Original Article

Transdermal patch incorporating salbutamol sulphate: *In vitro* and clinical characterization

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ABSTRACT: Eudragit patches containing salbutamol sulphate were prepared and evaluated as a rate-controlling membrane for transdermal use. The effect of different Eudragit polymers and various plasticizers on the permeability and mechanical properties of the prepared patches were studied. Drug patches of Eudragit polymers were prepared by a casting method employing methanol as a solvent and dibutylphthalate, polyethylene glycol 400, Propylene glycol, and triacetin as plasticizers. These patches were evaluated for weight and thickness uniformity, swelling index, tensile strength, percentage of elongation, and moisture absorption capacity. *In-vitro* release characteristics of these patches were studied and analyzed. The patches were found to have a uniform thickness. Patches prepared using Eudragit RS 100 (T_8) as well as RS100 + L100 in a ratio of 3:1 (T_{15}) plasticized with triacetin were found to have a tensile strength lower than that of other patches. Permeability characteristics of selected patches were studied. Patch formulations T_8 and T_{15} containing 10% oleic acid and 5% dimethyl formamide as penetration enhancers, respectively, displayed the highest permeability to salbutamol sulphate. These two formulations were selected for further clinical investigation and although both resulted in improvement in respiratory function tests, only the first formulation resulted in significant improvement.

Keywords: Salbutamol Sulphate, Transdermal patches, Asthma, Respiratory function

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1. Introduction

Salbutamol sulphate (SS) is widely used for the therapeutic management and prophylaxis of asthma and nocturnal asthma in particular (*1*). Although SS is considered to be the drug of choice for the treatment of asthma, it has several drawbacks such as its short biological half-life of about 4-6 hours (*2*) and its susceptibility to extensive first-pass metabolism, thus requiring frequent administrations by both oral and inhalation routes. It has a short duration of action, low peak plasma level of 1.2 µg/mL, and poor bioavailability of only 14.8% (*3*). These factors necessitated formulation of a controlled-release drug delivery system for SS.

A transdermal patch is a medicated adhesive patch that is placed on the skin to deliver a time-released dose of medication through the skin in order to treat systemic illnesses. Such a system offers a variety of significant clinical benefits over other methods of administration. For example, it provides controlled release of the drug to the patient and enables a steady blood-level profile, leading to reduced systemic side effects, and sometimes provides improved efficacy over other dosage forms (*4-6*). In addition, the dosage form of transdermal patches is user-friendly, convenient, and painless. The generally accepted view is that they offer improved patient compliance (*7*).

The present work is an attempt to incorporate SS into the transdermal drug delivery system (TDDS) employing various types of Eudragits. The aim is to monitor the release of SS to maintain its therapeutic levels and evaluate it clinically as well. Hence, SS was selected as it undergoes first-pass metabolism and has a short half-life, thus presenting a challenge in terms of achieving controlled transdermal delivery of SS.

2. Materials and Methods

2.1. *Materials*

SS was donated by Amoun Co., Cairo, Egypt. Eudragit L100-55, RS100, RL100, S100, and L100 were purchased from Rhom Pharm GmBH Weiterstadt, Germany. Triacetin, polyethylene glycol 400 (PEG 400), dibutylphthalate (DBP), *n*-octanol (NO), and dimethyl formamide (DMF) were purchased from Sigma-Aldrich, USA. Propylene glycol (PG), methanol, oleic acid (OA), sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, magnesium chloride, sodium nitrite, and potassium sulphate were purchased from Adwic, El-Nasr Chemical Co., Cairo, Egypt. Double-distilled water was used throughout the study.

2.2. *Preparation of salbutamol sulphate transdermal patches*

The transdermal patches of SS were prepared by a solvent casting technique using different types of Eudragit polymers (RS100, RL100, L100-55, L100 and S100) (*8*). An alcoholic solution of SS was prepared in which the weighed amount (5 mg) of the drug was dispersed in 10 mL methanol. Different Eudragit polymers (5%, w/v) were added to the alcoholic drug solution while stirring to ensure uniform distribution. Lastly, the plasticizer was added to protect the polymeric patches from brittleness upon storage. The plasticizers used were DBP, PEG 400, PG, and triacetin in different concentrations. The dispersion processes were prepared using a magnetic stirrer (Thermolyne Corporation, USA) providing constant stirring (500 rpm) at room temperature until clear solutions were obtained. The compositions of the tested transdermal patches are shown in Table 1.

Table 1. Composition of SS loaded Eudragit patches in each formula (%)

Formula	Eudragit type	Plasticizer	Plasticizer concentration (%)
T_1	RL100	PEG 400	40
T_2		PG	50
T_3		DBP	30
T_4		Triacetin	30
T_5	RS100	PEG 400	50
T_6		PG	50
T_7		DBP	30
T_8		Triacetin	20
T_9	L100-55	PEG 400	40
T_{10}		PG	50
T_{11}		Triacetin	30
T_{12}	RS100 + L100 (3:1)	PEG 400	40
T_{13}		PG	50
T_{14}		DBP	30
T_{15}		Triacetin	30
T_{16}	RS100 + S100 (3:1)	PEG 400	40
T_{17}		PG	50
T_{18}		DBP	30
T_{19}		Triacetin	30
T_{20}	RS 100 + RL100 (3:1)	DBP	30

Measured volumes (10 mL) of the polymeric solutions were poured onto a plastic substrate (circular dish of 57 mm^2 diameter and 8 mm depth) and dried on a level bench at room temperature for 24 h with an inverted funnel overhead to provide a uniform rate of evaporation. The formulated patches were allowed to equilibrate in a desiccator over anhydrous calcium chloride for another 24 h before the evaluation process to ensure total hydration and to exclude entrapped air (*9*). The patches were evaluated within one week of the casting date.

2.3. *In-vitro characterization of the prepared salbutamol sulphate transdermal patches*

2.3.1. *Uniformity of initial drug content*

For drug content determination, the total content of transdermal systems (n = 3) was placed in a 100 mL volumetric flask and dissolved in methanol. The solution was filtered through a Whatman filter membrane (0.45 µm) prior to spectrophotometric drug analysis at 276 nm (Shimadzu, model UV-1601 PC, Kyoto, Japan).

2.3.2. *Uniformity of patch weight and thickness*

Three randomly selected patches of each formulation were weighed and their average weight was calculated. Patch thickness was determined using calipers (Vernier Caliper, Shanghai, China) and recorded. Results were reported as the mean (± S.D.) of five measurements (the four corners and the center of each patch).

2.3.3. *Percent dissolution and swelling index (SI) of the transdermal patches*

The patches were dried in a desiccator over anhydrous calcium chloride at ambient temperature until a constant weight was obtained (W_1); then, they were immersed for 3 days in 100 mL distilled water at 37°C. Excess water present on the swollen patches was removed by careful patch blotting with filter paper. The patches were reweighed (W_2), returned to the dessicator, and dried to a constant weight; then, they were reweighed again (W_3) (*10*).

$$\% \text{ dissolution} = \frac{W_1 - W_3}{W_1} \times 100$$

The swelling index (SI) was determined from the amount of water absorbed per unit weight of undissolved patches retrieved from the distilled water after immersion (*10*).

$$SI = \frac{W_2 - W_3}{W_3} \times 100$$

Results were reported as the mean (± S.D.) of three replicates.

2.3.4. *Moisture absorption capacity of the patches*

The water absorption capacities of various films were determined at 33, 65, and 97% relative humidity (RH). Films were cut into 1 × 1 cm strips. The strips were conditioned by weighing; they were placed in a dessicator at 40°C for 24 h, removed, and exposed to conditions of 33% RH (containing saturated solution of magnesium chloride), 65% RH (containing saturated solution of sodium nitrate), and 97% RH (containing saturated solution of potassium sulphate) in different desiccators at room temperature. Weight was measured periodically every 48 h for 14 days until a constant weight was obtained. The moisture absorption capacity of the films (weight %) was calculated in terms of percentage increase in the weight of film over the initial weight of the specimen (*11*,*12*).

2.3.5. *Mechanical properties of salbutamol sulphate patches*

The tensile strength, the film's percentage elongation at break, and the modulus of elasticity (Young's Modulus) were determined using a tensile strength tester (TN-30 code N 9112-ID, India). Patch strips 1 cm in width were grasped using an upper and lower flat-faced metal grip laminated with a smooth rubber grip. The distance between the grips was set at 2 cm and this distance, therefore, represented the length of the film under stress. A speed of 5 mm/s was used for all measurements (*13*).

The load applied to the patch was automatically increased at a specific rate until the patch broke. Only results from films that were observed to break in the middle area of the test strip during testing were used. Results were reported as the mean (± S.D.) of five replicates. The tensile strength and elongation at break were calculated as below:

The percentage elongation at break, E_b, of tested films was determined, where E is the film's extension to break and L_0 is its original length (*14*).

$$E_b = [E/L_o] \times 100$$

The break strength, B, of tested films was determined, where F is the break force of the film and A_R is its cross sectional area (*14*).

$$B = F/A_R$$

The modulus of elasticity (M_E) of the patch was calculated from Hook's law (*15*).

$$B = M_E / (E/L_o)$$

2.3.6. *In-vitro release studies*

The *in-vitro* release of transdermal patches was performed with a paddle over disk method, in accordance with the US Pharmacopoeia (USP 27 apparatus 5) (*16*). Briefly, a volume of 250 mL freshly prepared Sorenson's phosphate buffer pH 5.5 (dissolution medium) was placed in the vessel and the temperature of the medium was equilibrated to 32 ± 0.5°C. A patch sample on its plastic substrate was covered with a stainless steel screen disc (mesh size 100 µm) of the same size, with the release surface facing up. The assembly was prevented from floating and hitting the rotating paddle by attaching a glass disc to the bottom of the plastic substrate using cyanoacrylate adhesive. The paddle was then rotated at 50 rpm.

At predetermined time intervals over a total period of 8 h, aliquots (5 mL) were withdrawn and replaced with fresh medium. The samples were filtered through 0.45 µm Whatman filter membranes and spectrophotometrically analyzed for drug content at 276 nm. The results were the mean values of three runs. Cumulative amounts of drug released were plotted against time for different formulations. The obtained data were subsequently analyzed to determine the order of release.

2.4. *In-vitro permeation studies of salbutamol patches*

Abdominal skin (approximately 1 mm in thickness) from male newborn mice (age 6 days or younger) was carefully excised (Cairo University Labs, Cairo, Egypt). All animals were treated in accordance with the principles of laboratory animal care (Guide for the Care and Use of Laboratory Animals, 1985) (*17*).

After removing the hypodermal adipose tissue, the skin was used as a barrier membrane for *in-vitro* transdermal permeation. When not used immediately, the skin was kept refrigerated (2-5ºC) and used within 3 days (*18*). *In-vitro* mice skin permeation studies were performed in vertical Franz-type glass single diffusion cells (Keshary-Chien type) (*19-21*). The volume of the receptor cell was 17 mL and the effective surface area available for permeation was 3.14 cm^2. Briefly, the freshly excised mice skin was mounted between the donor and receptor cells such that the epidermal surface faced the donor compartment. Each prepared patch was placed on the stratum cornium side of the skin, after which the receptor cells were filled with PBS and thermostated at 37ºC by placement in a water bath. The hydrodynamics of the receptor fluid were maintained by stirring the fluid at 600 rpm in order to prevent any boundary layer effects. At predetermined time intervals over a period of 24 h, the receptor solution was sampled (200 µL), filtered through a 0.25-µm filter membrane, and analyzed by HPLC in order to determine the extent of the permeated drug. Briefly, SS concentration was analyzed using a reverse-phase HPLC method in order to determine the extent of the permeated drug. A Shimadzu HPLC system including a solvent delivery

pump (Shimadzu LC-10AT), a controller (Shimadzu SCL-10A), and a UV detector (Shimadzu SPD-10A) was used in this study. A 3.9 × 150 mm long NOVA-Pack C_{18} 60A, 4U, cartridge column (Agilent C) with a particle diameter of 3.5 µm was used. During the assay, SS was eluted isocratically at a flow rate of 1.2 mL/min and monitored with a UV detector operating at 276 nm. The mobile phase for the assay consisted of a mixture of water, methanol, and acetonitrile (70:20:10, v/v) pH-adjusted to 2.5 by 10% phosphoric acid. The run time for the assay was 10 min, and the retention time for SS was 3.0 min (*22*).

The same volume of fresh PBS was supplied to the receptor after each sampling. Each permeation experiment was replicated at least 3 times.

Three permeation enhancers, namely 10% OA, 5% DMF, and 5% NO (%w/w of the dry polymer weigh), were incorporated separately into selected patch formulations (T_8 and T_{15}) that produced optimum results in all previous tests. Data on the permeation of SS through hairless mice skin was graphically plotted as the cumulative amount of the permeated drug per unit area as a function of time, from which the permeation parameters were calculated including the cumulative amount of the permeated drug per unit area after 24 h (Q_{24}), steady state flux (J_{ss}), apparent permeability coefficient ($P_{app.}$), lag time (t_{lag}), diffusion constant (D), and the enhancement ratio for the permeability coefficient (ER) (*23-25*).

2.5. *Determination of clinical efficacy of the selected formulations*

Two optimum formulations (according to the *in-vitro* parameters) were selected for further clinical investigation in asthmatic patients after they satisfied optimum physical, mechanical, and release parameters.

Subjects: Subjects were selected from 30 adult patients who were newly admitted to the Chest Department of Ain shams University Hospitals and the ICU of El-Kasr El-Aini Teaching Hospital complaining of asthmatic attacks. Patients were recruited to investigate the clinical efficacy of selected patches and all had to be diagnosed with acute asthma to serve as subjects. All selected asthmatics (mild and moderate) were non-smokers and met the criteria mentioned in the new Egyptian guidelines for the diagnosis and management of asthma (*26*). The study was approved by the Ethics Committee of both Ain Shams University Hospital and El-Kasr El-Aini Teaching Hospital and the research followed the tenets of the Declaration of Helsinki promulgated in 1964.

Protocol: The study adopted an open randomized, parallel design. All patients were subjected to the following after providing written consent:

An initial screening that included a medical history, physical examination, vital signs, ECG, plain chest X-ray, liver function tests, kidney function tests, and fasting and postprandial blood glucose levels.

Pulmonary function tests with a spirometer (Cosmed Pony Graphics version 3.2 E-MB) at the Pulmonary Function Laboratory of the Chest Department and Critical Care Unit at Ain-Shams University Hospital and Kasr El-Aini Hospital, respectively; tests included FVC, FEV_1, and FEV_1/FVC. All pulmonary function tests were performed for each subject before and after the study.

To objectively assess the impact of intervention, these parameters were scored and a composite score was then calculated. Heart rate and respiratory rate were counted and scored.

NB: The exclusion criteria included evidence of acute or chronic infection, pregnancy, breast-feeding or any chronic medical illness other than asthma, oral steroid therapy, and $FEV_1 < 60\%$ of the predicted value (Severe asthmatic patients).

The recruited patients were randomly classified into two groups:

Group I: 15 patients receiving formulation T_8/OA (equivalent to 5 mg Salbutamol).

Group II: 15 patients receiving formulation T_{15}/DMF (equivalent to 5 mg Salbutamol).

The transdermal patch was applied onto the anterior surface of the forearm near the elbow. The patients were instructed not to remove the patch and also to look for any sign of irritation at the site of application. The patch was removed after 24 h. At the conclusion of the study, a physical examination including vital signs and ECG were re-performed. The patients were discharged after suitable medication to ensure a reasonably safe FEV_1 and were asked to continue with their regular medication.

2.6. *Statistical analysis*

Unless indicated, results are presented as mean ± standard deviation (S.D.). One-way analysis of variance. (ANOVA) was used to determine significance among groups, after which post hoc tests with the Bonferroni correction were used for comparison between individual groups. Other statistical comparisons were done by the Mann-Whitney test for nominal continuous data, Wilcoxon signed rank test to compare mean differences in the data, and the Chi-squared (X^2) test for categorical data; a p value of < 0.05 was considered significant. Statistical Package for Social Science (SPSS) was used for data analysis.

3. Results and Discussion

3.1. *In-vitro characterization of the prepared salbutamol sulphate transdermal patches*

3.1.1. *Uniformity of initial drug content, weight, and thickness*

SS patches were evaluated for their physical parameters

Table 2. Physical characterization, drug content, % dissolution and SI of SS transdermal patches

Formula	Drug content (% ± S.D.)	Uniformity of weigh (gm) (average ± S.D.)	Uniformity of thickness (mm) (average ± S.D.)	% Dissolution (average ± S.D.)	SI (average ± S.D.)
T1	91.62 ± 0.877	0.547 ± 0.076	0.131 ± 0.003	14.82 ± 0.255	1.362 ± 0.172
T2	9.31 ± 0.990[a]	0.525 ± 0.033	0.154 ± 0.030	14.38 ± 0.566	1.858 ± 0.215
T3	94.47 ± 2.046	0.507 ± 0.068	0.144 ± 0.023	11.18 ± 2.574	0.403 ± 0.088
T4	88.24 ± 1.782	0.523 ± 0.016	0.135 ± 0.038	14.32 ± 0.976	0.175 ± 0.011
T5	N/A	N/A	N/A	N/A	N/A
T6	89.52 ± 1.810	0.678 ± 0.017	0.162 ± 0.055	5.57 ± 0.325	0.0814 ± 0.008
T7	92.61 ± 3.521	0.491 ± 0.014	0.156 ± 0.023	1.876 ± 0.461[b]	0.398 ± 0.020
T8	94.23 ± 1.541	0.603 ± 0.008	0.158 ± 0.011	3.955 ± 0.516	0.255 ± 0.059
T9	N/A[c]	N/A	N/A	N/A	N/A
T10	88.35 ± 0.495	0.563 ± 0.059	0.163 ± 0.003	24.02 ± 0.679[b]	0.503 ± 0.041
T11	89.24 ± 2.503	0.573 ± 0.008	0.146 ± 0.006	15.92 ± 0.551	0.529 ± 0.037
T12	90.24 ± 2.630	0.549 ± 0.038	0.138 ± 0.006	11.51 ± 0.707	0.438 ± 0.088
T13	93.61 ± 5.020	0.622 ± 0.013	0.129 ± 0.003	12.75 ± 0.650	0.282 ± 0.030
T14	92.57 ± 0.792	0.603 ± 0.012	0.157 ± 0.004	4.38 ± 1.032[b]	0.149 ± 0.038
T15	88.73 ± 1.457	0.628 ± 0.023	0.159 ± 0.001	4.74 ± 0.720	0.087 ± 0.018
T16	N/A	N/A	N/A	N/A	N/A
T17	N/A	N/A	N/A	N/A	N/A
T18	N/A	N/A	N/A	N/A	N/A
T19	96.23 ± 2.701	0.628 ± 0.054	0.162 ± 0.006	5.97 ± 0.481	0.25 ± 0.071
T20	94.36 ± 1.754	0.622 ± 0.027	0.148 ± 0.004	13.95 ± 0.525	1.355 ± 0.205

[a] Statistically significant, ANOVA, $p < 0.05$; [b] Statistically significant, ANOVA, $p < 0.001$; [c] Not applicable.

(thickness and weight uniformity, as well as drug content). The drug content analysis of the prepared formulations showed that the process employed to prepare patches in this study was capable of providing films with a uniform drug content and minimum batch variability. All the prepared patches complied with the pharmacopoeial limits for content uniformity (*27*). The prepared patches had a thickness ranging from 0.129 to 0.163 mm, and their weight was uniform, varying from 0.491 to 0.678 gm/patch (Table 2). These ranges are suitable for application to the skin as reported by Clearly (*28*).

3.1.2. *Percent dissolution and swelling index (SI) of the transdermal patches*

The incorporation of plasticizers in Eudragit patches has weakened its resistance to solubility in distilled water. This can be attributed to the fact that plasticizer molecules increase the flexibility of Eudragit molecules and render the patches more permeable to the water molecules (*29*). Of the plasticizers used, PG was found to be the most effective in reducing the water resistance of Eudragit patches while DBP was the least effective (Table 2).

As is apparent from Table 2, the percent dissolution increased with the incorporation of Eudragit RL100 (T_1-T_4) and L100-55 (T_{10} and T_{11}) compared to the patches prepared with RS100 (T_6-T_8). Moreover, addition of Eudragits L100, S100, and RL100 to RS100 in the prepared patches (T_{12}-T_{20}) in a ratio of 1:3 led to increased percent dissolution. This may be attributed to the increase in the freely permeable resin in water as a result of using these polymers (*30*).

The water uptake capacity of the patch was measured by the swelling index (SI). The data in Table 2 revealed that transdermal patches containing Eudragit L100-55 and RL100 exhibited the highest SI in comparison to other formulations. These results suggest that these patches would be more permeable to the drug. This may be due to the porosity generated in the remnants of the patches after dissolution (*31*).

3.1.3. *Moisture absorption capacity of the patches*

Moisture absorption of polymeric patches affects both the mechanical properties and the drug release pattern. Moisture absorption capacities under different humidity conditions (Figure 1) revealed that the moisture uptake of the patches depended on the type of both Eudragit and plasticizer used.

Moisture absorption in 97% RH is relatively high and the weight of most patches significantly increased in comparison to other levels of RH. The highest absorption capacities within 2 weeks were 4.221%, 7.325%, and 10.73% for those prepared using Eudragit L100-55 and PG while the lowest (1.811, 3.993 and 6.304) were recorded for Eudragit RS100 patches containing DBP at 33%, 65%, and 97% RH, respectively.

As is apparent, Eudragit L100-55 and RL100 formulated patches plasticized with any of the aforementioned plasticizers absorbed water to a greater extent than did patches containing Eudragit RS100. Also obvious is the fact that inclusion of Eudragits L100, S100, or RL100 in RS100 led to increased water absorbing ability of the prepared patches. This could be due to the hydrophilic nature of these Eudragits compared to Eudragit RS100 alone. This hydrophilic nature may be attributed to the fact that Eudragit RL polymers contain double the quaternary ammonium groups of Eudragit RS. Moreover, Eudragit L100-55 is a free-flowing powder that is redispersible in water and dissolves above pH 5.5. The higher methacrylic acid content of Eudragits L100 and S100 increases their hydrophilic characteristics (*32*).

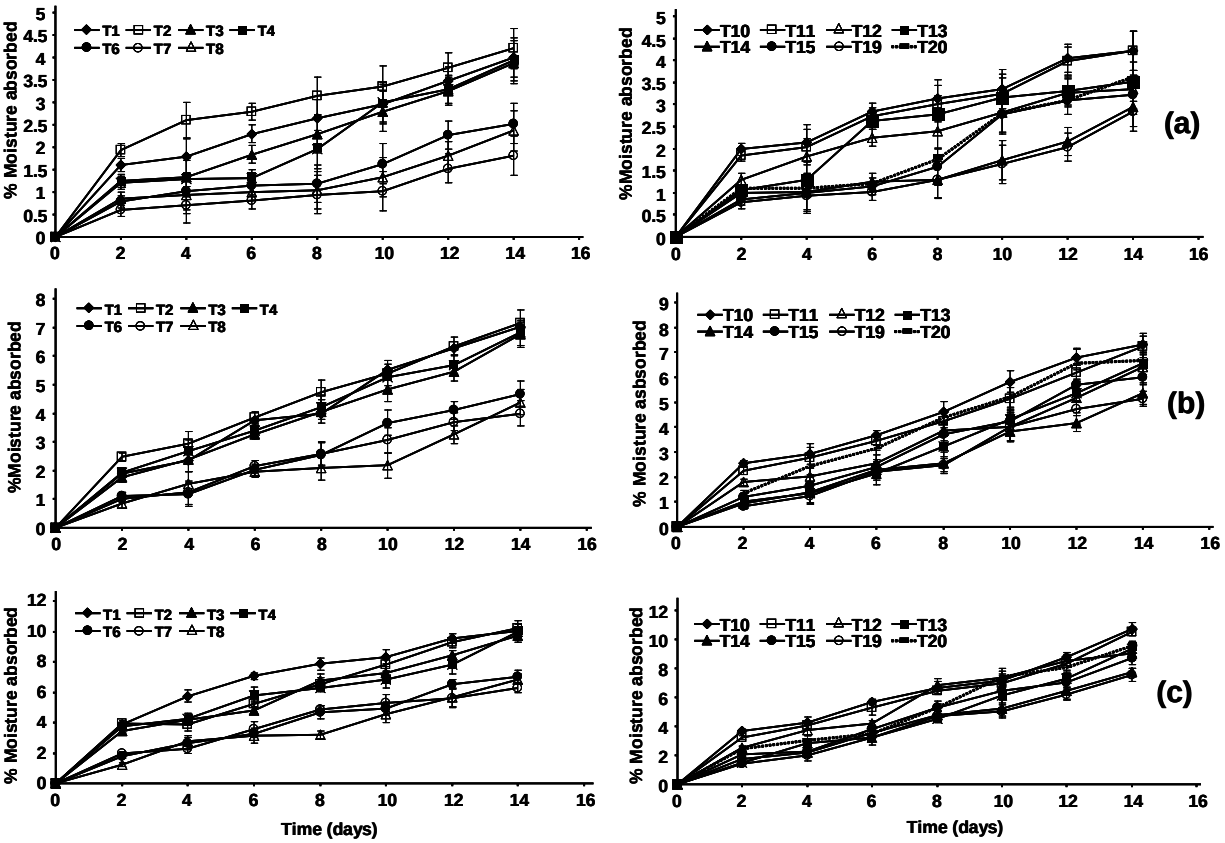

Figure 1. Moisture absorption capacity of salbutamol sulphate transdermal patches at a: 33 % RH, b: 65% RH and c: 97% RH.

Table 3. Mechanical properties of salbutamol sulphate transdermal patches

Formula	Elongation % (average ± S.D.)	Tensile strength (Kg/cm^2) (average ± S.D.)	Modulus of elasticity (average ± S.D.)
T_1	4.19 ± 0.438	1.205 ± 0.177[b]	28.75 ± 2.475[b]
T_2	9.27 ± 0.325	0.62 ± 0.042	6.62 ± 0.552[b]
T_3	24.95 ± 1.500	0.071 ± 0.016	0.284 ± 0.040
T_4	16.98 ± 0.480	0.105 ± 0.057	0.618 ± 0.1457
T_5	N/A[c]	N/A	N/A
T_6	76.03 ± 1.541[b]	0.0652 ± 0.008	0.0857 ± 0.008
T_7	91.85 ± 1.154	0.055 ± 0.014	0.0598 ± 0.003
T_8	208.22 ± 2.531[b]	0.015 ± 0.007	0.0072 ± 0.003
T_9	N/A	N/A	N/A
T_{10}	38.4 ± 0.990[b]	0.0659 ± 0.023	0.1716 ± 0.042
T_{11}	14.37 ± 1.1880	0.11 ± 0.109	0.765 ± 0.066
T_{12}	11.85 ± 0.976	0.3125 ± 0.141	2.637 ± 0.528
T_{13}	22.71 ± 1.640	0.72 ± 0.242	3.17 ± 0.467
T_{14}	87.11 ± 1.432	0.06 ± 0.038	0.0689 ± 0.017
T_{15}	197.99 ± 2.84[b]	0.04 ± 0.008	0.0202 ± 0.002
T_{16}	N/A	N/A	N/A
T_{17}	N/A	N/A	N/A
T_{18}	N/A	N/A	N/A
T_{19}	20.19 ± 1.782	0.075 ± 0.021	0.371 ± 0.058
T_{20}	12.303 ± 1.427	0.13 ± 0.143	1.0566 ± 0.514

[a] Statistically significant, ANOVA, $p < 0.05$; [b] Statistically significant, ANOVA, $p < 0.01$; [c] Not applicable.

Also of note is the fact that lower water absorption capacity was achieved in the presence of DBP than with the other plasticizers used. This is possibly attributed to the limited water affinity of DBP (*33*).

3.1.4. *Mechanical properties of salbutamol sulphate transdermal patches*

The physicomechanical properties of patches are among the factors that determine the suitability and acceptability of prepared patches. The tensile strength, % elongation, and modulus of elasticity were determined for the prepared patches. All results of mechanical properties are shown in Table 3. The tensile strength ranged from 0.015 kg/cm^2 for T_8 to 1.205 kg/cm^2 for T_1. Percent elongation ranged from 4.19% for T_1 to 208.22% for T_8. Optimum mechanical properties were clearly obtained from transdermal SS patches (T_8) containing RS100 and

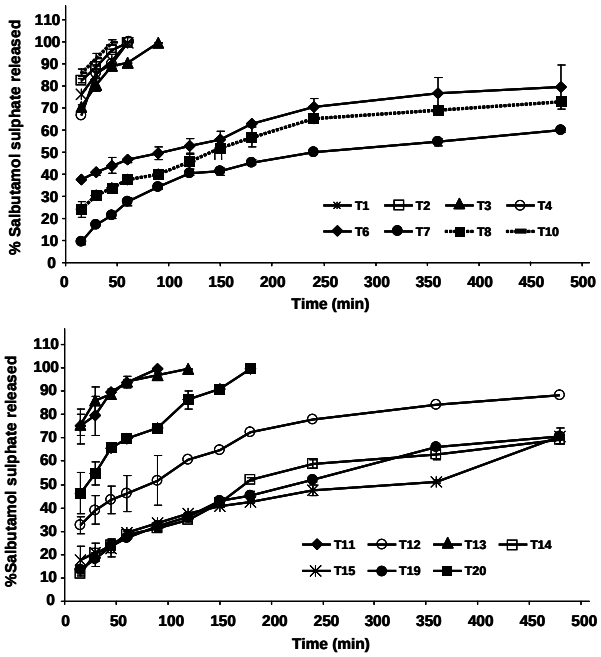

Figure 2. *In-vitro* release profiles of salbutamol sulphate in Sorensen's phosphate buffer (pH 5.5) from patch formulation (T_1-T_{20}).

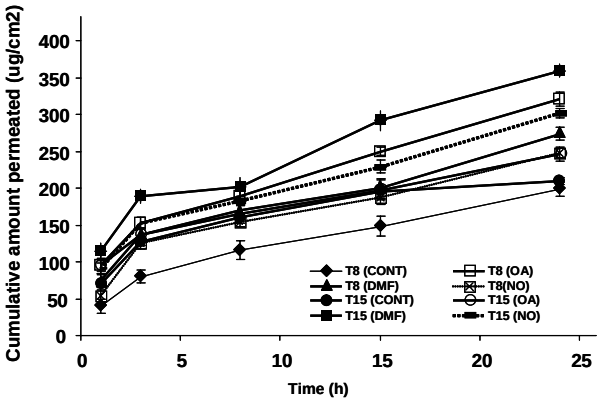

Figure 3. Permeation profiles of salbutamol sulphate from patches a) T_8 and b) T_{15} containing different enhancers through hairless mice skin compared to control.

triacetin, followed by patches (T_{15}) containing RS100 + L100 and triacetin. This could be due to the high affinity of triacetin for water, which contributes to its elongation ability.

3.1.5. *In-vitro release of salbutamol sulphate from the prepared patches*

Drug release testing is a crucial part of the development of transdermal patches as it helps to ensure the batch-to-batch uniformity of each drug delivery system and to evaluate the release rate of the drug from the prepared formulations (*34*). Even though body temperature is maintained at 37°C, the temperature of the skin surface is 32°C (*35*). This is why the temperature of the dissolution medium was kept at 32 ± 0.5°C. Sorensen's phosphate buffer of pH 5.5, used as a dissolution medium, simulated the pH of the skin surface (*36*).

As is apparent in Figures 2 and 3, the amount of SS released from patches T_{10} was significantly higher ($p < 0.001$) than that released from other patches as it reached ~100% within 45 min. This might be due to the high solubility of Eudragit L100-55 in solutions of pH 5.5 and the high water affinity of PG plasticizer. A point of note is that Eudragit RL100 patches had a higher release of drug than those prepared with Eudragit RS100. This could be attributed to the lower content of quaternary ammonium groups in Eudragit RS100 than in RL100, resulting in less swelling in aqueous media. Thus, it is extensively employed in the pharmaceutical industry because of its potential for the development of controlled-release drug delivery systems (*32*).

In addition, inclusion of Eudragit RL100 to RS100 (1:3) (T_{20}) led to a slight reduction in the release profile of the drug (~100% within 180 min) compared to the use of RL100 alone. In contrast, incorporation of Eudragit L100 and S100 in RS100 (1:3) (T_{20}) led to a great reduction in the release profile of the drug compared to the use of RL100 alone but still provided a higher release profile than patches containing RS100 alone. Moreover, PG plasticizer resulted in a higher release rate of the drug, followed by PEG and then triacetin and finally DPB. Patches T_7 and T_{14} plasticized with DBP clearly exhibited significantly lower release rates of the drug (59.975 and 69.425%, respectively). That said, a better release profile was provided by Eudragit RS100 plasticized with triacetin (T_8) and Eudragit RS100 + L100 in (3:1) ratio plasticized with triacetin (T_{15}), as they achieved 72.97 and 70.82% within 8 h, respectively.

Linear regression analysis of release data was done to determine the proper order of release. Zero-, first-, and Higuchi diffusion-controlled model equations were applied to all *in-vitro* release results, indicating that the drug is released from all transdermal patches *via* a diffusion-controlled mechanism.

3.2. *In-vitro drug permeation studies*

The *in-vitro* release studies conducted revealed that polymeric patches prepared using RS100 with 20% triacetin (T_8) and a combination of RS100 and L100 in a ratio of 3:1 (T_{15}) with 30% triacetin as a plasticizer were found to be the most suitable with respect to drug content and all of the physical parameters evaluated (Table 2). Thus, the patches T_8 and T_{15} were considered for further *in-vitro* permeation studies.

The major rate-limiting step for the transport of hydrophilic drugs is their permeation through the stratum cornium. Once they have permeated through the stratum cornium, they are rapidly absorbed into the systemic circulation. As a result, hydrophilic drugs elicit poor local pharmacological response due to low retentivity in the skin layers (*37*). Chemical enhancers are known to enhance the influx of hydrophilic drugs across the stratum cornium. Table 4 summarizes the

Table 4. Percutaneous penetration parameters of salbutamol sulphate across abdominal mouse skin from different transdermal patches

Formula	Q_{24} (µg/cm^2)	J_{ss} (µg/cm^2•h)	Lag time t_{lag} (min)	$P_{app.}$ (cm/h) × 10^{-2}	D (cm^2/h) × 10^{-4}	ER_{sal}
T_8 control[a]	198.52 ± 2.121	6.35 ± 0.212	52.79 ± 0.311	1.27 ± 0.071	3.83 ± 0.255	----
T_8 (OA)[a]	320.81 ± 1.853†	9.14 ± 0.184	49.61 ± 0.834	1.82 ± 0.240	4.08 ± 0.212	1.43 ± 0.099
T_8 (DMF)[a]	273.61 ± 3.677†	7.56 ± 0.775	49.69 ± 2.376	1.51 ± 0.240	4.07 ± 0.622	18 ± 0.198
T_8 (NO)[a]	238.32 ± 5.968	7.3 ± 0.962	51.34 ± 0.962	1.46 ± 0.325	3.94 ± 0.382	1.15 ± 0.228
T_{15} control[b]	209.73 ± 3.224	5.41 ± 0.410	54.69 ± 1.725	1.08 ± 0.116	3.70 ± 0.438	----
T_{15} (OA)[bc]	245.47 ± 1.626	5.96 ± 0.071	55.39 ± 2.022	1.19 ± 0.057	3.65 ± 0.495	1.10 ± 0.3
T_{15} (DMF)[bd]	349.36 ± 1.202†	9.86 ± 0.212	49.38 ± 1.047	1.97 ± 0.057	4.10 ± 0.297	1.82 ± 0.269
T_{15} (NO)[be]	301.84 ± 1.782†	8.26 ± 1.202	51.73 ± 2.786	1.65 ± 0.212	3.91 ± 0.297	1.52 ± 0.283

[a] T_8: polymeric patch prepared using RS100 + 20% triacetin; [b] T_{15}: polymeric patch prepared using combination of RS100 and L100 in the ratio of 3:1 + 30% triacetin; [c] OA: 10% oleic acid; [d] DMF: 5% dimethyl formamide; [e] NO: 5% *n*-octanol.

Table 5. Demographic data, heart rate and respiratory rate for the recruited patients and their pulmonary function tests both before and after patch application

Category	**T_8**		**T_{15}**	
No. of patients (%)	15 (55.56)		12 (44.44)	
No. of males (%)	5 (30)		4 (30)	
Mean of age in years (SD)[a]	34.1 (7.82)		28.0 (4.55)	
Mean disease duration/year (SD)	5.6 (2.22)		4.75 (2.31)	
No. of wheezing patients (%)	6 (40)		6 (50)	
Mean of H.R (bpm) (SD)	93.0 (11.04)		104.13 (12.74)	
Mean of R.R (bpm) SD)	23.95 (5.66)		26.56 (7.48)	
Pulmonary function tests	Before	After	Before	After
FVC%[d]	83.0 (5.40)	89.4 (7.00)[c]	84.0 (5.21)	87.0 (8.28)
FEV_1[e]	61.6 (3.91)	81.5 (17.0)[b]	64.75 (8.68)	78.63 (15.09)
FEV_1/FVC%	62.71 (3.91)	75.11 (10.34)[c]	62.56 (4.21)	75.23 (10.68)[b]

[a] SD = Standard deviation; [b] Statistical Significant at $p < 0.05$, Wicoxon signed ranks test; [c] Statistical Significant at $p < 0.01$, Wicoxon signed ranks test; [d] FVC: Forced vital capacity; [e] FEV_1: Forced expiratory volume in one second.

effect of enhancers (viz OA, DMF and NO) on the steady-state flux (J_{ss}) and permeability coefficient of SS as well as the lag time, diffusion coefficient (D), and enhancement factor (ER). Moreover, the cumulative amounts of the drug at different diffusion times are shown in Figure 1. Among the various types of enhancers studied, OA for formulation T_8 and DMF for patch formulation T_{15} provided a higher permeability coefficient and enhancement factor (ER).

An interesting finding is that OA interacts with and modifies the lipid domains of the stratum corneum because of its similar structure to these lipids (*21*). Electron microscopic studies have shown that OA in human stratum corneum exists as a separate phase (or as 'pools') within the bilayer lipids (*38,39*). The formation of such pools would result in permeability defects within the bilayer lipids, thus facilitating permeation of hydrophilic permeants through the membrane.

Considering the small, highly polar nature of DMF, it may interact with the head groups of some lipid bilayers to disrupt its backing geometry. Furthermore, DMF in skin may facilitate drug partitioning from the formulation into the skin; one study reported a 12-fold increase in the flux of caffeine permeating across human skin treated with DMF (*40*).

3.3. *Determination of clinical efficacy of the selected formulations*

The two finally selected patches, formulation T_8 containing 10% OA and T_{15} containing 5% DMF, were tried clinically on 30 acute asthmatic patients. These formulations were found to be the most appropriate with respect to drug content and all of the physical parameters evaluated, and they also exhibited superior *in-vitro* release behavior and a higher permeability coefficient and enhancement factor (ER).

Out of the 30 recruited patients, 27 completed the study and 3 dropped out because their condition was exacerbated and they required nebulizers. The 27 patients that completed the study had an average age of 31.4 ± 7.11 years, ranging from 23 to 50 years (median = 30.5 years). Patients' conditions were classified as mild to moderate bronchial asthma with average disease duration of 5.2 ± 2.24 years, ranging from 2 to 9 years (median = 5 years). Baseline characteristics are shown in Table 5. There was a preponderance of females (about 2:1) in both groups. The subjects' baseline demographic parameters, vital signs, and spirometric parameters were comparable ($p < 0.05$).

Twenty four hours after drug administration, all of the studied parameters (FEV_1%, FVC% and FEV_1/FVC) improved significantly ($p < 0.05$) in the first group (with patch T_8). Those patients treated with patch T_{15} showed significant improvement ($p < 0.05$) in their FEV_1/FVC alone and showed non-significant improvement in both FEV_1% and FVC% (Table 5).

None of the recruited patients experienced any untoward effect or discomfort during and up to a week after the study period. No signs of skin reactions were seen at the site of application in any of the patients.

Quite clear from the reported results is the fact that patches prepared using RS100 and L100 in a ratio of

3:1 (T_{15}) with 30% triacetin with OA as a penetration enhancer had optimum physiochemical properties together with the greatest clinical improvement among all the tested patches.

4. Conclusion

The films of SS obtained by the solvent casting method had acceptable mechanical characteristics and satisfactory % drug release. The prepared films were transparent and had a smooth surface without any interactions between the drug and polymer.

The study demonstrates the feasibility of formulating transdermal drug delivery systems to deliver SS as part of asthma management. The transdermal formulations were found to be safe and non-reactive. Transdermal delivery of SS appears to be a better route for patients who respond well to β-agonists. In light of the present results, formulations for the transdermal delivery of SS should be further improved for durations up to several days. This is especially relevant in a country like Egypt, where inhalers are too expensive devices to be routinely used by asthmatic patients and where the inhalation technique is not adequately implemented.

Acknowledgement

The authors would like to thank Amoun Co., Cairo, Egypt, for their donation of salbutamol sulphate.

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(*Received June 9, 2008; Revised September 5, 2008; Accepted September 13, 2008*)