Research Article

Development and evaluation of Metronidazole containing topical gel using different gelling agents

Urmistha Sarkar, Anusree Raha, Prosenjit Mukherjee, Monit Paul, Anindya Bagchi

Netaji Subhas Chandra Bose institute of Pharmacy, Chakdaha, Nadia, West Bengal, India.

Received: 19 July 2018 Revised: 24 August 2018 Accepted: 2 September 2018

Abstract

Objective: The purpose of the present study was to develop and evaluate the topical gel containing metronidazole using different gelling agents such as hydroxy methyl propyl Cellulose, Na-alginate, and Na-CMC in variable concentrations. **Material and Methods:** Nine formulated gels were prepared and evaluated physically in terms of colour, odour, homogeneity, pH, spreadibility, tube extrudability, drug content and in vitro diffusion study. **Results:** The results revealed that the surface pH of nine gel formulations were within the natural skin pH range, among them sodium alginate formulations (F4, F5, F6) showed exact skin pH. Drug content was high (76.38%) in HPMC gels and In-vitro release study indicated that % released at 180 minutes is 23.37%. Drug release from the HPMC gels increased with the addition of PG. In case of gels containing HPMC, Na-alginate, Na-CMC as gelling agents, additions of PG increased the release of drug from the gels. Also antibacterial spectrum have been measured for the Formulation called F3 which showed good result after comparing with marketed formulation. **Conclusion:** It can be concluded that PG is a good penetration enhancer and HPMC good gelling agent for metronidazole gels. ***Keywords:*** Metronidazole, topical gel, HPMC, Na-Carboxy methyl cellulose, Na-alginate, spreadibility

Introduction

Now a days many dosage forms are available; topical preparation is one of them. It avoids the GI-irritation and first-pass effect and increase the drug bioavailability (Jani et al., 2010).Gels are defined as a semi solid system consisting of two phases and produce cross linked three dimensional rigid networks like structure. The structural materials that form the gel network can be composed of inorganic or organic particles and polymers where the inorganic particles are not dissolve but merely dispersed throughout the continuous phase and organic particles are dissolved in continuous phase, randomly coiled in the flexible chain (Kaur et al., 2013).

The drug delivery system from vagina has advantages over oral drug delivery system because of ability to avoid first pass effect due to presence of dense network of blood vessels and ease of administration due to large surface area. In case of controlled release dosage form vaginal irritation is reduced and increased stability and prolonged release of drug decreasing frequency of dosing than the conventional drug delivery system. Where conventional vaginal drug delivery system such as pessaries, foams, creams are relatively for short period which limited effective drug levels at the targeted site because of the self-cleaning action of the vaginal tract and increase the frequency of dosing and that leads to patient inconvenience and toxic condition. This is the main reason to choose the controlled drug delivery system (Ciurba et al., 2015).

Among the all bacteriostatic substance metronidazole is an important active substance that has been used for antibiotic and antiprotozoal medication. It is used for the treatment of pelvic inflammatory disease, endocarditic, dracunculiasis, giardiasis, trichomoniasis, amebiasis, and most important in bacterial vaginitis. According to World Health Organisation's list of essential medicines metronidazole is most effective and safe medicine for human health system with few common side effects such as nausea, metallic taste, loss of appetite and headaches when taken it orally (Yellanki et al., 2010).

An attempt has been made, in the present work, to develop antibacterial gels of Metronidazole, using a bland of

Address for Corresponding Author:
Anindya Bagchi
Netaji Subhas Chandra Bose Institute of Pharmacy, West Bengal, India
Email id: tajuanindya@gmail.com
Mobile No: +91-9330954315

DOI: https://doi.org/10.31024/ajpp.2018.4.6.10

polymers such as HPMC, Na-alginate and Na-CMC. The objectives of the study were to investigate the performance of polymers and effect on the characteristics of the Metronidazole gels with the parameters like Colour, odour, homogeneity, pH, Spreadability, Tube Extrudability, Drug content and In vitro diffusion study.

Material and Methods

Materials

We collected Metronidazole from Yarrow Chem Products Manufacturer. The other chemicals which were purchased from Loba chemie PVT. LTD, those were Hydroxy Propyl Methyl Cellulose (HPMC), Sodium Alginate, Sodium Carboxy Methyl Cellulose (Na CMC), Propylene Glycol, Methyl Paraben, Propyl Paraben.

Preparation of gel

Nine types of gel formulations were prepared using HPMC, Sodium alginate, Sodium CMC as gelling agents. Required quantity of gelling agent was weighted accurately and dispersed into the small quantity of distilled water with continuous stirring to form homogeneous dispersion. The drug was dissolved in suitable solvent here propylene glycol and added to the above dispersion. Other excipients such as methyl paraben, propyl paraben were also added with continuous stirring. The final weight of gel formulations were adjusted to 10g with distilled water. The gel formulations were stored in wide mouthed bottles (Jani et al., 2010).

Characterization and Evaluation of gel

Physical Characterization

The nine metronidazole gel formulations using different gelling agents were tested by visual inspection for colour, odour, homogeneity, when the gels have been set in the containers (Swetha et al., 2013).

Measurement of Surface pH

The pH of the metronidazole gels were measured using the digital pH meter. 1g of gel was dissolved in 25 ml of distilled water in a beaker. Then the electrode was dipped into the beaker solution and allowing it to equilibrium for 1 min and constant reading was noted (Ciurba et al., 2015; Rao et al., 2013).

Spreadability

The spreadability is very important for the therapeutic potency and application purpose of the gel formulations. We can express the spreadability in terms of time in seconds taken by two slides to slip off from gel formulation which was placed in between the slides under the direction of certain load (20g) (Kaur et. al., 2013).

The formula for the calculation is:

$S = M^* (L/T)$

Where,

M = weight tied to upper slide (20g)

L = length of glass slide slipped

T = time taken to separate the slides

Tube Extrudability

For this test a closed collapsible tube containing gel formulation was taken. A stain less steel clip was applied at the end point of the tube to prevent any rollback. 500 g weight was applied on the tube and removed the cap. The metronidazole gel was extruded (Ciurba et al., 2015).

The formula for the calculation is:

$E = (M/A)$

Where,

E = tube extrudability

M = weight applied on the tube (500g)

A = area of the extrude gel

Drug Content Determination

1 g of metronidazole gel from each formulation were taken

Table 1. Composition of Formulations

Ingredients	F1 (gm)	F2 (gm)	F3 (gm)	F4 (gm)	F5 (gm)	F6 (gm)	F7 (gm)	F8 (gm)	F9 (gm)
Metronidazole	1	1	1	1	1	1	1	1	1
HPMC	1	0.5	1.5	-	-	-	-	-	-
Sodium Alginate	-	-	-	1	0.5	1.5	-	-	-
Sodium CMC	-	-	-	-	-	-	1	0.5	1.5
Propylene glycol	2	2	2	2	2	2	2	2	2
Methyl Paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled water	Upto 10	Upto 10	Upto 10	Upto 10	Upto 10	Upto 10	Upto 10	Upto 10	Up to 10

and dissolved into 100 ml of phosphate buffer pH 6.8 individually and kept for 24 h. Next day filtered the solutions after stirring and was analysed the solution using UV spectrophotometer at 341nm. The calculation was carried out using calibration curve (Ciurba et al., 2015).

In-vitro diffusion Study

The diffusion studies of nine formulated metronidazole gels were carried out in Franz diffusion cell for studying the diffusion release of gels from a cellophane membrane. Gel sample 1g was taken in cellophane membrane and used 100 ml of phosphate buffer pH 6.8 as dissolution medium at (37±1) °C. 5ml of each sample was withdrawn periodically at 15, 30, 60, 90, 120, 180 minutes and each sample was replaced with equal volume of fresh buffer. Then the samples were analysed using UV spectrophotometer at 341nm after suitable dilution (Kaur et al., 2013).

Antimicrobial assessment

Pharmacological evaluation involves testing of the microbial susceptibility to chemotherapeutic agents. Determination of antimicrobial effectiveness against specific pathogens is essential for proper therapy. Sensitivity of organisms to antimicrobials may be quantified by the minimum concentration require to inhibit their growth (Minimum inhibitory concentration, MIC). Since it is easier to measure and apply to both bactericidal and bacteriostatic drugs, MICs are frequently used. The formulation (F3) was tested for their *in-vitro* growth inhibitory activity against *Staphylococcus aureus*.

Preparation of cultures

Bacteria was cultured in sterile nutrient broth medium which had been autoclaved at 121°C under a pressure of 15 atmospheres for 15 min. and left to grow for 48 h at 37°C in an incubator. The bacterial cultures obtained were diluted with autoclaved Nutrient. This culture served as the inoculums for the antimicrobial experiments.

Preparation of agar plates for Antimicrobial activity

Nutrient agar plates were prepared by mixing Nutrient agar (28 g) in 1000 ml distilled water boiled to dissolve the medium completely. Nutrient agar solution was sterilized by autoclaving at 121.C for 15 min at 15 lb pressure. After cooling (45.C), agar solution (25 ml) were poured into sterilized Petri dishes and left to solidify. Agar plates were inoculated with an overnight bacterial culture, using spread plate method after appropriate serial dilutions. Nutrient agar plates were used for *Staphylococcus aureus.* The formulation (F3) was aseptically put into the wells (100 µl approx.) made in agar plates making lawns of *Staphylococcus aureus*. The Nutrient agar plates were then incubated at 37 ^{0}C for 24 h. The diameter of inhibitory zone surrounding disc was then measured after 24 hours for three days individually and compared with standard. Two cross sectional points and the average was taken as the inhibition zone and the size of the zone diameter was measured. The plates were then photographed individually.

Result and Discussion

Physical Characterization

After nine metronidazole gel formulations using different gelling agents had been set in the containers were tested by visual inspection for colour, odour, and homogeneity. All gel formulations were homogenous in nature and pleasant in odour. Formulation F1 to F3 contains HPMC and yellowish white in colour, formulation F4 to F6 contains Na-alginate and brown in colour, formulation F7 to F8 contains Na-CMC and creamy in colour.

Measurement of Surface pH

The pH of the metronidazole gels were measured using the digital pH meter. That the surface pH of nine gel formulations were within the natural skin pH range, among them sodium alginate formulations (F4, F5, F6) showed exact skin pH.

Table 2. Physical Characterization and surface pH of Metronidazole Gel

Formulation	Color	Odor	Homogeneity	Surface Ph
F1	Yellowish White	Pleasant	Homogenous	6.0
F2	Yellowish White	Pleasant	Homogenous	6.0
F3	Yellowish White	Pleasant	Homogenous	6.0
F4	Brown	Pleasant	Homogenous	5.5
F5	Brown	Pleasant	Homogenous	5.0
F6	Brown	Pleasant	Homogenous	5.8
F7	Creamy	Pleasant	Homogenous	7.0
F8	Creamy	Pleasant	Homogenous	6.5
F9	Creamy	Pleasant	Homogenous	7.0

Spreadability

It is a very important evaluation for topical gel formulation just because it spreads on application to the affected part of skin. HPMC gels showed superior spreadability where Na-CMC gels showed moderate spreadability.

Tube Extrudability

In extrudability study formulation F6 (Na-alginate containing metronidazole gel) showed superior extrudability than any other formulations and formulation F3 (HPMC containing metronidazole gel) showed lowest extrudability.

Drug content determination

Drug content of all gel formulations was found in between 58.46% to 76.38%. The drug content appears to be high in case of gel contains HPMC (F3). Drug content appears to be low in case of gel contains Na-alginate (F4).

In-vitro diffusion study

In-vitro release study indicated that % released at 180 minutes is 23.37% in case of HPMC gel formulation F3. Drug release from the HPMC gels increased with the addition of PG. A set of gels containing Na-alginate as gelling agent (F6) showed drug release 20.92% at 180 minutes. A set of gels containing Na-CMC as gelling agent (F9) showed drug release 16.00% at 180 minutes. In case of gels containing HPMC, Na-alginate, Na-CMC as gelling agents, additions of PG increased the release of drug from the gels. It is concluded that PG is a good penetration enhancer and HPMC good gelling agent for metronidazole gels.

Antimicrobial Activity

The results of antimicrobial activity were shown in table 3. Antimicrobial spectrum has been measured for the formulation F3. Results suggests that it could be very

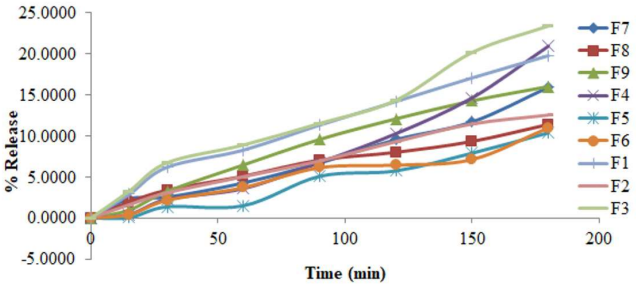

Figure 1. *In-vitro* diffusion study of metronidazole gels

Table 3. Spreadability, extrudability and drug content of Metronidazole Gel

Formulation	Spreadability (gm*cm/ sec)	Extrudability (gm/cm²)	Drug Content (%)
F1	21.4	86.80	63.66
F2	33.0	101.45	69.94
F3	26.7	80.00	76.38
F4	16.7	73.95	53.46
F5	17.4	125.00	70.03
F6	16.9	195.31	74.35
F7	20.0	138.50	72.98
F8	23.3	154.32	64.43
F9	17.0	94.51	73.91
SD	**5.227232**	**37.90679**	**6.794504**

Table 4. In-vitro Diffusion Study of metronidazole gel formulations

%drug release time	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
15	2.8868	1.6287	3.2638	0.4087	0.0328	0.4305	2.3377	1.9251	0.9648
30	6.1879	3.0772	6.7347	2.1617	1.3733	2.2841	2.5545	3.3939	3.2432
60	8.2702	5.0342	8.8874	3.7115	1.4941	3.5852	4.2761	5.0958	6.4667
90	11.3212	6.9485	11.5230	6.1324	5.0775	6.7752	6.7069	7.0575	9.5725
120	14.1862	9.2990	14.3307	6.4293	5.7433	10.2711	9.5417	7.9956	12.0778
150	17.0692	11.4499	20.1650	7.1261	7.8990	14.6281	11.7053	9.3289	14.2696
180	19.7695	12.5367	23.3784	10.9850	10.3758	20.9239	15.9170	11.3934	16.0095
S.D.	**5.598367**	**3.849169**	**6.685882**	**3.24953**	**3.519131**	**6.823032**	**4.70239**	**3.09324**	**5.220875**

promising topical alternative for treatment of vaginal infection as a controlled release drug delivery system.

Table 5. Zone of Inhibition (mm)

Day	Marketed Formulation	Prepared Formulation (F3)
1	23.8	24.5
2	23.7	24.7
3	24.6	25.7

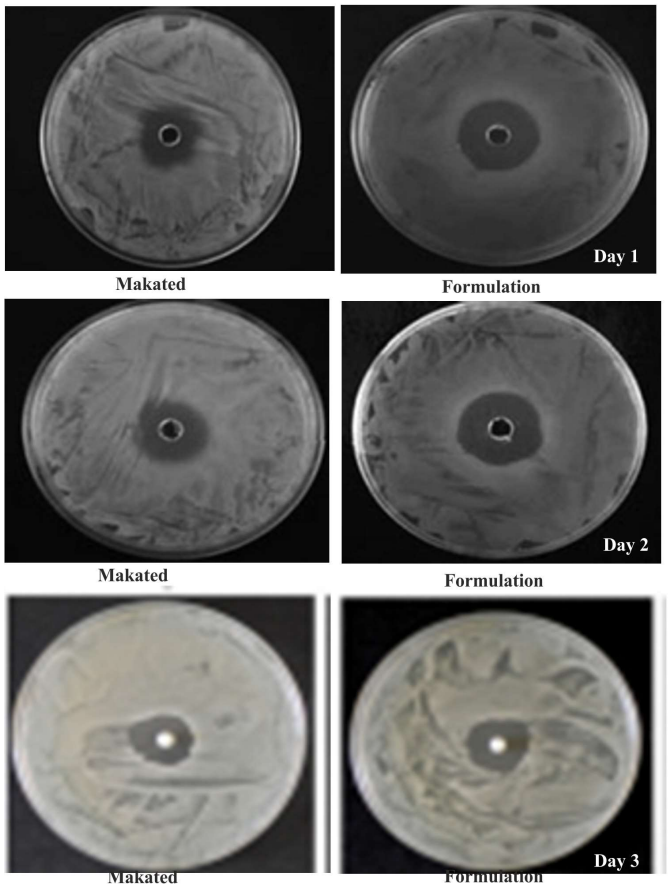

Figure 2. Zone of inhibition in three different days

Conclusion

From the above results, it is concluded that the topical gel of metronidazole prepared with different gelling agents HPMC, Na-alginate, Na-CMC were showed acceptable properties such as colour, odour, pH, homogeneity, spreadability, tube extrudability, drug content and in vitro diffusion study. A set of gels containing HPMC as gelling agent (F3) showed superior drug release 23.37% at 180 minutes. With an intention to enhance the release, PG was incorporated. Drug release increased with the addition of PG. Drug content appears to be high (76.38%) and uniformly distributed. A set of gels containing Na-alginate as gelling agent (F6) showed drug release 20.92% at 180 minutes. Drug content appears to be higher (74.35%) and uniformly distributed. Drug release increased with the addition of PG. A set of gels containing Na-CMC as gelling agent (F9) showed drug release 16.00% at 180 minutes. Drug content appears to be higher (73.91%) and uniformly distributed. With an intention to enhance the release, PG was incorporated. Here also drug release increased with the addition of PG. Results indicated that the HPMC gels show higher release of the drug compared to other gelling agents. Therefore we can conclude that HPMC is a potential gelling agent for Metronidazole gels. It could be very promising topical alternative for treatment of vaginal infection as a controlled release drug delivery system. Also antibacterial spectrum have been measured for the Formulation called F3.

Acknowledgement

The authors are thankful to the respected Principal Sir, Dr. Arnab Samanta, Netaji Subhas Chandra Bose Institute of Pharmacy, West Bengal for providing necessary facilities for the completion of research work.

Conflicts of interest: Not declared.

References

Adriana C, Luminita L, Paula A, Anca GM, Camil-Eugen V, Nicoleta T. 2015. In vitro/ In vivo Performance study of New Metronidazole Periodontal Gel Formulation. Farmacia, 63(1):11-19.

Jani R, Jani K, Setty M, Patel D. 2010. Preparation and Evaluation of Topical Gel of Valdecoxib. International Journal of Pharmaceutical Sciences and Drug Research, 2(1):51-54.

Kaur PL, Guleri TK. 2013. Topical Gel: A Recent Approach for Novel Drug delivery. Asian Journal of Biomedical and Pharmaceutical sciences, 3(17):1-5.

Nayak SB, Rout PK, Nayak UK, Bhowmik BB. 2010. Development and Characterization of Bioadhesive Gel of Microencapsulated Metronidazole for Vaginal Use. Iranian Journal of Pharmaceutical Research, 9(3):209-219.

Rao M, Sukre G, Aghav S, Kumar M. 2013. Optimization of Metronidazole Emulgel. Journal of Pharmaceutics, 1-9.

Swetha HC, Velmurugun S, Narayana RP, Reddy NG. 2013. Formulation and Evaluation of Clarithromycin Topical Gel. International Journal of Drug Delivery & Research, 5(4):194-202.

Yellanki SK, Singh J, Manvi FV. 2010. Formulation, Characterization and Evaluation of Metronidazole Gel for Local Treatment of Periodontitis. International Journal of Pharma and Bio Sciences, 1(2):1-9.