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FORMULATION AND EVALUATION OF TRANSDERMAL DRUG DELIVERY OF RALOXIFENE HYDROCHLORIDE

Iti Chauhan*[1] and Meenakshi Bajpai [2]

ITS Paramedical (Pharmacy) College [1], Ghazaibad (UP), India
Raj Kumar Goel Institute of Technology [2], Ghaziabad (UP), India

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Correspondence to Author:

Iti Chauhan

ITS Paramedical (Pharmacy) College, Ghazaibad (UP), India

ABSTRACT

The present investigation was taken up to prepare and evaluate a transdermal drug delivery system (patch) of Raloxifene Hydrochloride to increase its bioavailability. The matrix type patches were prepared using different ratios of Eudragit RL100, Poly vinyl Pyrrolidone K-30, HPMC, CAP and PEG6000 in different ratios. All the prepared formulations were subjected to physical studies (weight variation, thickness, moisture content, moisture uptake, flatness, Water Vapour transmission rate, folding endurance, tensile strength, and % elongation), thumb tack test, in vitro release studies, and in vitro diffusion studies. In vitro permeation studies were performed using artificial membrane and across skin derived from albino rat. The accelerated stability studies for the formulations were performed as per the ICH guidelines. The studies showed that formulation prepared with HPMC and CAP in ratio 5:2 with adhesive polyisobutylene and Eudragit RL100 & PVP in ratio 6:4 (self sticking) were the effective systems to achieve desired results. The interaction studies carried out by comparing the results of TLC, Infrared and UV analysis of pure drug and medicated patches indicated no chemical interaction between drug and excipients.

INTRODUCTION: Raloxifene hydrochloride is a Selective estrogen receptor modulator that produces estrogen-agonistic effects on bone and lipid metabolism and hence is used in treatment and prevention of postmenopausal osteoporosis [1]. Currently Raloxifene is available in tablet form only; but on oral administration it undergoes extensive first pass metabolism to hepatic glucuronide conjugates. The oral bioavailability of Raloxifene is 2.0% [2]. As the drug possess characteristics (poor bioavailability, lipophilic, smaller dose etc.) suitable for the formulation of an safe and alternative transdermal route, the aim of the present study was to develop different transdermal matrix patches with varied ratios of Hydroxypropylmethylcellulose (HPMC), Cellulose acetate phthalate (CAP), Poly Ethylene Glycol 6000 (PEG) using polyisobutylene as adhesive and Eudragit RL100 and PVP (Self sticking). Transdermal delivery offers added advantages such as improved patient convenience, Non-invasive, Constant dosing rate, Capacity to terminate drug effect.

MATERIALS AND METHODS: Raloxifene hydrochloride was obtained from Panacea Biotech.All other reagents used were of analytical grade. All the experiments were carried out at Raj Kumar Goel Institute of Technology, Ghaziabad in year 2007.

Preparation of Backing Membrane: The backing membrane was prepared with an aqueous solution of 4%w/v Poly vinyl alcohol. A weighed amount of poly vinyl alcohol was added to a requisite volume of warm, distilled water and a homogenous solution was made by constant stirring and intermittent heating at 60^0c for few seconds. The homogenous solution was poured into glass Petri dishes already wrapped with aluminum foil around open ends and were kept for drying at 60^0C for 6h, forming a smooth, uniform, transparent backing membrane [3, 4].

Fabrication of Medicated patch: The different placebo patches were prepared using various combinations of hydrophilic and lipophilic polymers by trial and error method. Those polymeric combinations that exhibited smooth, flexible films were selected for preparing the drug incorporated matrix system. All the matrix systems were prepared by Solvent Evaporation technique according to the formula given in (**table 1**) [5, 6].

TABLE 1: FORMULATION OF DRUG MATRIX FOR THE TRANSDERMAL PATCH

Compound	F1	F2	F3	F4	F5
Raloxifene	60	60	60	60	60
Eudragit RL100	240	180	150	120	60
PVP K-30	60	120	150	180	240
Di butyl phthalate	30%	30%	30%	30%	30%
Solvent *	4ml	4ml	4ml	4ml	4ml

Solvent*: Isopropyl alcohol and dichloromethane (40:60)

Compound	F3	F4	F5	F6	F7	F8	F9	F10	F11
Raloxifene	60	60	60	60	60	60	60	60	60
HPMC	-	180	180	180	180	180	180	180	180
CAP	180	36	45	120	72	90	72	90	-
PEG6000	-	-	-	-	-	-	72	72	72

Solvent: Methanol for HPMC & PEG; Acetone for CAP

Evaluation: All the formulations were evaluated for physical appearance, Thickness, Weight variation, Moisture content and uptake, Water vapor transmission rate (WVTR), Drug content, Flatness, Folding endurance, Tensile strength, and % elongation. Thumb tack test was performed for evaluating adhesion. Microscopic studies of Placebo and medicated patches were also done.

Thickness: It was assessed at different points of the patch using Screw Gauge. Moisture content and uptake was found out using the procedure as described [7].

WVTR: Glass bottles of 15mL were used as transmission cells. Anhydrous Calcium Chloride was filled into each bottle and an adhesive (Araldite) was spread over the brim of the bottle. The patch was fixed onto the bottle to ensure a tight seal. The entire assembly was accurately weighed and then placed in a dessicator containing 200 ml of saturated solution potassium chloride (84% RH). The bottles were weighed every day up to 7 days, and the difference in weight was noted [8].

Drug content: Individual patch was dissolved in methanol in a 100mL volumetric flask. Flasks were put on a wrist action shaker and kept for 24 hrs. The solutions were filtered and samples were analyzed spectrophotometrically for RLH content

Flatness: Longitudinal strips were cut out from the prepared patch, one from the centre and two from either side. The length of each strip was measured, and the variation in the length due to the non uniformity in flatness was measured [7].

Folding endurance: Folding endurance was determined by repeatedly folding the film at the same place until it brake. The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance [8].

Tensile strength and **%Elongation:** were determined using an apparatus fabricated in laboratory [9].

Thumb tack test: It was performed by lightly pressing a thumb on a patch for ~5 s and then quickly removing it. By varying the pressure and time of contact, and considering the difficulty of pulling the thumb from the adhesive, it was possible to guess how easily, quickly, and strongly the adhesive formed a bond with the skin [3, 4].

Microscopic study: Placebo and medicated films were evaluated for microscopic studies using optical microscope.

In-vitro **Dissolution Studies**: The dissolution of the patches was performed using USP basket-type dissolution apparatus (Phosphate Buffer, pH 7.4, 50 rpm). The samples withdrawn at different time intervals were analyzed for RLH content at 285.5 nm using UV spectrophotometer [5, 7].

In-vitro **Diffusion Studies**: The studies were carried out using artificial membrane i.e. Cellulose nitrate filter membrane (0.8 µm) using Franz diffusion cell. The receiver compartment was filled with Phosphate buffer, pH 7.4 (PBS) and maintained at 37±1^{0}C by a circulating water bath, stirring at 60 rpm by a Teflon coated magnetic bead on a magnetic stirrer. Area exposed for diffusion = 0.785 cm$^{2.}$ Sample was withdrawn at equal intervals for 12 hr and analyzed for RLH at 285.5 nm [7].

In-vitro **skin permeation studies**: The in vitro skin permeation studies were carried out in a similar manner as that of in vitro diffusion studies except that the membrane barrier used in this study was full thickness skin from abdominal portion of an albino rat after killing the animal [5].

Drug Excipient Interaction Study in Formulated Patches: The methanolic solutions of pure drug, medicated and placebo formulations were filtered through vacuum filter and scanned spectrophotometrically between 200-400nm using a UV spectrophotometer [5].

Stability studies: The stability studies were conducted according to ICH guidelines by storing the replicates of the TDDS at 40±0.5^{0}C and 75±5% Hrs. The samples were withdrawn at 3, 6, 9 weeks and analyzed for physical appearance, drug content, *in-vitro* diffusion studies [5].

RESULTS & DISCUSSION: In the present study, transdermal patches bearing Raloxifene Hydrochloride were formulated using various polymer ratios and combinations of HPMC, CAP, PEG6000, Eudragit RL100, PVP, and Sodium Alginate and characterized on the basis of physical characters, *in-vitro* drug release, *in-vitro* diffusion studies, *ex-vivo* skin permeation and stability studies. No interaction between the drug and excipients were found on the basis of TLC, UV and IR studies. Polymeric drug-free films of various ratios and combinations of polymers were prepared and evaluated as shown in **table 2**. Those polymeric combinations that exhibited smooth, flexible films were selected for preparing the drug incorporated matrix system.

The Thickness and weights of the patches were found to be uniform among different batches as shown in **table 3**. The data obtained of % Moisture Content/ Uptake and water vapor transmission rate are shown in **table 4**. Moisture Content/ Uptake were found to increase with increasing concentration of hydrophilic polymers, PVP, HPMC, PEG and Sodium Alginate. The small Moisture Content in the formulations helps them to remain stable and from being a completely dried and brittle film. WVTR were higher for patches formulated with HPMC, PEG, Sodium Alginate (1.160×10^{-1} - 1.753×10^{-1}) in comparison to films fabricated with Eudragit-PVP (0.272×10^{-1} - 0.608×10^{-1}). Good Uniformity in drug content among the batches was observed with all formulations and ranged from 98.7% to 100%.

Table 5 shows all the results of physical parameters of patch evaluation. The results of Flatness Study showed that none of the formulations had the differences in the strip lengths before and after their cuts. It indicates 100% flatness observed in the formulated patches. The increase in amount of cellulose acetate phthalate increases the tensile strength of patch. Sodium alginate patch was found to possess least Tensile strength. The elongation at break was found to vary between 4 to 35%. The formulations using the polymers Eudragit RL100 and PVP in an 8:2, 6:4, 5:5 ratios showed optimum tackiness with the thumb and good adherence capacity with human skin. The formulations using Polyisobutylene as adhesive also showed good adhesion.

TABLE 2: CHARACTERIZATION OF POLYMERIC DRUG FREE FILMS

Formulation code	Polymers	Ratio	Physical appearance	Thickness (mm)
1.	HPMC	100%	Transparent, flexible film	0.074
2.	HPMC:PVP	8:2	No film was formed	---
3.	CAP	100%	Hard, Brittle	0.085
4.	CAP:HPMC	5:1	No film was formed	---
5.	HPMC:CAP	8:2	Hard, Brittle	0.06
6.	HPMC:CAP	6:4	Hard, Brittle	0.065
7.	HPMC:CAP	5:2	Smooth, uniform, tough	0.093
8.	HPMC:CAP	2:1	Smooth, uniform, tough	0.105
9.	HPMC:CAP:PEG6000	5:2:2	Smooth, uniform, soft film	0.065
10.	HPMC:CAP:PEG6000	2:1:2	Smooth, uniform, soft film	0.08
11.	HPMC:PEG6000	5:2	No intact film	---
12.	Sodium Alginate	100%	Smooth, uniform, flexible	0.075

13	Eud: PVP	8:2	Smooth, Transparent, tough, flexible film	0.095
14	Eud: PVP	6:4	Smooth, Transparent, tough, flexible film	0.095
15	Eud: PVP	5:5	Smooth, Transparent, tough, flexible film	0.075
16	Eud: PVP	2:8	Smooth, Transparent, tough, flexible film	0.080
17.	Eud: PVP	4:6	Smooth, Transparent ,tough, flexible film	0.07
18.	Eud:PEG4000	6:4	Hard and brittle	0.035
18.	EC:PVP	8:2	Smooth, uniform, tough	0.01
19.	EC:HPMC	1:1	Non uniform film	---
20.	PVA: PEG6000	8:2	No film was formed	---
21.	PEG4000: PEG6000	5:5	No intact film	---
22.	PVA:PVP	8:2	Hard film; difficult to remove from substrate	---
23.	Eud: PVP+DMSO	6:4	Very sticky film	---
24.	HPMC:CAP+DMSO	5:2	Again very sticky film	---

TABLE 3: PHYSICAL CHARACTERIZATION OF DRUG LOADED PATCHES

Formulation code	Polymers	Ratio	Thickness	Weight variation
1.	HPMC	100%	0.082 ± 0.003	0.367 ± 0.03
2.	HPMC: CAP	5:2	0.107 ± 0.001	0.567 ± 0.04
3.	HPMC: CAP	2:1	0.111 ± 0.002	0.558 ± 0.035
4.	HPMC: CAP	5:3	0.115 ± 0.001	0.575 ± 0.02
5.	HPMC:CAP:PEG6000	5:2:2	0.075 ± 0.00	0.634 ± 0.025
6.	HPMC:CAP:PEG6000	2:1:2	0.09 ± 0.00	0.652 ± 0.02
7.	Eud RL100:PVP	8:2	0.1 ± 0.0003	0.379 ± 0.01
8.	Eud RL100:PVP	6:4	0.102 ± 0.002	0.383 ± 0.02
9.	Eud RL100:PVP	5:5	0.0825 ± 0.005	0.407 ± 0.04
10.	Eud RL100:PVP	4:6	0.103 ± 0.002	0.379 ± 0.03
11.	Eud RL100:PVP	2:8	0.072 ± 0.003	0.404 ± 0.06
12.	Sodium Alginate	100%	0.081 ± 0.004	0.383 ± 0.04

TABLE 4: % MOISTURE CONTENT & % MOISTURE UPTAKE

Formulation Code	Polymers	% Moisture Content	% Moisture uptake	Water Vapor Transmission Rate (gm/cm^2/day)
1.	HPMC	10.13 ± 2.1	76 ± 0.19	$1.398 \times 10^{-1} \pm 0.106$
2.	HPMC:CAP (5:2)	12.3 ± 0.15	75.1± 0.21	$1.297 \times 10^{-1} \pm 0.06$
3.	HPMC:CAP (2:1)	9.23 ± 3.6	73.6 ± 0.35	$1.213 \times 10^{-1} \pm 0.04$
4.	HPMC:CAP (5:3)	7.6± 1.3	70.06 ± 0.41	$1.160 \times 10^{-1} \pm 0.05$
5.	HPMC: CAP: PEG (5:2:2)	12.6 ± 1.21	77.2± 1.41	$1.266 \times 10^{-1} \pm 0.008$
6.	HPMC: CAP: PEG (2:1:2)	11.26 ± 1.8	48.7 ± 1.45	$1.346 \times 10^{-1} \pm 0.08$
7.	Sodium alginate	10.66 ± 1.01	89.1± 0.18	$1.753 \times 10^{-1} \pm 0.03$

8.	Eudragit: PVP (8:2)	4.95 ± 0.05	2.22 ± 0.46	$0.272 \times 10^{-1} \pm 0.09$
9.	Eudragit: PVP (6:4)	5.7 ± 0.1	3.7± 0.4	$0.296 \times 10^{-1} \pm 0.07$
10.	Eudragit: PVP (5:5)	6.55 ± 0.91	5.3 ± 0.1	$0.601 \times 10^{-1} \pm 0.09$
11.	Eudragit: PVP (4:6)	7.83 ± 0.31	9.06 ± 2.05	$0.520 \times 10^{-1} \pm 0.02$
12.	Eudragit: PVP (2:8)	8.56 ± 0.52	11.1±3.42	$0.546 \times 10^{-1} \pm 0.02$

TABLE 5: PHYSICAL PARAMETERS OF PATCH EVALUATION

Formulation Code	Polymers	Flatness	Folding Endurance	Tensile Strength (Kg/mm^2)	% Elongation
1.	HPMC	100%	6	970 ± 1.0	25 ± 2.2
2.	HPMC:CAP(5:2)	100%	6	1200 ± 2.1	30 ±2.0
3.	HPMC:CAP(2:1)	100%	6	1250 ±1.8	32.5 ±1.8
4.	HPMC:CAP(5:3)	100%	5	1300 ± 2.0	35 ±1.9
5.	HPMC: CAP: PEG (5:2:2)	100%	6	500 ±1.5	4 ±2.1
6.	HPMC: CAP: PEG (2:1:2)	100%	6	575 ± 3.0	5 ±2.5
7.	Sodium alginate	100%	7	175 ± 2.5	1.25 ±1.5
8.	Eudragit: PVP (8:2)	100%	6	985 ± 4.5	12.5 ±1.5
9.	Eudragit: PVP (6:4)	100%	5	950 ± 3.5	10.1±2.1
10.	Eudragit: PVP (5:5)	100%	5	25 ± 5.0	2.5±0.9
11.	Eudragit: PVP (4:6)	100%	5	625 ±2.5	7.5±1.5
12.	Eudragit: PVP (2:8)	100%	4	840 ± 3.0	6.2±2.5

The formulations with HPMC (100%), HPMC: CAP (5:2), Eudragit: PVP (8:2, 6:4) exhibited the greatest (99.94; 98.8; 95.62; 98.6% respectively) percentage of drug release values (**fig. 1**). A linear relationship was observed with cumulative drug release vs. square root time (0.9835 - 0.9963). As the concentration of hydrophilic polymers increased in the formulations, the dissolution rate increased substantially. An initial rapid release was observed in matrix controlled drug delivery systems, which could be accounted for direct exposure of matrix diffusion system to diffusion media and quick release of drug at the surface.

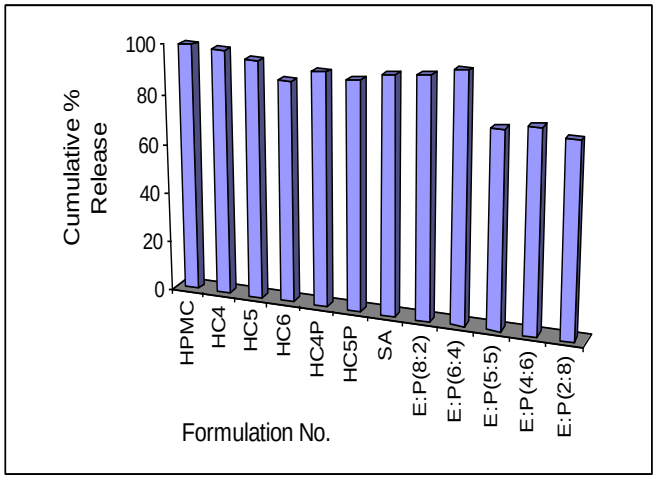

FIG. 1: COMPARATIVE STUDIES OF DISSOLUTION OF DIFFERENT FORMULATIONS

The cumulative % of drug permeated through membrane was found maximum for formulations containing HPMC, HPMC: CAP (5:2), Eudragit: PVP (6:4) with a value of 87.9%; 89.1%; 89.3% respectively (**fig. 2**). A linear relationship was observed with cumulative drug release vs. square root time (0.991-0.9963) as shown in **table 6.**

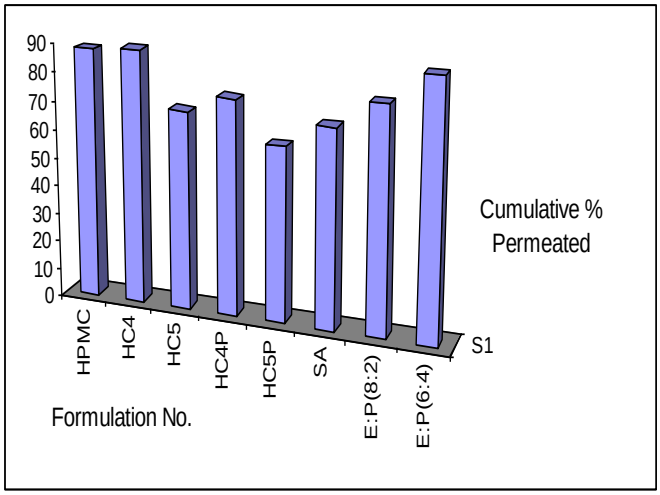

FIG. 2: COMPARATIVE STUDIES OF FORMULATIONS OF DIFFUSION ACROSS MEMBRANE

The cumulative % of drug permeated through rat skin was found maximum for formulations containing HPMC: CAP (5:2), Eudragit: PVP (6:4) with a value of 88.9%; 90.1% respectively. Flux was determined directly as the slope of the curve between the steady-state values of the amount of drug permeated (mg/cm^2) vs. time in hours and permeability coefficients were deducted by dividing the flux by the initial drug load (mg/cm^2).

The results are given in **table 7**. The release of drug from the transdermal film, when plotted against the square root of time yields a straight line, it indicates that the release pattern is obeying Higuchi's kinetics (**fig. 3**).

TABLE 6: *IN-VITRO* PARAMETERS FOR DRUG DIFFUSION THROUGH ARTIFICIAL MEMBRANE

Formulation	Flux (mg/cm^2/hr)	Permeability coefficient
HPMC	0.0897	0.065
HPMC:CAP(5:2)	0.0934	0.067
HPMC:CAP(2:1)	0.0719	0.05
HPMC: CAP: PEG (5:2:2)	0.0781	0.057
HPMC: CAP: PEG (2:1:2)	0.0705	0.051
Sodium alginate	0.0791	0.057
Eudragit: PVP (8:2)	0.0961	0.061
Eudragit: PVP (6:4)	0.0984	0.063

TABLE 7: IN VITRO PARAMETERS FOR DRUG SKIN PERMEATION

Formulation	Flux (mg/cm^2/hr)	Permeability coefficient
HPMC	0.0725	0.052
HPMC:CAP(5:2)	0.0858	0.062
Eudragit: PVP (8:2)	0.0985	0.063
Eudragit: PVP (6:4)	0.1008	0.065

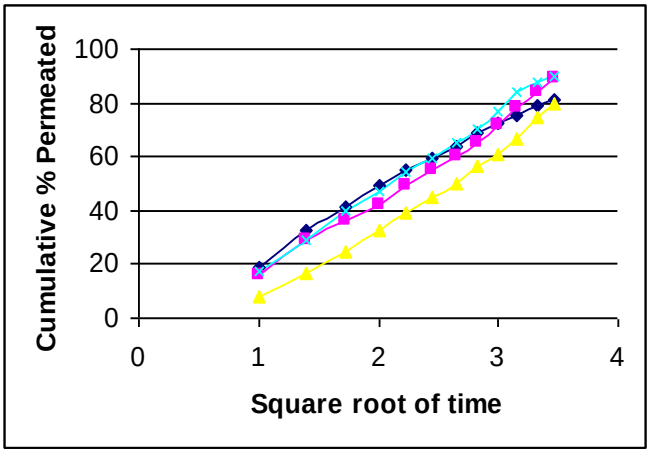

FIG 3: COMPARATIVE STUDY OF IN VITRO SKIN PERMEATION FROM FORMULATIONS SELECTED AFTER IN VITRO DIFFUSION STUDIES ACROSS MEMBRANE

◊HPMC; ■ (HPMC: CAP; 5:2); ▲ (Eudragit: PVP; 8:2); × (Eudragit: PVP; 6:4)

In our experiments, a linear relationship was observed with cumulative drug release vs. square root time (0.992-0.998). This observation thus supports that the patches released the drug by diffusion dominated mechanism. The formulation with HPMC was physically unstable as tensile

strength was decreased due to increased moisture which in turn affects the flexibility of the film as well as drug release. The formulation HPMC: CAP (5:2), Eudragit: PVP (8:2; 6:4) were found physically stable. The overall performance of TDDS after storage was tested through in vitro diffusion studies. The studies showed an increase in diffusion rate through artificial membrane and this may be due to increase in moisture content.

CONCLUSION: The studies showed that formulation prepared with HPMC and CAP in ratio 5:2 with adhesive polyisobutylene (**fig. 4 & 6**) and Eudragit RL100 & PVP in ratio 6:4 (self sticking) (**fig. 5 & 7**) were the effective systems to achieve desired results. The transdermal patches developed in the study have great utility and are a viable option for effective and controlled management of osteoporosis in post menopausal women.

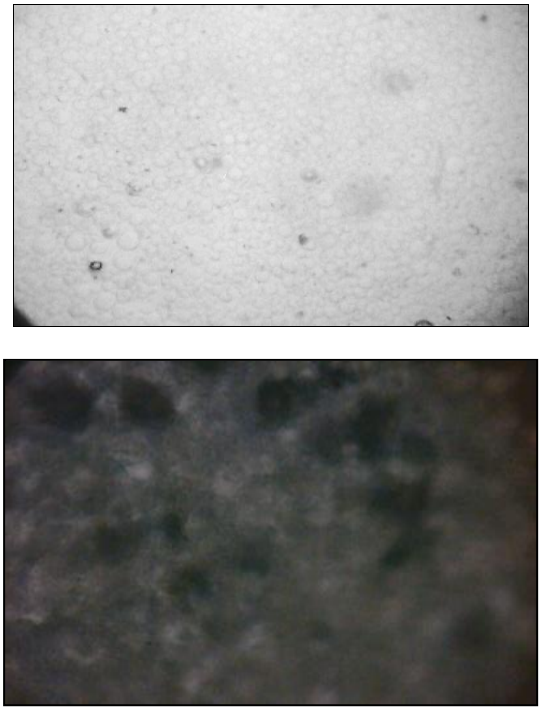

FIG. 4: OPTICAL IMAGES OF PLACEBO AND MEDICATED PATCHES OF HPMC: CAP (5:2)

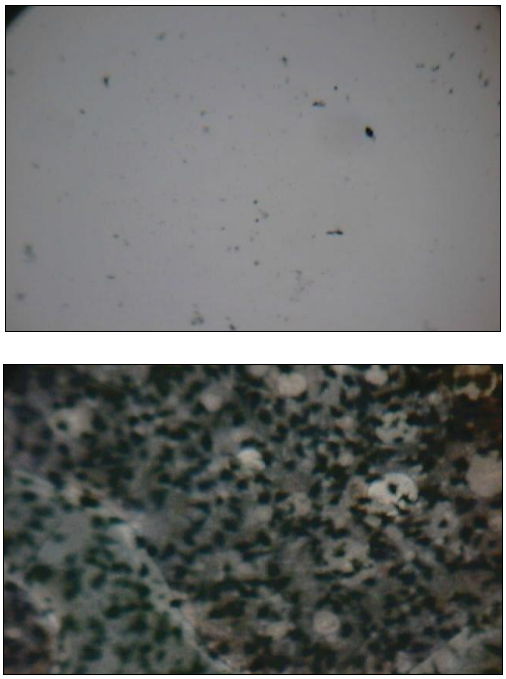

FIG. 5: OPTICAL IMAGES OF PLACEBO AND MEDICATED PATCHES OF EUDRAGIT: PVP (6:4)

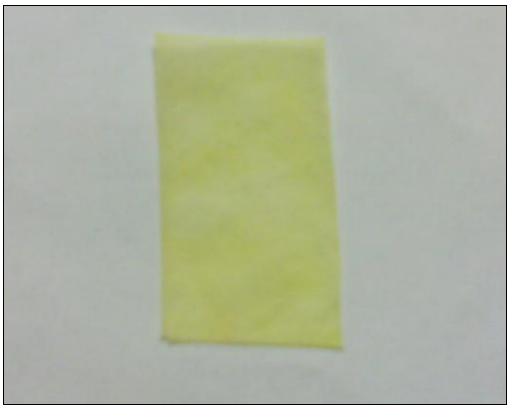

FIG. 6: PATCH OF RLH WITH HPMC: CAP (5:2)

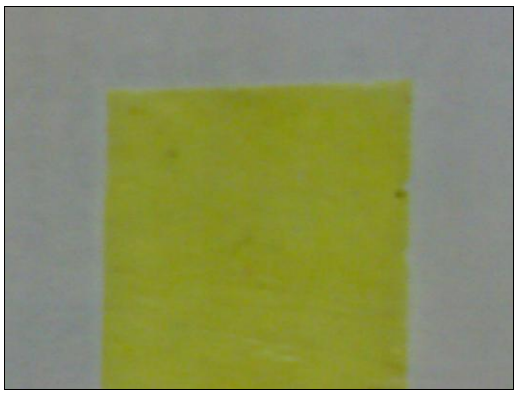

FIG. 7: PATCH OF RLH WITH EUDRAGIT RL100: PVP (6:4)

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