

In vitro Evaluation of Tinidazole Bioadhesive Vaginal Gels

Zainab T.Salih*¹

*Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

Many trials were made to prepare Tinidazole 2% as bioadhesive vaginal gels using different gel bases including hydroxypropyl methyl cellulose (3 and 4% w/w), methylcellulose (3 and 4% w/w) and carboxymethylcellulose (2 and 3% w/w). Swelling index of the polymers, pH, viscosity, bioadhesive force, and in-vitro drug release to the simulating vaginal fluid (S.V.F.) were investigated for all the prepared bioadhesive gels. The mechanism of drug release from the gel bases was also investigated.

The results revealed that C MC 3% gave the highest viscosity and bioadhesive strength with the lowest release rate while lowest viscosity and bioadhesive force was obtained with HPMC gel base with the highest release rate. The mechanism of drug release was affected by the type of gel base. Fickian diffusion was obtained with all gel bases.

Key words: Tinidazole, bioadhesive polymers, gels

الخلاصة

تبنيدازول 2% حضر على شكل هلام مهبلي مخاطي الالتصاق باستخدام قواعد هلامية مختلفة تشمل هيدروكسي بروبيل ميثيل سيليلوز، الميثيل سيليلوز والكاربوكسي ميثيل سيليلوز والتي تم قياس نسبة الانتفاخ لها. كما تم قياس الاس الهيدروجيني واللزوجة وقوة الالتصاق وتحرر الدواء من التراكيب الهلامية المحضرة في الوسط المشابه للمحيط المهبلي باس هيدروجيني 4.2. وقد اظهرت النتائج ان تحرر الدواء يتأثر بنوع وتركيز البوليمر المستخدم، وأن 3% كاربوكسي ميثيل سيليلوز اعطى أعلى لزوجة وأعلى التصاق وأعلى نسبة انتفاخ وأبطأ تحرر. اما الهيدروكسي بروبيل ميثيل سيليلوز فقد اعطى أعلى تحرر للدواء ولم يعطي أي نسبة انتفاخ في التركيز المستخدم.

Introduction

The vaginal route of administration has many advantages compared to other routes ⁽¹⁾. The vaginal epithelium is permeable to a wide range of drugs like hormones, peptides, proteins and antimycotics agents that need to reside at the site of infection for a prolonged period to be more effective. The vagina provides a promising site of systemic drug delivery because of its large surface area and its rich blood supply. In addition, a prolonged contact of a delivery system with the vaginal mucosa may be achieved more easily than at other route of drug absorption site like rectum or intestinal mucosa ⁽²⁾. Tinidazole (TND) is {1- [2-(ethylsulphonyl) ethyl] - 2 - methyl - 5 - nitroimidazole} has been proved to be very effective for the therapy of amoebiasis, trichomonas, lamblasis and anaerobic infections ⁽³⁾. The drug is well tolerated and widely used in clinical practice in the form of I.V infusion, tablets and vaginal pessaries ⁽⁴⁾. The efficacy of TND in the treatment of trichomonas vaginalis has been well documented ⁽⁵⁾. TND is similar to metronidazole intravaginal gel can be used to treat bacterial vaginosis ^(6,7), it is effective, safe, easy to apply and has fewer side effect compared to oral dosage forms of

this medication ^(8,9) patients tolerate gels better than vaginal inserts or ointments. ⁽¹⁰⁾

This study was conducted to formulate TND in a suitable mucoadhesive vaginal gel through studying different variables affecting the physicochemical properties and the in vitro release of the drug from these preparations.

Materials and Methods

Materials

Tinidazole (TND) was supplied by Sigma chemical co., methylcellulose (MC) and carboxymethylcellulose (CMC) from (BDH limite (HPMC) from (Shin-Etsu chemical co.- Germany). All other materials used were of analytical grade.

Methods

Preparation of simulating vaginal fluid (S.V.F)

Simulating vaginal fluid (S.V.F) was prepared by dissolving 3.51gm of sodium chloride, 1.4gm of potassium hydroxide, 0.22gm of calcium hydroxide, 0.018gm of bovine serum albumin, 2 gm of lactic acid, 1gm of acetic acid, 0.16gm of glycerol, 0.4gm of urea and 5gm of glucose in 1 liter distilled water then the pH was adjusted at 4.2 ⁽¹¹⁾.

1Corresponding author E- mail : Zainabthabit@yahoo.com

Received : 3/5/2010

Accepted : 18/9/2010

Preparation of TND vaginal aqueous gel bases

The tested aqueous gel bases were prepared by dispersing the polymers powder of 3 and 4%(w/w) MC, 2 and 3%(w/w) CMC and 3 and 4%(w/w) HPMC in cold freshly distilled water with the aid of a high speed stirrer, until a solutions were complete⁽¹²⁾. The products were refrigerated for 1hour before use to obtain a suitable consistency. TND gels were prepared by dispersing 2% drug in different gel bases as shown in table(1).

Table 1:Composition of TND bioadhesive vaginal gel

Materials (gm)	F1	F2	F3	F4	F5	F6
Tinidazole	2	2	2	2	2	2
Methylcellulose (Mc)	3	4	-	-	-	-
Carpoxymethylcellulose (CMC)	-	-	2	3	-	-
Hydroxypropylmethylcellulose (HPMC)	-	-	-	-	3	4
H ₂ O Q.S	100	100	100	100	100	100

In vitro dissolution of TND from gel bases

The release study was carried out in a USP apparatus type I at 100 rpm, a basket of 2.5 cm in diameter was enclosed with a multifold filter paper (dialysis cell) in order to be filled with 1gm of each base containing 2% w/w TND. After connecting to a stirrer motor, the dialysis cell was immersed to about 1 cm of its surface in 500 ml of simulating vaginal fluid pH4.2 at 37°C for 5 hours. Samples were withdrawn after 0.5, 1, 2, 3, 4 and 5 hours and replaced with an equal volume of the same fluid solution. The samples were analyzed for their drug content at its λ_{max} 320nm^(13, 14).

The effect of polymer type on the release of TND from the gel bases

The effect of MC, CMC and HPMC bases on the release of TND from gel bases was studied.

The effect of polymer concentration on the release of TND from the gel bases

The effect of 3 and 4% w/w MC, 2 and 3% w/w CMC and 3 and 4% w/w HPMC on the release of TND from gel bases was studied.

Measurement of the swelling index of dry polymer in S.V.F

The swelling index is the volume in ml occupied by 1 gm of polymer after it has swollen in aqueous liquid (S.V.F used in the study) for four hours. The process is carried out by placing 1gm of dry polymer in 25ml graduated cylinder, moisten it with 1ml (ethanol 96%) and with an addition of 25 ml S.V.F shaking vigorously every 10 minute for one hour, then allowing to stand for three hours. After 1.5 hours from beginning the test any volume of liquid retained in the layer of the polymer and any particles of polymer floated at the surface of the liquid were released⁽¹⁵⁾. The swelling was measured according to this equation:

$$\text{Swelling} = \frac{(\text{final volume} - \text{initial volume})}{\text{initial volume}}$$

Mechanism of TND release from gel bases

To understand the mechanism of the drug release, the release profiles from different gel bases, the % fraction drug released verses time profiles were plotted using linear fitted equations proposed by Koresmeyer - Peppas⁽¹⁶⁾

$$F = Mt/Mo = kt^n \quad \text{at time } t \quad (1)$$

$$\ln F = \ln k + n \ln t \quad (2)$$

The equations express the fraction (F) release of drug from gel. Where Mt is the amount released at time t , Mo is the initial amount of drug, K is the rate constant and n is the releasing exponent value indicative the mechanism of drug release (if n is 0.5 or less (fickian) holds the release, while when n greater than 0.5 this indicate non-fickian (anomalous) releasing mechanism), n value could be result from the slope obtained by the drawing of \ln . Fraction released verses \ln . Time.

Viscosity measurement

A Brook Field digital viscometer with a suitable sample adaptor was used to measure the viscosity in centipoises of the bioadhesive prepared gel.⁽¹⁷⁾

Determination of pH

The pH of the bioadhesive gels were determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode dipped into gel formulation for 30 minute. The measurements of pH of each system were replicated three times⁽¹⁸⁾

Determination of mucoadhesive force

The mucoadhesive potential of each system was determined by measuring the force required to detach the gel from sheep vaginal mucosal tissue by using a modified chemical balance. A section of vaginal mucosa was cut from the sheeps vaginal cavity and instantly fixed with mucosal side out onto each glass vial using a rubber band. The vials with vaginal mucosa were stored at 37°C for 5 minutes. Then next vial with a section of mucosa was connected to the balance in inverted position, while first vial was placed on a height adjustable pan. Fixed amount of sample of each gel system were placed onto the vaginal mucosa of first vial. Then the height of second vial was adjusted so that mucosal surfaces of both vials come in intimate contact. Five minutes contact time was given to ensure intimate contact between tissues and the sample. Then weight was kept rising in the pan until vials get detached. The bioadhesive forces as the detachment stress in dyne/cm², was determined from the minimal weights that detached the tissues from the surface of each gel using the following equation⁽¹⁹⁾:

Detachment stress (dyne/cm²) = $m \times g / A$

Where, m = weight required for detachment of two vials in grams.

g = Acceleration due to gravity [980cm/s²].

A = area of tissue exposed.

The vaginal mucosa was changed for each measurement. Measurements were repeated three times for each of the gel system.

Results and Discussion

In order to fortify the adhesion of administered drug onto the mucosal surface, TND was formulated in a suitable mucoadhesive polymers, MC, CMC and HPMC bases to take a full advantageous of

the contact time, the drug can be dispersed in the gel giving a concentration that is higher than corresponding to the solubility of the drug⁽²⁰⁾, as well as, it can be considered as a way for sustaining the release of the drugs from gel dosage form. MC, CMC and HPMC were used as a gel base because of their safety use for vaginal application as well as their compatibility with TND, with other vaginal secretion.

The effect of polymer type and concentration on the releasing of TND from the gel bases

Figures 1, 2 and 3 showed the amount of TND released from gel bases in S.V.F medium. It was found that the amount of TND released from gel bases were significantly influenced ($p < 0.05$) by the type of the base and the concentration the polymer used.⁽²¹⁾ In general it was found that highest amount of TND was released from CMC gel base and decreased with an increasing in polymer concentration.⁽²²⁾ also the drug release rate was decreased with the increase in the polymer concentration.⁽²³⁾

Estimation of swelling capacity

The swelling index (capacity) for 1gm dry MC and CMC are 50% and 80% respectively. HPMC is soluble and non-swollen polymer at the concentration used. The high swelling % of CMC in S.V.F indicates that the swelling of this polymer takes place at a slower rate. The straight lines obtained from plotting of t/v (t : time in hour, v : volume of aqueous S.V.F absorbed per volume of dry polymer) against time t in hour as shown in figures (4 and 5) were utilized to calculate the kinetic of swelling including initial rate of swelling (reciprocal of the intercept) and swelling equilibrium (reciprocal of slope)⁽²⁴⁾, as shown in table 2

Table 2 : The initial rate of swelling (reciprocal of intercept) and swelling equilibrium (reciprocal of slope)

Polymer type	Swelling to equilibrium size (ml of fluid/ml of dry polymer)	Initial rate of swelling (ml of fluid solution /hr.ml of dry polymer)	Correlation coefficient r
MC 3%	1.797	74.626	0.99
MC 4%	1.496	12.987	0.9885
CMC 2%	1.951	11.454	0.9944
CMC 3%	1.500	14.619	0.995

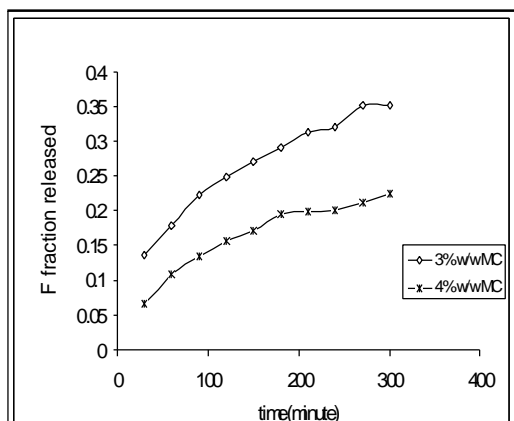


Figure 1 : The effect of MC Concentration on the release profile of TND at pH 4.2 and 37 ° C

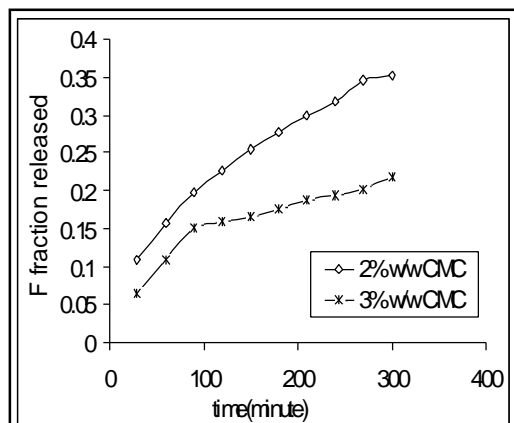


Figure 2: The effect of CMC on the release profile of TND at pH 4.2 and 37 ° C

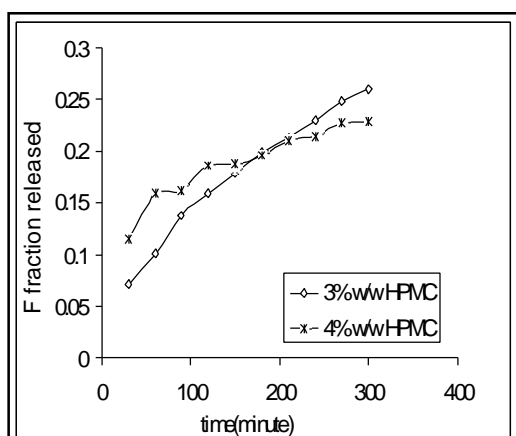


Figure 3 : The effect of HPMC on the release profile of TND at pH 4.2 and 37 ° C

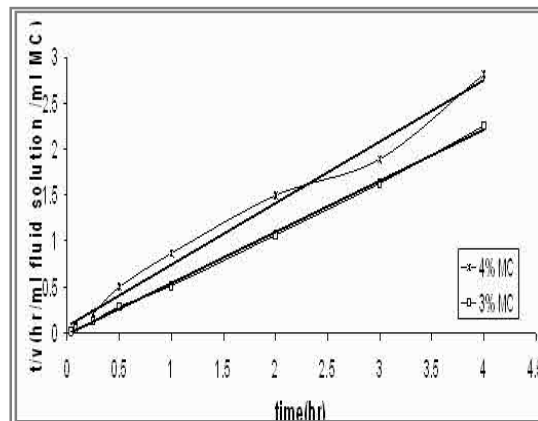


Figure 4 : The swelling mechanism of 3 & 4% w/w MC in simulating vaginal fluid pH 4.2 and 37 ° C

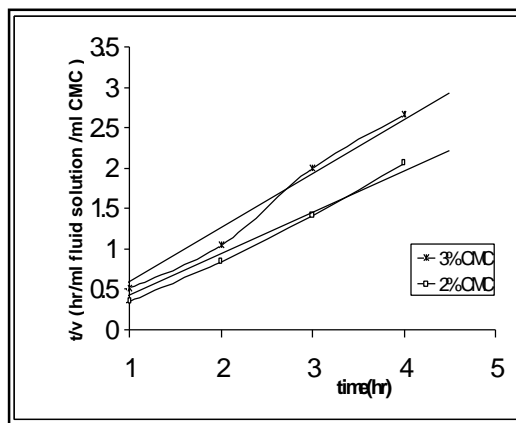


Figure 5 : The swelling mechanism of 2 & 3% w/w C MC in simulating vaginal fluid pH 4.2 and 37 ° C

Releasing mechanism of TND from gel bases

It was found that formulation with higher swelling index retard the release of drugs more than those with lower swelling indices, this swelling also depends on the pH of the medium and the presence of electrolytes, where the S.V.F pH 4.2 has a lot of electrolytes content such as: sodium chloride, potassium hydroxide, calcium hydroxide⁽²⁵⁾. This swelling is important since it significantly decreases the releasing rate of the drug and increases in the amount of the drug release after five hours, as shown in table-3. The reason behind this increasing in the amount released due to erosion of the gel as a result of severe hydration or relaxation of the polymer, so interchain intermolecular force will no longer be able to resist any external forces, once gel erodes, it breaks up into smaller and smaller particles, more surfaces will be exposed to the fresh swelling medium

and hence more drug will be released leads to an increasing in the amount of the drug released from CMC gel base more than other

swollen MC gel base after five hours⁽¹⁸⁾. As a result, CMC gel bases hold a Fickian release mechanism.

Table 3 :The release rate, amount released and the releasing mechanism of the TND from gel bases

Polymer type and concentration	n exponent release value	K % min ⁿ releasing rate	amount release (mg/5hr) (mean±SD,n=3)	Mechanism of TND release
MC 3%	0.41	5.3974	7.05±0.14	Fickian
MC 4%	0.49	3.5744	4.466±0.13	Fickian
CMC 2%	0.509	4.1594	7 ±0.15	Fickian
CMC 3%	0.52	3.582	4.35±0.12	Fickian
HPMC 3%	0.29	6.1472	5.2±0.03	Fickian
HPMC 4%	0.5	3.1864	4.58±0.11	Fickian

Viscosity measurment

Viscosity is an important parameter for characterizing the gel as it affects the spreadability ,extrudability and release of drug⁽²⁶⁾. CMC gel base show the highest viscosity, and as the polymer concentration increase ,the viscosity will be increased as shown in table 4. On the other hand , minimum level of viscosity was seen with F5 and F6 explain the rapid release of drug from this gel system and this due to non swelling property of HPMC polymer at these concentration .

Table 4:The viscosity and pH of the prepared TND bioadhesive vaginal gel

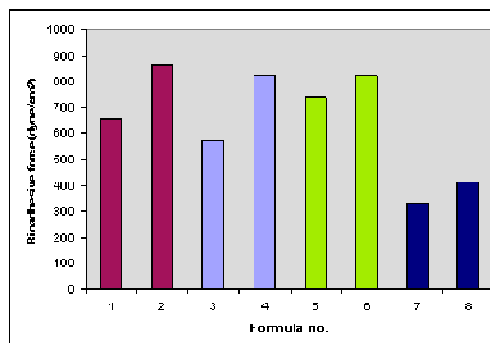
Formula No.	Viscosity (cps)	pH
F1	1450	6.02
F2	3240	5.2
F3	1700	5.03
F4	6800	5.17
F5	14.5	5.02
F6	21.6	5.2

Determination of pH

The pH of gels were found to be within the range of 5-6.2 which is within the limit of semisolid specification and at this pH the gel will be non irritant to vagina⁽¹²⁾. The physical appearance of the gel was evaluated with naked eye . The gel was smooth without any lumps and of uniform color ,no color change /liquification /separation were observed .

Determination of mucoadhesive force
Scheme 1 indicates the vaginal bioadhesive properties of the prepared gels in sheep vagina, and the results showed that all vaginal bioadhesive strengths were found in the

following order F2,F4>F3>F1>F6>F5
Indicating that (F2)and (F4) showed the highest bioadhesive properties.



Scheme 1: Bioadhesive strength of TND Bioadhesive vaginal gels

Conclusion

Results of this study confirm that, the physical properties of the prepared gel were affected by the type and concentration of the polymer used in the preparation. The more sustained preparation with highest bioadhesive force and highest viscosity was obtained by the use of 3% CMC as a gel base. In addition to that the mechanism of drug release from tinidazole 3%CMCgel base was diffusion mixed with erosion.

References

1. Richardson JI Illum L The vaginal route of peptide & protein drug delivery .Adv. drug delivery rev 1992 ; 8: 341-366 .
2. Claudia, KV, Constantiae, Irene H. , Andreas, SB . Development and *in vitro* evaluation of a mucoadhesive vaginal delivery system for progesterone. J. controlled release 2001; 77: 323-332.

3. Trac JW, Webster LT. Drugs used in the chemotherapy of protozoal infection .In:Hardman, J.,G., Limbird,L., E.,the pharmacological basis of therapeutics 9th ed. Mc Graw –Hill,New York 1996 ; 995-998
4. Martindale. The complete drug reference, 32 ed., pharmaceutical press 1999; 594: 1099, 1541.
5. BNF, British national formulary, British medical association and Royal Pharmaceutical society of Great Britain 2004; 47 ed.: 287, U.K.
6. Milani,M., Barcellona,E., Agnello,A., Efficacy of the combination of 2g oral tinidazole and acidic buffering vaginal gel in comparison with vaginal clindamycin alone in bacterial vaginosis :a randomized ,investigator -blinded, controlled trial .Euro .J. of obst. and gyne 2003 ;109: 67-71.
7. Livengood, . G. H. Mc Gregor , ., J.,A., Soper, D., E, Newton, E., Thomason, J.,L., Bacterial vaginosis : efficacy and safety of intravaginal metronidazole treatment .Am.J.Obstet.Gynecol 1994 ;170: 759-764.
8. Hanson, JM., McGregor, JA, Hiller, SL., Eschenbach, DA., Kreutner A.K., Galask, RP., Martens, M.. Metronidazole for bacterial vaginosis. Acomparision of vaginal gel vs. oral therapy .J.Reprod.Med 2000; 45: 889-896.
9. Dubonchet, L. , McGregor, J.A. , Ismail , M. , McCormack, W.M. , A pilot study of metronidazole vaginal versus oral metronidazole for tretmeant of trichomonas vaginalisvaginitsis ,sex. Transm. di 1998 ;25:176-179.
10. Edsman, K. Carlfors J. Peterson R. Reological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur.J.Pharm.Sci. 1998; 6: 105-112.
11. Owen,DH., Peters JJ., Lavine L.L., Katz D.F., Effect of temperature and pH on the contraceptive gel viscosity .Contraceptive 2003;67: 57-64 .
12. Farhan, J.A , Mohd ,A .A., Zeenat .I K , Roop, K. K. and Mushir, A . Development and in vitro evaluation of an acid buffering bioadhesive vaginal gel for mixed vaginal infection . Acta pharm. 2008 ; 58: 407-419 .
13. Mariee, N.K. Factors affecting the in vitro diffusion of diclofenac sodium from ointment. Iraqi .J. of pharmacy 2001; 1: 62-71
14. Hui, H.W., Robinson, J.R., Effect of particle dissolution rate on ocular drugbioavailability .J. pharma. sci. 1986; 75: 280-287.
15. BP ,British Pharmacopoeia 2008 ;vol. IV, Appendix XIE A273.
16. Martin A.N. “Physical Pharmacy”, 4th ed , Waverly, New Delhi. 1993; 497.
17. Ritger, PL, Peppas, NA. Modelling of water transport solute release in physiologically sensitive gels. J.control release 1987; 5:37-40 .
18. Benoy, B.B.,Bhalani, S.N. and Arkenduc . Formulation development and characterization of metronidazole microencapsulated bioadhesive vaginal gel. Int. J. of pharmacy and pharmaceutical sciences 2009; 1, Issue1.
19. Yong, C S, Choi, T S, Quan, Q Z . Rhee, J D, Kim, CK , Lim, S J, Effect of Na chloride on the gelation temperature, gel strength and bioadhsive force of poloxamer gels containing diclofenac Na. Int. J .pharm. 2001; 226:195-205.
20. Veyries ,M.,L., Couarraze,G., Geiger,S., Agnely,F., Massias,L., Kunzli,B., Faurisson,R., Rouveix,B. , Controlled release of vancomycin from poloxamer 407 gels .Int.J.Pharm. 1999; 192: 183-193.
21. Mirchandani HL ,Chien YW ,Bruce PD, Senshang L ,Effect of HPMC and carbopol on the release and floating properties of gastric floating drug delivery system using factorial design Int.J.Pharm. 2003; 253: 3-22.
22. Jaleh, V, Nasser T., Sharona ,S .,Development and physical characterization of a periodontal bioadhesive gel of metronidazole, Drug deliv.2002; 9, issue 2: 127-133.
23. Martinez, N Vazquez, Antoniocruz, R .del C ,Alvarez Castillo A ,Mendoza, A M , Martinez, A B, Morales Cepeda , Swelling kinetic of hydrogels from methyl cellulose and poly acrylamide .I S S N 2007 ; 6 : 337-345.
24. Naim, S., Samuel, B., Chauhan, B., Paradkar, A. Effect of potassium chloride and cationic drug on swelling, erosion and release from κ -carrageenan matrices. AAPS PharmSciTech. 2004; 5(2): article 25.
25. Omidian, H, Park, k., Swelling agents and devices in oral drug delivery .J.drug .del.sci.tech. 2008; 18(2): 83-93.
26. Sanap, G.S., Smita, T., Tarannum, S. and Sangita, G. Formulation and evaluation of mucoadhesive beads of glipizide by 2³ factorial design .jou. of phar. Res. 2009; 2(5): 934- 938.