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Formulation and evaluation of mucoadhesive vaginal tablets of tenofovir disoproxil fumarate

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ABSTRACT

Mucoadhesive vaginal tablets of tenofovir disoproxil fumarate (TDF) have been formulated using hydroxypropyl methylcellulose (HPMC K100) as the matrix forming retarding agent with various mucoadhesive polymers such as carbopol 934, 940, chitosan and sodium carboxymethylcellulose and evaluated. Wet granulation method was employed to make the tablets. Drug-excipient compatibility, precompression parameters such as percentage yield, bulk density and tapped density of the granules, Carr's index, Hausner's ratio, angle of repose, post compression parameters such as colour, shape, physical dimensions, weight variation, hardness, friability, swelling index, assay, in vitro dissolution study and ex vivo mucoadhesion studies were performed. Based on the evaluation studies, B1 was selected as the best formulation and evaluated further for release kinetics and curve fitting analysis. B1 followed zero- order kinetics. Accelerated stability study was conducted as per ICH guidelines by keeping B1 at 40 $^{o}C \pm 2\ ^{o}C$ and 75 % RH ±5% RH. After accelerated stability study, based on the evaluation tests, it was found that B1 remained stable for a period of six months.

Keywords: HIV, mucoadhesive, tenofovir disoproxil fumarate, vaginal tablets.

INTRODUCTION

It is stated in the UNAIDS 2013 report that HIV continues to be driven by gender inequalities and pernicious societal practices that lead to a higher susceptibility of the female population acquiring AIDS. The report further highlights the fact that in low and middle income countries, women account for 52% of the total HIV infections while men account for 48%. While, in sub- Saharan Africa, the epicentre of the global HIV pandemic, women account for as high as 57% of the total infected population. The gender injustices faced by women include sexual abuse and unprotected sex with promiscuous husbands and partners. Although the data is meagre, the report states that 12% of the female sex workers are infected with HIV, with numbers rising up to 30% in areas of medium to high HIV prevalence [1]. The aim of the research embodied in this article was to formulate viable mucoadhesive intravaginal topical dosage forms that can offer pre-exposure prophylaxis against HIV. Mucoadhesive intravaginal tablets have been investigated as they are easy to formulate, contain the precise dose and can be scaled up for mass industrial production. Tenofovir disoproxil fumarate (TDF), a nucleotide reverse transcriptase inhibitor (NtRTI) was chosen as the drug as it is 100 times more potent in its antiviral activity compared to its prodrug, Tenofovir (TFV). TDF also has enhanced permeability than TFV leading to a reduction in dose requirement [2]. TDF has been successfully used in the treatment of HIV infected people since its FDA approval in October 2001 [3]. TDF has been

reported to be well tolerated in patients with no long term toxicity reported so far. TDF does not interact with cytochrome P 450 system [4].

Hydroxypropyl methylcellulose (HPMC K100) was used as the rate- retarding matrix forming polymer. HPMC K100 has been successfully tried as a rate- retarding polymer in the formulation of various vaginal dosage forms, such as matrix tablets and long acting gels [5,6]. All the mucoadhesive polymers such as carbopol 934, 940, chitosan and sodium carboxymethylcellulose have been safely used in various vaginal formulations. The goal of the research was to find out the best formulation amongst the various combinations of HPMC K100 and mucoadhesive polymers mentioned above.

MATERIALS AND METHODS

Materials:
Drug (TDF) was a gift sample from Strides Arcolab Limited, *Strides* House, Bilekahalli, *Bannerghatta Road*, Bangalore – 560076; Chitosan was a gift sample from Central Instiute of Fisheries Technology (CIFT), CIFT Junction, Willingdon Island, Matsyapuri P.O, Kochi- 682029. Carbopol 934, Carbopol 940, sodium carboxymethylcellulose (NCMC) , hydroxypropyl methylcellulose (HPMC), microcrystalline cellulose (MCC) glacial acetic acid, sodium chloride, Albumin powder, lactic acid, calcium hydroxide, potassium hydroxide, glycerol, urea, glucose were purchased from Loba Chemie Pvt. Ltd. Jehangir Villa, 107, Wode House Road, Colaba, Mumbai- 400 005.

Instruments:
Shimadzu UV VIS double beam 1700, Shimadzu FTIR, USP disintegration test apparatus (Remi equipments, Bangalore), Rimek 10 station rotary tablet punching machine, Dissolution apparatus (Labindia DS 8000).

Methodology
The animal experiments were conducted after obtaining certification from the institutional animal ethics committee of Krupanidhi College of Pharmacy, vide letter, Ref No: KCP/IAEC-02/2012-13.

Drug: polymer compatibility studies: The study was conducted by keeping TDF and the excipients (Carbopol 934/ Carbopol 940/ chitosan/ NCMC / HPMC K100 / MCC at a ratio of 100 mg: 100 mg in individual tightly sealed glass vials at 40 ^{o}C ± 2 ^{o}C and 75 % RH ± 5% RH for 12 weeks. At the end of 12 weeks, the samples were subjected to FTIR analysis.

Formulation of mucoadhesive vaginal tablets: The tablets were prepared by wet granulation method according to table.1. Briefly, the drug and the polymers were weighed according to formulation chart and triturated together in a mortar. 20% w/v of polyvinyl pyrrolidone in ethyl alcohol was used as binder solution. The binder solution was added dropwise with continuous kneading to obtain a coherent mass. The coherent mass was passed through sieve no.12, collected on butter paper and dried in a tray dryer at 40 ^{o}C for 30 minutes. The dried granules were repassed through sieve no.14 and weighed. The granules were then blended with 2% talc and 1% magnesium stearate and after performing precompression evaluation studies were punched into ovoid shaped tablets using Rimek 10 station rotary tablet punching machine.

Table 1. Formulation chart

CODE	TDF	HPMC K100	CP 934	CP 940	CS	NCMC	MCC
B1	25	125	75	-	-	-	75
B2	25	125	25	-	-	-	125
B3	25	125	-	75	-	-	75
B4	25	125	-	25	-	-	125
B5	25	125	-	-	75	-	75
B6	25	125	-	-	25	-	125
B7	25	125	-	-	-	75	75
B8	25	125	-	-	-	25	125

All the quantities in ‘mg’. TDF- tenofovir disoproxil fumarate (drug), HPMC- hydroxypropyl methylcellulose, CP- carbopol, CS- chitosan, NCMC- sodium carboxymethyl cellulose, MCC- microcrystalline cellulose.

Evaluation of mucoadhesive vaginal tablets:
Pre-compression evaluation:
The following equations were used:

$$\% \text{ Yield} = \frac{\text{practical yield}}{\text{theoretical yield}} \times 100$$

$$\text{Bulk density} = \frac{\text{Mass of granules}}{\text{bulk volume}}$$

$$\text{tapped density} = \frac{\text{Mass of granules}}{\text{tapped volume}}$$

$$\text{Carr's Index} = \frac{(\text{tapped density} - \text{bulk density})}{\text{tapped density}} \times 100$$

$$\text{Hausner's ratio} = \frac{\text{tapped density}}{\text{bulk density}}$$

$$\text{Angle of repose } (\alpha) = \tan^{-1}\frac{h}{r}$$

Where, h = fixed height of the pile, r = radius of the base of the pile.

Post-compression evaluation:
Organoleptic properties:
Colour: The colour of the tablets was inspected visually.
Shape: The shape was inspected visually.

Physical dimensions:
The length, width, and thickness of the tablet were measured using Vernier's Calliper.

Weight variation:
The test was carried out according to I.P 2007.Twenty tablets were weighed individually and all together. Average weight was calculated from the total weight of all tablets. The individual weights were compared with the average weight. The percentage difference in the weight variation should be within the permissible limits prescribed by I.P 2007.

$$\text{Percentage deviation} = \frac{(\text{individual weight of tablet} - \text{Average weight})}{\text{Average weight}} \times 100$$

Hardness: The hardness was measured by using Monsanto hardness tester. Three tablets from each batch were checked for hardness and the average hardness was reported.

Friability: The friability was checked using Roche friabilator. 10 tablets were randomly selected and weighed together and the initial average weight (W_1) was recorded. After 100 RPM in the friabilator, the tablets were reweighed and the final weight (W_2) was recorded.

$$\% \text{ friability} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Swelling index:
The swelling index study was carried out at room temperature in simulated vaginal fluid pH 4.2 (SVF). One tablet each was selected from each of the batches and weighed (W_0). Then each of the tablets was kept in Petri dish filled with SVF. Then the swollen tablets were removed carefully, excess water removed by tissue paper, and weights recorded at the first, second, third, fourth, fifth and sixth hours. The swelling index was calculated by the following formula:

$$\text{swelling index } (S.I) = \frac{(W_t - W_0)}{W_0} \times 100$$

W_t = Weight of the tablet at time t, W_0 = Weight of tablet at time zero.

Assay:
Three tablets were taken together in a mortar and crushed to a fine powder. The weight equivalent to 25 mg of drug was taken in a 100 ml volumetric flask. Methanol was added to the flask, and sonicated for 60 min. Then the volume was made up with methanol and the flask was shaken. Then it was filtered with the help of Whatman filter paper no.1. Then, 5ml of the filtrate was taken and again diluted to 100 ml with SVF and analyzed spectrophotometrically at 260.5 nm. SVF was taken as blank. The readings were taken in triplicate and the average absorbance was taken for further calculation.

$$\text{calculated amount of drug} = \frac{\left(\frac{\text{Abs} - \text{intercept}}{\text{slope}}\right)(*\,20 * 100)}{1000}$$

$$\%\ \text{assay} = \frac{\text{calculated amount of the drug}}{\text{theoretical amount of the drug}} \times 100$$

***In vitro* dissolution study:**
The *in vitro* dissolution studies were carried using USP XXIV paddle type dissolution apparatus. One tablet was taken from each batch and introduced into100 ml dissolution medium of SVF pH 4.2 maintained at 37°±0.5 °C at a rotation speed of 50 RPM. 1 ml of aliquots was withdrawn at predetermined time intervals and an equivalent volume of fresh medium was replaced to maintain sink condition. The aliquots were diluted and analyzed spectrophotometrically at 260.5 nm. The average of three readings was taken.

***Ex vivo* mucoadhesive studies**:
Mucoadhesive force required to detach the tablet from the excised sheep vaginal tissue was determined by using the modified balance set- up. The freshly excised sheep vaginal tissue was procured from the local slaughter house and kept in a tightly closed thermostatic flask with SVF at – 8^{o}C in the freezer. When required, the sample was thawed and the unwanted submucosa and fibrous tissues were removed. The mucosal tissue was fixed on to the base of one side the two- pan balance with the help of a cello tape. 9 cm^2 of the mucosal tissue was exposed for the experiment. It was kept hydrated with SVF throughout the experiment. Similarly, another tissue was fixed on the platform with a cello tape. Then the pan with the vaginal tissue at its base was allowed to adhere with the tablet. A preload of 50 g was kept for 1 min on this pan to ensure proper adhesion. After 1 min, the preload was removed and incremental weights were added to the other pan till there was detachment. The average of three readings was taken. The force of detachment was calculated using the formula:

Force of detachment (dyne/cm^2) = $\frac{m \times g}{A}$
m = mass of the weights added in gram; g = acceleration due to gravity (980 cm/s^2)
A = area of the tissue exposed.

Mucoadhesion retention time:
The vaginal tissue was washed with SVF pH 4.2 and attached on a glass slide with cellophane tape. A tablet from each batch was pressed onto the tissue for 30 s. This glass slide was tied to the arm of a USP disintegration test apparatus. It was then given a reciprocating motion in 900 ml of SVF medium at 37 ^{o}C. It was observed for signs of erosion and detachment periodically. When it was visually confirmed that the entire tablet was eroded or detached, the apparatus was stopped. The time taken for complete erosion or detachment was taken as the mucoadhesion retention time. The average of three readings was taken.

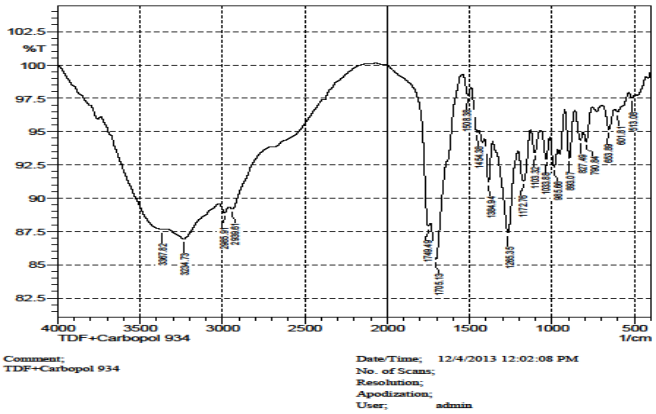
Release kinetics of the selected formulation:
The release kinetics and curve fitting was studied using an MS- Excel based software program.

Accelerated stability study:
The selected formulation B1 was kept in glass vials at 40 ^{o}C ± 2 ^{o}C and 75 % RH ± 5% RH for a period of six months.

RESULTS

Drug:excipient compatibility studies:
FTIR of TDF+ carbopol 934 is shown in fig.1.

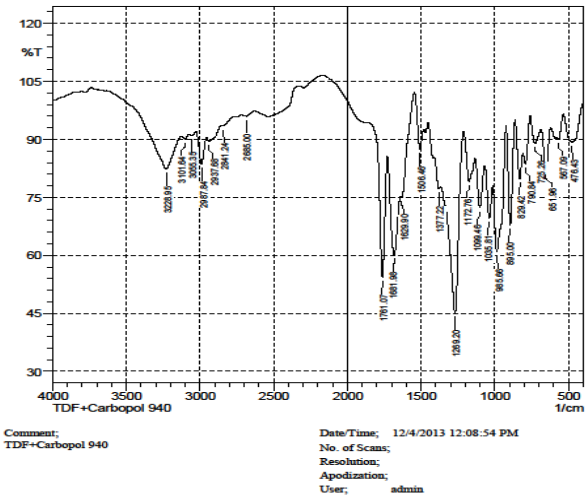
Fig.1. FTIR of TDF+carbopol 934



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	513.08	97.545	0.312	534.3	491.86	0.428	0.029
2	601.81	96.458	0.463	619.17	534.3	1.138	0.126
3	653.89	95.157	1.634	698.25	619.17	1.347	0.233
4	790.84	94.239	1.324	808.2	754.19	1.111	0.127
5	827.49	94.361	1.196	862.21	808.2	1.149	0.14
6	893.07	93.001	3.631	922	862.21	1.364	0.474
7	985.66	92.314	1.741	1012.66	964.44	1.464	0.192
8	1033.88	92.938	1.835	1068.6	1012.66	1.496	0.206
9	1103.32	93.407	1.702	1128.39	1068.6	1.516	0.214
10	1172.76	91.302	2.899	1205.55	1128.39	2.535	0.575
11	1265.35	87.399	6.409	1359.86	1205.55	6.174	1.948
12	1384.94	91.194	3.153	1410.01	1359.86	1.604	0.337
13	1419.66	94.95	0.922	1491.02	1442.8	0.803	0.097
14	1508.38	97.725	0.916	1541.18	1491.02	0.358	0.093
15	1705.13	85.395	4.588	1737.92	1541.18	6.831	1.124
16	1749.49	87.892	0.733	2011.82	1737.92	3.358	-4.131
17	2939.61	89.199	0.469	2956.9	2700.43	9.498	-0.366
18	2955.91	88.908	0.529	3022.55	2958.9	3.153	0.073
19	3234.73	86.953	1.386	3344.68	3022.55	17.856	0.977
20	3367.82	87.645	0.544	3738.17	3344.68	16.312	1.704

FTIR of TDF+carbopol 940 is shown in fig.2.

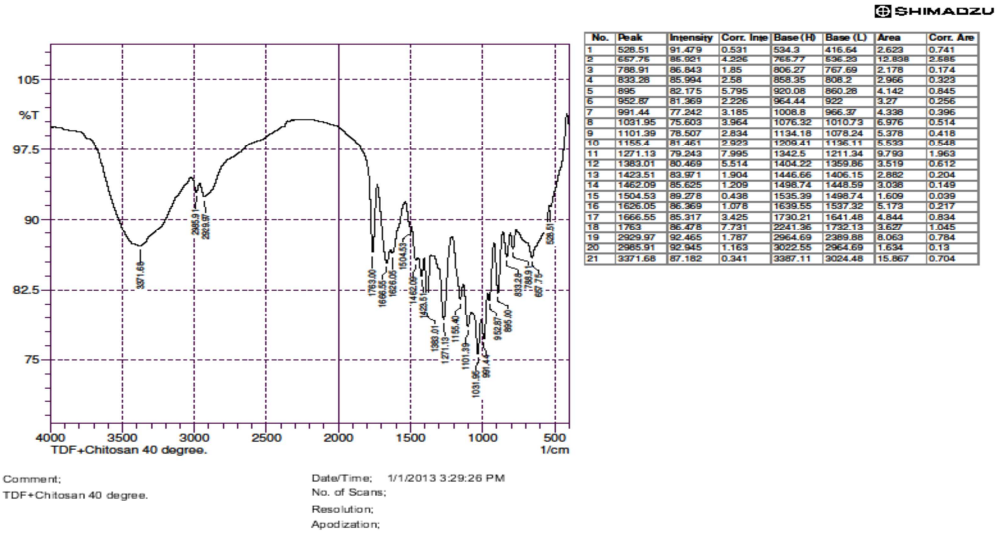
Fig. 2. FTIR of TDF+ carbopol 940



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	476.43	89.199	8.519	534.3	399.28	4.482	3.244
2	567.09	90.083	1.916	578.66	534.3	1.528	0.214
3	651.96	81.95	10.778	686.68	619.17	3.892	1.676
4	725.26	89.033	5.31	759.98	686.68	2.899	1.007
5	790.84	83.636	6.267	808.2	759.98	2.728	0.781
6	829.42	79.924	9.931	862.21	808.2	3.644	1.35
7	895	67.79	26.442	922	862.21	5.612	4.09
8	985.66	60.898	21.666	1012.66	922	13.504	7.271
9	1035.81	69.639	10.549	1066.67	1012.66	6.701	1.619
10	1099.46	72.316	11.802	1132.25	1066.67	6.985	2.057
11	1172.76	79.138	9.559	1209.41	1132.25	6.068	1.956
12	1269.2	45.095	41.452	1365.65	1209.41	28.261	16.889
13	1377.22	77.21	2.507	1410.01	1365.65	4.245	0.292
14	1506.46	87.386	8.895	1545.03	1481.38	1.958	1.168
15	1629.9	75.106	2.318	1637.62	1545.03	5.181	-0.136
16	1681.98	60.106	20.17	1728.28	1637.62	14.449	5.77
17	1761.07	54.382	32.762	2169.99	1728.28	12.107	3.224
18	2685	95.983	0.672	2715.86	2629.06	1.358	0.129
19	2841.24	93.461	0.366	2852.81	2760.23	2.202	0.015
20	2937.68	89.447	1.55	2956.97	2852.81	4.283	0.493
21	2987.84	83.577	7.533	3028.34	2956.97	4.082	1.232
22	3055.36	91.012	0.575	3072.71	3028.34	1.745	0.068
23	3101.64	90.102	0.872	3126.71	3072.71	2.33	0.116
24	3228.95	82.362	10.709	3635.94	3126.71	15.28	7.29

FTIR of TDF+Chitosan is shown in fig.3.

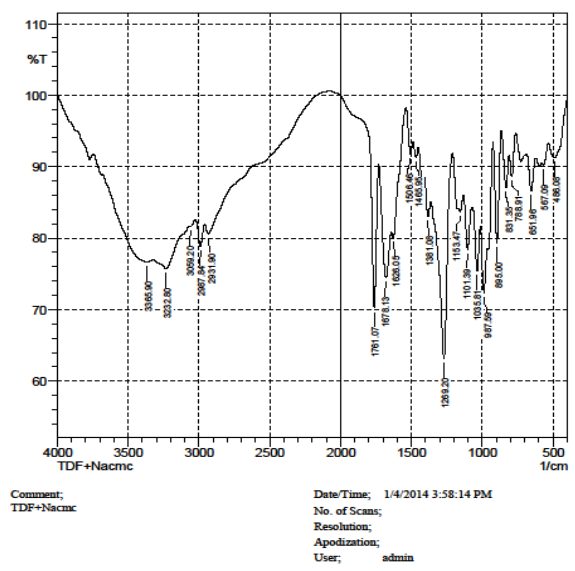
Fig 3. FTIR of TDF+Chitosan



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	528.51	91.479	0.531	534.3	416.64	2.629	0.741
2	687.75	86.561	4.336	765.77	636.23	15.898	2.686
3	788.91	86.843	1.85	806.27	767.69	2.178	0.174
4	833.28	85.994	2.58	858.35	808.2	2.966	0.323
5	895	82.175	5.795	920.08	860.28	4.142	0.845
6	952.87	81.369	2.226	964.44	922	3.27	0.256
7	991.44	77.242	3.185	1008.8	966.37	4.338	0.396
8	1031.95	75.603	3.964	1076.32	1010.73	6.976	0.514
9	1101.39	78.507	2.834	1134.18	1078.24	5.378	0.418
10	1165.4	81.461	2.929	1269.21	1136.11	5.539	0.548
11	1271.13	79.243	7.995	1342.5	1211.34	9.793	1.963
12	1383.01	80.469	5.514	1404.22	1359.86	3.519	0.612
13	1423.51	83.971	1.904	1446.66	1406.15	2.882	0.204
14	1462.09	85.625	1.209	1498.74	1448.59	3.038	0.149
15	1504.53	89.278	0.438	1535.39	1498.74	1.609	0.039
16	1626.05	86.369	1.078	1639.55	1537.32	5.173	0.217
17	1666.55	85.317	3.425	1730.21	1641.48	4.844	0.834
18	1763	86.478	7.731	2241.36	1732.13	3.627	1.045
19	2929.97	92.465	1.787	2964.69	2389.88	8.063	0.784
20	2985.91	92.945	1.163	3022.55	2964.69	1.634	0.13
21	3371.68	87.182	0.341	3387.11	3024.48	15.867	0.704

FTIR of TDF+NCMC is shown in fig.4.

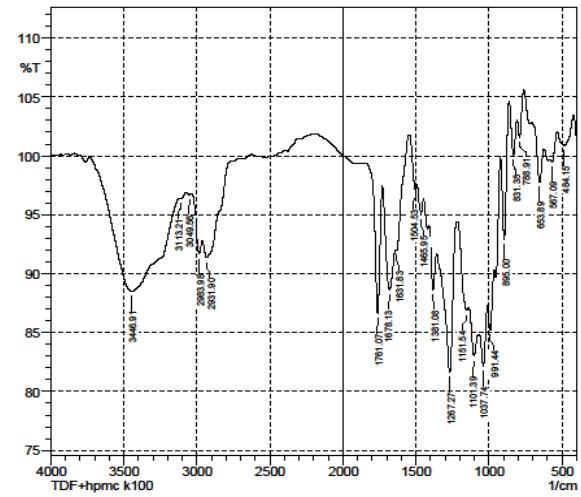
Fig.4. FTIR of TDF+Chitosan



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	486.08	91.273	4.177	532.37	399.28	3.836	1.679
2	567.09	90.201	0.959	578.66	532.37	1.83	0.124
3	651.96	86.633	4.814	684.75	621.1	3.177	0.708
4	788.91	88.61	4.328	808.2	761.91	1.881	0.462
5	831.35	87.05	6.054	862.21	808.2	2.41	0.792
6	895	79.283	14.862	922	862.21	3.609	2.064
7	987.59	72.308	7.86	1012.66	960.58	6.11	1.089
8	1035.81	75.382	7.392	1070.53	1012.66	5.676	1.002
9	1101.39	78.433	6.748	1132.25	1070.53	5.297	0.996
10	1153.47	83.699	1.177	1166.97	1132.25	2.551	0.114
11	1269.2	63.108	26.112	1361.79	1209.41	16.833	8.694
12	1381.08	83.009	3.557	1406.15	1361.79	3.144	0.409
13	1421.58	91.345	1.674	1487.17	1448.59	1.366	0.16
14	1506.46	91.707	3.551	1537.32	1487.17	1.315	0.376
15	1626.05	80.085	3.308	1641.48	1537.32	5.642	0.415
16	1678.13	74.454	10.283	1730.21	1641.48	8.69	2.622
17	1761.07	70.232	20.994	2075.47	1730.21	8.252	1.04
18	2931.9	80.619	1.886	2958.9	2387.95	31.54	-0.985
19	2987.84	78.87	3.332	3026.41	2958.9	6.266	0.534
20	3059.2	81.658	0.149	3064.99	3026.41	3.329	0.027
21	3232.8	75.707	2.891	3323.46	3064.99	27.757	1.68
22	3365.9	76.652	1.697	3682.23	3323.46	33.334	3.733

FTIR of TDF+HPMC K100 is shown in fig.5.

Fig.5. FTIR of TDF+HPMC K100



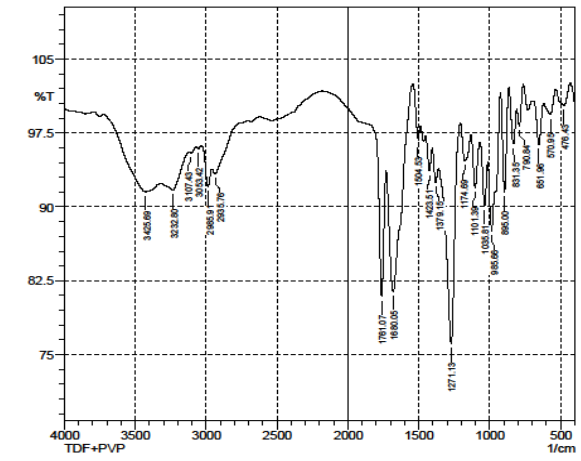
No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	484.15	100.857	1.803	532.37	420.5	-0.758	0.563
2	567.09	99.482	0.935	582.52	532.37	-0.084	0.102
3	653.89	97.779	3.658	705.97	621.1	-0.122	0.494
4	788.91	101.586	2.555	808.2	761.91	-0.613	0.242
5	831.35	100.078	3.693	860.28	808.2	-0.429	0.426
6	895	92.91	9.003	920.08	860.28	0.523	1.104
7	991.44	85.229	3.227	1008.8	964.44	2.654	0.359
8	1037.74	82.153	3.952	1068.6	1008.8	4.462	0.562
9	1101.39	82.995	2.924	1138.04	1068.6	5.019	0.462
10	1151.54	86.878	1.507	1215.19	1138.04	3.661	0.383
11	1267.27	81.613	11.808	1357.93	1215.19	7.822	3.356
12	1381.08	88.649	4.129	1410.01	1357.93	2.155	0.49
13	1465.95	94.99	1.866	1492.95	1446.66	0.816	0.193
14	1504.53	97.187	1.286	1545.03	1492.95	0.194	0.112
15	1631.83	91.997	0.636	1637.62	1545.03	1.195	-0.118
16	1678.13	88.647	5.791	1728.28	1637.62	3.535	1.386
17	1761.07	86.585	11.355	1855.58	1728.28	2.617	1.734
18	2931.9	91.357	2.478	2962.76	2760.23	4.689	1.2
19	2983.98	91.651	2.308	3036.06	2962.76	1.994	0.285
20	3049.56	96.713	0.11	3070.78	3036.06	0.494	0.009
21	3113.21	96.356	0.046	3117.07	3070.78	0.703	0.013
22	3446.91	88.515	9.755	3734.31	3117.07	20.139	15.08

Comment;
TDF+hpmc k100

Date/Time; 12/4/2013 12:14:17 PM
No. of Scans;
Resolution;
Apodization;
User; admin

FTIR of TDF+PVP is shown in fig.6.

Fig.6. FTIR of TDF+ PVP



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	476.43	100.301	1.986	534.3	430.14	-0.487	0.52
2	570.95	99.358	1.954	617.24	534.3	-0.059	0.38
3	651.96	96.355	4.267	688.61	617.24	0.4	0.593
4	790.84	98.188	2.658	808.2	761.91	0.03	0.261
5	831.35	96.389	4.424	864.14	808.2	0.303	0.545
6	985.66	91.497	10.354	922	864.14	0.846	1.311
7	985.66	87.523	9.109	1012.66	922	3.061	2.267
8	1035.81	90.109	5.231	1068.6	1012.66	1.735	0.618
9	1101.39	91.903	5.023	1132.25	1068.6	1.544	0.677
10	1174.69	94.694	3.323	1205.55	1132.25	1.314	0.645
11	1271.13	76.078	20.466	1359.86	1205.55	8.708	6.087
12	1379.15	92.457	2.32	1404.22	1359.86	1.247	0.24
13	1423.51	93.629	2.87	1450.52	1404.22	0.99	0.295
14	1504.53	97.01	2.565	1541.18	1487.17	0.188	0.265
15	1680.05	81.422	14.587	1730.21	1541.18	7.977	6.308
16	1761.07	80.897	13.316	2181.56	1730.21	3.904	-0.804
17	2935.76	93.326	0.97	2958.9	2708.15	3.898	-0.264
18	2985.91	92.043	2.703	3030.27	2958.9	1.967	0.386
19	3053.42	95.927	0.203	3070.78	3030.27	0.711	0.019
20	3107.43	95.496	0.185	3120.93	3070.78	0.959	0.025
21	3232.8	91.7	2.051	3317.67	3120.93	6.21	0.881
22	3425.69	91.538	2.696	3728.53	3317.67	10.104	2.503

Comment;
TDF+PVP

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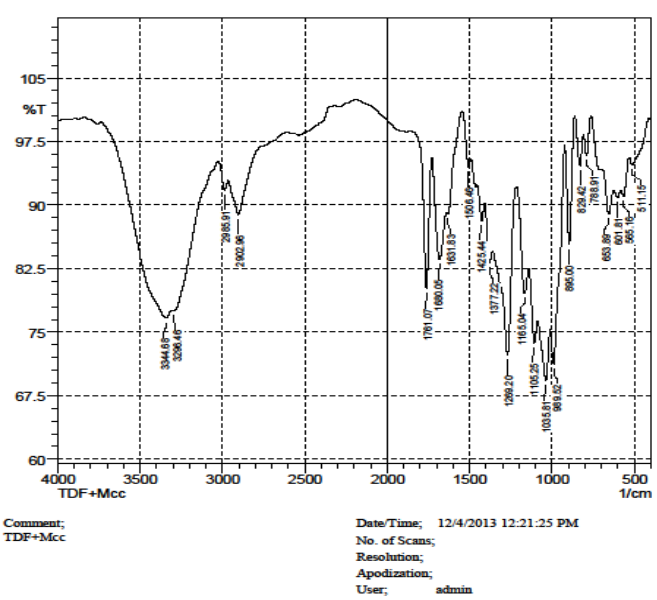
Resolution;
Apodization;
User; admin

FTIR of TDF+MCC is shown in fig.7.

Fig.7. FTIR of TDF+MCC



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	511.15	94.738	1.61	530.44	408.92	1.761	0.64
2	565.16	90.993	1.878	580.59	530.44	1.652	0.214
3	601.81	90.85	0.837	624.96	580.59	1.762	0.09
4	653.89	88.918	3.733	700.18	624.96	2.911	0.513
5	705.97	95.824	3.403	808.2	761.91	0.451	0.333
6	829.42	94.669	4.531	860.28	808.2	0.672	0.538
7	895	85.323	13.324	922	860.28	2.088	1.777
8	989.52	71.266	9.26	1010.73	922	8.475	2.452
9	1035.81	69.245	6.42	1084.03	1010.73	10.249	1.442
10	1105.25	73.738	4.973	1139.97	1084.03	6.38	0.775
11	1165.04	79.582	6.158	1215.19	1139.97	5.522	1.049
12	1269.2	72.318	16.972	1359.86	1215.19	13.129	5.276
13	1377.22	83.157	3.425	1408.08	1359.86	3.261	0.423
14	1425.44	88.181	2.863	1452.45	1408.08	2.064	0.304
15	1506.46	94.499	2.502	1545.03	1491.02	0.653	0.221
16	1631.83	86.979	1.089	1639.55	1545.03	2.05	-0.106
17	1680.05	83.593	8.483	1728.28	1639.55	5.106	2.02
18	1761.07	80.183	16.201	1861.37	1728.28	4.142	2.47
19	2902.96	88.895	4.982	2964.69	2609.77	8.107	1.315
20	2985.91	91.771	1.931	3024.48	2964.69	1.843	0.244
21	3296.46	77.545	0.248	3300.31	3024.48	16.902	-1.286
22	3344.68	76.652	3.164	3736.24	3300.31	27.152	2.933

PRE-COMPRESSION EVALUATION

Table.2. Results of precompression parameters

Formulation code	% Yield	Bulk Density	Tapped Density	Carr's Index	Hausner's Ratio	Angle of Repose α^o
B1	90	0.506	0.540	6.296296	1.067194	36
B2	89	0.322	0.533	39.58724	1.65528	48
B3	72	0.393	0.500	21.4	1.272265	43
B4	89	0.320	0.400	20	1.25	43
B5	77	0.329	0.431	23.66589	1.31003	44
B6	80	0.267	0.360	25.83333	1.348315	44
B7	80	0.284	0.394	27.91878	1.387324	46
B8	78	0.292	0.368	20.65217	1.260274	42

Post-compression evaluation:

The results of the post- compression evaluation are presented in table.3.

Table.3. Results of post- compression evaluation

Formulation code	Colour	Shape	Physical dimensions			Weight Variation	Hardness	Friability
			Avg. L mm*	Max.W mm*	Avg. T mm*	Average Weight mg	Kg/cm^{2}*	%*
B1	white	ovoid	12.3	7.5	4.2	200.3	5.5	0.6
B2	white	ovoid	12.3	7.5	4.2	199.5	5	0.5
B3	white	ovoid	12.3	7.5	4.2	199.6	6	0.55
B4	white	ovoid	12.3	7.5	4.2	200	5	0.6
B5	white	ovoid	12.3	7.5	4.3	199.5	6	0.5
B6	white	ovoid	12.3	7.5	4.3	200.5	5.5	0.55
B7	white	ovoid	12.3	7.5	4.3	199.6	5.5	0.7
B8	white	ovoid	12.3	7.5	4.3	200.3	5	0.4

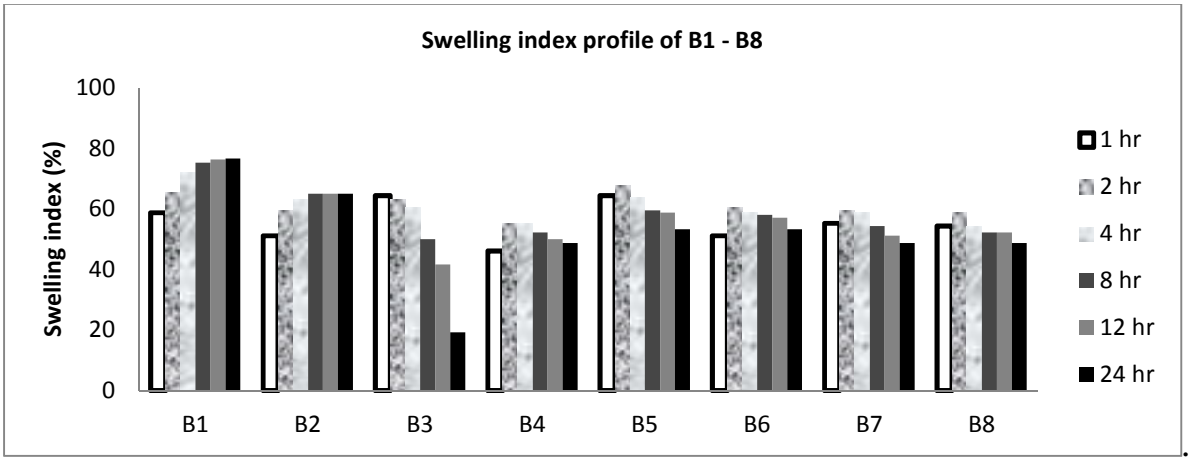
**Average of three readings; L- length, W- width, T- thickness.*

The results of swelling index study are presented in table.4 and fig.8 depicts the graph.

Table.4. Results of swelling index study

Formulation code	Swelling index (%)					
	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
B1	58.82	65.57	72	75.29	76.40	76.67
B2	51.16	59.62	63.16	65.00	65.00	65.00
B3	64.41	63.16	60.38	50.00	41.67	19.23
B4	46.15	55.32	55.32	52.27	50.00	48.78
B5	64.41	67.69	63.79	59.62	58.82	53.33
B6	51.16	60.38	58.82	58.00	57.14	53.33
B7	55.32	59.62	58.82	54.35	51.16	48.78
B8	54.35	58.82	54.35	52.27	52.27	48.78

Fig.8. Swelling index profile of B1-B8



The results of the assay are presented in table.5.

Table.5. Results of assay

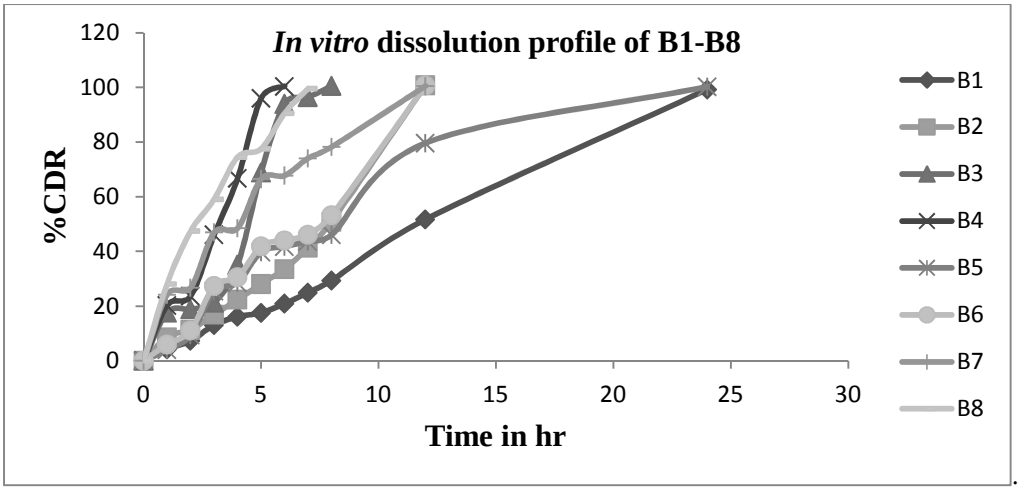
Formulation	Amount of drug 'mg'	% w/w
B1	25.15	100.61
B2	24.52	98.06
B3	24.79	99.15
B4	24.64	98.55
B5	24.64	98.55
B6	24.52	98.06
B7	24.76	99.03
B8	25.21	100.85

The results of the *in vitro* dissolution study are presented in table.6 and fig.9 depicts the graph.

Table.6. Results of the *in vitro* dissolution study

Time →	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr	12 hr	24 hr
Code ↓	%CDR	%CDR	%CDR	%CDR	%CDR	%CDR	%CDR	%CDR	%CDR	%CDR
B1	4.16	7.30	13.03	16.09	17.49	20.87	24.92	29.34	51.70	99.25
B2	8.65	11.38	16.96	22.38	28.06	33.60	41.40	50.96	100.86	
B3	17.60	18.88	21.30	35.21	68.87	94.02	96.48	100.58		
B4	23.68	46.07	66.78	96.05	100.44					
B5	4.06	9.36	25.43	28.57	39.78	41.75	43.64	46.08	79.64	100.28
B6	5.93	11.11	27.45	30.60	41.87	44.04	46.12	53.25	100.69	
B7	24.23	26.76	46.99	48.49	66.48	67.77	74.09	78.23	100.52	
B8	28.13	47.45	59.11	74.52	77.58	90.54	99.59			

Fig.9. *In vitro* dissolution study of B1-B8



The results of *ex vivo* mucoadhesion studies are presented in table.7.

Table.7. Results of *ex vivo* mucoadhesion studies

Code	Force of detachment (dyne/cm^2)	Mucoadhesion retention time (hr)
B1	32.66×10^2	12
B2	28.31×10^2	7
B3	22.86×10^2	4
B4	18.51×10^2	3
B5	31.57×10^2	10
B6	27.22×10^2	9
B7	30.48×10^2	10
B8	27.22×10^2	8

The results of release kinetics and the kinetics data for B1 are presented in table.8 in table.9 respectively.

Table.8. Release kinetics for B1

Time in hr	Log Time	SQRT Time	% Cumula tive Release	Log % Release	% Drug remaining	Log % Drug remaining
0		0	0		100	2
1	0.000	1.000	4.157609018	0.619	95.84239098	1.982
2	0.301	1.414	7.300167492	0.8633	92.69983251	1.967
3	0.477	1.732	13.02935272	1.1149	86.97064728	1.939
4	0.602	2.000	16.08519535	1.2064	83.91480465	1.924
5	0.699	2.236	17.49462481	1.2429	82.50537519	1.916
6	0.778	2.449	20.86763361	1.3195	79.13236639	1.898
7	0.845	2.646	24.92011451	1.3966	75.07988549	1.876
8	0.903	2.828	29.33905895	1.4674	70.66094105	1.849
12	1.079	3.464	51.69808631	1.7135	48.30191369	1.684
24	1.380	4.899	99.24984854	1.9967	0.750151456	-0.125

Table.9. Kinetics data for B1

	Zero order	Korsmeyer-Peppas	Higuchi	First Order
$K_{0(Slope)}$	4.167091	0.9994		-0.084
R^2	0.9944	0.9904	0.8586	0.8471
	4.1671			K_1= 0.195

Fig.10, fig.11, fig.12 and fig.13 represent the zero- order, first order, Korsmeyer- Peppas and Higuchi models respectively.

Fig.10. Zero- order profile of B1

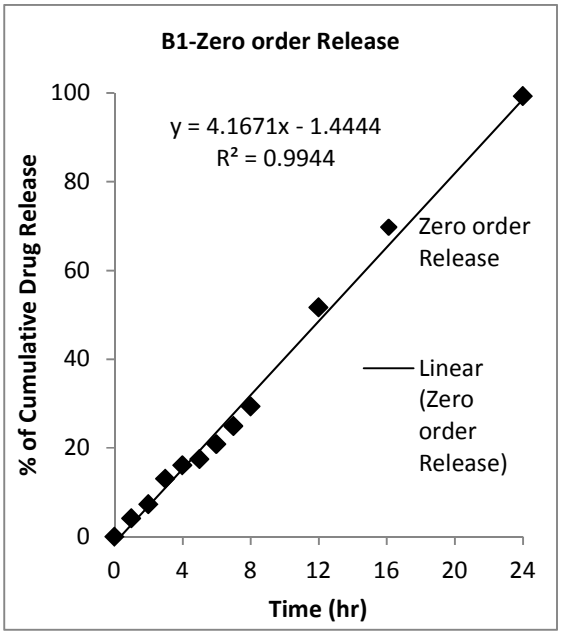


Fig.11. First order profile for B1

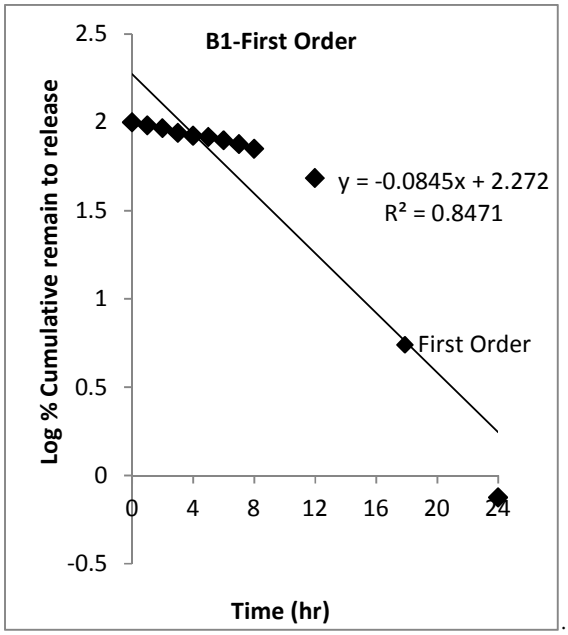


Fig. 12. Korsmeyer-Peppas profile for B1

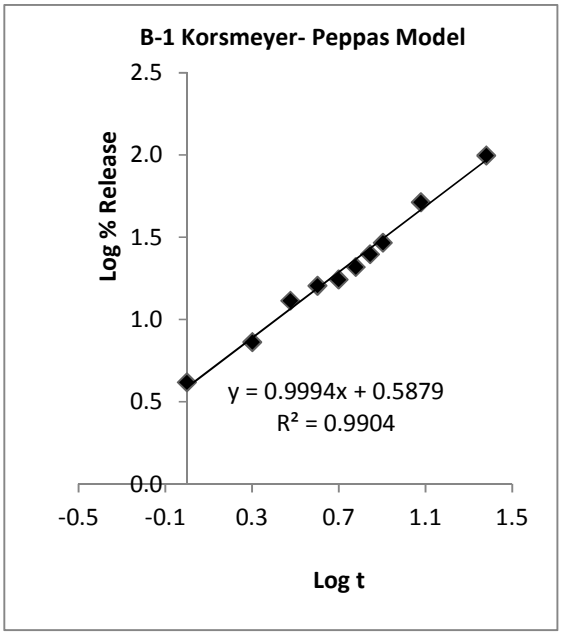
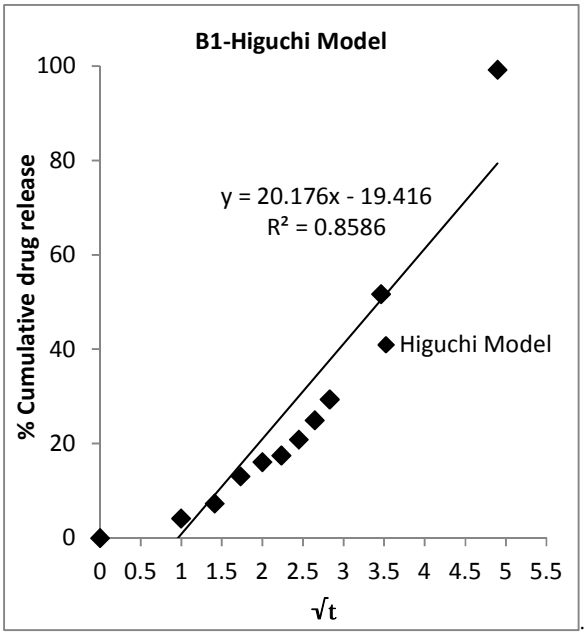


Fig.13. Higuchi profile for B1

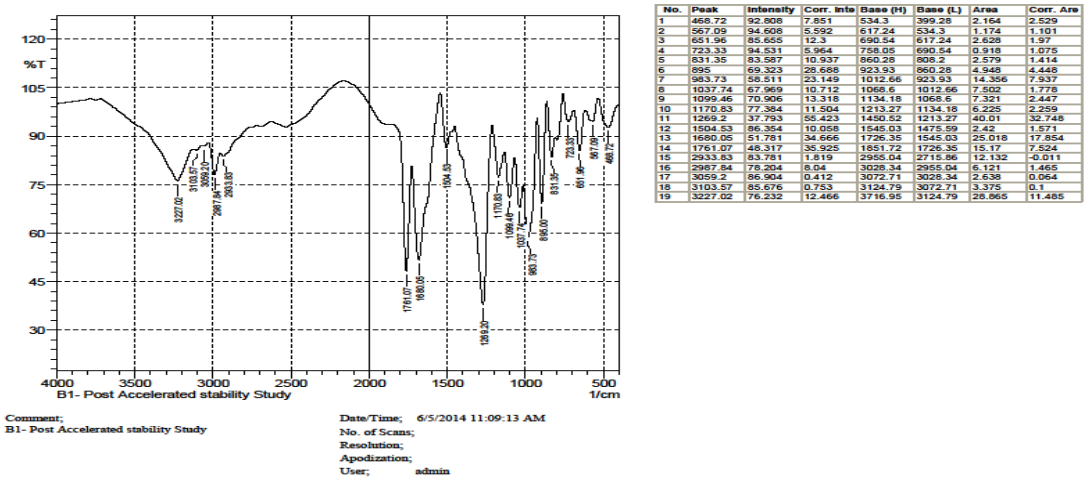


Results of accelerated stability studies for the selected B1 mucoadhesive vaginal tablets after keeping for 6 months at 40 °C ± 2 °C /75% ± 5 RH:

The colour of B1 was found to be white. The average hardness for B1 was reported to be 5 kg/ cm^2 and for C1 it was reported to be 5.5 kg / cm^2. The drug content was found to be 24.79 mg (99.15% w/w) as compared to 25.15 mg (100.61% w/w) before accelerated stability study. B1 had a % CDR of 100.653 at the end of the 24^{th} hr, compared to 99.25% before accelerated stability study. The force of mucoadhesion of B1 was found to be 30.48 $\times 10^2$ dyne/cm^2,

compared to 32.66×10^2 % before accelerated stability study. The mucoadhesion retention time of B1 was found to be 10 hours whereas it was 12 hours before accelerated stability study. The graph depicting the FTIR of B1 formulation after the accelerated stability study is shown in fig.14.

Fig. 14.FTIR of B1 post study



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	468.72	92.608	7.851	534.3	399.28	2.164	2.529
2	567.09	94.608	5.592	617.24	534.3	1.174	1.101
3	651.96	85.655	12.3	690.54	617.24	2.628	1.97
4	723.33	94.531	5.964	758.05	690.54	0.918	1.075
5	831.35	83.587	10.937	860.28	808.2	2.579	1.414
6	895	69.323	28.688	923.93	860.28	4.948	4.448
7	983.73	58.511	23.149	1012.66	923.93	14.356	7.937
8	1037.74	67.969	10.712	1068.6	1012.66	7.502	1.778
9	1099.46	70.906	13.318	1134.18	1068.6	7.321	2.447
10	1170.83	77.384	11.504	1213.27	1134.18	6.225	2.259
11	1269.2	37.793	55.423	1450.52	1213.27	40.01	32.748
12	1504.53	86.354	10.058	1545.03	1475.59	2.42	1.571
13	1680.05	51.781	34.666	1726.35	1545.03	25.018	17.854
14	1761.07	48.317	35.925	1851.72	1726.35	15.17	7.524
15	2933.83	83.781	1.819	2955.04	2715.86	12.132	-0.011
16	2987.84	78.204	8.04	3028.34	2955.04	6.121	1.465
17	3059.2	86.904	0.412	3072.71	3028.34	2.638	0.064
18	3103.57	85.676	0.753	3124.79	3072.71	3.375	0.1
19	3227.02	76.232	12.466	3716.95	3124.79	28.865	11.485

DISCUSSION

Drug: excipients compatibility studies: The drug: excipients compatibility studies showed no interaction as the major peaks of the drug TDF, were still manifested in the FTIR after having been mixed with the excipients at an elevated temperature and humidity of 40 ^{o}C ± 2 ^{o}C and 75 % RH ± 5% RH for 12 weeks. The interpretation of the FTIR graphs is discussed in table.10.

Table.10. Interpretation of drug: excipients compatibility studies

	Peaks cm^{-1}
TDF + Carbopol 934	3234– NH_2 (overlapping with -OH); 2985,2939- CH stretching; 1749- C=O group, 1705- C=O stretching; 1384-CH_3 weak, 1265- CH_3- strong peak; 1172- P=O stretching;1103,1033-C-O stretching (ether)
TDF + Carbopol 940	3228- NH_2 ; 2987, 2937- CH stretching;1761- C=O group; 1377-CH_3 weak, 1269- CH_3- strong peak; 1172- P=O stretching;1099,1035-C-O stretching (ether).
TDF+ HPMC K100	3446- OH peak overlapping –NH_2; 2983,2931- CH stretching;1761- C=O group, 1705- C=O stretching; 1381-CH_3 weak, 1267- CH_3- strong peak; 1151- P=O stretching;1101,1037-C-O stretching (ether)
TDF+MCC	3296- NH_2, OH peak overlapping; 2985,2902- CH stretching;;1761- C=O group; 1377-CH_3 weak, 1269- CH_3- strong peak; 1165-P=O stretching;1105,1035-C-O stretching (ether)
TDF+NCMC	3232- NH_2, OH peak overlapping; 2987,2931- CH stretching;;1761- C=O group; 1381-CH_3 weak, 1269- CH_3- strong peak; 1153-P=O stretching;1101,1035-C-O stretching (ether)

The oral prophylactic dose of TDF is 300 mg whereas, the topical dose is kept at 25 mg. TDF, when topically applied intravaginally at a dose of 10 µg/ ml resulted in >90% inhibition of HIV. Also, according to scientific literature, at this concentration, no deleterious effects were observed to the vaginal epithelial cells [2]. Based on the scientific literature, local volume of distribution of the vaginal compartment was assumed to be 100 ml [7], and the desired inhibitory concentration to be 10 µg/ ml. Hence, the dose = 100 × 0.01= 1mg. For 24 hr duration it is 24 mg, which was rounded off to 25 mg.

Pre-compression evaluation: The % yield varied from the lowest value of 77% for B5 to the highest value of 90% for B1. The bulk density was lowest for B6 at 0.267 and highest for B1 at 0.506.The tapped density varied from lowest for B6 at 0.360 to highest for B1 at 0.540. The Carr's index was lowest for B1 at 6.29 which indicated excellent flow and highest for B2 at 39 which indicated poor flow. The Hausner's ratio was lowest for B1 at 1.067 indicating good flowability and highest for B2 at 1.65 indicating poor flow.

The angle of repose values ranged from lowest value of 36^o for B1, indicating a fair flow and highest value of 48^o indicating poor flow.

Post-compression evaluation: The colour of tablets was white for formulations B1- B4, whereas, it was off-white for B4-B8 because of chitosan and sodium CMC being creamish to buff coloured polymers. The shape of all the tablets was ovoid as this shape would help in the easy administration into the vaginal cavity. The length of all the tablets was 12.3 mm, the maximum width was 7.5 mm and the average thickness was between 4.2mm - 4.3 mm. All the tablet formulations passed the weight variation test according to I.P. The average weight was around 200 mg. The hardness varied from a minimum of 5 kg/cm^2 for B2, B4, B8 and maximum of 6 kg/cm^2 for B3 and B5. All the tablet formulations passed the friability test (loss NMT 0.8%). The friability was least for B8 at 0.4% and highest for B7 at 0.7%. The swelling index study results showed that B1 had the maximum swelling index at the end of 24th hr at 76.67%, which may be due to the combined swelling effect of the hydrophilic polymers HPMC K100 and carbopol 934. B3 had the minimum swelling index at 19.23% which may be due to the fact that carbopol 940 eroded in water rather than swelling. This erosion of Carbopol 940 maybe due to its structure which is more open and comprises linear acrylic acid chains whereas carbopol 934 is composed of cross-linked fuzz balls or mini gels. The assay results were highest for B8 at 25.21 mg and 100.85% w/w and lowest for B6 at 24.52 mg and 98.06% w/w. The *in vitro* dissolution study showed that B1 had a % CDR of 99.25% at the end of 24th hr; B2 had a % CDR of 100.86 at the end of 12th hr. This prolonged release may be due to the higher ratio of polymers HPMC and Carbopol 934. B3 had a % CDR 100.58% at the end of 8th hr; B4 had a % CDR of 100.44 at the end of 6th hr. This faster release may be due the fact that carbopol 940 erodes in water. B5 had a % CDR of 100.28 at the end of 24th hr, due to higher ratio of HPMC K100 and chitosan. B6 had a % CDR of 100.69 at the end of 12th hr, due to a lower ratio of HPMC K100 and chitosan. B7 had a % CDR of 100.52 at the end of 12th hr, due to higher ratio of the HPMC K100 and NCMC. B8 had a % CDR of 99.59 at the end of 7th hr due to a lower ratio of HPMC K100 and NCMC. The mucoadhesion force of detachment was lowest for B4 at 18.51×10^2 dyne/cm^2 and highest for B1 at 32.66×10^2. The mucoadhesion retention time was lowest for B4 at 3 hr and highest for B1 at 12 hr. B1 was selected as the best formulation due to its better swelling index, more prolonged release, and superior mucoadhesion force and retention time. According to the release kinetics, B1 had R^2 of 0.994, 0.847, 0.990, and 0.858 for zero-order, first-order, Korsmeyer –Peppas and Higuchi models respectively. Hence, it is understood that B1 followed zero-order kinetics. The accelerated stability data showed no significant changes in the formulation as indicated by the results. The FTIR of B1 showed the major peaks of TDF as indicated in table. 11.

Table.11. FTIR peak table of B1

Peak (wavenumber cm^{-1})	Interpretation
3227	NH_2 (overlapping with -OH);
3103, 2987	-CH stretching
1761	-C=O stretching
1269	-CH_3 strong peak
1170	- P= O stretching
1099,1037	-C-O stretching (ether).

CONCLUSION

The present investigation deals with the formulation of mucoadhesive vaginal tablets of the microbicide, tenofovir disoproxil fumarate using various combinations of polymers. The aim of this study was to provide mucoadhesive tablets having prolonged release and good mucoadhesion. These tablets were made with the intention to provide topical prophylactic dose of TDF in the vagina against HIV. The mucoadhesive tablets of TDF were found to have varying degrees of mucoadhesion, retention and *in vitro* release based on their formula. B1 showed the desired mucoadhesion, retention and prolonged release for 24 hr. Hence, it was selected for release kinetics, where it followed zero- order release kinetics. As the appearance, hardness, drug content, *in vitro* release studies, force of mucoadhesion, and FTIR of B1 batch of vaginal mucoadhesive tablets did not reveal significant differences, it can be said that B1 batch of tablets were stable at the end of the accelerated stability study.

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REFERENCES

[1] Global Report: UNAIDS report on Global Aids pandemic. Joint United Nations Programme on HIV/AIDS (UNAIDS), **2013**.
[2] P.M.M Mesquita, R.Rastogi, T.J Segarra, R.S. Teller, N.M Torres. A.M.Huber, P.F. Kiser, B.C. Herold, *J. Antimicrob. Chemother*., **2012,** 67, 1730 –38.
[3] US FDA. Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations [Internet],USA,Office of Generic drugs, Division of labeling and Program support; **2001 Oct 26 [updated 2013 July 25],** Available from: http://www.accessdata.fda.gov/scripts/cder/ob/docs/obdetail.cfm?Appl_No=021356&TABLE1=OB_Rx**.**
[4] J.E Gallant, S. Deresinski, *CID*, **2003,** 3, 944-950.
[5] G.M. Patel, M.M. Patel, *Acta Pharm.*, **2009,** 59, 273–288.
[6] F.T. Demiroz, F.Acarturk, D. Erdogan, *Int. J. Pharm.*, **2013,** 457, 25-39.
[7] D.A. Woolfson, M.L. Umrethia, V.L. Kett, K.R. Malcolm, *Int. J. Pharm.*, **2010,** 388, 136-143.