CHEMICAL STABILITY STUDIES OF BIOADHESIVE TOPICAL GEL

LALIT KUMAR[1,*] AND RUCHI VERMA[2]

[1,2]Department of Pharmacology, Sikkim Manipal Institute of Medical Sciences, Sikkim Manipal University, 5th Mile, Tadong, Gangtok, E. Sikkim – 737102, India Email: lalitkumar_pharma@yahoo.co.in; lalitkumar.pharma@gmail.com

ABSTRACT

The main purpose of this work was to study the chemical stability of optimized bioadhesive topical gel. For that Bioadhesive topical gel formulation was optimized on the basis of bioadhesive strength and *in-vitro* drug permeation study of prepared bioadhesive topical gel. To achieve the objectives of present study, BNTGs were prepared using natural bioadhesive polymer A*egel marmelos* (plant Bale) and hydroxyethyl cellulose (HEC). Bioadhesive strength of BNTG3 was found to be 1.72 ± 0.023 gm/cm^{2}. BNTG3 permeates only $89.00\% \pm 0.59\%$ over 24 h. The shelf life of BNTG3 was found to be 0.439 years and 0.302 years at $25 \pm 2°C$ and $40 \pm 2°C$, respectively. Degradation half-life of BNTG3 was found to be 2.902 years and 1.996 years at $25 \pm 2°C$ and $40 \pm 2°C$, respectively. Activation energy was found to be 4631.7003 cal/mol. All the data was found to be extremely significant by studying one way ANOVA at $p < 0.05$ level. In last it was concluded that, BNTG3 will be more effective and stable if it is stored at or below $25 \pm 2°C$. So, BNTG3 was successfully developed and standardized for improved topical therapeutics.

Keywords: Gel, Bioadhesion, Stability Study, etc.

INTRODUCTION

Nimesulide is a second generation non-steroidal anti-inflammatory drug, which is widely used in the long term therapy of rheumatoid arthritis, in alleviating pain and inflammation. Its biological half-life have been reported to be 3 to 4 h, requires multiple daily dosing for maintaining therapeutic effect throughout the day which means more fluctuation[1]. But bioadhesive topical gels avoid this drawback by increasing the contact between the formulation and biological membrane, so as to avoid the fluctuation of formulation and act as a sustained release formulation[2].

Topical application of the drug prevents these side effects and offers potential advantage of delivering the drug at the site of action[3]. Most importantly controlled release of drug from these BNTGs avoids the more fluctuation, reduces the cost of the therapy and improves the patient compliance.

Gels are transparent or translucent semisolid formulations containing a high ratio of solvent/gelling agent[4].

Stability is officially defined as the time lapse during which the drug product retains the same properties and characteristics that it possessed at the time of manufacture. The stability of the product is expressed as the expiry period or technically as shelf-life. Shelf-life is the time required to reduce the concentration of the reactant to 90 percent of its initial concentration.

Order of a reaction is defined as the number of concentration terms on which the rate of a reaction depends when determined experimentally. Drug decomposition or degradation occurs during storage because of chemical alteration (reaction) of the active ingredients or additives. The chemical kinetics is helpful to predict the rate of such reactions and furthermore to evaluate the shelf-life. The speed of many reactions increases about two or three times with every 10° rise in temperature. *Arrhenius equation* explains the effect of temperature on rate of reaction[5].

In this study, Nimesulide topical gels were formulated using natural bioadhesive polymer and were evaluated with different *in-vitro* evaluation studies.

MATERIALS AND METHODS

Materials

Natural polymer was extracted from the ripe fruit of Bale (*Aegle Marmelos*) and Nimesulide was obtained as a gift sample from Sun Pharm, Ahmedabad. Dimethyl sulfoxide (DMSO) and acetone was purchased from the SD Fine-chemical Ltd., Mumbai, India and Hydroxy ethylcellulose was purchased from Sisco research Lab (P)

Bombay, India. Triethanolamine was purchased from Universal Lab. Pvt. Ltd, Mumbai, India.

Preparation of BNTGs

Gels were prepared by cold mechanical method described by *Schmolka (1972)[6]* and *Chaudhari et al. (2009)[7]*. Required quantity of polymer (Natural polymer and Hydroxyethyl cellulose) was weighed and it was sprinkled slowly on the surface of purified water for 2 h. After which it was continuously stirred by mechanical stirrer, till the polymer gets soaked in the water. With continuous stirring, triethanolamine was added to neutralize the gel and it also maintained the pH of the gel. Now the appropriate quantity of DMSO (Dimethyl sulfoxide) was added to the gel, which behaves as the penetration enhancer, followed by the required quantity of methyl paraben as a preservative. Finally the Nimesulide was added to the gel with continuous stirring till drug get dispersed in gel completely. Six formulations of bioadhesive topical gel were prepared by using Natural polymer and Hydroxyethyl cellulose in different ratio. The prepared gels were packed in wide mouth glass jar covered with screw capped plastic lid after covering the mouth with an aluminum foil and were kept in dark and cool place[8-9].

In–vitro drug permeation study of bntgs:

The *in-vitro* drug permeation study of topical gel through the hairless goat skin membrane was performed using a Keshary-Chien (KC) type glass diffusion cell at $37 \pm 2°C$. Fresh hairless goat skin membrane was mounted between donor and receptor compartments. 1.0 gm of topical gel was kept in donor compartment with 1 ml of 0.1N NaOH. The entire surface of skin membrane was in direct contact with receptor compartment containing 85 ml of phosphate buffer pH 7.4. The receptor compartment was continuously stirred at 50 rpm. The temperature maintained was $37 \pm 2°C$. The samples were withdrawn for 24 h at predetermined period of time and same volume was replaced with freshly prepared phosphate buffer pH 7.4. The absorbance of withdrawn sample was analyzed by UV-Visible Spectrophotometer at $\lambda_{max} = 392$ nm using a placebo as a blank[10-11].

Ex-vivo Bioadhesive Strength Measurement of BNTGs using Hairless Goat Skin

A modified balance method was used for determining *ex-vivo* bioadhesive strength[12]. The procedure employed in this study was approved by Institutional Ethical Committee (HPI/09/60/1AEC/0085). Fresh goat hairless skin was obtained from a local slaughter-house and used within 2 h of butcher. Skin was separated by removing underlying fat and loose tissues. The membrane was washed with distilled water and then with phosphate buffer pH 7.4[13].

The modified Patel et al *(*2007)[13] method was used for the measurement of bioadhesive strength. The fresh skin was cut into two equal pieces and washed with phosphate buffer pH 7.4. Two pieces of skin were tied to the two glass slide separately from that one glass slide was fixed on the wooden piece (fixed portion) and other piece was tied with balance on right hand side. The right and left pans were balanced by adding extra weight on left-hand pan. 1 gm of topical gel was placed between these two slides containing hairless skin pieces, and extra weight from left pan was removed to sandwich two pieces of skin and some pressure was applied to remove the presence of air and this will improve the bioadhesion. Balance was kept in this position for 5 min. Weight was added slowly at 100 mg/min to the left-hand pan until patch detached from skin surface. The weight (in gram) required to detach the gel from skin surface gave the measure of bioadhesive strength[12-17].

Bioadhesive strength was calculated by using following:

$B_s = W_g/A$

Where, B_s is bioadhesive strength, W_g is weight required (in gms) and A is area (in cm^2).

Chemical stability of BNTG3

Stability studies of gel were done according to the International Conference on Harmonization (ICH) harmonized guidelines on stability testing of new drug substance and product. Formulated gel were filled in the sterile lacquered collapsible aluminium tubes and stored at different temperature condition viz. 25±2°C (refrigerator temperature) and 40±2°C (condition of accelerated stability testing) for a period of six months and studied for variation in drug content[5, 17-19].

Determination of parameters in chemical stability

For determination of Nimesulide stability in the topical gel different parameters was determined as given below:

Order of reaction

In order to investigate the order of reaction, stability data of BNTG3 was analyzed with the following mathematical models: zero-order reaction (equation-5) and first-order reaction (equation-6)[5, 17].

$$C_t = C_0 - k_0 t \qquad (5)$$

Where, C_0 is initial concentration, C_t is concentration of drug in BNTG3 at time t and k_0 is specific rate constant for zero-order. Equation-5 represents a linear expression, when C_t is plotted on y-axis against t on x-axis. The line gives a negative slope and the magnitude is equal to k_0.

$$ln\ C_t = ln\ C_0 - k_1 t \qquad (6)$$

Where, k_1 is specific rate constant for first-order. Equation-6 represents a linear expression, when $ln\ C_t$ is plotted on y-axis against t on x-axis.

Degradation half-life

In order to study the half-life (or degradation half-life) of first-order (because from the above study was found that degradation of drug in the gel follows first-order reaction), specific rate constant for first-order (k_1) was used with equation-7[5, 17].

$$t_{1/2} = 0.693/k_1 \qquad (7)$$

Where, $t_{1/2}$ is degradation half-life.

Shelf-life of BNTG3

Shelf-life of first-order (because from the above study was found that the degradation of drug in the gel follows first-order reaction) was also investigated, by using the relationship as given in equation-8[5, 17].

$$t_{90} = 0.105/k_1 \qquad (8)$$

Where, t_{90} is the shelf-life of BNTG3.

Arrhenius factor

Arrhenius equation explains the effect of temperature on the rate of a reaction. *Arrhenius factor* is defined as the frequency of collisions which can occur between molecules. *Arrhenius factor* was calculated by using equation-9[5].

$$log\ k = log\ (A) - Ea/2.303RT \qquad (9)$$

Where, k is specific rate constant for first-order, A is *Arrhenius factor*, R is ideal gas constant (1.987 cal/mol.deg.) and T is temperature used for the storage of formulation.

Estimation of energy of activation

For the estimation of energy of activation the data for two different temperatures are substituted in equation-10 and Ea was solved[5].

$$log\ (k_{1b}/k_{1a}) = Ea/2.303R[1/T_1 - 1/T_2] \qquad (10)$$

Where, k_{1a} is specific first-order rate constant at temperature T_1 (25±2°C), k_{1b} is specific first-order rate constant at temperature T_2 (40±2°C), Ea is energy of activation and R is an ideal gas constant (1.987 cal/mol.deg.).

Statistical analysis

Statistical analysis was carried out using INSTAT software; all values were expressed in mean ± SEM (Standard Error of Mean). All parameters were statistically analyzed at 95% confidence level. One way ANOVA Analysis of variance (ANOVA) was performed and differences was considered statistically significant if $p<0.05$[10].

RESULTS

Formulation design of BNTGs

Topical gels were prepared by using cold mechanical method using Natural Polymer and Hydroxyethyl cellulose in different ratio with other ingredients and solvents as given in Table 1. All the prepared topical gel formulations contain different drug: polymer ratio and coded as BNTG1, BNTG2, BNTG3, BNTG4, BNTG5 and BNTG6.

Table 1: Formulation design for the preparation of topical gel

Form. code	Drug (mg)	Natural Polymer (mg)	HEC (mg)	TEL (gm)	DMSO (gm)	Methyl Paraben (mg)	Distilled Water (gm)	Bioadhesive Strength (gm./cm²)
BNTG1	100.00	100.00	-------	0.23	2.20	15.00	100.00	1.17±0.009
BNTG2	100.00	200.00	-------	0.23	2.20	15.00	100.00	1.28±0.009
BNTG3	100.00	300.00	-------	0.23	2.20	15.00	100.00	1.72±0.023
BNTG4	100.00	----------	100.00	0.23	2.20	15.00	100.00	1.06±0.021
BNTG5	100.00	----------	200.00	0.23	2.20	15.00	100.00	1.13±0.015
BNTG6	100.00	----------	300.00	0.23	2.20	15.00	100.00	1.14±0.006

In–vitro drug permeation study of BNTGs

It was of immense interest that the release profile of formulation BNTG3 showed an auxiliary controlled release throughout the study period as was expected. The release rate was steady from the initial hour, and only 88.00±0.58% of drug was released up to 24 h and it was extremely significant ($p<0.001$, single factor ANOVA) like all the BNTGs. It means null hypothesis is nullified and alternative

hypothesis is accepted i.e. the variation in formulations in polymeric type and content (BNTG1 – BNTG6) have significant effect on drug release.

Data for the *in-vitro* drug permeation study are established in Table 2 and all these are concluded on basis of triplicates sampling.

Table 2: *In-vitro* drug permeability study

Time (hours)	% Cumulative release					
	BNTG1	BNTG2	BNTG3	BNTG4	BNTG5	BNTG6
0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
0.5	11.74±0.33	10.97±0.06	11.44±0.51	16.78±0.13	15.35±0.66	15.16±0.17
1.0	17.12±0.05	15.56±0.63	14.54±0.36	29.61±0.20	26.58±0.39	27.01±0.43
2.0	29.25±0.68	28.58±0.57	25.93±0.14	39.87±0.21	37.03±0.30	34.58±0.38
4.0	39.67±0.34	36.78±0.82	34.70±0.51	50.31±0.33	52.52±0.27	48.88±0.32
6.0	52.88±0.20	48.83±0.39	47.31±0.25	71.60±0.66	67.80±0.33	63.11±0.26
8.0	67.95±0.54	59.03±0.23	57.39±0.37	80.37±0.36	79.26±0.56	76.48±0.67
10.0	79.93±0.54	71.76±0.31	68.10±0.14	90.20±0.56	89.41±0.46	88.05±0.15
12.0	91.82±0.56	78.98±0.16	75.32±0.84	98.27±0.23	95.11±0.13	92.21±0.37
24.0	98.52±0.29	94.68±0.33	88.00±0.58	100.66±0.32	99.30±0.49	97.61±0.40

All values are expressed in mean ± SEM (n = 3), $p < 0.05$

Bioadhesive strength measurement of BNTGs

Bioadhesive strength of BNTGs was in between the range of 1.06±0.021 to 1.72±0.023. BNTG3 showed the highest bioadhesive strength as in Table 1. Bioadhesive strength studies are carried out in triplicates. The overall effect of polymer on bioadhesive strength (BNTG1 – BNTG6) was also extremely significant ($p < 0.05$, single factor ANOVA). It means null hypothesis is nullified and alternative hypothesis is accepted i.e. the variation in formulations in polymeric type and content (BNTG1 – BNTG6) have significant effect on bioadhesive strength.

Chemical stability of BNTG3

All the data for degradation of concentrations are presented here on the basis of triplicates sampling. Table 3 shows the concentration, potency and log percent concentration of BNTG3 for six months, and Table 4 shows the parameters determined for the stability of BNTG3. Shelf life (in year) at different temperatures like 25±2°C and 40±2°C result were found to be 0.439 years and 0.302 years, respectively. Degradation Half-life of BNTG3 was also calculated (in year) at different temperatures like 25±2°C and 40±2°C and the results were found to be 2.902 years, and 1.996 years for the respective temperatures. From the stability studies it was found that the energy required for the collisions of the molecule in the product is about 4631.7003 cal/mol. The overall effect of temperature on stability studies (BNTG1 – BNTG6) at both temperatures were also extremely significant ($p < 0.05$, single factor ANOVA). It means null hypothesis is nullified and alternative hypothesis is accepted i.e. the variation in formulations in polymeric type and content (BNTG1 – BNTG6) have significant effect on stability of gel.

Table 3: Stability studies (Drug concentration, potency & log % concentration) of BNTG3

Temperatures	25±2°C			40±2°C		
Time (in days)	Conc. (in mg/gm gel)	Potency (%)	Log % Conc.	Conc. (in mg/gm gel)	Potency (%)	Log % Conc.
0	1.08±0.005	100.00	2.000	1.08±0.001	100.00	2.000
7	1.08±0.006	100.00	2.000	1.06±0.001	98.15	1.992
14	1.06±0.001	98.15	1.992	1.03±0.001	95.37	1.979
30	1.07±0.001	99.07	1.996	1.01±0.001	93.52	1.971
60	1.04±0.001	96.29	1.984	1.00±0.006	92.59	1.967
90	1.01±0.005	93.52	1.971	0.98±0.005	90.74	1.958
120	1.03±0.001	95.37	1.979	0.96±0.006	88.89	1.949
150	0.99±0.001	91.67	1.962	0.93±0.005	86.11	1.935
180	0.96±0.002	88.89	1.949	0.91±0.005	84.26	1.926

All values of Concentrations are expressed in mean ± SEM (n=3), $p < 0.05$

Table 4: Parameters determined for the stability studies of BNTG3

Parameters	25±2°C	40±2°C
Zero-order (r^2)	0.9226	0.9462
First-order (r^2)	0.9258	0.9530
First-order rate constant (k_1) (in day^{-1}) $\times 10^{-4}$	6.542	9.513
Degradation Half-life (in year)	2.902	1.996
Shelf life (in year)	0.439	0.302
Energy of Activation (Ea) (cal/mole)	4631.7003	4631.7003
Arrhenius factor (A) (in day^{-1})$\times 10^{-4}$	1.6244	1.6229

DISCUSSION

In-vitro drug permeation study shows that BNTG3 releases less amount of drug over a period of 24 h, i.e. beneficial for an ideal controlled release formulation. As the concentration of polymer (both natural and hydroxyethyl cellulose) increases bioadhesive strength also increases, but bioadhesive strength increases more in case of natural polymer. BNTG3 has greater shelf-life and degradation half-life (in years) at 25±2°C as compared to 40±2°C. So it will require more time for degradation of drug. Hence, it will be surely helpful for both manufacturer and consumer, if they will store this formulation below or at 25±2°C. It was also concluded that the topical gel BNTG3 should be store at or below 25±2°C for increasing the life-time of formulation. So, it was successfully developed and standardized for improved topical therapeutics.

ACKNOWLEDGEMENT

providing gift sample of Nimesulide and evaluation study of gel, respectively.

REFERENCES

1. Jawahar N, Jayaprakash S, Maria Gerald Rajan NS, Nagarajan M, Dhachina Moorthi D, Jubie S, Manivannan R. Design and evaluation of sustained release suppositories of Nimesulide. Indian J Pharm Sci 2005; 67(5): 558-561.
2. Sudhakar Y, Kuotsu K, Bandyopadhyay AK. Buccal bioadhesive drug delivery – a promising option for orally less efficient drugs. J Control Rel 2006; 114: 15-40.
3. Derle DV, Sagar BSH, Pimpale R. Microemulsion as a vehicle for transdermal permeation of Nimesulide. Indian J Pharm Sci 2006; 68(5): 622-625.
4. Temu MJ, Damian F, Kinget R, Mooter GVD. Intra-vaginal gels as drug delivery systems. J Women Health 2004; 13: 834-844.
5. Subrahmanyam CVS. Text book of Physical Pharmaceutics. 2nd ed. New Delhi: Vallabh Prakashan; 2000.
6. Schmolka IR. Preparation and properties of Pluronic PF-127 gels for the treatment of burns. J Biomed Mater Res 1972; 6: 571-582.
7. Chaudhari P, Ajab A, Malpure P, Kolsure P, Sanap D. Development and *in-vitro* evaluation of thermo reversible nasal gel formulations of Rizatriptan benzoate. Indian J Pharm Edu Res 2009; 43: 55-62.
8. Saleem MA, Sanaullah S, Faizan S. Formulation and Evaluation of Gatifloxacin topical Gel. The Indian Pharmacist 2006: 88-92.
9. Gupta GD, Gaud RS. Release rate of Nimesulide from different gallants. Indian J Pharm Sci 1999; 61: 227-230.
10. Chatterjee A, Kumar L, Bhowmik BB, Gupta A. Microparticulated anti-HIV vaginal gel: *In vitro-in vivo* drug release and vaginal irritation study. Pharm Develop Tech 2010: 1-8.
11. Sanjay, Jain BD, Padsalg A, Patel K, Mokale V. Formulation, development and evaluation of Fluconazole gel in various polymer bases. Asi J Pharm 2007; 1: 63-68.
12. Gupta A, Garg S, Khar RK. Measurement of bioadhesive strength of mucoadhesive buccal tablets: design of an *in-vitro* assembly. Indian Drug 1992; 30: 152-155.
13. Patel VM, Prajapati BG, Patel HV, Patel K. Mucoadhesive bilayer tablets for Propranolol Hcl. AAPS PharmSciTech 2007; 8: E1-E6.
14. Kumar L. An-overview on preparation and evaluation of microparticulated intra-vaginal gel. Res J Pharm Tech 2009; 2: 48-51.
15. Kumar L, Verma R. Preparation and evaluation of intra-vaginal gel: a review. Res. J. Pharm. Dosage Form Tech 2010; 2: 7-13.
16. Kumar L, Bhowmik BB, Chatterjee A, Solanki D. Development of vaginal microcapsulated tablet and preclinical bioadhesive testing for prevention of transmission and treatment of HIV, presented in International conference on fundamental and translational research on HIV/AIDS: Global perspectives, 5th-8th Oct. 2008. Mumbai, India. P-22 to P-23.
17. Chatterjee A, Bhowmik BB, Kumar L. Formulation, *In-vitro* evaluation and stability of prolonged release anti-HIV bioadhesive microencapsulated vaginal gel. J Pharm Res 2010; 3: 183-192.
18. http://www.ich.org/LOB/media/MEDIA419.pdf
19. Lyer S. Stability testing of existing drug substances and products. Guidelines on GMP and Quality of Pharmaceutical Products. 2nd ed. Mumbai: D.K. Publications; 2003.