

IN VITRO EVALUATION OF 5-FLUOROURACIL DISSOLUTION PROFILES FROM VAGINAL BIOADHESIVE TABLETS

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Abstract

The paper assesses the dissolution profiles for twelve original formula of vaginal bioadhesive tablets containing 100mg of 5-fluorouracil (5-FU)/tablet. For the quantitative determination of 5-FU from the vaginal bioadhesive tablets, as well as for its *in vitro* release using the dissolution test, a high performance liquid chromatography (HPLC) method was used; the method was elaborated and validated. The results of the research pointed out that P1, P4, P5, P6 formulations, consisting of acrylic acid derivatives with various reticular degrees, in the first three hours released 80-99% of the total 5-FU, while the formulations including cellulose derivatives of various viscosity degrees, P7-12 released the substance slower, reaching the concentration level of 35-60% in 6 hours and 60-80% in 10 hours.

Rezumat

Obiectivul lucrării a constat în evaluarea profilurilor de dizolvare pentru douăsprezece formule originale de comprimate bioadezive vaginale cu 100 mg 5-fluorouracil (5-FU)/comprimat. Pentru determinarea cantitativă a 5-FU din comprimatele bioadezive vaginale cât și pentru evaluarea cedării *in vitro* a acestuia prin testul de dizolvare, s-a folosit o metodă de analiză prin cromatografie de lichide de înaltă performanță (HPLC) elaborată și validată de autori. Rezultatele cercetărilor au evidențiat faptul că formulările P1, P4, P5, P6, care conțin derivați de acid acrilic cu grade diferite de reticulare, în primele 3 ore cedează între 80-99% din totalul cantității de 5-FU, în timp ce formulările ce conțin derivați de celuloză cu vîscozitate diferită, P7-12 cedează mai lent, atingând la 6 ore concentrații de 35-60%, iar la 10 ore de 60-80%.

Keywords: *in vitro* release, 5-fluorouracil delivery, vaginal bioadhesive tablets

Introduction

The pharmaceutical release phase of the drug substance from the formulation is extremely important within the absorption process and for its therapeutic effect.

The *in vitro* dissolution test represents an important quality parameter for tablets, as it can be used for the assessment of the dissolution

profile, which represents a bioavailability criterion of the substance in question [1,2,3].

To assess the dissolution and release profiles of the twelve original formulae of vaginal bioadhesive tablets with 100 mg of 5-fluorouracil (5-FU)/ tablet, formulae developed, physically and chemically characterized by the authors [4,5] it has been created and validated an analysis method based upon high performance liquid chromatography [6]; this method is used also for the quantitative determination of 5-FU from the bioadhesive tablets and for the evaluation of its *in vitro* release using the dissolution test.

Material and Method

We designed 12 bioadhesive vaginal tablets formulations containing 100mg 5-FU/comp.: 3 formulations based on NF Carbopol 71 P noted P1, P2, P3, 3 formulations based on Carbopol 974 P NF noted P4, P5 and P6, 3 formulations based on Metolose 90 SH 4000 noted P7, P8, P9 and 3 formulations based on Metolose 90 SH 100 000 noted P10, P11 and P12. The percentage of the matrix polymer varied for each series of formulations ranging from 20 to 50%. Pharmacological and technical characteristics of tablets ranged within the limits allowed by the quality regulations.

HPLC Thermo Surveyor chromatographic system foreseen with Surveyor LC –Pump, Autosampler Surveyor LC-Plus and UV-VIS detector

The column had as *stationary phase* octadecylsilane silica gel for chromatography (C₁₈) (5µm) Thermo Fisher – Scientific Betasil C18 with the following dimensions $l = 0.15\text{ m}$, $\Phi = 4.6\text{ mm}$ of stainless steel at a temperature of 37°C. The *mobile phase* was a mixture of orthophosphoric acid, water, tetrahydrofuran (V/V)(0.5: 97.5:2) with a rate of 0.7mL/min.

Detection was performed using a UV-VIS detector set at the wavelength of 270nm, band width 4; reference wavelength 450nm, band width 80nm. The ***injected volume*** was of 10µL. ***The internal standard solution (SI)*** consisted of 10mg pyridine 3-carbohydrazide (P3C) dissolved in water and diluted at 10mL of the same solvent (C_{IS} = 1.0mg/mL).

Dissolution Test Station SR8 Plus (AB & L Jasco) devise (Apparatus 2)

The dissolution environment used was acetate buffer solution pH4.2, 900 mL. ***The rotational speed*** of the mixer was set at 60rpm. ***The temperature*** was set at 37°C±0.5°C. ***The sample interval*** was initially of 30 minutes, it was then changed to each hour for 8 hours, while the last samples were taken at 10 and 12 hours, respectively. ***The sample volume*** was of 0.5 mL analysed solution which was replaced with the same volume

of the receiver. The sample of 0.5mL solution analysed was diluted with water (R), 0.1mL of internal standard solution was added and it was brought to 10mL with the same solvent (ST). The tests were performed on 6 tablets and the results indicate the average of the six determinations.

The 5-fluorouracil content [6] was calculated using the linear regression equation and it is related to the concentration of the internal standard with the formula:

$$\% 5FU = \frac{(A_{ST} - a)}{A_{SI}} \times C_{SI} \times F_{ST}$$

where,

- A_{ST} = peak area corresponding to 5-fluorouracil in the solution analyzed

- A_{SI} = peak area corresponding to the internal standard in the solution analyzed

- C_{SI} = concentration of the internal standard in the solution analyzed

- F_{ST} = the dilution factor corresponding to the solution analyzed

- b = straight line calibration curve

- a = intercept through the origin of the calibration curve

For the statistical analysis of the results the ANOVA test was used. The comparison of the dissolution profiles can be performed using independent and dependent mathematical methods.

The independent model relies, for comparison, on the difference factor (f_1) and the similarity factor (f_2). Using the average values of dissolved concentrations at each period of time, the difference factor (f_1) and similarity factor (f_2), respectively were calculated [7], with the formula:

$$f_1 = \left\{ \left[\sum_{t=1}^n (R_t - T_t) \right] / \left[\sum_{t=1}^n (R_t) \right] \right\} \times 100$$

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^n (R_t - T_t)^2 \right] \right\}^{-0.5} \times 100$$

where: n = the number of sampling time points,

R_t = released 5-FU percentage of the reference formulations at time point t ;

T_t = released 5-FU percentage of the test formulation at time point t ;

If a dissolution profile is similar to a reference profile, f_1 factor must be as close to 0 as possible, while f_2 factor must be as close to 100 as possible. Generally the values ranging between 0-15 for f_1 and those higher than 50 (50 – 100) for f_2 ensure the similarities of the two dissolution profiles. For the 12 analyzed systems it was considered the NxM type

matrix, where N represents the number of analyzed systems and M, the sum of the sample times.

Results and Discussion

The amount of 5-FU released by the twelve formulations of vaginal bioadhesive tablets, in a period of 12 hours, is presented in figure 1.

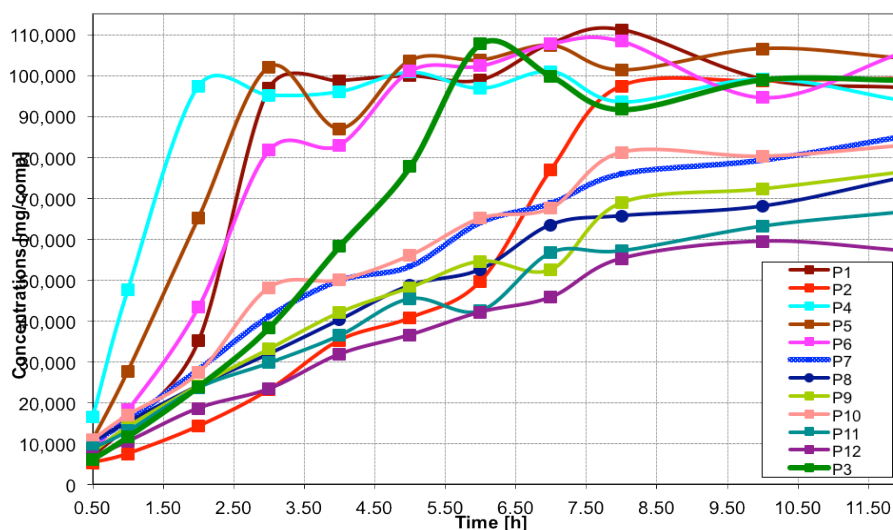


Figure 1

Dissolution profiles of 5-FU from vaginal bioadhesive tablets
(the concentration is expressed in mg/tablet)

The Anova test, with only one factor, applied to the data series, relies on the nul hypothesis: each sample presents the same distribution probability of concentrations within the time interval considered 0-720 minutes. The alternative hypothesis considers that there is no similar distribution probability of concentrations with a insecurity level of 95%.

Table I

Statistic study based on the Anova test

Variation source	SS	df	MS	F	P-value	F critical
Within the same group	38959.79819	11	3541.799835	3.934629054	6.97281E-05	1.869290423
Between different groups	108019.3264	120	900.1610537			
Total	146979.1246	131				

All the tests were statistically relevant given the conditions of the determinations: 37°C and an angular rotation of 100rpm with $p < 0.05$. The

statistic test has a value $F = 3.94$ higher than the value of F statistic critical that is to 1.87. The value of individual standard variations indicates a statistically significant difference between the release rates of 5-FU for the 12 systems studied. The use of the calculation formulae for f_1 difference factor and f_2 similarity factor lead to the following correlation matrix (tables II and III).

Table II
Matrix of difference factor f_1

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
P1	-	37.30	20.16	16.12	11.22	7.46	34.96	43.75	43.20	33.74	49.18	55.34
P2		-	32.42	74.66	67.79	57.47	27.41	26.92	27.47	29.07	30.48	32.65
P3			-	36.00	29.98	22.56	24.06	32.82	31.98	24.30	38.93	45.90
P4				-	12.27	17.28	39.00	47.22	47.36	37.46	52.67	58.75
P5					-	9.02	37.73	46.12	46.27	36.20	51.68	57.89
P6						-	33.51	42.56	42.22	31.98	48.16	54.72
P7							-	13.51	13.71	4.05	22.41	32.38
P8								-	5.85	18.48	10.33	21.85
P9									-	18.81	12.21	21.64
P10										-	24.32	34.04
P11											-	12.85
P12												-

Table III
Matrix of similarity factor f_2

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
P1	-	20.34	31.29	32.72	46.73	55.24	23.96	19.72	19.72	25.13	17.39	15.12
P2		-	31.36	16.61	18.71	22.29	42.08	38.93	39.17	40.86	34.60	31.35
P3			-	24.02	28.88	36.33	35.02	28.79	28.56	35.35	24.90	22.08
P4				-	44.01	34.39	21.01	17.68	17.62	21.75	15.84	13.66
P5					-	49.09	22.58	18.56	18.54	23.57	16.38	14.09
P6						-	26.20	21.46	21.37	27.30	18.80	16.28
P7							-	54.51	54.56	75.50	43.75	36.90
P8								-	69.89	50.39	62.45	48.34
P9									-	51.21	57.99	48.19
P10										-	41.51	35.40
P11											-	60.40
P12												-

Correlating the two tests we can notice the following: the release profile for P1 is similar to the profile corresponding to P6; the profile

corresponding to P7 is similar to the profiles corresponding to P8, P9 and P10; the profile corresponding to P8 is similar to the profiles corresponding to P9 and P11; the profile corresponding to P9 is similar to the profile corresponding to P11; the profile corresponding to P11 is similar to the profile corresponding to P12. The analysis of intergroup variation for the 12 samples is presented in figures 2 and 3.

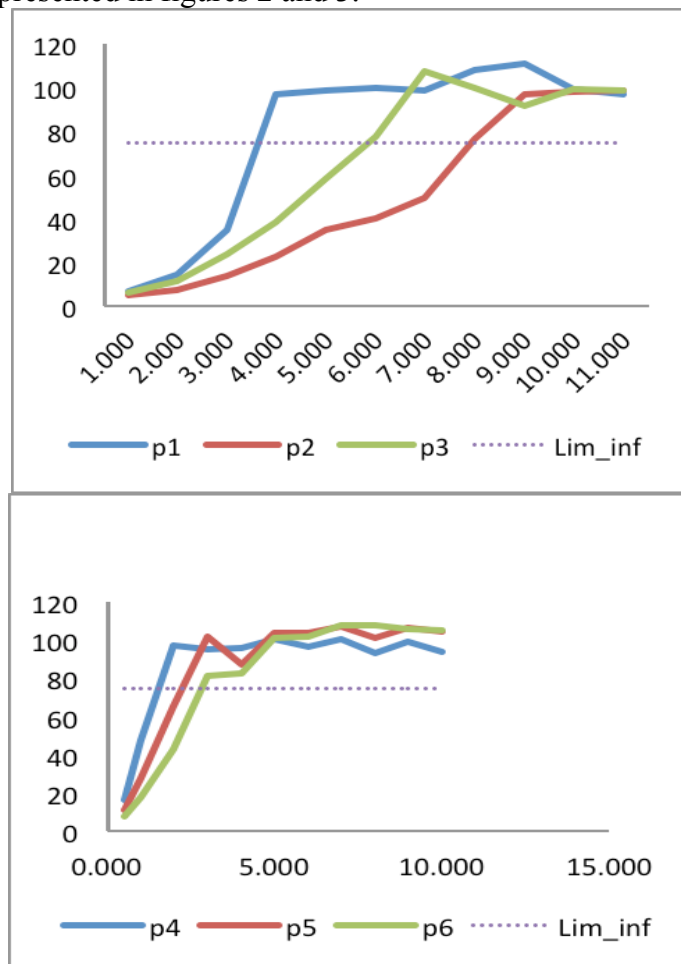


Figure 2
Release profile for P1, P2, P3, P4, P5 and P6

Considering the factor analysis it is noticed that in case of sample P1 the minimum concentration of 90% is reached after 3 hours, while for P3 is reached after 6 hours, while for P2, in 8 hours. For P4 the concentration level of 97% is achieved in 2 hours, while for P5 and P6, in 3 and 5 hours, respectively.

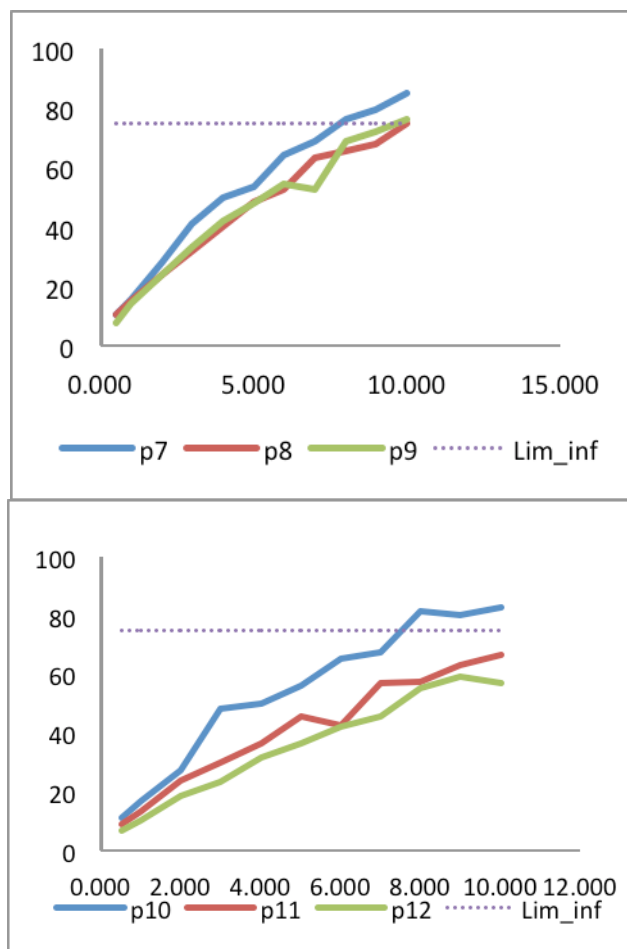


Figure 3

Release profile for P7, P8, P9, P10, P11 and P12

Release profile for P7 corresponds to a linear regression, the correlation coefficient being equal to 0.97. A major similarity is noticed for samples P8 and P9.

The release profile for P10, P11 and P12, corresponds to a first order statistics. The variations regarding the concentration level, depending on the sample time present a minimum correlation coefficient of 0.94. Under these circumstances, it is noticed that only for sample 10, the minimum level of 75% is reached within the considered release interval.

Conclusions

The results of the performed researches underlined that the formulations consisting in Carbopol presented higher 5-FU release levels,

with a very good general dissolution and release profile. The samples with Carbopol 71 C reached the minimum concentration of 90 % for P1 in 3 hours, while for P3 it was reached in 6 hours, and for P2, in 8 hours. The samples with Carbopol 974 C reached the concentration level of 97% for P4 in 2 hours, for P5 in 3 hours and for P6 in 5 hours.

The introduction of Metolose in the formulation decreased the release rhythm of 5-FU, modifying the general dissolution and release profile, reaching the minimum level of 75%, within the estimated time limit, only for samples P7, P8, P9 and P10.

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