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RESEARCH PAPER

In Vitro and Ex Vivo Permeation Studies of Chlorpheniramine Maleate Gels Prepared by Carbomer Derivatives[#]

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ABSTRACT

The antihistaminic chlorpheniramine maleate (CPM) is used for symptomatic relief of hypersensitivity reactions and in pruritic skin disorders. At present, the drug is marketed in tablet, capsule, syrup, cream, and injectable dosage forms. Chlorpheniramine maleate has some side effects when taken orally. Due to its first pass effect, only 25%–45% of the orally administered dose reaches the blood circulation. To bypass these disadvantages, we aimed to investigate percutaneous absorption of CPM from gel formulations prepared with different carbomer derivatives (Carbopol 934, 940, 941, 2984, 980, and 981; main differences are related to presence of a comonomer and cross-link density). Cellulose membrane was used as the diffusion barrier for all the formulations' drug-release studies. The release of active substance from carbopol derivatives, which have the least cross-linking density (Carbopol 941 and 981) was found to be numerically higher than the others. The formulation (F8; 1% Carbopol 941) that exhibited the maximum drug release through the cellulose membrane was further studied for drug release by using polyurethane membrane, excised rat skin, and human skin. The penetration of the active substance through different diffusion barriers was found to be statistically different ($p<0.05$) when compared. Of all the different diffusion barriers, rat skin gave the closest results to human skin. Thus topical application of CPM in the carbomer gel may be of potential use for local activity. The type and concentration of carbomers can affect drug release. The synthetic membranes are useful in assessments of formulations in quality

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assurance but they do not give definite indication of how a formulation will behave when it is used on skin.

Key Words: Chlorpheniramine maleate; Carbomers; Gels; Percutaneous absorption; Rat skin.

INTRODUCTION

Antihistamines are a diverse group of drugs that possess the ability to inhibit various histamine actions in the body. They bear certain structural resemblances to histamine and act principally through a reversible competitive antagonism of histamine at the receptor sites.[1] One of the alkylamines approved by the U.S. Food and Drug Administration (US FDA) is Chlorpheniramine maleate (CPM). It has the known side effects of all antihistamines when given orally. The most common ones are sedation, varying from slight drowsiness to deep sleep, dizziness, muscular weakness, and gastrointestinal disturbances.[2] It is well absorbed from the gastrointestinal tract; however, due to the first pass effect; only 25%–45% of the orally administered dose reaches the blood circulation.[3] In order to bypass these disadvantages; semisolid formulations have been proposed as topical applications. We preferred the gels (semisolid formulations) since CPM has no official trademarked gel dosage forms. For the development of effective topical formulations, it is important to determine the diffusion properties of drugs in the semisolid vehicles, especially when the release of drugs at the application site is likely to be rate-limited by the diffusion of the drugs in those vehicles. In recent years it has been shown that the skin is a useful route for drug delivery to the systemic circulation. Advances in the sciences of drug design, drug delivery technology, penetration enhancement, and topical vehicle formulation have allowed the stratum corneum, classically considered to be a total barrier to the ingress of chemical substances, to be used as a portal for the delivery of a select group of drugs. The increased interest in topical drug delivery systems has necessitated the development of new experimental procedures for the assessment of these products.[4]

The therapeutic efficacy of a topically applied drug depends on its ability to penetrate the skin and to be accumulated in the deeper layers of the skin. The extent of this absorption varies depending on both the physicochemical properties of the penetrant and its formulation. The vehicle composition can affect both drug release and skin permeability properties.[5]

Carbomers have been used to prepare gel formulations for topical use by several workers.[6–8] Carbomer is a polymer of acrylic acid and forms hydrogel in water or alkaline solution due to hydration of the carboxyl groups in its structure.[9] They exhibit high viscosity at low concentrations. Moreover, they are quite stable to heat with negligible batch-to-batch variability. They are also unaffected by aging, do not support bacterial or fungal growth, and are nonirritating.[10] Some of the carbomer derivatives contain benzene residue which are greater than approved limits at the ppm degrees.[11] Of course ppm levels of benzene residue will not affect the drug release but we wanted to approach the subject from the viewpoint of toxicity and safety of use.

The purpose of this investigation was to study the effect of carbomer type, carbomer concentration, and different diffusion barriers such as, cellulose dialysis membrane, polyurethane membrane, excised rat skin, and human skin on in vitro and ex vivo drug release from gels prepared using six different carbomers and to evaluate the mechanism of drug release from the gels.

EXPERIMENTAL

Materials

The following materials were used as received: CPM and propylene glycol (Sigma Chemical Company, USA), Carbopol 934, 940, 941, 2984, 980, and 981 from (B.F. Goodrich Chemical Company, USA), Ethanol (96 %), thimerosal (Merck, Germany), triethanolamine (Carlo Erba, Italy), cellulose membrane (Travenol Lab. Inc., USA), and polyurethane membrane (Omiderm, Omicron Scientific Ltd., Israel).

Equipment

Franz diffusion apparatus (Ildam, Turkey), constant temperature thermostatic water bath and circulators (GCA Precision Scientific, USA), UV spectrophotometer (Schimadzu 2100 S, Japan), viscosimeter (Brookfield Rotational Digital Viscosimeter DV II RVTDV-II USA), stirrer (Heidolph SO111, Germany), and pH meter (Philips PW 9422 Great Britain) were used.

Table 1. Compositions of the formulations (w/w%).

Code of formulation	Crb. 934	Crb. 940	Crb. 941	Crb. 2984	Crb. 980	Crb. 981	EA	PG	TMS	TEA	CPM	Distilled water q.s. to make
F1	0.5						30	15	0.01	q.s.	1	100
F2	1						30	15	0.01	q.s.	1	100
F3	2						30	15	0.01	q.s.	1	100
F4		0.5					30	15	0.01	q.s.	1	100
F5		1					30	15	0.01	q.s.	1	100
F6		2					30	15	0.01	q.s.	1	100
F7			0.5				30	15	0.01	q.s.	1	100
F8			1				30	15	0.01	q.s.	1	100
F9			2				30	15	0.01	q.s.	1	100
F10				0.5			30	15	0.01	q.s.	1	100
F11				1			30	15	0.01	q.s.	1	100
F12				2			30	15	0.01	q.s.	1	100
F13					0.5		30	15	0.01	q.s.	1	100
F14					1		30	15	0.01	q.s.	1	100
F15					2		30	15	0.01	q.s.	1	100
F16						0.5	30	15	0.01	q.s.	1	100
F17						1	30	15	0.01	q.s.	1	100
F18						2	30	15	0.01	q.s.	1	100

Crb.: carbopol; EA: ethyl alcohol (96%); PG: propylene glycole; TEA: triethanolamine; TMS: thimerosal q.s.: quantum sufficit.

Preperation of Gel Formulations

All the carbomer derivatives were used at the percentages of 0.5–1–2.[12] Required amounts of water, propylene glycol, and ethanol were mixed together. This mixture was then divided into two equal parts. An appropriate amount of carbomer resin was added to one part. After standing overnight appropriate amounts of triethanolamine were added and well mixed until the gel was formed. The CPM and thimerosal were dissolved in the rest of the mixture and were added slowly to the gel formulations by mixing gently. Prepared formulations are given in Table 1.

Stability Studies

Hydrogels were stored in glass containers (well stoppered) for six months in the dark at room temperature (23±1°C). They were checked after preparation and bimonthly throughout a six-month period. Physical evaluation of stability of the samples was carried out by visual inspection and rheological tests.

Viscosity Measurements

A Brookfield Rotational Digital Viscosimeter DV II RVTDV-II was used to measure the viscosity (in cps) of the gels. The spindle (numbers of 2, 3, 7, TF 96) was rotated at 10 rpm. Samples of the gels were allowed to settle over 30 minutes at the assay temperature (25±1°C) before the measurements were taken.

pH Measurements

The pH was measured in each gel, using a pH meter, which was calibrated before each use with buffered solutions at pH 4, 7, and 10.

Drug Content Studies

Drug content of the gels was determined by dissolving an accurately weighed quantity of gel (about 100 mg) in about 50 mL of pH 6.0 phosphate buffer saline. These solutions were quantitatively transferred to volumetric flasks and appropriate dilutions were made with the same buffer solution. The resulting solutions were then filtered through 0.45 μm membrane filters[13] before subjecting the solutions to spectrophotometric analysis for CPM at 261 nm.

In Vitro Diffusion Studies

Franz diffusion cells with a receiver compartment volume of 14 mL and effective diffusion area of 1.86 cm^2 were used in this study. The receptor phase (pH 6.0 phosphate buffer saline) was continuously

Table 2. CPM% content studies of the formulations through six months.

Code of formulation	Months							
	0		2		4		6	
	CPM%	SD±	CPM%	SD±	CPM%	SD±	CPM%	SD±
F1	98.89	0.11654	98.75	0.13598	99.03	0.12582	98.97	0.14359
F2	99.65	0.12367	99.51	0.11327	99.43	0.12934	99.75	0.14325
F3	99.74	0.12547	99.84	0.16547	99.76	0.09865	99.87	0.00974
F4	90.96	0.09865	91.16	0.12591	91.25	0.11384	91.07	0.13240
F5	93.85	0.08624	93.79	0.07935	93.94	0.07836	93.76	0.09841
F6	95.26	0.16329	95.14	0.15124	95.35	0.11621	95.23	0.09625
F7	98.75	0.23101	98.63	0.19648	98.51	0.09965	98.84	0.10341
F8	96.56	0.30120	96.25	0.25343	96.43	0.19654	96.16	0.17856
F9	98.75	0.20341	98.53	0.15321	98.62	0.12871	98.84	0.11654
F10	97.25	0.12348	97.32	0.16141	97.16	0.15222	97.02	0.14325
F11	97.34	0.35214	97.67	0.29784	97.54	0.26140	97.21	0.21354
F12	94.52	0.13265	94.65	0.14231	94.72	0.13587	94.44	0.12958
F13	97.25	0.32145	97.51	0.29875	97.63	0.25412	97.36	0.19652
F14	96.84	0.21387	96.72	0.19542	96.64	0.18624	96.97	0.09856
F15	93.85	0.36219	93.54	0.29624	93.452	0.21359	93.51	0.16984
F16	94.35	0.26159	94.71	0.23154	94.28	0.19587	94.55	0.16254
F17	95.12	0.23154	95.34	0.21534	95.18	0.20154	95.27	0.19328
F18	98.24	0.26874	98.45	0.26514	98.33	0.24137	98.41	0.23654

SD: standard deviation.
Note: each reading is an average of three determinations.

stirred and kept at a temperature of 37±0.5°C during the experiments. One gram of gel formulation was placed at the donor compartment. At appropriate times, 300 μL of the sample was withdrawn from the receiver compartment and the same amount of fresh buffer solution was added to keep the volume constant. This dilution of the receiver content was taken into account when evaluating the penetration data. Each experiment was run on three independent cells. The samples were analyzed spectrophotometrically at a wavelength of 261 nm and the concentration of CPM in each sample was determined from a previously calculated standard curve. Each data point represented the average of three determinations. In vitro release studies were observed for a four hour period.

The sample with optimum drug release through the cellulose membrane was further studied with different diffusion barriers. For this use we preferred the polyurethane membrane as a synthetic membrane because of its use as synthetic skin for burns. Rat skin was chosen as a natural membrane, since it appears to be as good as pig skin in modeling absorption through human tissue.[4] Rat skin and human skin were obtained as follows:

Male Sprague–Dawley rats weighing 180–200 g, were anesthetized with an intraperitoneal injection of ketamine HCl and their hair was removed using an electric clipper. For regeneration of the damaged skin we waited one week. At the end of this period, the rats were again anesthetized with the same method and their skin was carefully excised. The adhering fat and debris were carefully removed from the skin samples.

Samples of adult human skin were obtained from breast reduction operations. Membranes consisting of stratum corneum and epidermis were obtained by heat separation technique, which requires the skin to be heated to 60°C for 2 min (by simple immersion in water). Then the human skin sample consisting of stratum corneum plus epidermis was separated from dermis by blunt dissection.[4] The skin samples were then soaked in normal saline solution and waited in the deep freeze until used in the diffusion studies.

In vitro release studies with the excised rat and human skin lasted for 24 hours.

Data Analysis

The cumulative amount of CPM-permeated per-unit-area was plotted against time, and the slope of the linear portion of the plot was estimated as steady state flux (J_{SS}). The permeability coefficient (K_p) was

Table 3. pH values of the formulations measured for six months.

Code of formulation	Months							
	0		2		4		6	
	pH	SD±	pH	SD±	pH	SD±	pH	SD±
F1	8.148	0.05892	8.049	0.00264	8.088	0.00548	8.192	0.00245
F2	6.956	0.00356	6.789	0.00564	6.765	0.00561	6.807	0.00487
F3	5.633	0.00614	5.592	0.00462	5.562	0.01234	5.502	0.03121
F4	7.551	0.05214	7.406	0.00129	7.392	0.00387	7.461	0.00397
F5	6.853	0.00658	6.891	0.00987	6.795	0.02131	6.808	0.00432
F6	5.485	0.02341	5.374	0.00879	5.407	0.00356	5.398	0.00621
F7	7.595	0.05321	7.605	0.00624	7.552	0.00431	7.498	0.06276
F8	6.790	0.00568	6.698	0.00246	6.704	0.00798	6.662	0.06954
F9	5.780	0.02481	5.729	0.01452	5.693	0.05214	5.754	0.02547
F10	7.751	0.03124	7.603	0.00587	7.654	0.06871	7.591	0.05741
F11	6.449	0.04121	6.503	0.00421	6.398	0.06541	6.472	0.00847
F12	5.960	0.00459	5.804	0.00328	5.856	0.01254	5.911	0.00583
F13	7.778	0.00654	7.741	0.00789	7.693	0.00684	7.651	0.00547
F14	7.146	0.00889	7.096	0.00742	7.025	0.00598	7.083	0.00689
F15	6.069	0.00387	5.942	0.00598	5.984	0.00874	6.087	0.01457
F16	7.823	0.00213	7.848	0.00284	7.795	0.00541	7.804	0.00627
F17	7.019	0.00687	7.101	0.00987	6.903	0.00231	6.984	0.00531
F18	6.138	0.00687	6.054	0.00897	6.087	0.00618	6.117	0.00861

SD: standard deviation.
Note: each reading is an average of three determinations.

calculated as $K_p = J_{ss}/C_v$ where C_v is the total donor concentration of the gel.[14]

Statistical comparisons were made using one way ANOVA and Kruskal Wallis analysis of variance. The level of significance was considered when $p<0.05$.

RESULTS AND DISCUSSION

Stability Studies

Carbomer gels presented good stability. Therefore, no macroscopical physical changes were observed during storage. Drug content, pH, and viscosity values of the formulations carried out at six months showed no significant differences (Tables 2–4).

Drug Release Studies

Effect of Polymer Concentration and Viscosity of the Formulation

The viscosity values of the all formulations increased with the increasing carbomer concentrations (Table 5). There is an inverse relationship between viscosity and diffusion.[2] This relationship could change the process of releasing CPM from the formulations. The release process of drug from the preparation depends on the dissolution from the gels in aqueous media, and more viscous gels release CPM more slowly than less viscous gels ($p>0.05$).[15]

Drug release from the formulations at the higher polymer concentrations decreased (Fig. 1) except for the formulation prepared with 1% Carbopol 941 ($p>0.05$). At the same drug concentrations, the release rate is conditioned by the concentration of the polymer in strict relation with the gel viscosity. The lower the release rate, the greater the polymer concentration, and therefore, the more compact the network structure of the gel and the higher its viscosity.[16] It is possible that at the higher polymer concentrations the active substance is trapped in smaller polymer cells and it is structured by its close proximity to the polymer molecules. This increases the diffusional resistance more than expected.[17] It was shown that when diffusion of drugs occurs primarily through the aqueous channels in the gels, their diffusivity is an inverse function of polymer content. Diffusion of methotrexate decreased with increasing Carbopol concentration in

Table 4. Viscosity values of the formulations measured for six months.

		Months							
		0		2		4		6	
Code of formulation	Spindle number	Viscosity 10^3 cps	SD±	Viscosity 10^3 cps	SD±	Viscosity 10^3 cps	SD±	Viscosity 10^3 cps	SD±
F1	2	1.27	0.00002	1.27	0.00005	1.28	0.00004	1.27	0.00002
F2	TF 96	21	0.00324	21.1	0.00265	21	0.00124	20.9	0.00187
F3	TF 96	53	0.00583	53	0.00421	53.05	0.00364	52.98	0.00405
F4	3	6.84	0.00112	6.83	0.00097	6.85	0.00092	6.84	0.00094
F5	TF 96	48	0.00029	48.07	0.00031	48.06	0.00018	48.05	0.00012
F6	TF 96	83	0.00007	83.05	0.00004	82.97	0.00002	83.10	0.00001
F7	7	10.80	0.00094	10.83	0.00084	10.79	0.00076	10.81	0.00074
F8	TF 96	23	0.00134	23.04	0.00127	23.02	0.00116	23.04	0.00097
F9	TF 96	44	0.00010	43.99	0.00005	44	0.00004	44.01	0.00003
F10	2	1.36	0.00045	1.35	0.00057	1.36	0.00051	1.36	0.00049
F11	TF 96	40	0.00021	40.02	0.00016	40.01	0.00010	40.05	0.00009
F12	TF 96	113	0.00001	113.01	0.00001	112.98	0.00002	113.01	0.00001
F13	7	8.40	0.00092	8.41	0.00082	8.39	0.00078	8.41	0.00079
F14	TF 96	76	0.00015	76.05	0.00019	76.03	0.00017	76.02	0.00014
F15	TF 96	85	0.00005	85.01	0.00002	85	0.00001	85	0.00002
F16	7	10.40	0.00072	10.41	0.00034	10.39	0.00024	10.40	0.00022
F17	TF 96	31	0.00011	31.04	0.00005	31.05	0.00004	31.02	0.00001
F18	TF 96	56	0.00032	56.03	0.00029	56.02	0.00027	56.02	0.00014

Measurements were done at 10 rpm and at 25±1°C.
SD: standard deviation. cps: cantipoise TF96: t spindle. TB92: t spindle.
Note: each reading is an average of three determinations.

Table 5. Viscosity and the percentage amount of drug released by using cellulose membrane at the end of the four hour period.

Code of formulation	Polymer concentration %	Viscosity (10^3 cps±S.D.)	Spindle number	Amount of drug released (% ± S.D.)
F1	0.5	1.27±0.00002	2	10.865±0.02008
F2	1	21±0.0324	TF96	9.263±0.00354
F3	2	53±0.00583	TF96	7.220±0.01722
F4	0.5	6.84±0.00112	3	10.693±0.02013
F5	1	48±0.00029	TF96	10.484±0.01345
F6	2	83±0.00007	TF96	7.136±0.01642
F7	0.5	10.8±0.00094	7	11.622±0.01025
F8	1	23±0.00134	TF96	12.376±0.02132
F9	2	44±0.00010	TF96	10.076±0.00215
F10	0.5	1.36±0.00045	2	11.557±0.01003
F11	1	40±0.00021	TF96	8.996±0.01752
F12	2	113±0.00001	TF96	7.749±0.01699
F13	0.5	8.40±0.0092	7	11.670±0.0105
F14	1	76±0.00015	TF96	9.527±0.03121
F15	2	85±0.00005	TF96	7.829±0.01229
F16	0.5	10.4±0.00005	7	11.632±0.01350
F17	1	31±0.00011	TF96	10.340±0.00663
F18	2	56±0.00011	TF96	7.293±0.0523

Measurements were done at 25±1°C with the spindle speed of 10 rpm.
Note: each reading is an average of three determinations.

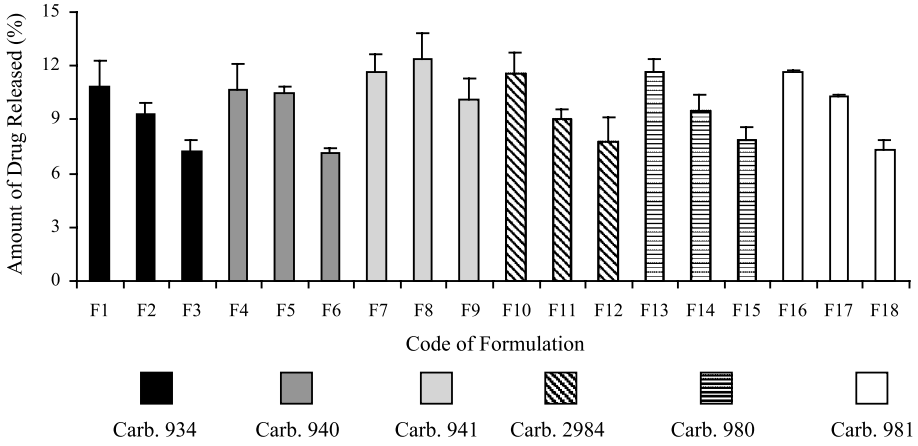

Figure 1. Cumulative amount of drug released (%) from the tested formulations at the end of the four hour period by using cellulose dialysis membrane.

the range of 1%–3%, which is in close agreement with the effect of carbopol content on diffusivity.[18] The permeability coefficient of the active substance also decreased with the increase of the polymer concentration ($p>0.05$) (Fig. 2).

Effect of Carbopol Type

According to the European Pharmacopoeia, carbomer derivatives must not contain more than 2 ppm benzene residue.[19] But all traditional polymers (Carbopol 934, 940, 941) do not comply with this new European Pharmacopoeia rule, since they are 50 years old and still polymerized in benzene. They, therefore, contain a benzene residue that is higher than the 2 ppm[11] allowed by the European Pharmacopoeia. In many scientific studies these traditional polymer derivatives have been used up today. Since benzene penetrates normal intact human skin more rapidly than many small organic molecules, and, therefore, is potentially toxic, the skin should be considered a portal of entry for benzene.[20] Percutaneous absorption of benzene has been studied and risk of exposure has been reported by many scientific workers.[21–25] Of course ppm levels of benzene residue will not affect the drug release but we just wanted to approach the matter from the viewpoint of toxicity and safety use. As expected Carbopol 2984, 980, and 981 (benzene-free residue derivatives) have exhibited the same drug release profiles as Carbopol 934, 940, and 941 (Figs. 1 and 3). Benzene-free residue carbomer derivatives can be used instead of the traditional ones. Thus the risk of

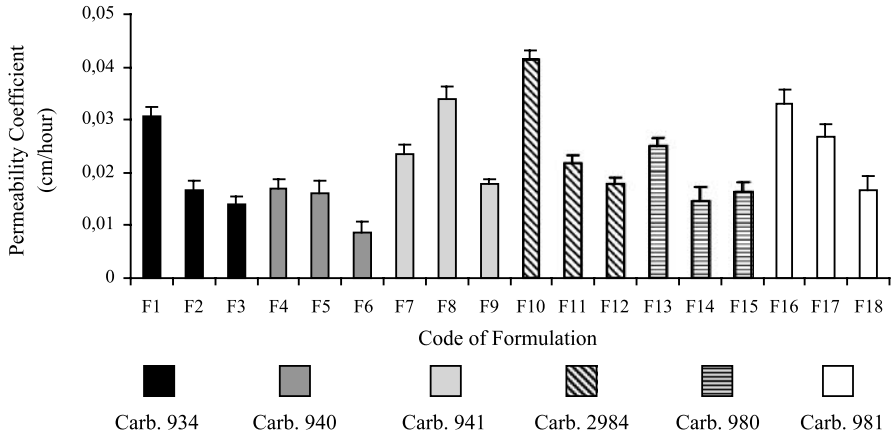

Figure 2. Permeability coefficient of chlorpheniramine maleate calculated from the in vitro data by using cellulose dialysis membrane.

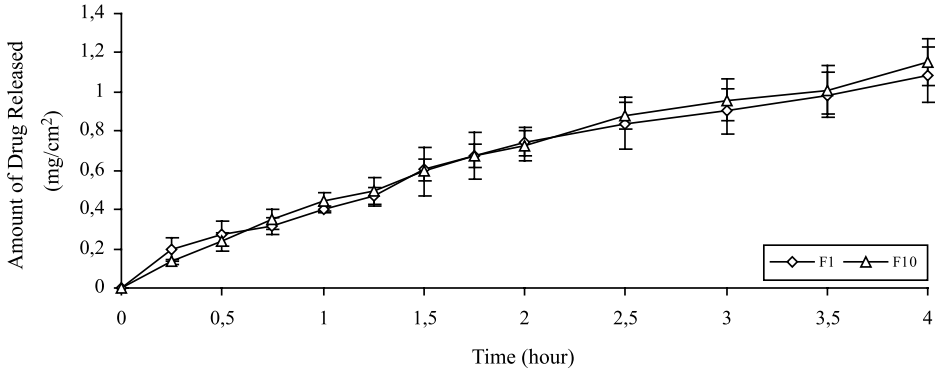

Figure 3. Comparision of in vitro drug release from the formulations prepared with Carbopol 934 and 2984 at the percentage of 0.5.

percutaneous absorption of benzene can be prevented, especially in the use of long-term dermal therapy of carbomer-based semisolid formulations.

No significant differences in drug-release characteristics were observed for all the carbomer derivatives, but there seems to be a trend in the release profile showing fastest drug release from gels prepared with Carbopol 941 and Carbopol 981 (Fig. 1), because these carbomer derivatives have the least cross-linking density. Decreasing cross-linking density of a polymer decreases the tortuosity of the matrix through which the drug has to diffuse, thus increasing the drug release.[10] On the other hand the cross-linking effect in the drug diffusion can be interpreted in terms of: a) mobility of the polymeric chain; b) average pore size; and c) mobility of the aqueous medium in the gel. Probably the decrease of drug release with the cross-linking, fundamentally, leads to a reduction of average pore size in the gel. Another phenomenon that explains the decrease with the increment in cross-linking density is that the mobility of the solvent inside the gel decreases.[26]

Effect of Different Diffusion Barriers

The F8 coded formulation that exhibited the maximum drug release through the cellulose membrane was further studied for drug release by using polyurethane membrane, rat skin, and human skin. The rate of drug release from the polyurethane membrane was found to be the highest ($p<0.05$) (Figs. 4 and 5) because of its hydrophilic nature as the active substance and its thinnest structure (about 20 μm). Thus, polyurethane membrane is not an ideal membrane for mimicking the human skin in in vitro percutaneous absorption studies; although, it can be used as synthetic human skin in burns.[27]

The developed formulation must be evaluated by performing actual studies in humans. This is, however, costly and time consuming; and it requires efficient safeguards and approved clinical protocols. Instead, the in vitro skin permeation studies correlate with in vivo performance as they dictate the amount of drug available for absorption. Further, these are economic, readily available, and remarkably similar in absorption

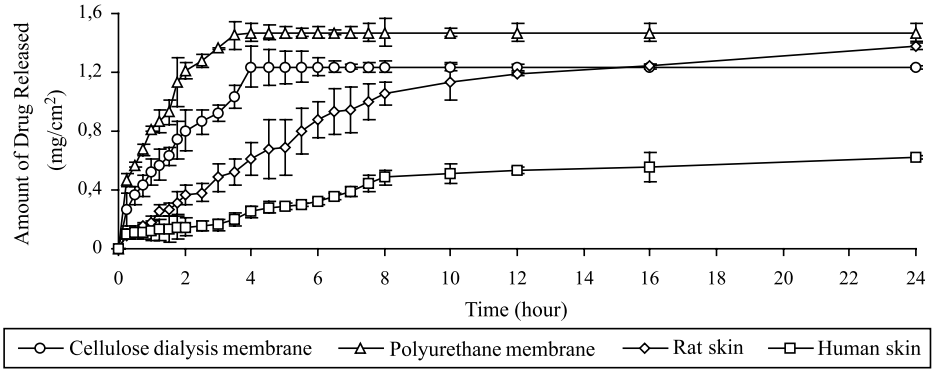

Figure 4. In vitro release of chlorpheniramine maleate from the F8 coded formulation by using different diffusion barriers.

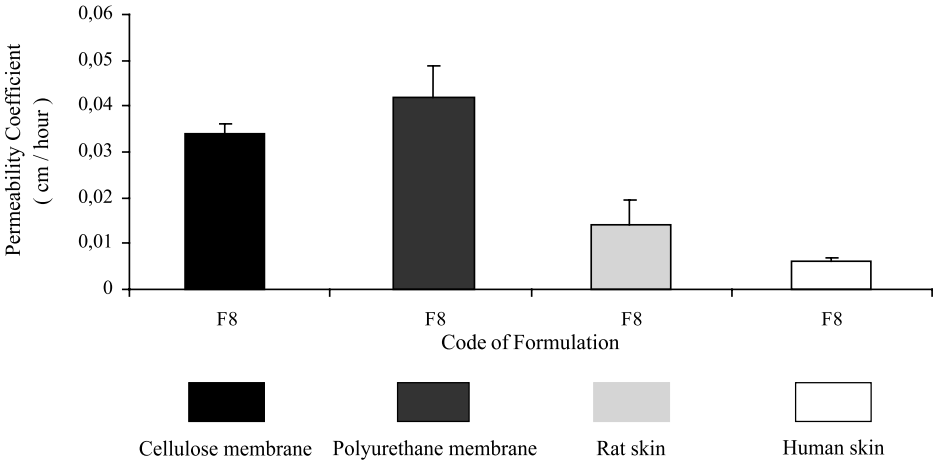

Figure 5. Permeability coefficient of chlorpheniramine maleate calculated from the in vitro data by using different diffusion barriers (All treatments were found statistically different when compared with each other, $p<0.05$.)

to the skin.[28] The rat skin was found to be more permeable than human skin ($p<0.05$) (Figs. 4 and 5). This can be attributed to the fact that CPM is an ionic molecule and penetrates the skin by trans-appendagel routes by means of hair follicules and sweat ducts.[29] As is known, rat skin has a greater number of hair follicules than human skin. Penetration enhancing effects of ethanol have been observed, especially with the use of rat skin. It was demonstrated that ethanol could enhance the skin flux of compounds primarily by a) increasing the drug solubility in the donor phase; b) increasing skin lipid fluidity, and c) forming new pores in the stratum corneum.[30] Thus the high lipid content of the rat skin may be easily perturbated by ethanol, which is incorporated in the formulations at a ratio of 30%. This make the active substance penetrate more easily from the rat skin compared to the human one.[14,31] But within the

Table 6. Kinetic parameters of the active substance calculated from the in vitro data by using cellulose dialysis membrane.

	1st order kinetic			0 order kinetic			$Q\sqrt{t}$ kinetic		
Code of formulation	k_0	r^2	SWSD	k_r	r^2	SWSD	K	r^2	SWSD
F1	0,2409	0,9695	0,4136	2,5772	0,9731	0,3778	1,3876	0,9844	0,5810
F2	0,1858	0,9784	0,4934	0,1971	0,9810	0,4695	1,0206	0,9836	0,1625
F3	0,1708	0,9932	0,2969	1,7789	0,9938	0,2469	4,8949	0,9759	0,6437
F4	0,2160	0,9798	0,4800	2,3070	0,9823	0,4475	1,2456	0,9886	0,2880
F5	0,1856	0,9782	0,3451	2,3041	0,9901	0,4214	1,2263	0,9869	0,3003
F6	0,1586	0,9870	0,1122	1,6555	0,9882	0,1037	5,3928	0,9799	0,3295
F7	0,2479	0,9876	0,4213	2,6648	0,9896	0,3801	1,4702	0,9867	0,5629
F8	0,2378	0,9783	0,8162	2,5705	0,9799	0,7630	1,6825	0,9796	0,3086
F9	0,2048	0,9604	0,6848	2,1869	0,9639	0,6527	1,2944	0,9835	0,1861
F10	0,2637	0,9740	0,3019	0,0283	0,9782	0,2650	1,4763	0,9956	0,8549
F11	0,1877	0,9848	0,3005	1,9845	0,9864	0,2809	8,9015	0,9826	0,2871
F12	0,1680	0,9955	0,1281	1,7603	0,9958	0,1175	6,1080	0,9749	0,3711
F13	0,2502	0,9979	0,3394	2,6885	0,9982	0,3003	1,4180	0,9764	0,7358
F14	0,2073	0,9891	0,2330	2,1973	0,9904	0,2116	9,6720	0,9813	0,4940
F15	0,1638	0,9898	0,1496	1,7170	0,9894	0,0139	6,0517	0,9599	0,3784
F16	0,2708	0,9646	0,4300	2,9259	0,9644	0,3808	1,6499	0,9381	0,1373
F17	0,1979	0,9665	0,3502	2,1039	0,9651	0,3250	9,9009	0,9453	0,5620
F18	0,1605	0,9812	0,5363	1,6710	0,9816	0,4783	4,7180	0,9717	0,5134

SWSD: sum of weight of squared deviation; r^2: determination coefficient; k_0: first order rate constant; k_r: zero order rate constant; K: $Q\sqrt{t}$ kinetic rate constant.

different diffusional barriers rat skin gave the closest results to human skin (Figs. 4 and 5). No correlation has been found between permeability characteristics of synthetic membranes and human skin. The poor drug release through the rat and human skin (Fig. 5) could be attributed to the fact that the complexity of the composition of the skin offered more resistance to the penetrating drug molecules during the diffusion process.[3]

In order to develop an ideal kinetic model to interpret diffusion rate data in terms of meaningful parameters, various kinetic models were applied to obtain the best fit of the data. The diffusion rate data were treated with first order, zero order, and $Q\sqrt{t}$ kinetics models. It has been found that generally the releases occur in accordance with zero order and $Q\sqrt{t}$ kinetics (Table 6). The fact that it is emitted in accordance with first order kinetics shows that the agent is distributed homogeneously in the formulations. According to the model developed by Higuchi, this harmony among kinetics is seen in case of homogenous distribution of the active agent in the formulations.[32]

In conclusion, CPM seems to be a suitable drug entity for possible development of gel dosage forms. By applying CPM via the skin route some side effects can be easily eliminated and desired therapeutic concentration of the active substance at the application site can be achieved. Carbomer type and concentration affected the drug release. The use of low concentrations of carbomer derivatives gaves the maximum drug release profiles. The penetration enhancer effect of ethanol has been observed, especially with the use of rat skin. Natural membranes must be used at the in vitro diffusion studies to predict the real drug release characteristic. The reasonable similarity between rat and human skin indicate that this species play an important role in research of transdermal absorption. As expected ppm levels of benzene residue did not affect the drug release characteristic. Thus, the use of benzene-free residue carbomer derivatives seems to be good alternative to benzene-residue derivatives for safe use, but long term toxicologic studies must be done.

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