripolyposphate is used for cross-linking.

Table No 2: Parameters used in batches of sustained release microspheres of metronidazole

Batch	Temperature	Speed(rpm)	Drug: polymer ratio	Internal organic phase
F1	35°C	2000	1:1	Acetic acid
F2	35°C	2000	1:1.5	Acetic acid
F3	35°C	2000	1:2	Acetic acid
F4	35	2000	1:2.5	Acetic acid
F5	35	2000	1:3	Acetic acid

In this method , the polymer solution in aqueous acid media is added to oil phase consisting of both light and heavy liquid paraffin in equimolar ratios containing surfactants and stirred to form an W/O emulsion. Continue stirring at high speed while adding measured quantity of aqueous glutaraldehyde solution drop wise at different time intervals. Microspheres obtained are separated and washed, first with petroleum ether and then with distilled water to remove the adhere liquid paraffin and glutaraldehyde. Vary the volume of glutaraldehyde to achieve effective cross-linking efficiency. Dry microsphere are finally vacuum desiccator

Evaluation of microparticles:

Micrometric properties:

Determination of particle size:

The particle size was determined using stage micrometer. The diameters of about 300 microspheres were measured and the average particle size determined.

Percentage yield:

The percentage yield of different formulations was determined by weighing the floating microspheres after drying. The percentage yield was calculated as follows.

$$\% \text{ Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

Drug entrapment:

The various batches of the sustained release microspheres were subjected to estimation of drug content. The sustained release microspheres equivalent to 50 mg of metronidazole from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved in methanol (10 ml) in volumetric flask (100ml) and made the volume with 0.1 N HCl. This solution is then filtered through Whatmann filter paper No. 44. After filtration, from this solution accurate quantity (10 ml) was taken and diluted up to 100 ml with 0.1 N HCl. From this solution, accurate volume (2 ml) was pipette out and diluted up to 10 m1 with 0.1 N HCI and the absorbance was measured at 277 nm against 0.1 N HCI as a blank. The percentage drug entrapment was calculated as follows

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Precisely weighed (10 mg) microspheres were crushed and dispersed into 25 ml phosphate buffer (pH 6.8) without any material loss for the determination of encapsulation efficiency. The prepared mixture was shaken for 24 h. After 24 h, the solution was filtered, and the filtrate was analyzed for the drug content by a UV spectrophotometer (UV-3000+, Lab India Instruments, Mumbai, India) at 277 nm after suitable dilution.

Percent drug content:

The microspheres was powderd accurately weighed a quantity of the powder equivalent to 25mg of metronidazole , transfer to 500 ml volumetric flask using 300 ml of methanol the resulting suspension was heated at 60° and shaked for 15 min cool, dilute with 500 ml of methanol a suitable volume of filtrate with sufficient methanol to produce a solution containing 0.01%w/v of metronidazole measure the absorbance of resulting solution at maximum at 277nm.

In vitro dissolution study:

The study was carried out using dissolution apparatus USP Type-I (Rotating Basket type)
Dissolution Medium : 0.1 N Hydrochloric acid (pH 1.2), 900ml.
Speed of Paddle : 100 rpm.
Temperature of Dissolution Medium : 37^0C ±0.5^0C
Accurately weighed microsphere equivalent to 200 mg of metronidazole were taken in muslin cloth and it was kept in baskets. Dissolution study was carried out in 0.1 N Hydrochloric acid (pH 1.2) at 100 rpm at temp 37 ºC ± 0.5^0C. During dissolution study 10 ml aliquot was withdrawn at a time intervals of 1 to 12 hrs. and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatmann filter paper and absorbances were measured at 277 nm. Drug concentration in the samples was determined from the standard calibration curve. Cumulative percent of drug dissolved was found out at each time point.

Surface Morphology:

SEM is microscope that uses electrons rather than light to from an image. There are many advantages of using SEM instead of a light microscope. SEM has a large depth of field, which SEM instead of a light microscope. SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time.
This study was performed at Diya Labs, Mumbai by Scanning Electron Microscopy (SEM) using JSM 6380 A(JOEL, Japan).The microspheres were coated with Platinum by ion sputtering using Auto fine coater JFC-1600 (JOEL, Japan) The microspheres were kept on the sample holder and the scanning electron micrographs were taken.

Characterization of Optimized Formulation by IR, XRD

IR spectrum of optimized batch was determined by Fourier Transform Infrared Spectrophotometer by using KBr dispersion method. Microsphere of optimized batch were recorded with X ray diffractometer using PRS measurement program.

Accelerated Stability Studies:

Stability study of formulation was carried out to point out any visual physical or chemical changes made in the formulation after storing it at elevated temperature and humidity condition. chemical and physical stability of SD_s formulation was assessed at 40± 2^0C and 75±5% RH per ICH Guidelines. SD_D formulation was filled in sealed vial with aluminum foil and stored for 3 month in stability chamber. sample were analyzed for physical parameter and drug content during time period of 3 months

Results and Discussions.

Solubility determination of metronidazole

Table no 3: Data for solubility of metronidazole in solvents

Solvent	Solubility(mg/ml)	Mean	%RSD
Distilled water	4.525	40579	0.15451
0.1 N HCl	2.105	2.164	0.05412
Phosphate buffer pH 6.8	0.0256	0.02656	0.00087
Phosphate buffer pH 7.8	0.0294	0.0303	0.00134

UV spectroscopic determination:

Determination of λmax in 0.1 N HCl
The UV spectrum obtained is shown in figure no.2 the wavelength of maximum absorbance (λ max) was found to be 276 nm in 0.1N HCI.

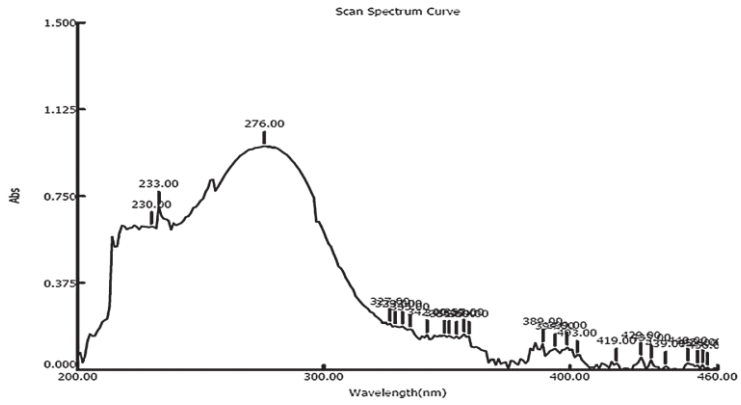

Fig. No.1 Scanning curve for metronidazole in (0.1) N HCl in wavelength range 200nm-400nm

The graph of absorbance vs. concentration for pure metronidazole is shown in figure 2 it was found to be linear in concentration range of 1-10ug/ml at 276nm. Hence drug obeys the beer's-lambert law in this range for metronidazole.

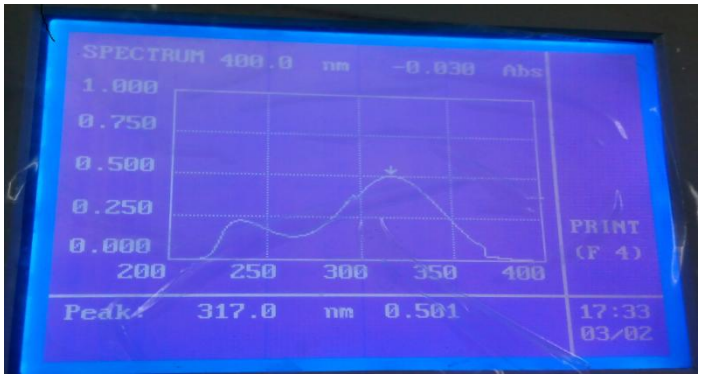

Fig. No. 2 Scanning curve for metronidazole in phosphate buffer (pH 6.8) wavelength range 200nm-400nm

The UV spectrum obtained is shown in figure 3 the wavelength of maximum absorbance λmax was found to be 317 nm in phosphate buffer pH 6.8.

Preparation of calibration curve of metronidazole

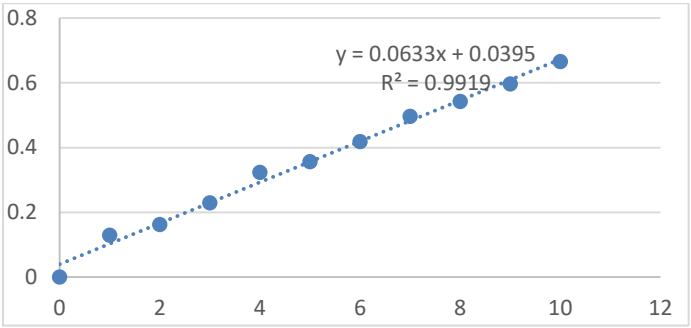

Fig No. 3 Reading of the absorbance of metronidazole in 0.1N HCI obtained from UV Spectroscopy

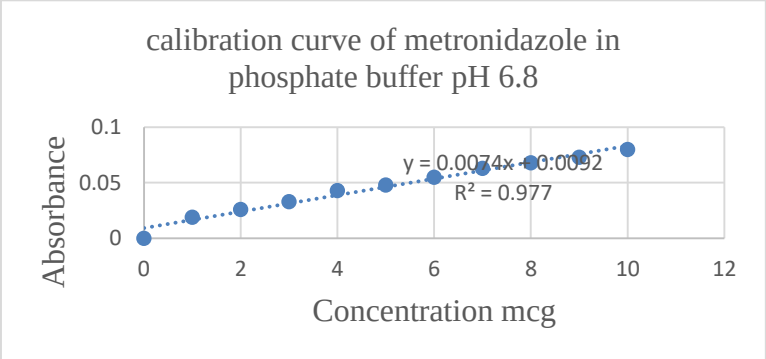

Fig No.4 Reading of the absorbance of metronidazole in phosphate buffer pH6.8 obtained from UV Spectroscopy

The graph of absorbance vs. concentration for pure metronidazole is shown in Graph no. 5 it was found to be linear in concentration range of 1-10ug/ml at 317nm. Hence drug obeys the beer's-lambert law in this range for metronidazole

IR spectrum interpretation:

The IR spectrum of metronidazole is shown in figure 6 the interpretation of IR frequencies are shown in table 12.

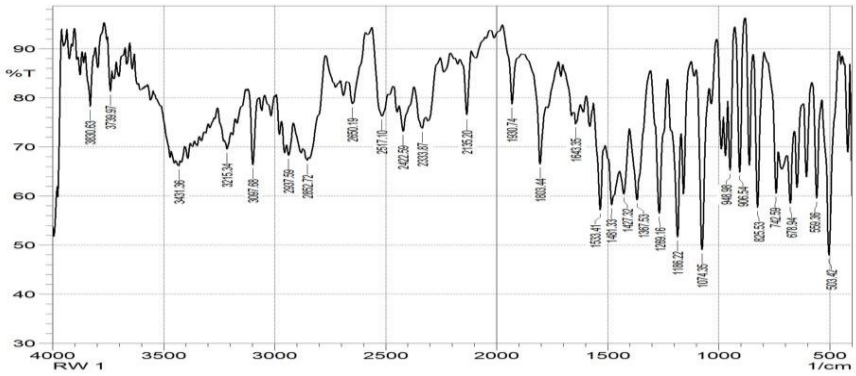

Fig. No. 5 FT-IR spectrum of metronidazole

In FT-IR Spectrum of pure metronidazole the presence of peak were characteristic to that of a pure drug, and showed no change in the individual peak of MTZ

Determination of X-Ray Diffraction pattern

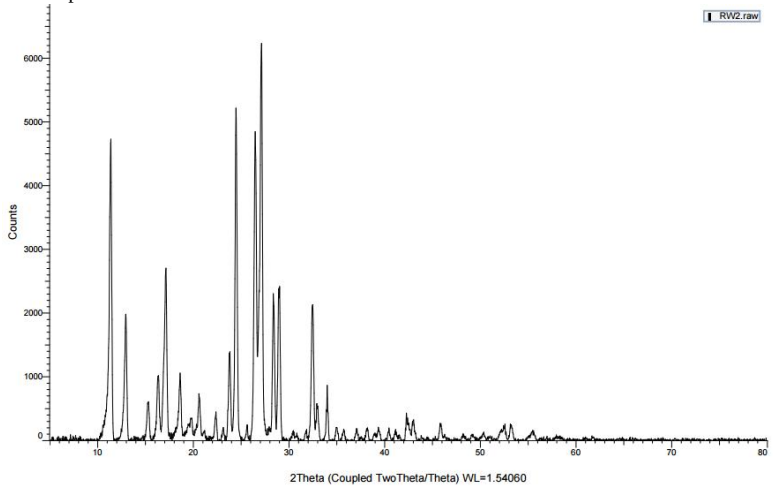

Fig no 6 XRD diffractogram of metronidazole

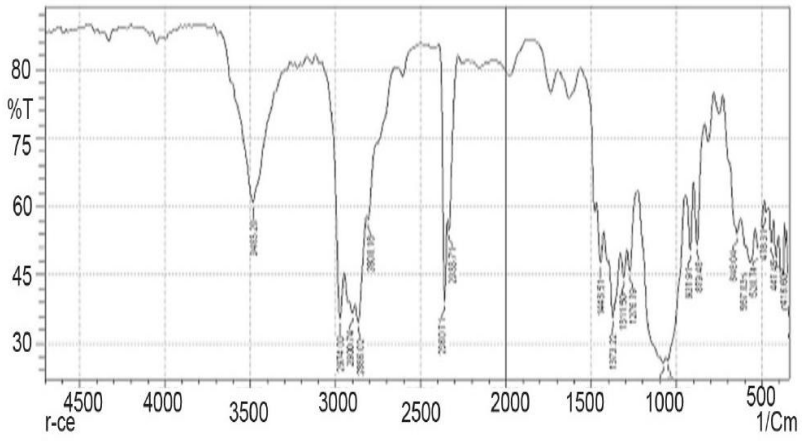

Fig. No. 7 FT-IR spectrum of ethylcellulose

EC showed characteristic peaks at 2974 cm-1 and 2869 cm-1 due to C–H stretching vibration peak. The –OH stretching vibration peak was observed at 3485 cm-1 in the control EC. The other important peaks at 1091, and 1373 cm-1 corresponded to C-O–C stretching and C–H bending respectively [35]. The FT-IR spectrum of treated EC sample showed important peaks for –CH stretching at 2873, 2976 cm-1 and –OH stretching peak was evidenced at 3485 cm-1. Vibration peaks at 1066 and 1375 cm-1 were mainly due to CO–C stretch and C–H bending, respectively. The result showed that C-O-C stretch present in control EC at 1091 cm-1 was shifted downward to 1066 cm-1 in treated EC. Hence, it is assumed that bio field treatment may reduce the bond strength and force constant of C-O-C bond with respect to controlled.

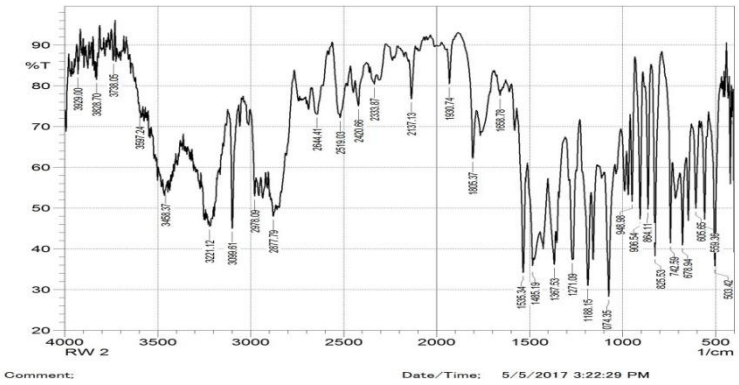

Fig no. 8 FT-IR spectrum of physical mixture of metronidazole: ethyl cellulose

FT-IR spectra of physical mixture containing drug and polymers. IR study demonstrates that no change in the individual peak of MTZ and EC and physical mixture of both. It showed that drugs have no incompatibility problem.

Determination of X-Ray Diffraction pattern

The XRD study suggested that the X-ray diffractogram of metronidazole indicated the presence of crystalline material with sharp principle peaks and whereas polymers, ethylcellulose, were found to be amorphous in nature. The X-ray diffractogram of microspheres of metronidazole effect of the amorphous polymer

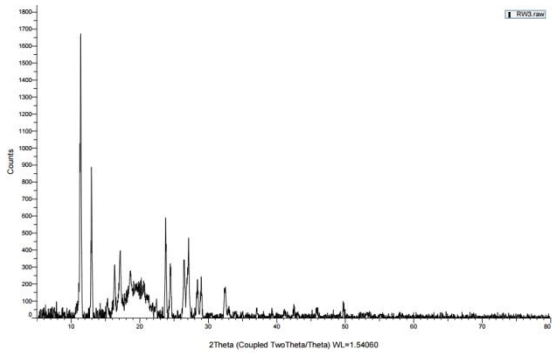

Fig no 9 XRD diffractogram of metronidazole: ethylcellulose

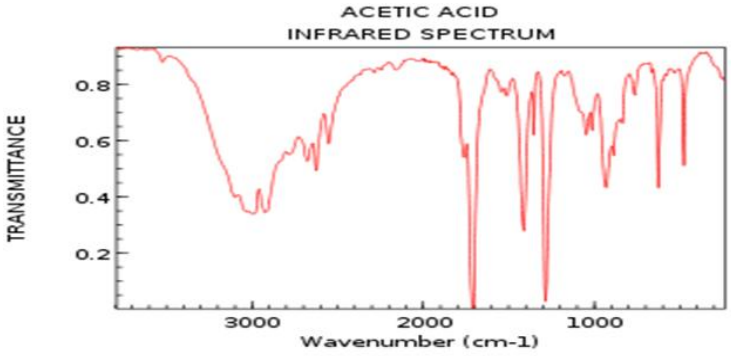

Fig. No. 10 FT-IR spectrum of acetic acid

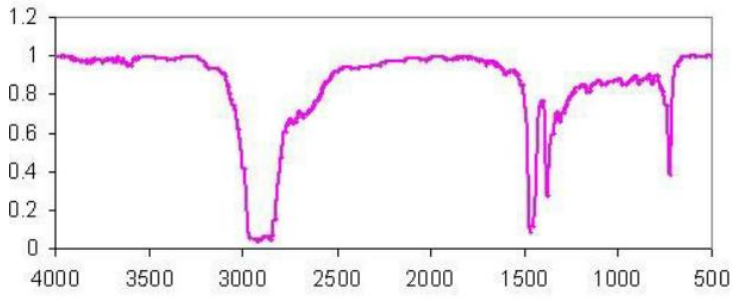

Fig. No. 11 FT-IR spectrum of liquid paraffin

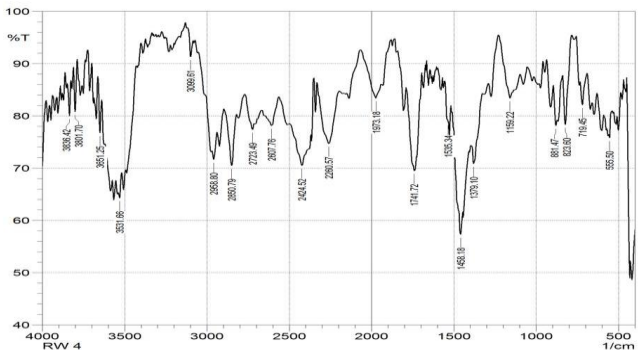

Fig no 12 FT- IR spectrum of formulation F1ratio

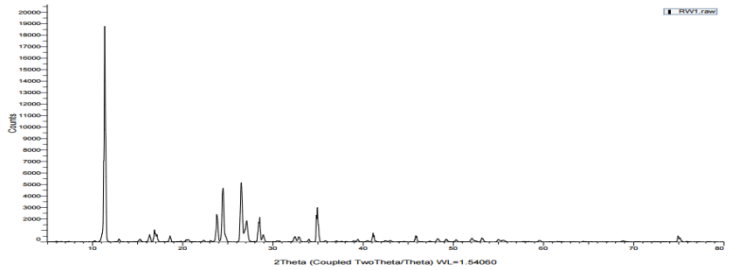

Fig no 13 XRD diffractogram of formulation F1

The XRD study suggested that the X-ray diffractogram of metronidazole indicated the presence of crystalline material with sharp principle peaks and whereas polymers, ethylcellulose, were found to be amorphous in nature. The X-ray diffractogram of microspheres of metronidazole effect of the amorphous polymer.

Evaluation of microsphere for micrometric properties:

Table no.4 Data for average particle size

Sr.no	Formulation code	Average particle size
1	F1	44.73±0.57
2	F2	45.02±0.81
3	F3	47.065±0.37
4	F4	112.032±1.11
5	F5	132.452±0.37

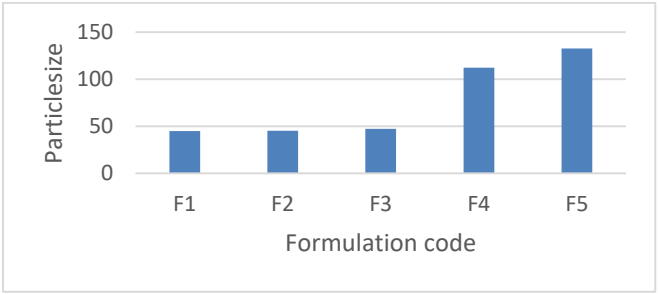

Fig No 14. Average Particles

.Table no. 5 Data for evaluation of microspheres

Formulation code	Angle of repose	Bulk density	Taped density	Carr's compressibility index	Hausner's ratio
F1	15.69±0.754	0.124± 0.032	0.205± 0.064	17.443± 0.024	1.085±0.0652
F2	16.31±0.633	0.132 ±0.078	0.173± 0.083	20.516 ±0.036	1.076±0.0416
F3	19.46±0.352	0.172± 0.122	0.193 ± 0.054	28.113± 0.56	1.041±0.0323
F4	28.21±0.32	0.768 ±0.026	0.85± 0.08	39.62±0.12	1.670±.042
F5	34.21± 0.01	0.961 ±0.03	0.89± 0.009	12.76 ±0.023	1.18±0.021

Determination of particle size:

By keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity increases which influences the size distribution of particle. If there was increase in the amount of polymer concentration, there was increase in relative viscosity so as result increases in mean particle size. The average particle size of microspheres is found to be within 112-171um.

Angle of repose:

All the formulation show angle of repose value in the range of .these values for angle of repose (<30) indicated good flow properties.

Bulk density and tapped density:

Bulk density depends upon particle size, shape, and tendency of particles to adhere together. The values for bulk density were found to range from 0.142 to 0.451 The values for tapped density were found to range from 0.105 to 0.520.

Compressibility index.

These values were found in the range of 15.44 – 20.91 respectively. These values for compressibility index indicated good flow properties of microcapsules.

Hausner's ratio;:

It was ranging from 1.18±0.021 to 1.670±0.42i.e , all the preparation showed that they had good flow properties

Percentage yield

Table. No. 6 Percent yield of different batches of microspheres

Sr. No.	Formulation Code	% yield
1	F1	72.33± 3.94
2	F2	71.00± 2.41
3	F3	45.36± 0.21
4	F4	32.88± 0.62
5	F5	34.45± 0.46

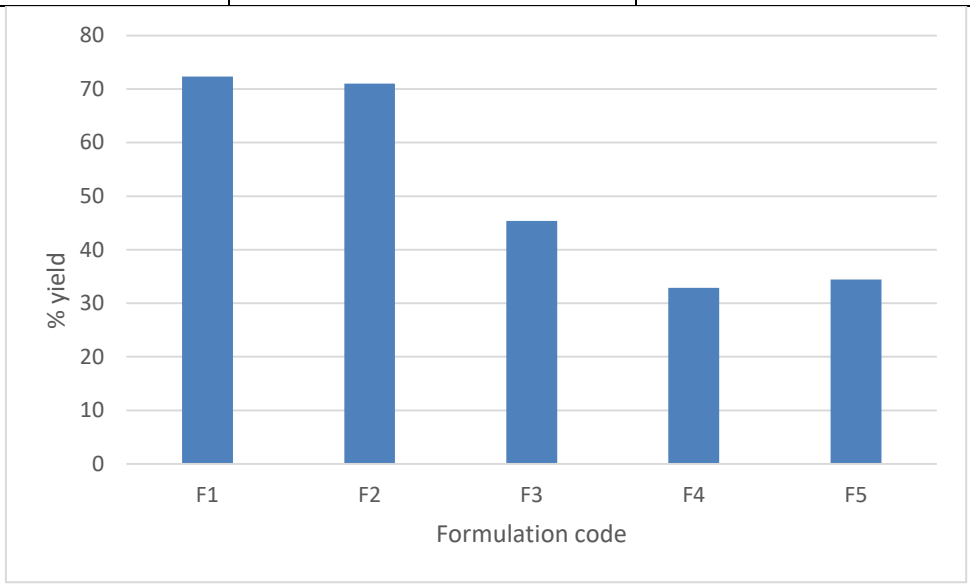

Fig No. 15 Percentage yield of microsphereDrug entrapment efficiency:

The drug % encapsulation efficiency of ethyl cellulose microsphere is shown in table .the drug: polymer ratio showed significant effect on the encapsulation efficiency of microspheres. The increase in concentration of polymer showed the increase in drug encapsulation efficiency. The microcapsules formulated using acetic acid as internal organic

phase or solvent showed brtter encapsulation efficiency than other formulation .the % encapsulation efficiency is found to be in the range of 63.74±0.88 and 98.76±4.68.

Table. No. 7 Entrapment efficiencyof different batches of microspheres

Sr. No.	Formulation code	Entrapment efficiency (%)
1	F1	63.74 ±0.88
2	F2	79.02± 4.88
3	F3	86.96± 2.49
4	F4	90.11± 1.78
5	F5	98.76± 4.68

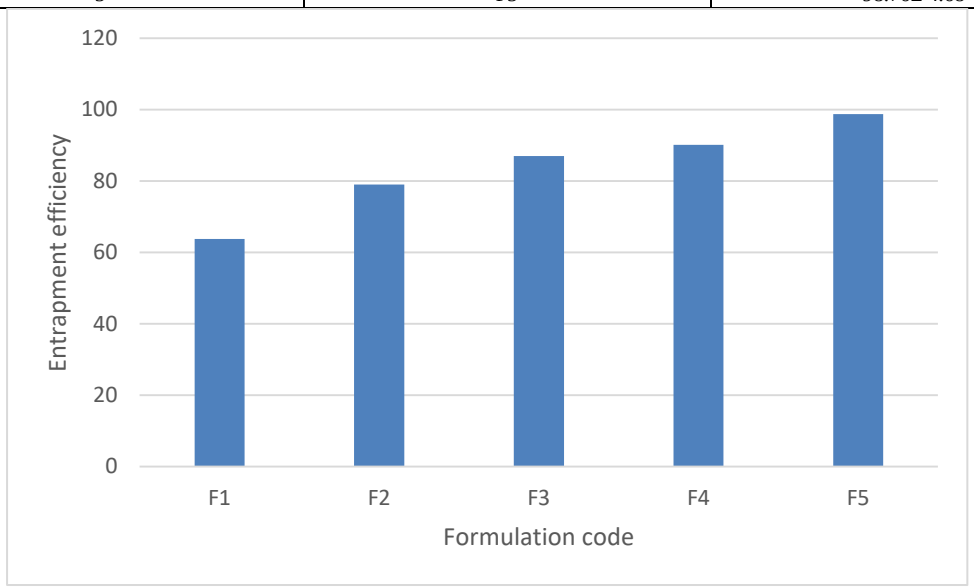

Fig No 16 Entrapment efficiency of different batches of microspheres

Percent drug content

Table. No.8 Percent drug content of different batches of microspheres

Sr. No.	Formulation code	% Drug content
1	F1	70.64± 2.67
2	F2	72.33± 3.74
3	F3	50.44± 4.08
4	F4	35.11 ±2.48
5	F5	29.02± 4.71

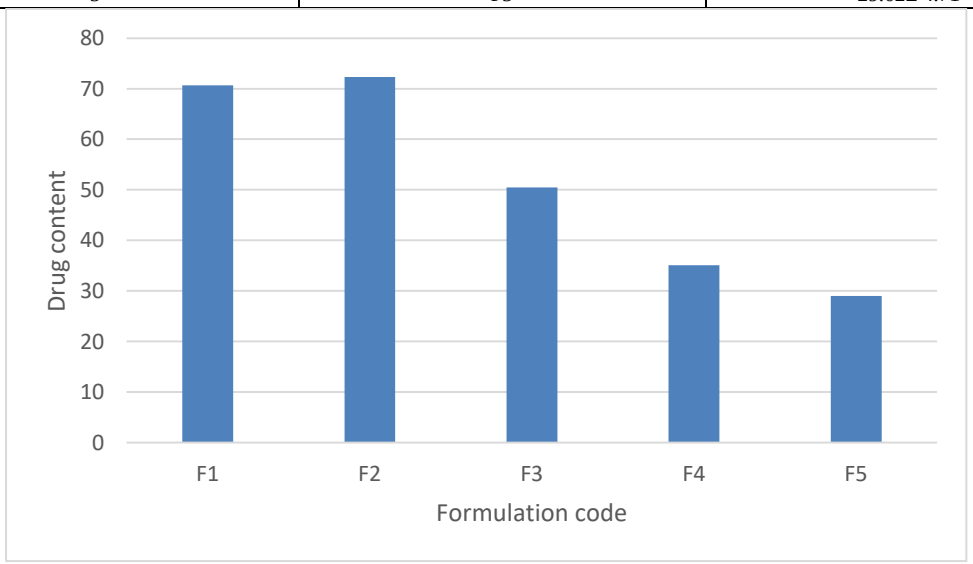

Fig No.17 Percent drug content of different batches of microspheres

The results of the determination of microsphere drug content for various polymers: drug ratios are shown in table from the five formulations F2 has the highest milligram of the drug content following by other formulations. Because it may be due to the highest amount of theoretical drug content and

highest percentage yield in this ratio

In vitro drug release study

Release of metronidazole from sustained release microspheres was evaluated in 0.1N HCl (pH 1.2). Polymer ethyl cellulose is of low permeability and insoluble in water sustained microspheres release the drug in acidic condition and the drug release found to be approximately linear. The drug release from the microspheres was controlled by the polymer. Ethyl cellulose is not a water soluble polymer and it does not show, pH dependency.as the polymer content was increased and the drug loading was decreased, the release of drug was decreased significantly.
In order to increase release rate of drug, the ratio of drug and polymer is decreased and increased respectively. Formulation F1 showed best appropriate balance of drug release has been shown for preparation.

Table no. 9 *In-vitro* drug release from all formulation of microspheres

Hrs.	Cumulative percent drug release ± SD				
	F1	F2	F3	F4	F5
1	20.17 ±1.0	11.12 ±0.34	19.23 ±0.10	12.42± 0.46	16.16 ±0.35
2	27.75 ±1.2	18.53 ±0.65	24.91 ±0.19	19.92 ±0.24	25.35 ±0.46
3	33.9 ±2.1	25.52 ±0.34	32.95 ±0.14	26.74± 0.57	33.85± 0.24
4	41.49 ±1.8	32.11± 0.76	41.01 ±0.84	32.69 ±0.18	42.82 ±0.47
5	47.17 ±2.2	40.66 ±0.54	50.49 ±0.16	39.91±0.49	50.27 ±0.57
6	54.27 ±2.2	47.15 ±0.78	58.53 ±0.23	47.06 ±0.36	59.80± 0.35
7	59.95 ±2.8	54.10 ±0.43	65.17 ±0.08	52.42 ±0.16	66.20± 0.24
8	67.53± 2.7	59.39 ±0.78	69.90 ±0.81	58.25 ±0.35	72.34± 0.75
9	74.17 ±3.2	63.02± 0.34	74.64 ±0.14	63.78 ±0.57	78.42± 0.24
10	78.42 ±3.4	65.69 ±0.65	78.91 ±0.4	68.44 ±0.42	81.69 ±0.64
11	82.6± 3.1	67.65 ±0.28	80.33 ±0.01	70.06 ±0.46	83.37 ±0.24
12	85.11 ±3.2	68.772± 0.58	84.08 ±0.10	71.882 ±0.74	84.771 ±0.74

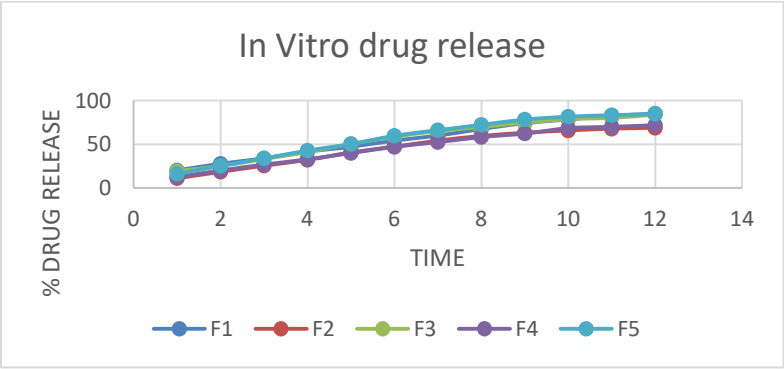

Fig no 18 In-vitro drug release profile of formulation

Surface morphology study

The SEM images of microsphere showed a nearly spherical shape with a smooth and rigid uniform surface (Figure 20) Formulation shows that some of

the microspheres have depressions on the surface which might be due to evaporation of acetic acid..

The particle were mostly discrete, round, and spherical, free flowing.it was found that increasing the rate of sterring from 900 rpm to 2000 rpm the size of microsphere reduces. But on increasing stering rate the drug entrapment is reduced.

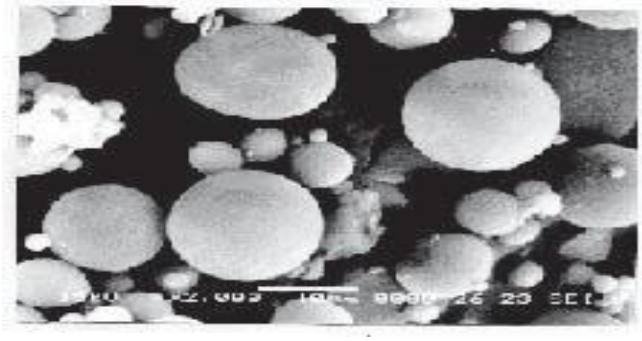

Fig No. 19 SEM Photograph of Microsphere Formulation 1:1

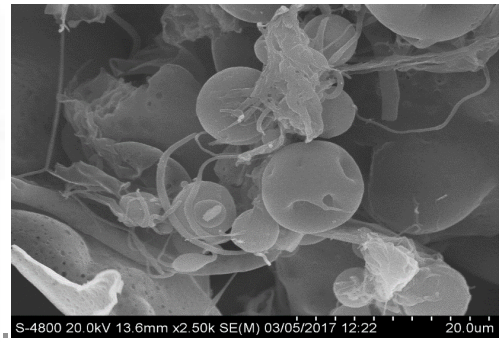

Fig No.20 SEM Photograph of Microsphere Formulation 1:1.5

Selection of optimized formulation:

Characterization of optimized formulation:

The word optimized means 'to make as perfect, effective or functional as possible' optimization is often used in pharmacy for formulations and there processing and practically, it may be consider as the search for a result that satisfactory and at a same time the best possible within a limited field of search.

The formulation was optimized on the basis of SI, matrix integrity and in vitro drug release .the phenomenon of swelling resulted in the retardation or slows the the drug release the batch F1 fulfilled all these all these requirements. For optimized batch F5, in vitro drug release which indicated that the microsphere show good sustained release behavior for 12 h. the percentage release of optimized batch was found to be at 1 hrs. and at 12hr.

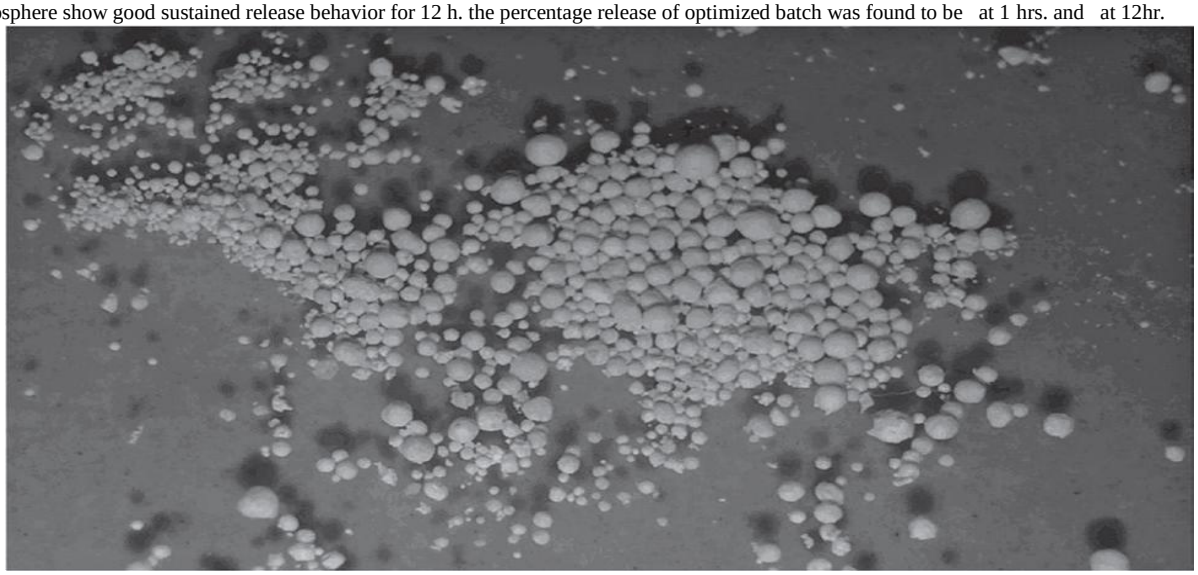

Fig No; 21 Photomicrograph of metronidazole content Ethylcellulose microsphere of optimized batch

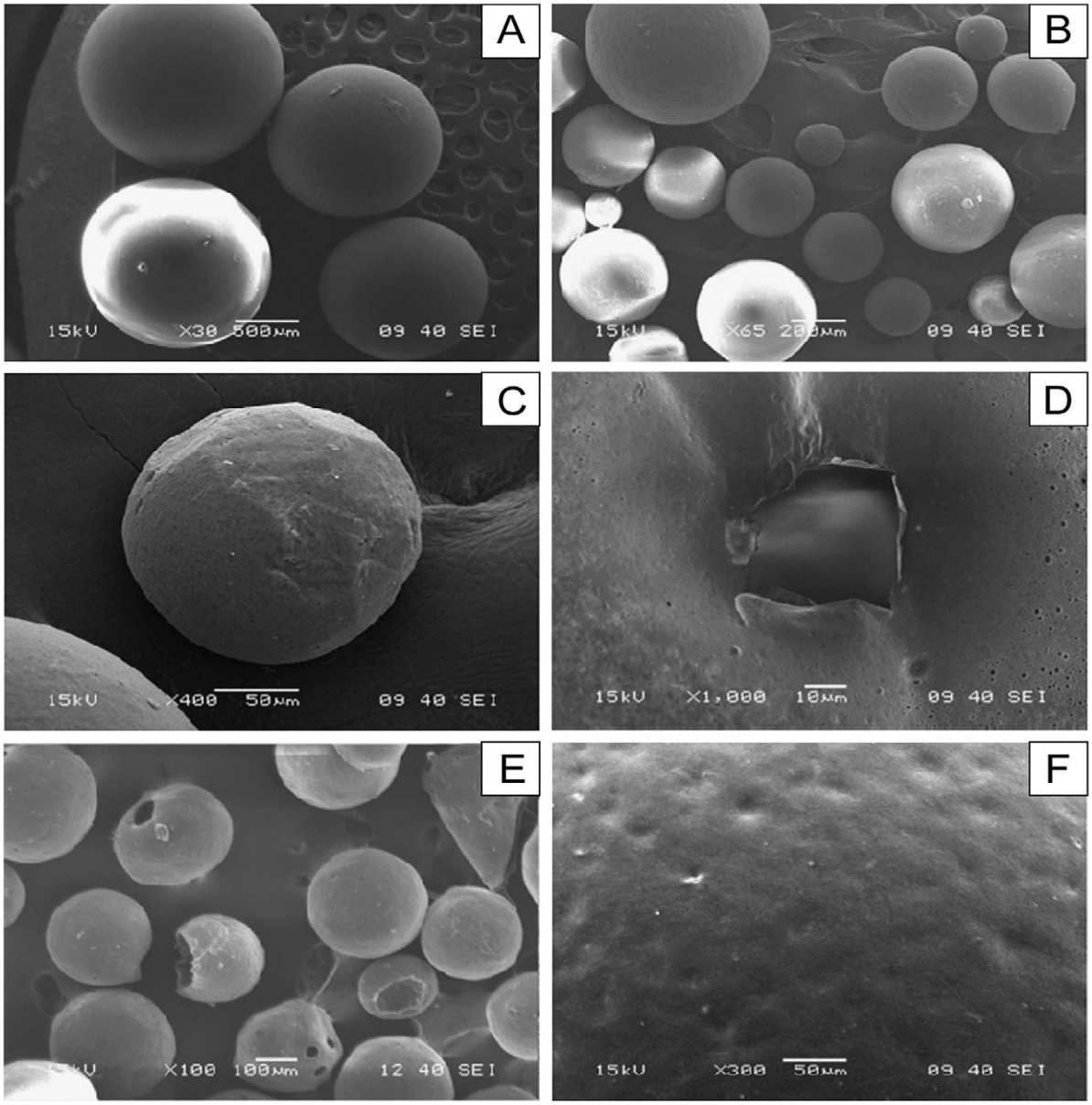

Fig. No. 22 SEM of optimized batch microspheres(A), microsphere of varying size(B), surface morphology (C),perforated microsphere(D), (,E),and smooth surface of microspheres

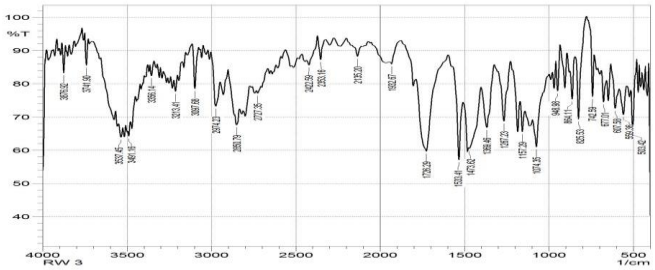

Fig no 23 FT-IR spectrum of formulation F1 ratio

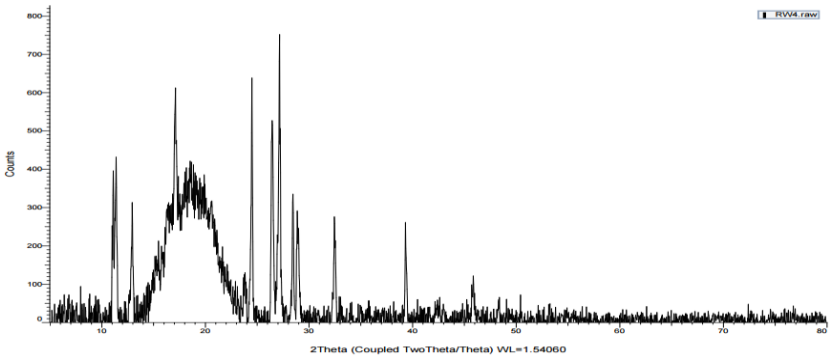

Fig no 24 XRD diffractogram of formulation F1

Accelerated stability studies of optimized formulation:

The stability result reveal that after 3 month showed the percentage drug release of optimized batch, there was no change in physical appearance in optimized batch of microspheres .there was no significant change in drug release after 3 months indicating the stability of formulation.

Table. No. 10 Accelerated stability study data for formulation F1

Sr. No	Days	Colour changes	% max drug release
1	0	White	85.11±0.1
2	30	White	85.3±0.89
3	60	White	85.75±0.49
4	90	White	85.92±0.13

Summary and Conclusions :

Metronidazole is drug from antimicrobial,antiprotozoal used in treatment of amoebiasis and giardiasis .literature survey revealed that it is having half-life 8 hrs had needed frequent dosing which decreases that patent compliance. Thus in this study the attempt was taken for preparation of modified release microsphere by using Ethylcellulose which will reduce the dosing frequency. The preformulation study was carried out using raw material .microsphere where formulated by using glutaraldehyde crosslinking method. In the present work the effect of cross linking agent on the various properties of microspheres where studied the study was carried out on micrometric properties of microspheres, encapsulation efficiency, *In-vitro*drug release from microspheres.

From the study, it is evident that promising sustained release microspheres of Metronidazole were developed by glutaraldehyde cross linking technique by using ethyl cellulose as matrix polymer for sustaining the drug release .From the results obtained by preformulation studies, it can be concluded that there was no incompatibility between drug and polymers. The evaluation studies such as particle size analysis, percentage drug entrapment, sustained release behavior, *in vitro* drug release and stability studies showed that formulation F2 is the optimized formulation. In vitro release kinetics study of formulation F2 showed that it follows Zero order diffusion kinetics. Stability studies showed that there was no change in the formulation after 90 days.

Thus the aim of the study to formulate sustained microspheres of Metronidazole was achieved. Microsphere preparation at optimum sped 2000 rpm with drug concentration with cross linking agent show good percentage drug entrapment and sustained release , diffusion of drug from cross linked structure depends on the degree of cross linking agent .hence while formulating microsphere rpm should be taken into account as a important criteria. Hence method employed for preparing microsphere and the parameter observed were reproducible EC microsphere loaded with metronidazole can be used for treatment of hepatcamoebiasis were action is needed.

To study the effect of crosslinking agent on properties of microspheres, cross linking agent selected namely as glutaraldehyde. Microspheres were prepared using different speed at three different drug: polymer ratio, (1:1, 1:1.5, and 1:2) In case of micrometric properties all the formulation show angle of repose value in the range of 15.69±0.745 to 34.21±0.61. these value for angle of repose <40 indicated good flow properties of microspheres the values of bulk density were found to range from 0.124±0.032 to 0.961±0.03 .the values for tapped density were found to range from 0.205± 0.064 to 0.89 ±0.009 .the value of compressibility index found in range of 17.443±0.024 to 12.76±0.023 these values for compressibility index .indicated good flow properties the values for Hausner's ratio 1.085±0.0652 to 1.18±0.021.i.e. all the preparation show that they had good flow property the average particle size of microspheres is found to be within 44.73±0.57 to 132.452±0.37µm.

The percent encapsulation efficiency is found to be in the range of 63.74±0.88 to 98.76±4.68 .percentage drug loading was found to be better in microspheres formulated with acetic acid and highest in batch F1 and from *In-vitro* drug release study it can be seen that the F1 batch, show in cumulative percentage drug release 84.77 % in the 12 hrs. From these result formulation F1 was selected as optimized formulation and evaluated forFTIR XRD,SEM .The IR data of metronidazole loaded ethyl cellulose microsphere batch F5 also show than bands as bands in IR data of pure drug ,these suggest that no chemical interaction between ethyl cellulose and metronidazole.

The DSC result also support IR spectrometry result indicating absence of drug- polymer interaction .XRD data indicate that metronidazole disperse at the molecular level in the blend polymeric matrix. SEM photograph showed that microcapsule were spherical in nature and had smooth surface and uniform distribution of drug within microspheres.

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