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Design and Evaluation Of Metronidazole Vaginal Tablet For Once Daily Administration

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Abstract : In this study bioadhesive sustained release tablets of metronidazole for once daily administration were formulated. This formulation helps to increase the localized effect of metronidazole by formulating the vaginal bioadhesive tablet and to increase the ease of application when compared to various types of vaginal gels and creams that are available in the market.Metronidazole is a nitro imidazole derivative class of anti-protozoal drug used to treat amoebiasis, vaginitis, trichomonal infections, trepenomal infections and giardisis. The objective of the present study is to formulate the bioadhesive controlled release drug delivery system which would remain in contact with the vaginal tissue for prolonged period of time in view to maximize the bioavailability and therapeutic efficacy of the drug.Bioadhesive tablets were prepared using metronidazole and sodium alginate in different proportions by wet granulation method. The prepared tablets were evaluated for weight variation, hardness, friability, dissolution and swelling studies.The release of drug from various vaginal bioadhesive tablets exhibited the following order F4>F1>F3, but F2 exhibited faster drug release compared to other formulations, which is not a desired characteristic for the treatment of vaginosis. By observing the above results, more ca^{+2} ions became available to bind with sodium alginate during the wet granulation stage of the preparation. As a result better and stronger gel was formed when high amount of calcium carbonate was used. As the concentration of ca^{+2} ions increases, stronger gel of calcium alginate is formed that delay the influx of the dissolution medium and efflux of the dissolved drug out the matrix. As a result drug is released in amore sustained manner.

Keywords : metronidazole, sodium alginate, wet granulation.

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1.Introduction:

From many years research was made in the field of sustained release dosage forms due to their enormous applications for the drug delivery . these dosageforms increased therapeutic activity, decreased wastage of drug, escaping the drug from first pass metabolism etc. Buccal, sublingual rectal, nasal, vaginal drug delivery systems made a significant role in the present era. Vagina is an important application site for drug delivery, especially for the local treatment of different bacterial, fungal and protozoa infections, for HIV prevention, delivery of contraceptives, spermicides or labor-inducers and for the treatment of precancerous lesions. It may also serve an alternative route for systemic drug delivery like contraceptives[1,2]. high patient compliance and adherence to therapy, the dosage forms or delivery systems should also be easy to administer and not cause discomfort or irritation compared to semisolid dosageforms like creams , ointments and gels. The vaginal route offers high efficiency based on an even distribution and long retention time of the drug in the vagina.

Different types of conventional v a g i n a l dosage forms, such as creams, gels, ointments, foams , pessaries[3] have been investigated for vaginal drug delivery, most of which has major drawback, and have been described as messy, uncomfortable, leaking in the underpants etc., resulting in low compliance. The low retention time is a well- known problem encountered in the formulation of drugs for vaginal application, and can also be attributed to the self-cleansing action of the vaginal tract which limits effective drug levels for short period and fluctuation in drug dose levels leads to increased dose frequency of the drug. This leads to the toxic condition[4].Inclusion of Bioadhesive polymers in the formulation to increase the retention time on the mucosal tissue. So, in the present investigation, bioadhesive polymers are added to increase the retention time.

The most widely investigated group of bioadhesive polymers are hydrophilic polymers containing numerous hydrogen bond forming groups, such as carbomers, chitosan, sodium alginate and cellulose derivatives. Being water-soluble, the polymers become adhesive on exposure to moisture, and will readily cohere to surfaces. They are known to produce high viscosity at low concentrations, but most of the polymers are pH sensitive and may therefore behave differently depending on the vaginal pH.

Solid formulations have the advantage of high dose accuracy and long term stability, as compared to semi-solid systems. However, the vaginal disintegration of conventional vaginal tablets is often slow, and the tablets are often rapidly cleared due to gravity combined with the self-cleansing action of the vagina. This may be circumvented by use of bioadhesive polymers in the formulation.

Metronidazole is an anti-bacterial drug, it was incorporated into a hydrophilic polymer matrix which is composed of sodium alginate. In this formulation various proportions of starch and calcium carbonate are used as diluents. In this investigation 250 mg of metronidazole was used, the total weight of prepared bioadhesive vaginal tablets was 400 mg. Here sodium lauryl sulphate was used as surfactant, talc acts as flow promotor and magnesium stearate acts as lubricant. Among various routes of drug delivery, the vaginal route offers many advantages due to its large permeation area, rich vascularization, avoidance of first pass metabolism and relatively low enzymatic activity. Bacterial vaginosis (BV) is the most common cause of vaginal symptoms including vaginal discharge among women. The prevalence in the United states is estimated to be 21.2 million (29.2%)[5.] Among women ages 14-49, based on a nationally representative sample of women who participated in NHANES 2001-2004.

Vaginitis is one of the most common gynaecological disorders[5,12], and it is defined as a infectious or non-infectious inflammation of the vaginal mucosa, sometimes with inflammation of the vulva.BV is caused by an over growth of various bacteria. It is advised to have antibiotic treatment in case of pregnancy because of possible complications like early labour, miscarriage, having a low birth weight baby or developing an infection of the uterus (womb) after child birth. A usual oral dose of 400-500 mg twice daily administration of metronidazole for atleast one week is prescribed in BV. In pregnancy oral 250 mg thrice daily administration is advisable but oral dose have various side effects like nausea, vomiting, metallic taste and risk associated with pregnancy and breast feeding.For the treatment of vaginosis various oral and topical metronidazole dosage regimens are available metronidazole gel 0.75%, one full applicator (5 g), intra-vaginally once a day for 5 days is recommended and has become available over the counter. Topical vaginal therapy is as effective as oral metronidazole conventional vaginal delivery systems such as creams, foams, pessaries and jellies reside at the targeted site for relatively shorter duration because of

self-cleaning action of the vaginal tract which limits effective drug levels for a shorter period and fluctuation in drug dose level leads to increased dose frequency of the drug this causes patient inconvenience and toxic conditions. So, to overcome the above mentioned disadvantages bioadhesive vaginal metronidazole tablets were prepared. In the present study metronidazole was used as a model drug due to its bacteriostatic and bactericidal activity against gram negative bacteria. The rationale behind the usage of metronidazole is low molecular weight offering greater permeation benefit through vaginal epithelial membrane.

Table no.1: Dosage Regimen of Metronidazole[6]:

S.NO	DRUG	DOSAGE REGIMEN
1.	Metronidazole	500 mg orally twice daily
2.	Metronidazole 0.75%	5 g intravaginal once daily
3.	Clindamycin 2% vaginal	5 g intravaginally once daily
4.	Metronidazole	250mg thrice daily

Table no.2: Trade Names of Some Market Available Metronidazole Dosage Forms[7]

S.NO	TRADE NAME	DOSAGE REGIMEN	DOSE
01	Flagyl	Tablets	250 mg
02	Flagyl	Tablets	500 mg
03	Flagyl ER	Tablets, sustained release	750 mg
04	Flagyl 375	Capsules	375 mg
05	Metro cream	Cream	0.75%
06	Metrogel	Gel	0.75% & 0.1%
07	Metro-gel vaginal	Gel	0.75%
08	Noritate	Cream	1%
09	Metronidazole	Injection	Solution 5mg/ml
10	Vandazole	Gel (vaginal)	0.75%

2. Materials and methods:

Metronidazole was a gift sample from medreich laboratories, Bengalore. Sodium alginate was purchased from SD fine chemicals Ltd., Starch was obtained from SD fine chemicals Ltd., Talc and magnesium stearate was obtained from Oxford laboratory. Calcium carbonate was obtained from SD fine chemicals Ltd., sodium lauryl sulphate was obtained from Oxford Laboratory.

2.1 Preformulation Studies:

Preformulation studies are an important component of drug development. It providesthe scientific basis of formulation development. A comprehensive preformulation study helps in investigation of physico-chemical properties of a drug molecule it also gives the formulation for designed to determine the compatibility of initial excipients with the active substance for a biopharmaceutical, physicochemical, and analytical investigation in support of promising experimental formulations. Efforts spent on preformulation provide cost saving in the long run, by reducing the challenges during formulation development.

Fundamental preformulation properties are specific to the drug molecule and are dependent on the chemical structure of the drug molecule. derived preformulation properties for solid oral dosage form like tablet include characterisation of particle properties like morphology and particle size, bulk density, flow properties and compaction behaviour. The last activity performed in preformulation studies is compatibility studies wherein the physical and chemical of the drug molecule is studied in presence of excipients, obviously the choice of excipients is dictated by the type of dosage form to be developed.

2.2 Determination of $\lambda_{max}$of metronidazole in ph-6 citrophosphate buffer:

Stock solution (100µg/ml) of metronidazole was prepared inPH-6 phosphate buffer. This solution was appropriately diluted to obtain a concentration of 10µg/ml. The resultant solution was scanned in the range of 200nm to 360nm on Shimadzu 1800 UV- Visible spectrophotometer. The drug exhibited aλ_{max} at 319.0nm inPH-6 phosphate buffer.

2.3. Preparation Of Standard Calibration Curve Of Metronidazole:

0.1g of metronidazole was accurately weighed and dissolved in 100ml of pH6 phosphate buffer to get a concentration of 1mg/ml(stock-solution-1). From the stock solution- I, aliquots . were taken and suitably diluted with pH6 phosphate buffer to get concentrations in the range of 2 to 20 µg/ml. The absorbance of these samples wereanalysed by using Shimadzu 1800 UV-Visible Spectrophotometer at 319.0nm against reference solution pH 6 phosphate buffer.

2.4. Drug And Excipient Compalibity Studies:

The drug and theexcipients chosen for the formulations were screened for compatibility study. The compatibility study was studied using FTIR. Drug and excipients interaction was checked by comparing the FTIR spectra of pure drug metronidazole and FTIR spectra of physical mixture and excipients. In the present study, potassium bromide pellet method[18] was employed. The samples were thoroughly blended with dry powder potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the IR spectrophotometer and the spectrum was recorded.

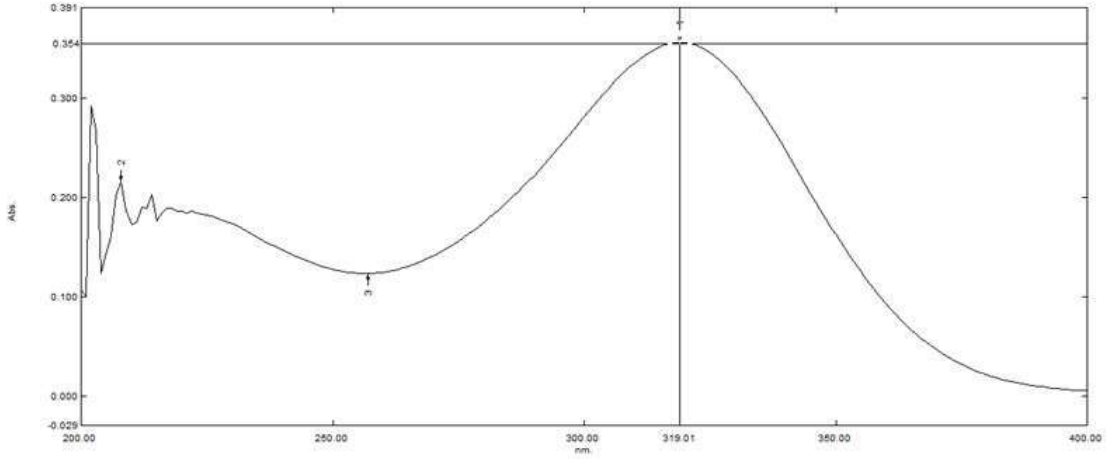

FIG_1__LAMDA_MAX_OF_METRODAZOLE

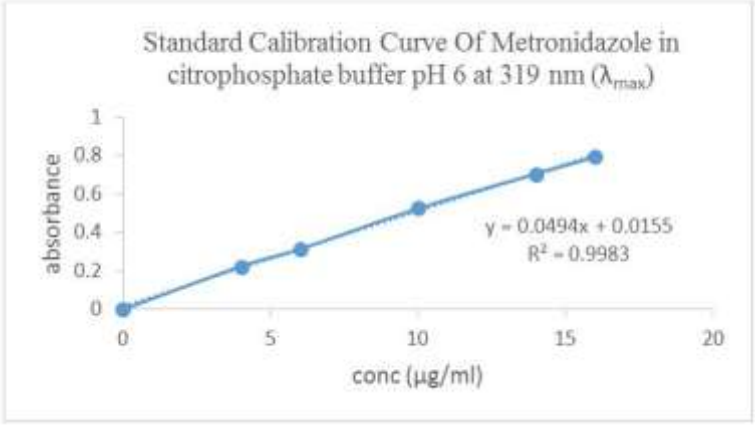

FIG_2_STANDARD_CALIBRATION_CURVE_OF_METRONIDAZOLE

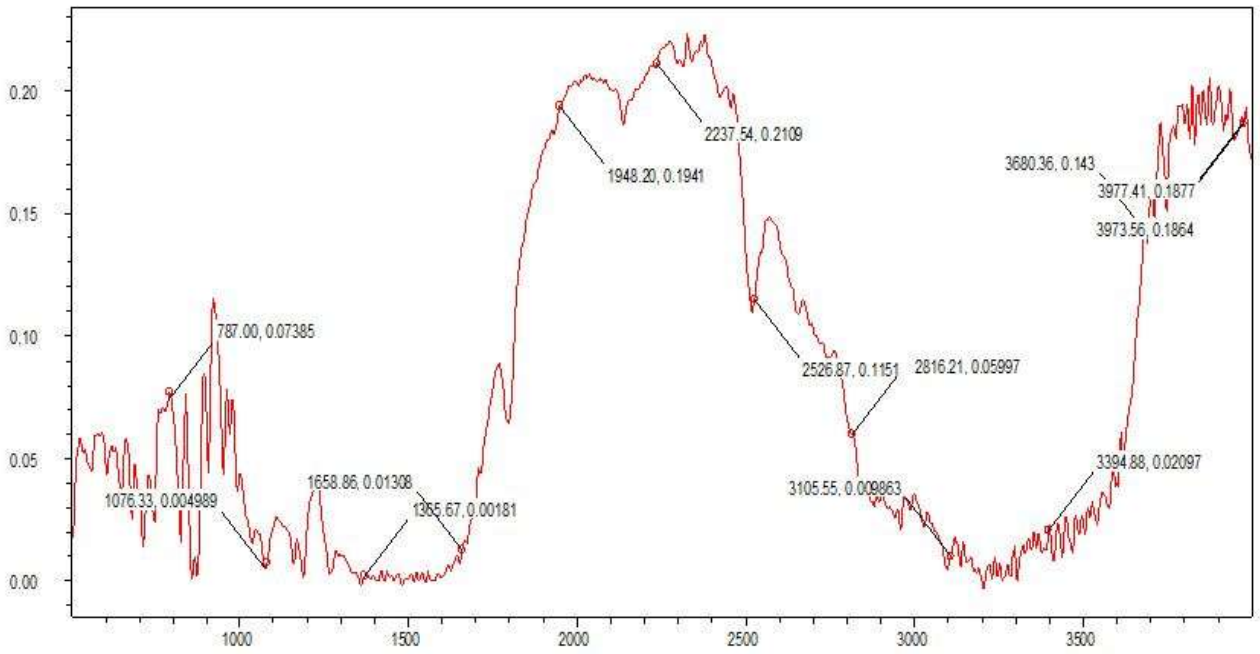

FIG_3_FTIR_OF_PURE_METRONIDAZOLE

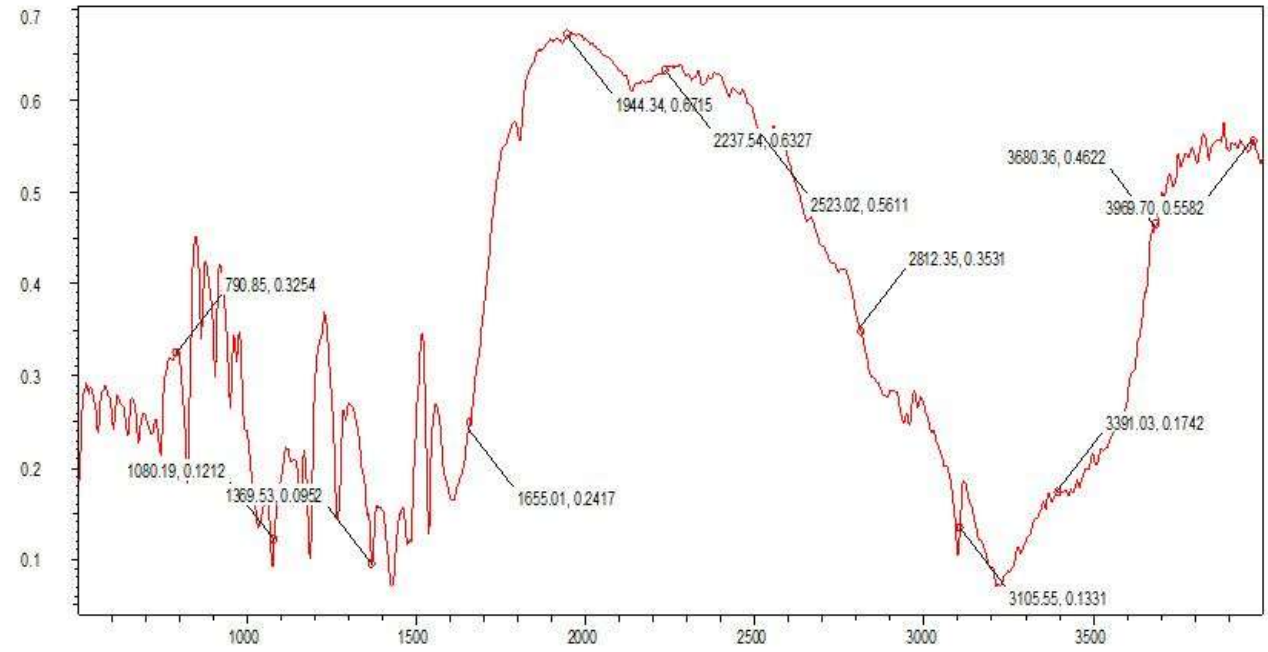

FIG_4_FTIR_OF_METRONIDAZOLE__SODIUM_ALGINATE

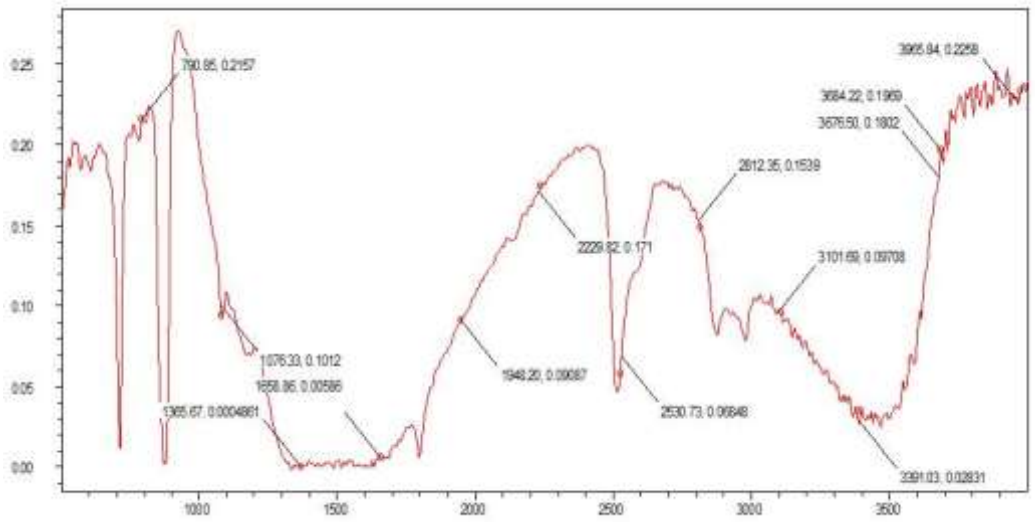

FIG_5_FTIR_OF_METRONIDAZOLE_SODIUM_ALGINATE_CALCIUM_CARBONATE

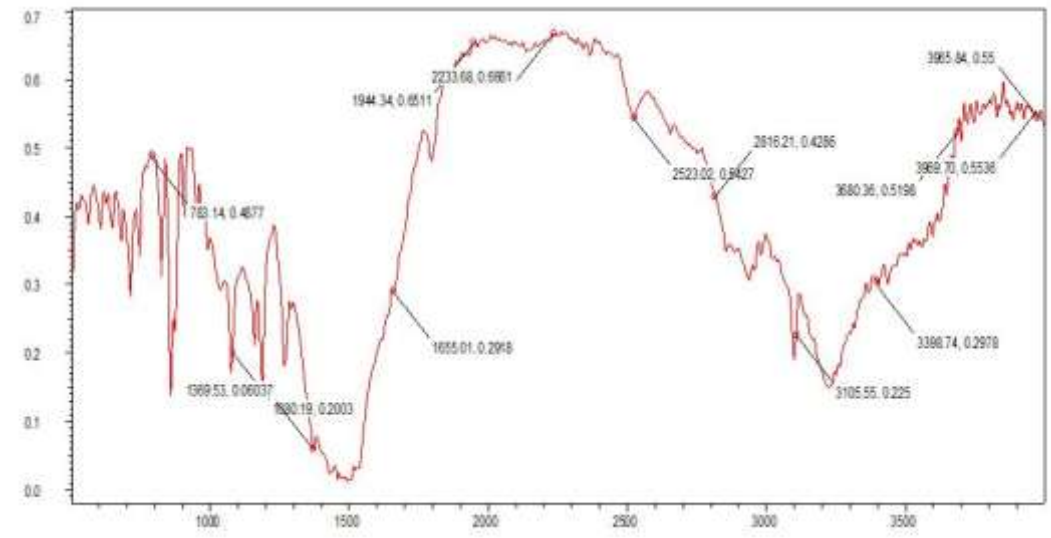

FIG_6_FTIR_OF_METRONIDAZOLE_SODIUM_ALGINATE__CALCIUM_CARBONATE__STARCH

2.5. Precompression Parameters[8]:

2.5.1. Angle Of Repose (º):

Frictional force leads to improper flow these forces are measured by using angle of repose.Angle of repose is defined as maximum angle possible between the surfaces of a pile of the powder on horizontal plane. The angle of repose experiment is determined by using funnel and burette stand. The funnel is fixed at height on the burette stand and the powder was passed through the funnel which from a pile. This region is encircled to measure radius of the pile. The process is done for multiple times, the average value is taken.

The angle of repose is calculated using the equation.

$$\textbf{Angle of repose (º)} = \textbf{Tan}^{-1}\textbf{(h/r)}$$

Where,

h = height of the pile
r = radius of the base of the pile
º = angle of repose

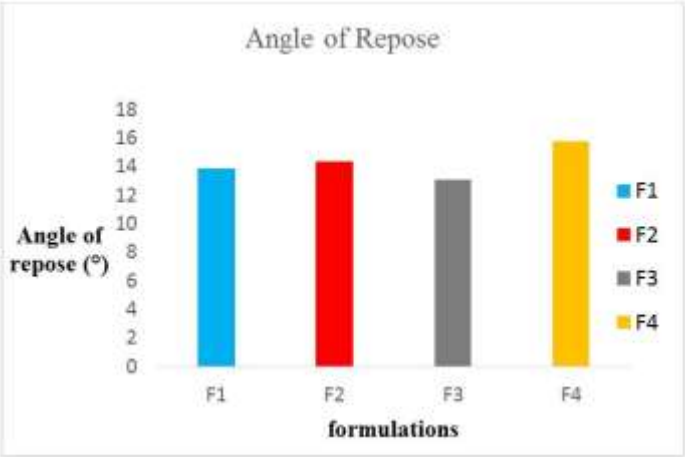

FIG_7_ANGLE_OF_REPOSE

2.5.2. Bulk Density:

The bulk density of a powder is the ratio of the mass of an untapped powder sample(W) is taken in a graduatedmeasuring cylinder and volume(V_0) including the contribution of the inter-particulate void volume. Hence the bulk density depends on both density of powder particles and spatial arrangement of particles in the powder bed. The bulk density can be expressed in grams per millilitre (g/ml).

Bulk density is calculated using the equation

Bulk density(BD)= weight of the powder/ volume of powder

2.5.3. Tapped Density:

The tapped density is obtained by tapping a measuring cylinder containing a powder sample and the volume is measured as initial volume. Measuring cylinder was fixed in "TAPPED DENSITOMETER" and tapped for 750-1250 times until the difference between succeeding measurements is less than 2%. The final reading was denoted by (I_f). The tapped density can be expressed in gram per millilitre(g/ml).

Tapped density (TD) = W/I_f

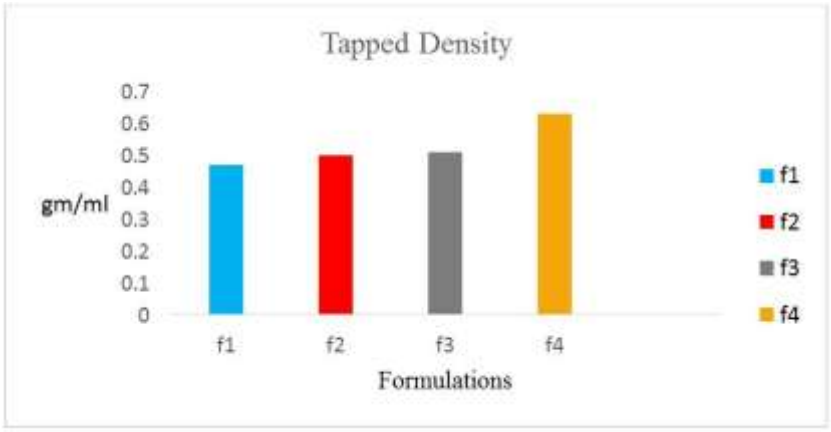

FIG_8_TAPPED_DENSITY

2.5.4. Carr's Index:

Compressibility index is an important measure that can be obtained from the bulk and tapped densities. In the theory, the less compressible a material the more flow able it is. A material having values of less than 20 – 30% is defined as the free flowing material.

The carr's index was calculated by using the formula:

Carr's index = [(tapped density – bulk density)/ tapped density] X 100

Table no.4: Carr's index and corresponding flow properties

Carr's index	Flow
5-15	Excellent
16-18	Good
18-21	Fair to passable
23-25	Poor

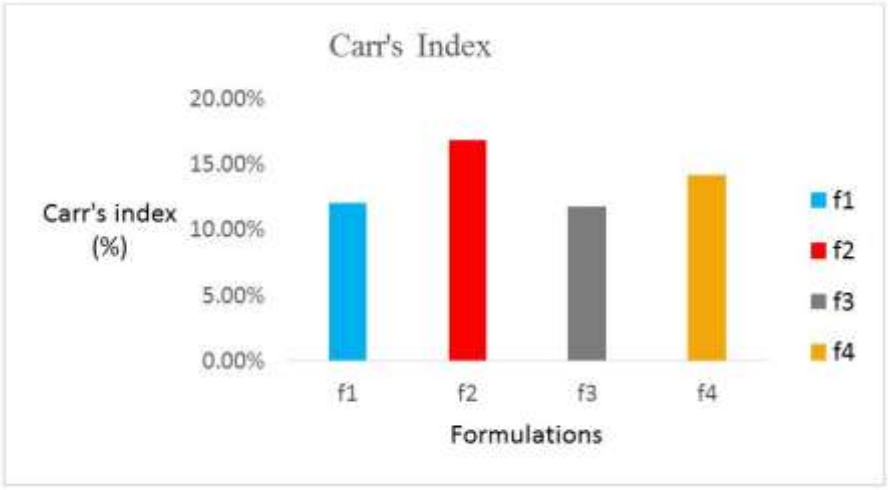

FIG_9_CARRS_INDEX

2.5.5 Hausner's ratio:

It indicates the flow properties of the powder and is measure by the ratio of tapped density to the bulk density.

Hausner's ratio was calculated by using the formula.

Hausner's ratio = (tapped density) / (bulk density)

Table no.5: Hausner's ratio and corresponding flow properties.

Hausner's ratio	Property
0-1.2	Free flowing

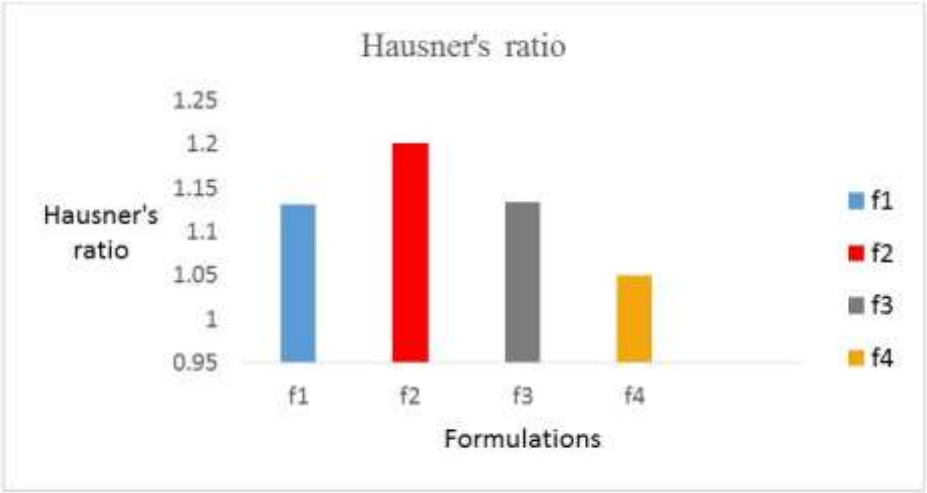

FIG_10_HAUSNER_RATIO

2.6. Preparation of Vaginal Tablets:

Bioadhesive vaginal tablets, each containing metronidazole 250 mg were prepared by wet granulation method. Formulations containing metronidazole and sodium Alginate in different combinations i.e. 1:0.5,1:0.4 1:0.3 were used. All the ingredients of tablets were blended in geometrical ratio in mortar with a pestle for 15 min. to obtain uniform mixture the blended powder was mixed with 10% starch paste to obtain coherent mass, this mixture was passed through sieve no. 18, dried at 50^0c moisture content of the granules were calculated, the granules were mixed with 15% fines and finally with talc and magnesium stearate. The blended granules were compressed into approximately 400 mg tablets using single punch tablet compression machine with 0.95 cm round shape punch. The composition of the prepared formulations was represented in table no.6.

The tablets were prepared under the following considerations.

1. Amount of drug (250 mg) was constant, the ratio of drug and sodium alginate was changed according to the invitro drug release.
2. Amount of starch and calcium carbonate were varied depending on the tablet integrity and drug release behaviour.

Table no.6: Composition of Metronidazole vaginal tablets

S.NO	INGREDIENTS	F1	F2	F3	F4
1.	Metronidazole	250mg	250 mg	250 mg	250 mg
2.	Sodium alginate	125mg	125 mg	84 mg	62.5 mg
3.	Starch	9mg	9.5 mg	-	-
4.	Calcium carbonate	10 mg	9.5mg	60mg	81.5 mg
5.	Sodium lauryl sulphate	3mg	3 mg	3 mg	3 mg
6.	Talc	1.8mg	1.8 mg	1.8 mg	1.8 mg
7.	Magnesium stearate	1.2mg	1.2 mg	1.2 mg	1.2 mg
8.	Total tablet weight	400mg	400mg	400mg	400mg

3. Evaluation

The vaginal bioadhesive tablets were subjected to the following evaluation tests[9]:

3.1. Physical evaluation:

The prepared tablets were observed visually for colour, surface texture.

3.2. Weight variation:

Twenty tablets were selected at random and weighed individually and the average weight was determined in a digital balance. Then percentage deviation for the average weight was calculated. weight limits as per IP/BP and USP values were showed in the table no.7.

Table no. 7: Standard Limit Values in Weight Variation Test

Average weight of a tablet IP/BP	**Percentage deviation limit**	**Average weight of a tablet USP**
80mg or less	±10	130 or less
>80 and <250 mg	±7.5	130 mg to 324mg
250 mg or more	±5	More than 324 mg

3.3. Hardness:

Tablet hardness of each formulation was determined using a monsantohardnesstester. The tablet was placed in between a fixed and moving jaw. The scale was adjusted to zero. Load was slowly increased until the tablet broken the value of the load at that point given a measure of the tablet. Hardness was expressed in kg/cm^2.

3.4. Thickness:

Three tablets from each batch were selected and measured for thickness using Vernier callipers. The extent to which the thickness if each tablet deviated from ± 5% of the standard value was determined.

3.5. Friability:

Friability can be performed by picking five to ten pre-weight tablet from each formulation were placed in to the Rochefriabilator operated 100 revolutions. Tablets were removed dusted and weighed again. Conventional compressed tablets that loss <0.5 – 1.0% of their weight are considered acceptable.

% friability was calculated by the formula.

% friability = [(initialwt – final wt) /initialwt] X 100

3.6. Swelling index:

Swelling characteristics of vaginal bioadhesive tablet were evaluated by dynamic swelling studies. Each tablet was studied for swelling behaviour. Tablets were weighed individually, initial weight was considered as w_1 i.e., dry tablet weight in grams it was placed separately in a petri dish containing 20 ml of citro -phosphate buffer pH 6. Solution. The tablets were immersed in buffer solution at regular intervals 1,2,3,4,5,6,7 till 48 hours. The tablets were carefully removed from petri dish and excess buffer was removed using tissue paper the swollen tablets were reweighed as W_2 gm. This experiment was performed in duplicate. The degree of swelling was calculated as follows[10,17]

Swelling index (S.I) = $[(W_2-W_1)/W_1*100$

Where W_1 is dry tablet weight and

W_2 is the weight of wet tablet.

3.7. Invitro drug release studies[10,16]

Drug release studies are performed using USP type two dissolution apparatus (paddle) the dissolution medium containing a pH of 6 citro phosphate buffer. The medium was maintained at 37° c (900 ml of citro phosphate buffer). The study was conducted over 24hour period the test tablet (400 mg) was placed in the dissolution medium, rotating at 25 rpm. samples (10 ml) were withdrawn at suitable time intervals and fresh dissolution medium (10 ml) was used to replenish the dissolution medium immediately after the withdrawal of test sample. The samples were analysed at 320 nm using uv spectrophotometer at a wave length of 319 nm. No interferences were occurred due to tablet excipients at this wavelength.

3.8. Determination of bioadhesive properties.

Invitro mucoadhesion studies were carried out using modified balance method[24]. vaginal tissue was obtained from slaughter house and was fixed to a supportive material with cyanoacrylate adhesive. This was kept in a beaker and the buffer was added to the upper surface of the tissue to maintain the mucosal viability. The tablet compressed was attached to the upper clamp with adhesive. The beaker was then slowly raised until the substrate comes in contact with the tablet. A preload of 50g was placed on the clamp for 5 min (pre-load time) so that the adhesion could be established. After this time, the preload was removed and water was added into the beaker at a constant rate of 100 drops/min. the addition of water was stopped when mucoadhesive system was detached from mucosa[11]. Weight required to detach the system from mucosa was noted and equivalent adhesion force was then calculated in g/cm^2.

Table no.8: Standard Calibration Curve of Metronidazole in pH 6 Phosphate Buffer

S.No	Concentration(µg/ml)	Absorbance
01	0	0
02	4	0.224
03	6	0.314
04	10	0.525
05	14	0.705
06	16	0.793

Table No.9 : Interpretation Of FTIR Spectra

S.NO	FUNCTIONAL GROUPS	FREQUENCY RANGE	PURE DRUG	DRUG+ STARCH	DRUG+CALCIUM CARBONATE
01	C=C (Stretching)	1620-1650	1658	1658.01	1658.86
02	-C=N (stretching of imine)	2400-2600	2526.87	2523.02	2530.73
03	$-CH_2-N-$ (CH deformation vibration)	1350-1400	1365.67	1369.53	1365.67
04	Alkyl/aliphatic CH_3 stretching	2880-2860	2816.21	2816.21	2812.35
05	N-H stretching	3221-3101	3105.55	3105.55	3101.69
06	O-H stretching vibration	3200-3700	3680.36	3680.66	3676.50
07	C=O stretching vibration	1030-1085	1076	1080.19	1076.33

Table No10: Results of Pre-Compression Parameters

S.NO	Evaluated parameter	F1	F2	F3	F4
01	Angle of repose (°)	13°.83±0.6	14°.32±0.6	13°.04±	15°.708±
02	Bulk density(gm/ml)	0.415±0.2	0.416±0.2	0.4517±0.2	0.54±0.2
03	Tapped density(gm/ml)	0.4718±0.2	0.5±0.2	0.512±0.2	0.6290±0.1
04	Carr's index	12.039±0.5	16.8±0.6	11.77±0.8	14.14±0.7
05	Hausner's ratio	1.13±0.2	1.2±0.4	1.133±0.2	1.049±0.1
06	Moisture content(%)	19.23±0.7	21.030.8	16.15±0.7	18.13±0.8

(mean±SD; n=3)

Table.no.11: Post compression parameters of F1-F4 Formulations

S.No.	Formulation	Thickness (mm)	Weight Variation(mg)	Hardness (Kg/Cm^2)	Friabilty(%)
1.	F1	3.3±0.3	398±0.8	7.0±0.4	0.32±0.2
2.	F2	3.3±0.1	401±0.7	6.8±0.4	0.34±-.2
3.	F3	3.3±0.2	398±0.8	6.5±0.3	0.25±0.1
4.	F4	3.3±0.1	399±0.8	7.0±0.2	0.22±0.09

4.Results And Discussion

4.1 Standard Calibration Curve For Metronidazole:

The calibration curve of metronidazole in PH 6 phosphate buffer was derived from the concentration and corresponding absorbance. The value of linear regression analysis gave the equation for the line of best fit as Y=0.0494x+0.155. Linearity was observed in the concentration range between 4µg/ml to 18µg/ml. the values were shown in table no.10 and represented graphically in figure no.3.

4.2 Drug Excipient Compatibility Studies:

The compatibility study was performed using FTIR for drug polymer mixture. The peak of pure drug was found to be $1658cm^{-1}$ for C=C (Stretching), 2526 cm^{-1} for -C=N (stretching of imine), 1365 cm^{-1} for $-CH_2$-N- (CH deformation vibration), 3680 cm^{-1} for O-H stretching vibration, 1076 cm^{-1} for C-O stretching vibration, 3105 cm^{-1} for N-H stretching. From the FTIR graph of drug polymer mixture it was found that the same peaks of the drug is available indicating that there is no incompatibility with the excipients.

4.3 Flow Properties:

The prepared granules of all the 4 formulations granules were taken to study the flow properties. The flow properties of each formulation such as bulk density, angle of repose, tapped density, Carr's index, and Hausner's ratio are determined and the 4 formulations are found to have satisfactory flow properties.

4.4.Evaluation Of Tablets:

The prepared bioadhesive metronidazole tablets were evaluated for weight variation, hardness and friability. The values are in the range of 7.3 to 10.3 kg/cm^2. The values of weight variation are in the range of 398 mg to 401 mg, which are in the limits. The range of friability is 0.22 to 0.34.

4.4.1.Invitro Drug Release Studies:

In this study various proportions of sodium alginate were taken. F1 consists of 0.5:1 ratio of sodium alginate and drug and different proportions of calcium carbonate and starch. F2 consists of 0.5:1 ratio of sodium alginate and drug but equal ratios of calcium carbonate and starch. F3 consists of 0.3:1 ratios of sodium alginate and drug and it contains only calcium carbonate. F4 formulation consists of 0.25:1 ratio of drug and sodium alginate and this formulation also contains only calcium carbonate.

Drug release study was carried out in citrophosphate buffer pH 6 for 24 hours. In this study the influence of sodium alginate concentration and concentration of starch and calcium carbonate were evaluated. Hydrocolloids like alginate can play a significant role in the design of a controlled release product. The alginate molecule will undergo an almost immediate hydration to create a hydrocolloidal layer of high viscosity. This makes up a diffusion barrier decreasing the migration of small molecules retarding drugs at higher alginate concentrations[29]. F1 and F2 formulations were prepared from starch and calcium carbonate combinations. F1 formulation exhibited 50.4% of drug release at 6^{th} hour, but F2 formulation exhibited 57.6% of drug release in 60^{th} min itself. F1 formulation showed 90.1 % drug release in 24^{th} hour, but F2 formulation showed 83.2% in 2^{nd} hour itself. It mainly is due to the concentration difference in the starch and calcium carbonate. In F2 dissolution study, from the time of 1^{st} sample to 6^{th} hour sample, there is a slow release of drug from the sodium

alginate polymer matrix. This phenomenon is observed throughout the dissolution study. For the 1st hour 36% of drug release was observed and at the 24th hour 90% of drug release was observed. But for F2 formulation which consists of equal amount of calcium carbonate and starch that is 9.5 mg at the time of first sample 57% of drug release was seen, it was higher % of drug release when compared to the F1 formulation. This might be contributed to the higher concentration of the calcium carbonate in the matrix tablet becausenF1 &F2 formulation consists of 0.5:1 ratio of drug and sodium alginate and drug. The increase in the calcium carbonate contributed to the sustained release effect on the polymer matrix. This effect may be due to the easy hydration and gelation of the sodium alginate which leads to the early clogging of pores present in the polymer matrix so that the tablet disintegration and dissolution become slow[3]. It is also observed with increase in the starch concentration the upper part of the tablet was detached and the integrity of the tablet was lost. Under static conditions , fracture formation was more and under agitation, of dissolution study the tablet exhibited rapid disintegration , exposing the large exposed surface area to the dissolution medium. So, this factor may contributed for the faster release of drug compared to the other formulations. But, due to presence of high concentration of the calcium carbonate the integrity of the tablet was maintained throughout the study, water uptake was slow. This might be contributed for the slow release of drug. This overall effect resulted in the different pattern of dissolution profile of F1 formulation compared with the F2 formulation.

So, F1 and F2 formulations were observed physically in the dissolution medium during dissolution studies. It was observed the tablet containing higher amount of starch compared to the calcium carbonate exhibited the higher uptake of water and they are losing their tablet integrity. With increase in the starch concentration, the formation of fracture or cracks in the tablet was increased and sustained drug release was observed at the concentration of 10 mg for calcium carbonate and 9mg for the starch in combination.

Bioadhesive vaginal tablets of F3 formulation were prepared to change the drug release pattern of metronidazole. In this formulation 0.3:1 ratio of sodium alginate and drug were taken omitting the starch because, presence of starch increased the fracture and crack formation in the dissolution study. In view of increasing the physical integrity, starch was eliminated and the remaining weight was adjusted using calcium carbonate making the total weight of the tablet to 400mg, which is similar to that of F1& F2 formulation. In this formulation 84mg of sodium alginate was taken, the dissolution study was carried out for 24 hours. At the time of 1st sample (1 hour) 37.4% of drug release was happened, which is increased by 1% compared to F1 formulation. So it can be concluded that the sustained effect of drug release was contributed due to the amount of calcium carbonate. At the 2nd hour, it was increased by 2% compared to 1st hour and 48% of drug release at 5th hour and finally at 8th hour only 68.56% was reached and at 24th hour 84.6% of drug was released. In F3 formulation during 24th hour of dissolution study only 84.6% of drug was released. so, F4 formulation was prepared using 0.25:1 ratio of sodium alginate and drug. Here, 62.5mg of sodium alginate was taken. F4 formulation was also prepared using only calcium carbonate, by observing the dissolution studies of F1,F2,F3. It was concluded that sustained release effect was due to change in calcium carbonate concentration. In this formulation sodium alginate concentration was decreased from 84mg to 62.5mg. for F4 formulation 36% of drug was released at the 1st sampling period (1 hour) which is similar to that of F1 formulation. At the 2nd hour the drug release was slowly increasing from the 38.2%.40.18%, 40.94%,44.02%, 48.65%. At the end of the 6th hour, 48.6% was achieved which is almost 50% of drug release. After 7th hour the drug release was decreased, at the 8th hour only 67.88% of drug was released. The similar drug release pattern was observed in F3 formulation also but the sustained effect was particularly observed after 5th hour onwards, this is due to the change in sodium alginate concentration and calcium carbonate, because F3 consists of 84&60mg, F4 consists of 62.5, 81.5mg of sodium alginate and calcium carbonate respectively. For the formulation, at the end of 24th hour 99.2% of drug was released. By observing the dissolution studies of F1,F2,F3 &F4, it can be reported that combination of starch and calcium carbonate is altering hydrophilicity of the vaginal matrix tablet. With increase in the calcium carbonate and decrease in the starch, the reverse effect is seen. The starch concentration is also modifying physical integrity and disintegration pattern. With increase in the sodium alginate concentration, sustained drug release effect was provided. Fracture formation or crack formation was decreased with calcium carbonate content increment. For F3 and F4 formulations nearly sustained effect was seen from 6th and 7th hour, after release of 50% drug respectively. This may be due to the formation of gel layer around the tablets. Formation of gel barrier around a matrix tablet controls the drug release behaviour[3,11], with increase in the polymer density, in gel barrier formation. Consequently, porosity of gel layer was decreased which is helpful for solvent imbibition. Decrease in the porosity decreases the penetration of dissolution medium into the tablet. This effect is seen from 6th& 7th hour for F3 &F4 formulations. So, porosity, hydrophilicity and gel barrier formation contributes sustained release effect. The release of drug from various vaginal bioadhesive

tablets exhibited the following order F4>F1>F3, but F2 exhibited faster drug release compared to other formulations, which is not a desired characteristic for the treatment of vaginosis.

By observing the above results, more ca^{+2} ions became available to bind with sodium alginate during the wet granulation stage of the preparation. As a result better and stronger gel was formed when high amount of calcium carbonate was used. As the concentration of ca^{+2} ions increases, stronger gel of calcium alginate is formed that delay the influx of the dissolution medium and efflux of the dissolved drug out the matrix. As a result drug is released in amore sustained manner . As the ratio of sodium alginate to the calcium carbonate was decreased. swelling is dependent on the degree of crosslinking between sodium alginate and calcium ions.

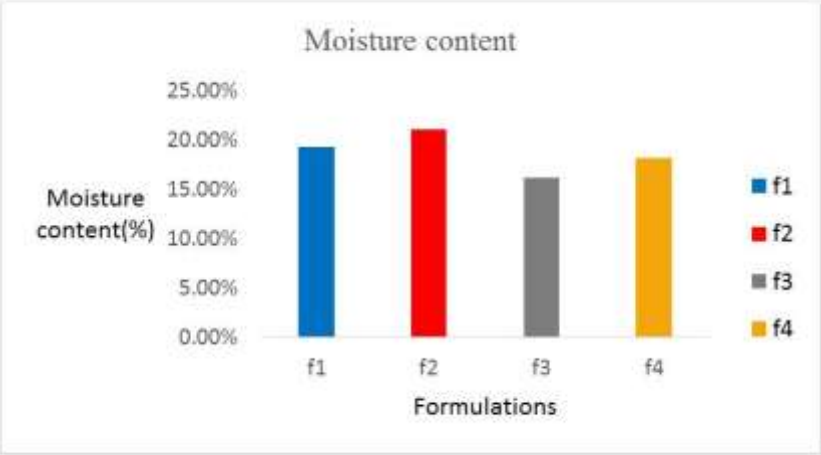

FIG_11_MOISTURE_CONTENT

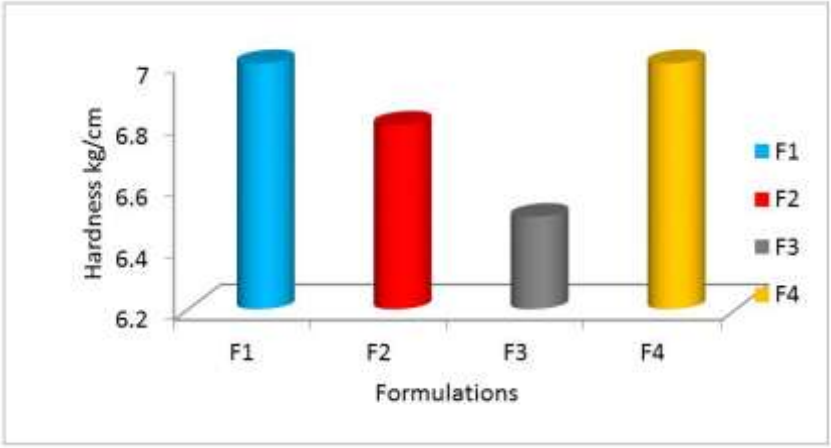

FIG_12_HARDNESS

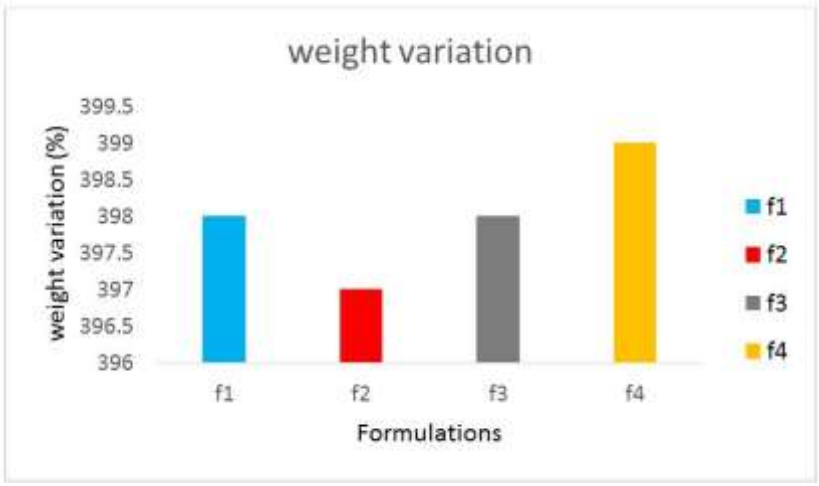

FIG_13_WEIGHT_VARIATION

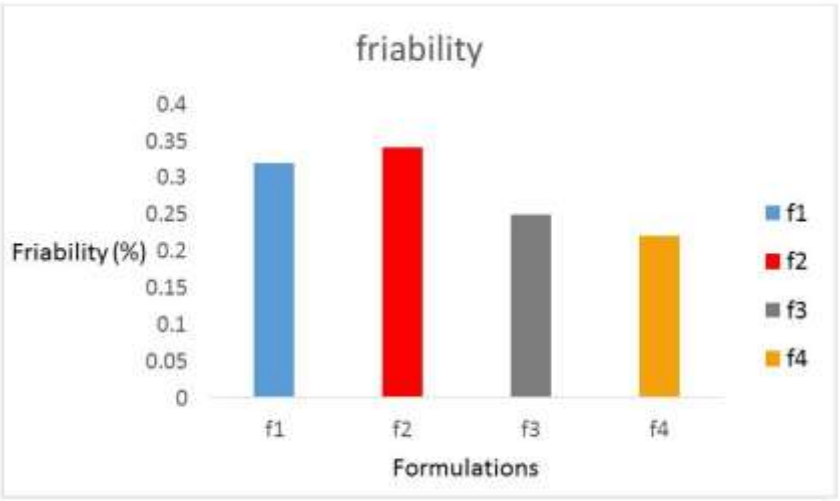

FIG_14_FRAIBILITY

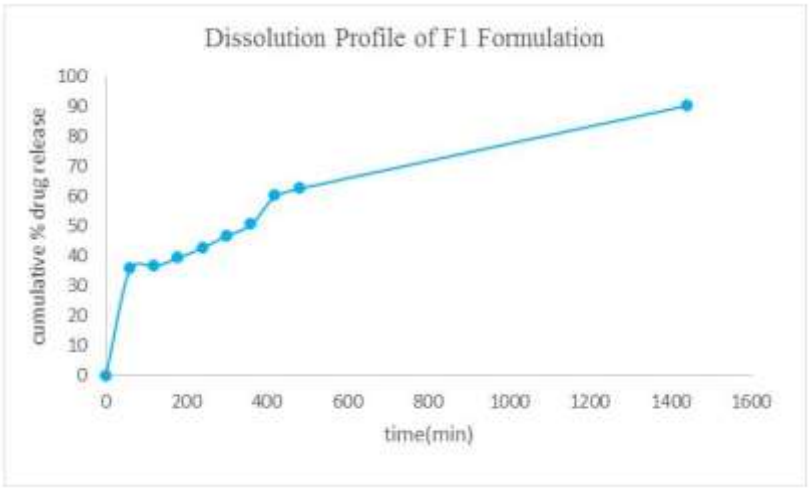

FIG_15_DISSOLUTION_PROFILE_OF_F1_FORMULATION

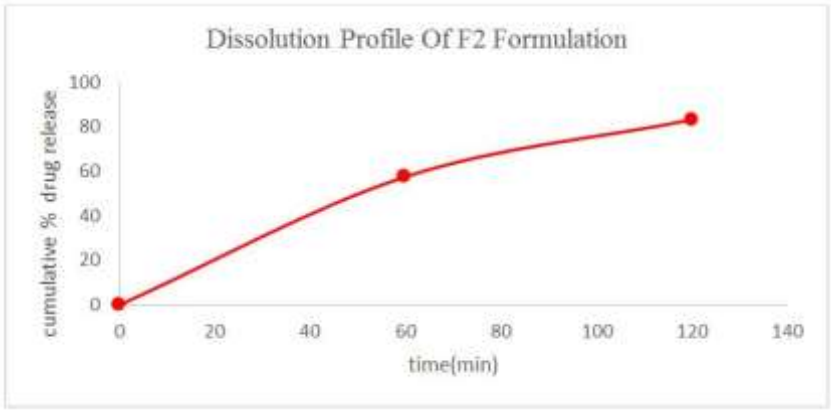

FIG_16_DISSOLUTION_PROFILE_OF_F2_FORMULATION

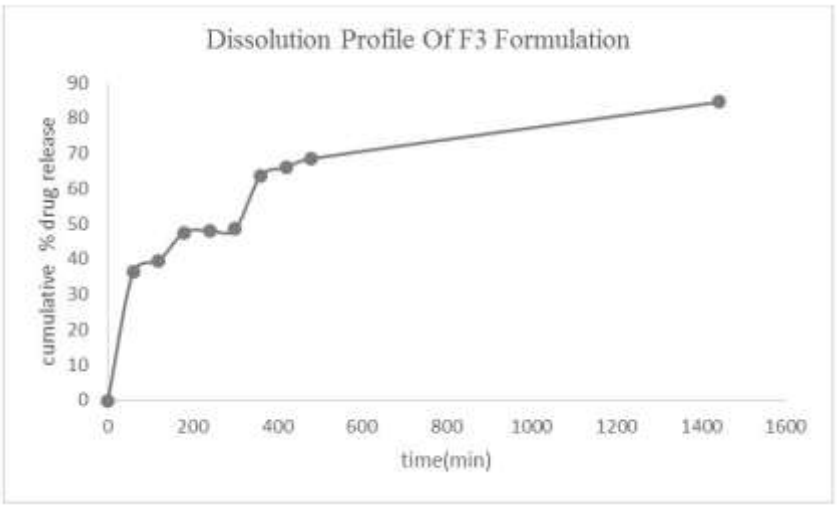

FIG_17_DISSOLUTION_PROFILE_OF_F3_FORMULATION

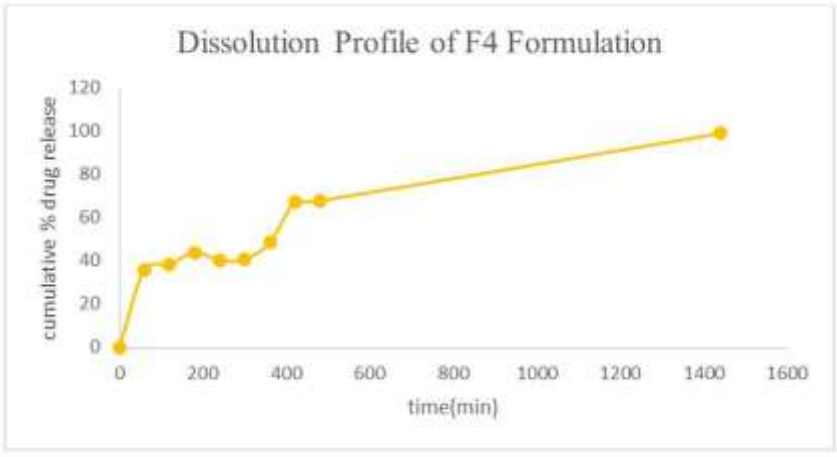

FIG_18_DISSOLUTION_PROFILE_OF_F4_FORMULATION

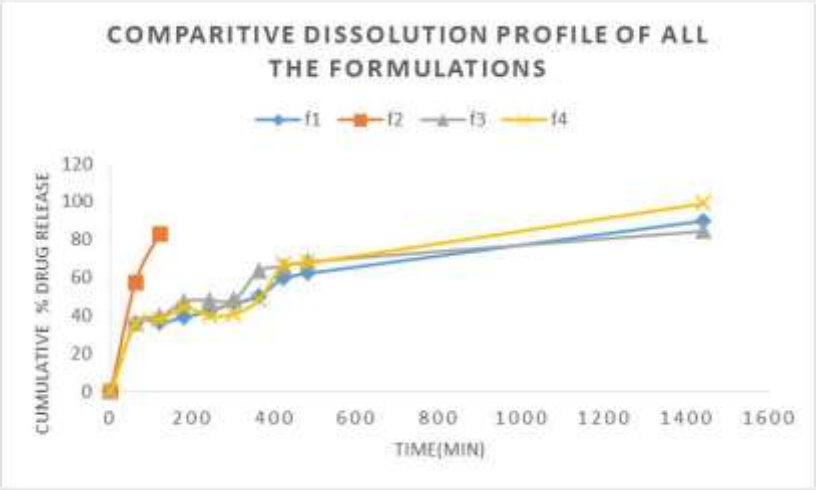

FIG_19_COMPARATIVE_DISSOLUTION_PROFILE_OF_ALL_FORMULATIONS

Table. No:12: Invitro Drug Release Study ofF1 Formulation

time in minutes	absorbance	µg/ml	mg/10ml	mg/900ml	drug content in mg	%drug release
0	0	0	0	0	0	0
60	0.07	99	0.99	89.1	89.1	35.64±0.3
120	0.072	100	1	90	90.99	36.39±0.4
180	0.074	107	1.07	96.3	98.29	39.31±0.4
240	0.082	115	1.15	103.5	106.56	42.64±0.4
300	0.087	125	1.25	112.5	116.71	46.68±0.5
360	0.105	140	1.4	126	126	50.4±0.6
420	0.119	165	1.65	148.5	149.9	59.96±0.6
480	0.121	170	1.7	153	156.05	62.42±0.6
1440	0.176	245	2.45	220.5	225.25	90.1±0.8

(mean±SD; n=3)

Table.no:13. Invitro drug release study of F2 formulation

time in minutes	absorbance	µg/ml	mg/10ml	mg/900ml	drug content in mg	%drug release
0	0	0	0	0	0	0
60	0.116	160	1.6	144	144	57.6±0.3
120	0.165	230	2.3	207	208.6	83.2±0.6

(mean±SD; n=3)

Table.no:14: Invitro Drug Release Study of F3 Formulation

time in minutes	absorbance	µg/ml	mg/10ml	mg/900ml	drug content in mg	%drug release
0	0	0	0	0	0	0
60	0.076	110	1.1	99	99	36.44±0.2
120	0.072	100	1	90	91.1	39.6±0.3
180	0.094	130	1.3	117	119.1	47.64±0.5
240	0.093	130	1.3	117	120.4	48.16±0.5
300	0.125	135	1.35	121.5	126.2	48.6±0.6
360	0.125	175	1.75	157.5	157.5	63.54±0.7
420	0.129	180	1.8	162	163.75	66.04±0.5
480	0.132	185	1.85	166.5	170.05	68.56±0.5
1440	0.167	235	2.35	211.5	211.5	84.6±0.2

(mean±SD; n=3)

Table.no:15: Invitro Drug Release Study of F4 Formulation

time in minutes	absorbance	µg/ml	mg/10ml	mg/900ml	drug content in mg	%drug release
0	0	0	0	0	0	0
60	0.072	100	1	90	90	36±0.3
120	0.085	105	1.05	94.5	95.5	38.2±0.2
180	0.082	120	1.2	108	110.05	44.02±0.3
240	0.077	108	1.08	97.2	100.45	40.18±0.5
300	0.096	110	1.1	99	103.33	40.94±0.4
360	0.097	135	1.35	121.5	121.5	48.6±0.7
420	0.134	185	1.85	166.5	167.85	67.14±0.6
480	0.132	185	1.85	166.5	169.7	67.88±0.7
1440	0.195	270	2.7	243	248.05	99.2±0.2

(mean±SD; n=3)

4.4.2.Swelling Index Studies:

Swelling index is an important parameter to be studied before considering mucoadhesion. The swelling results were expressed in terms of swelling index. The swelling index of all the formulations were given in table no: 16. F1 and F2 formulations contain starch and calcium carbonate in varying concentrations and the same concentration of sodium alginate. The formulation with calcium carbonate in higher concentration F1 possess high swelling index. when starch concentration was increased the integrity of the tablet was disturbed. This was observed in F2 when compared toF1. Sodium alginate gets converted into alginic acid in acidic medium, which swells in water and may act as a tablet disintegrant. The swelling index of the formulations were in the following order F3>F4>F1>F2. When F3 and F4 were compared the swelling index of F3 was high indicating that the decrease in free polymer concentration decreased the swelling index of the dosage form and higher kinetic energy of the water molecules inside the polymer matrix[14].

Table no.16: Results of Swelling Index Study

S.NO	FORMULATION	SWELLING INDEX(%)
1.	F1	10.2±0.4
2.	F2	9.6±0.5
3.	F3	15±0.5
4.	F4	12±0.6

(mean±SD; n=3)

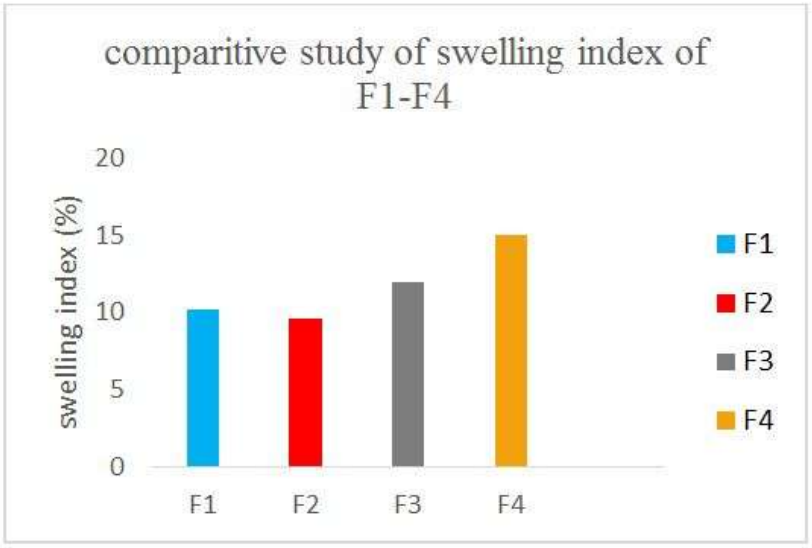

FIG20_SWELLING_INDEX_STUDY_OF_F1_-F4

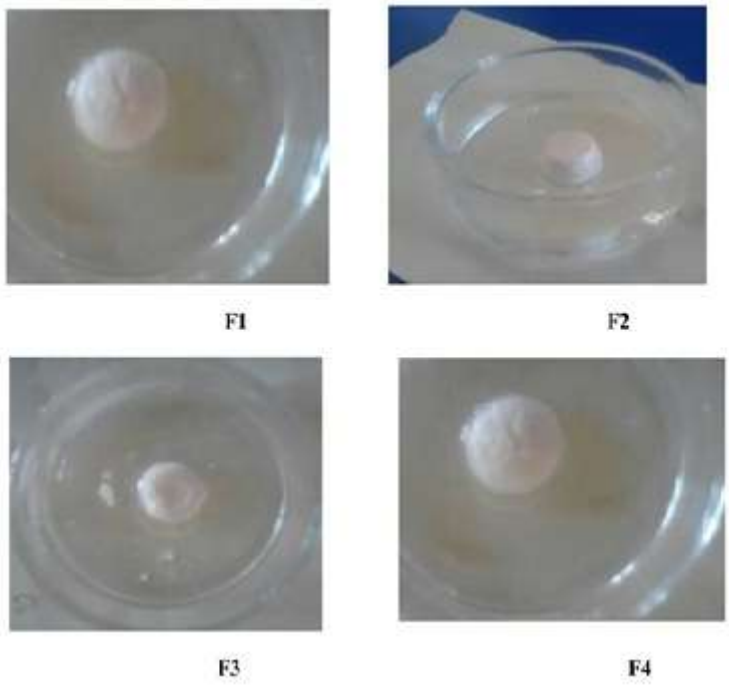

FIG_21_SWELLING_STUDIES_OF_PREPARED_FORMULATIONS

4.4.3. Bioadhesion Study:

It was suggested that the initial interaction between the polymer and the biological surface is through electrostatic interaction followed by mechanical interlocking of the polymer chains[13]. Invitro mucoadhesion testing for dosage forms was evaluated by detachment force measurements[15]. Increase in the polymer concentration caused an increase in the mucoadhesive strength. Hydration of the mucoadhesive polymer is essential to initiate the mucoadhesive bonding process. The cohesive forces arise when water from the space between the mucosa and the polymer is taken up, this plays a vital role in the establishment of an effective mucoadhesive bond[10]. F1 & F2 formulation were having similar bioadhesive strength as they have similar polymer concentration. F3 formulation having 1:0.3 ratios of drug and polymer was having high bioadhesion when compared to F4 formulation which have drug and polymer in 1:0.4 ratio.

Table no.17: Results of bioadhesion studies of F1-F4 formulations.

S.No	Formulation	Bioadhesion (g)
1	F1	60±0.1
2	F2	62±0.8
3	F3	80±0.8
4	F4	78±0.9

(mean±SD; n=3

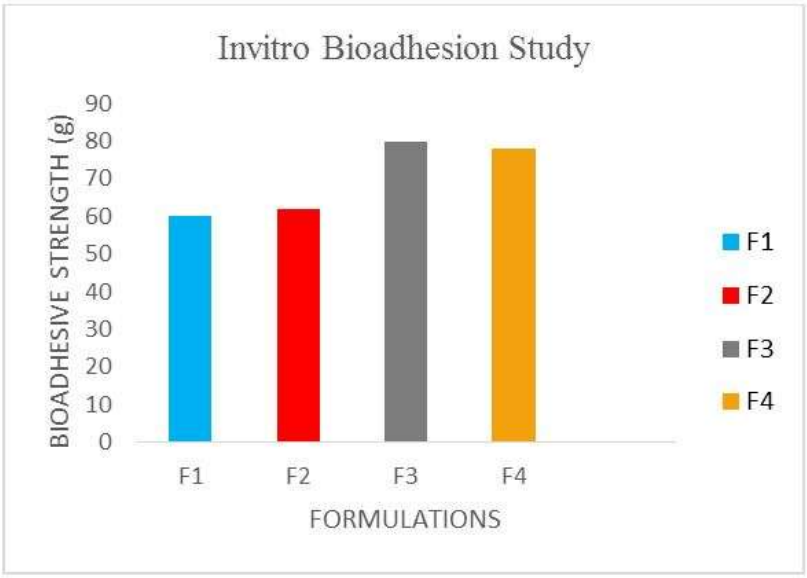

FIG_22_BIOADHESION_STUDIES_OF_ALL_FORMULATIONS

5. Conclusion

To derive maximum therapeutic benefits from certain drug substances, it is desirable to formulate them into sustained release dosage forms. This also helps in minimising the adverse effects of the drug. Various techniques have been employed to develop the bioadhesive vaginal tablets of metronidazole.

From the above experimental results, it can be concluded that:

- Sustained release and bioadhesive properties of metronidazole can be achieved by using sodium alginate as polymer.
- The IR spectra revealed that, there was no interaction between polymer and drug and also other excipients used.
- From this study it is evident that a promising bioadhesive vaginal tablet of metronidazole can be developed.
- The study also indicated that the amount of drug release changes with change in polymer concentration and concentration of calcium carbonate and starch.
- From various evaluation tests performed it was clear that the drug is safe for use as they do not have much weight variation. The hardness of the tablet indicates its stability.
- The invitro drug release studies were a proof indicating that the formulation is available for once daily administration.
- From the evaluation of bioadhesive nature of the formulation it was revealed that formulation has good bioadhesive properties.

Metronidazole bioadhesive vaginal tablets formulated in this research were found to be a promising drug delivery system for once daily administration.

6.Acknowledgment:

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7. References:

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