*Available online www.jocpr.com*

**Journal of Chemical and Pharmaceutical Research, 2021, 13(8):32-53**

**Research Article**

**ISSN : 0975-7384**
**CODEN(USA) : JCPRC5**

# Formulation and *In Vitro* Evaluation of Salbutamol Transdermal Patches

**Ali BA[1*], Ali MA[2]**

[1]*Department of Pharmacy, University of Al-Gand, Aden, Yemen*

[2]*Department of Pharmaceutics, University of Science and Technology, Sanaa, Yemen*

---

***ABSTRACT***

*Peroral salbutamol has been widely used to treat bronchospasm which acts by stimulating β-adrenergic receptors. Peroral salbutamol undergoes first pass metabolism extensively to inactive metabolite, shorter biological half-life and administered four times daily that leads to patient incompliance. The low dose, low plasma concentration and low molecular weight with pharmacokinetic limitations make it ideal candidate for transdermal drug delivery systems. However, its low permeability is the main barrier for using it as transdermal patches but it may be solved by adding penetration enhancers. Hence, the aim of this study was to formulate, develop, in vitro evaluate and optimize transdermal patches of salbutamol using different concentrations of film forming and bio adhesive polymers with different penetration enhancers and plasticizer for controlled release of salbutamol and thus to increase the bioavailability of the drug.*

*The evaluation of the possible interactions between salbutamol and the excipients used in this study was carried out using UV spectrophotometry. Then penetration enhancers (anise and eucalyptol oils) effects of different concentrations were evaluated by modified Franz diffusion cell of salbutamol solution through the rabbit skin.*
*The eight transdermal films were prepared by solvent evaporation technique using film-forming polymer as hydroxypolymethyl cellulose, in combination with bioadhesive polymer as sodium alginate with penetration enhancers (anise and eucalyptol oils) and propylene glycol as plasticizer in different ratios. Effects of polymer type, proportion and combination with the effect of penetration enhancer were studied on the physicochemical characterizations, bioadhesion, drug release and skin irritation. The physicochemical parameters like thickness, drug content, weight variation, moisture absorption and loss were evaluated for the prepared patches. In addition, the in vitro drug release studies were done using dissolution apparatus v. The obtained UV results showed that salbutamol was compatible with all excipients used in this study and showed that the characteristic λ max of the drug at 200 nm and for mixtures showed neither peak shift nor peak intensity changes were recorded. The diffusion results of salbutamol solution through rabbit skin were most satisfactory when using only 1% for both anise and eucalyptol oils. The preliminary formulated films were good appearance with suitable elasticity and any defected films were excluded.*

*All eight designed transdermal films showed good physical properties that showed no cracks or any other problems. No significant difference in thickness, average weight and in the drug content among the patches of the same formula. All formulations had good physicochemical characteristics and exhibited suitable bioadhesion strength. Results of moisture content and uptake indicated that the films had higher values due to propylene glycol so they must be stored in airtight containers. The percentage release of salbutamol was optimum for F3 and F6 films that was less than 70% at the end of 12 hours. On the other hand, other films showed rapid release after 8 hrs. The in vitro release data was treated with kinetic equations and it followed Higuchi's diffusion mechanism. It was observed that no skin irritation and hence patient compliance was expected. The present study can be concluded that transdermal patch of salbutamol can extend the release of drug for many hours with better expected bioavailability*

Ethyl alcohol was purchased from Al-Shamel Chemicals Co. Sana'a, Yemen. All aqueous solutions were prepared from distilled water. All other chemicals were of analytical grade and provided by Al-Hikma university laboratories, Taiz, Yemen [2].

UV spectrophotometer, Dissolution tester type V, Electronic Balance, Water purifier, Electronic Digital Caliber, Modified Physical Balance (Personal modification), Local Modified Franz Cell, Digital pH Meter, Dry Heat Oven, Heat Magnetic Stirrer and all glassware used were supplied by Al-Hikma university laboratories, Taiz, Yemen.

**Solubility studies**

The solubility of salbutamol in different solvents separately was studied at room temperature of 25°C. It was carried out by adding excess amount of drug in each case and keeping the excess drug containing flasks on a water bath shaker at 25°C.

**Determination of the λ max and the calibration curve of salbutamol in phosphate buffer solution pH 7.4:** Small amount of salbutamol was dissolved in phosphate buffer solution pH 7.4. Then by scanning for wavelength from 180 nm -400 nm on the UV spectrophotometer, λ max was measured and recorded. Salbutamol stock solution was prepared in phosphate buffer solution pH 7.4 and then further diluted with phosphate buffer solution to obtain a series of solutions of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/ml respectively. The absorbance of these solutions was measured spectro-photometrically at determined λ max, using phosphate buffer solution pH 7.4 as a blank. The standard curve was plotted for entire range of concentrations 1 to 10 µg/ml.

**Permeation test of salbutamol through rabbit skin:** The permeation tests through rabbit skin were carried out in a modified Franz diffusion cell as diagramed in Figure 1. The skin was carefully shaved by using electronic clipper and insures that all skin layers to be excised except the subcutaneous fat with other blood vessels that were removed and then stored in saline solution for next steps. The skin was rinsed with soap solution, then with purified water for many times [3]. The skin then placed between the donor and receptor compartments of the cell. The dermal side of the skin was directing into the receptor compartment and the stratum corneum facing the donor compartment. The receptor compartment of the diffusion cell was filled with 200 ml of phosphate buffer solution pH 7.4. The donor compartment contained 1 ml solution of salbutamol (10 mg/ml) in phosphate buffer solution pH 7.4. The diffusion cell with fitted rabbit skin was kept inside the beaker containing receptor fluid. The temperature of diffusion medium was maintained at 37°C ± 0.5°C by putting it on controlled hot plate. This whole assembly was kept on a magnetic stirrer and the medium of the receiver compartment was continuously stirred at 50 rpm. The donor compartment was covered to prevent evaporation of the solution. The samples were withdrawn at different time intervals and stored at room temperature until analysis. An equal amount of phosphate buffer solution pH 7.4 was replaced each time.

**Figure 1: Modified Franz diffusion cell**

Permeation studies were carried out over 12 hours and samples were withdrawn at 1, 2, 3, 4, 5, 6, 9 and 12 hours from the sampling port. Absorbance of the sample was measured by UV-spectrophotometer at λ max 200 nm. The

| | | | | | | |
|---|---|---|---|---|---|---|
| F5 | 30 | 50 | 100 | 0.15 | | 0.1 |
| F6 | 30 | 30 | 100 | 0.3 | | 0.1 |
| F7 | 30 | 50 | 100 | 0.3 | | 0.1 |
| F8 | 30 | 30 | 100 | 0.15 | | 0.1 |

**Evaluation of physicochemical characters of transdermal films:** The polymer types and excipients have an effect on the physicochemical properties as well as the release rate and permeability of drugs. Therefore, the formulated films were examined in order to select the most satisfactory films. Transdermal films were taken out from each casted film after complete drying and then evaluated for the following physicochemical properties [8].

**Visual examination of the prepared transdermal films:** Formulated films were evaluated and estimated by visual inspection for their physical appearance including color, elegance, clarity, homogeneity, stickiness, texture, uniformity, smoothness, flexibility, transparency, entrapment of any air bubble or precipitation of drug, which on a large part determines patient acceptability of the film, physical resistance during preparation and storing and also therapeutic efficacy [9].

**Film thickness test:** It was measured at different points for each film piece by using an electronic digital micrometer (0.001 mm). For each formulation, the thickness of three films was measured and the average of six readings was calculated and standard deviation was determined.

**Film flatness test:** The flatness was measured manually for the prepared films. An ideal film should be formulated in such a way that it possesses a smooth surface and it should not constrict with time. Flatness studies were performed to assess smoothness and contractibility properties. Longitudinal strips were cut out from each film, one from the center and two from either side. The length of each strip was measured and the variation in the length because of non-uniformity in flatness was measured by determining percentage constriction, considering 0% constriction is equivalent to 100 % flatness [10]. Flatness was determined using below given formula:

$$\%\text{Constriction}=[(L1-L2)/\ L2] \times 100$$

Where, L1=Initial length of each strip, L2=Final length of each strip after cutting. The flatness was measured in triplicate and average reading was considered.

**Weight variation test:** The weight of different prepared films was determined in which three dried films (1 $cm^2$ area) of each formula were taken and weighed individually on a digital balance and the average weights were calculated with standard deviation.

**Drug content uniformity test:** The salbutamol transdermal films of each formulation of specified area were cut and weighed accurately and then taken into volumetric flask (200 ml) of phosphate buffer solution pH 7.4 and continuously stirred on a magnetic stirrer [11]. Then the solution was filtered, diluted with the same medium if required. The absorbance values of drug was measured by UV spectrophotomer at λ max 200 nm and the mean values of drug content of three films was calculated and expressed as percentage.

**Folding endurance test:** Three films of each formulation of size (1 cm × 2 cm) were cut by using sharp blade. The folding endurance of the films was determined by repeatedly folding a strip at same point till it broke or up to 300 times. The number of times for film could be folded at the same place without breaking gave the value of folding endurance.

**Bioadhesion test:** The bioadhesive properties of the patches were successfully tested using the rolling ball tack test. In this test, stainless steel ball of 7/16 inches in diameter was released on an inclined track so that it rolled down and came into contact with horizontal, upward facing adhesive. The distance the ball traveled along the adhesive provides the measurement of tack, which is expressed as average of the stopping distance measurements that shall be reported in millimetres [12].

**Percentage moisture content test:** To determine the weight loss, the films were weighed individually and the average weight was calculated that was considered as an initial weight. Then all the films were kept in a desiccator containing anhydrous calcium chloride powder at normal room temperature for 24 hr. The final weight was noted when there was no further change in the weight of individual film [13]. The percentage moisture content was calculated as a difference between initial and final weight with respect to final weight. The result weights then calculated according to the following formula:

$$\text{Percentage moisture content} = [(\text{Initial weight} - \text{Final weight}) / \text{Final weight}] \times 100$$

**Percentage moisture absorption test:** The films were weighed separately and the average weight was calculated and this weight was considered as an initial weight. Then these films were kept in a desiccators containing saturated solution of potassium chloride (Relative humidity of 75%) at room temperature for 24 hr. The final weight was noted when there was no further change in the weight of individual patch. The percentage moisture absorption was calculated as a difference between final and initial weight with respect to initial weight [14]. The percentage moisture absorption was determined using below formula:

$$\text{Percentage moisture absorption} = [(\text{Final weight} - \text{Initial weight}) / \text{Initial weight}] \times 100$$

***In vitro*** **evaluation of salbutamol transdermal films**

***In vitro*** **drug release studies:** The paddle over disc method (USP apparatus V) as in Figure 2 was determined to evaluate the drug release from the formulated transdermal patches. Dry transdermal patches were cut into certain shape, weighed, and fixed over a glass plate with Alamir glue to fix it as shown in Figure 2. The glass plate was then placed at the bottom of the dissolution vessel filled with a 200 mL of the dissolution medium of phosphate buffer solution pH 7.4, allowing drug release only from the upper side of the film and the equipment was controlled to 37°C ± 0.5°C. The paddle was placed on a distance of 2.5 cm from the glass plate at the bottom of vessel and operated at a speed of 50 rpm. Samples can be withdrawn at predetermined time periods up to 12 hours and recompensed with blank phosphate buffer solution. Then the samples were filtered and analyzed after convenient dilution by UV spectrophotometer at λ max 200 nm. The samples were in triplicate and the mean values were reported and the cumulative percent released can be calculated and plotted versus time [15].

Data of *in vitro* drug release were fit into different kinetic equations and models to determine the most corresponding manner of drug release from the transdermal patches. The kinetic models analyzed were zero order, first order and Higuchi model. For all films, the obtained data from the various formulations were plotted in various different kinetic models and results were interpreted to determine the type of rate order.

To know more accurately the effect of the polymeric mixture on the release of salbutamol, the results must be analyzed regarding to the semi-empirical Korsmeyer-Peppas equation [16].

**Figure 2: Schematic diagram of the equipment utilized for the *in vitro* dissolution studies**
**Source: (USP, 2009)**

**Skin irritation test:** Four male rabbits weighing 2-3.4 kg were selected and were fed with standard nutrients and water. The dorsal part of each rabbit was hair removed by an electric razor. The films of area (2 $cm^2$) of blank films (control) and films containing salbutamol were placed to the dorsal shaved part of the skin and fixed by adhesive

**Solubility studies**

Solubility of baclofen was evaluated in different solvents. The results are mentioned in Table 2. Attempts were made at this point to know whether the media phosphate buffer, pH 7.4 was able to maintain sink condition in diffusion as well as in permeation studies. The results of solubility studies revealed that phosphate buffer solutions pH 7.4 was able to maintain sink conditions in dissolution as well as in permeation studies. Thus, phosphate buffer solutions were chosen as the dissolution and permeation media on this study because sufficient amount of drug could be dissolved in it, that is necessary to maintain sink condition [17]. These values of solubilities may be due the dielectric constants of the different solvents as when the dielectric constant increase of solvent increase (as for buffer solutions), the ability to solubilize increase. All the results were complied with that approved by British pharmacopeia.

**Table 2: Solubility data of salbutamol in different solvents**

| Solvent type | Solubility |
|---|---|
| Phosphate buffer (pH 7.4) | Soluble |
| Distilled water | Slightly soluble |
| Ethanol | Soluble |
| Chloroform | Very soluble |

**Determination of the λ max and the calibration curve of salbutamol in phosphate buffer solution pH 7.4:** The UV maxima of resultant solution were measured with UV Spectrophotometer. The UV maximum of salbutamol in the solution was found to be one single peak at 200 nm which was suitable for the preparation of standard curve and determination of salbutamol in various formulations. Figure 6 shows the UV spectrograph of baclofen in phosphate buffer pH 7.4.

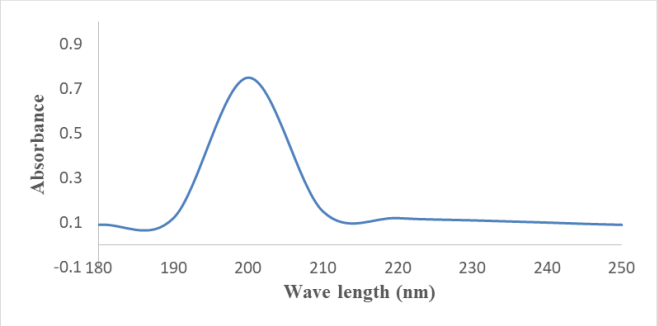


**Figure 6: UV absorbance spectrum of salbutamol in phosphate buffer solution pH 7.4**

The calibration curve of salbutamol in phosphate buffer pH 7.4 was prepared by measuring the absorbance of the solutions in the range of concentrations 1 to 10 µg/ml at the wavelength 200 nm. The concentration of tested samples and the corresponding absorbance were depicted in Table 3, graphically represented by Figure 7 and the procedural constant (K) was calculated.

**Table 3: The calibration table of salbutamol in phosphate buffer solution pH 7.4 at λ max 200 nm**

| | | | | | |
|---|---|---|---|---|---|
| 5 | 900 | 1167 | 890 | 1309 | 850 |
| 6 | 1289 | 1780 | 1000 | 2060 | 1067 |
| 9 | 1845 | 3020 | 1960 | 4590 | 1309 |
| 12 | 2050 | 5890 | 2100 | 6870 | 1698 |

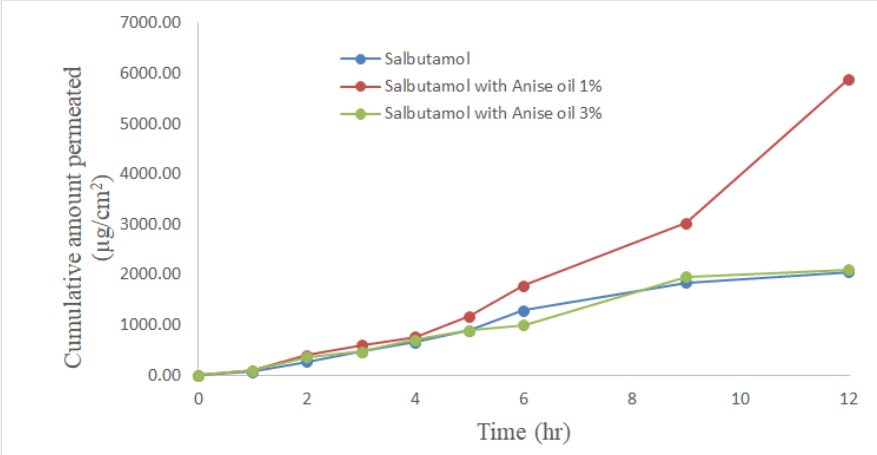


**Figure 9: Permeation of salbutamol solution through abdominal rabbit skin using Anise oil as penetration enhancer at different concentration**

**Figure 10: Permeation of salbutamol solution through abdominal rabbit skin using Eucalyptol oil as penetration enhancer at different concentration**

**Visual examination of the prepared transdermal films:** The formulated films were shown in Figures 11 and it was observed that when using HPMC polymer as a film forming polymer and sodium alginate as a bio-adhesive polymer, the optimum prepared films (from F1 to F8) were uniform, smooth, soft, high to moderate flexible and transparent appearance. They were easy to remove from the petri dish surface, had uniform edges without cracks and no air bubbles or drug precipitation. As seen in the photographs showed in figures, it was observed that, all the formulas meet the ideal properties of physical appearance of patch.

The drug loaded films had the same properties and appearance of the drug free films except the transparency in which the drug-loaded films were transparent with some turbidity and this ensures uniform dispersion of salbutamol in patches. Therefore, based on the physical appearance, it was concluded that only eight formulas were appeared physically acceptable to complete the other physical characterization and others were excluded**.**

**Figure 11: Photographs of prepared transdermal films F2**

**Figure 12: Photographs of prepared transdermal films F6**

**Figure 13: Photographs of prepared transdermal films of different formulas**

limit for the percentage deviation of all the films of less than (mg) is ± 10% (B.p, 2009). The average percentage deviation of all formulations was found to be within the pharmacopoeial limit, and hence all the formulation passed the test for weight variation as per official requirements.

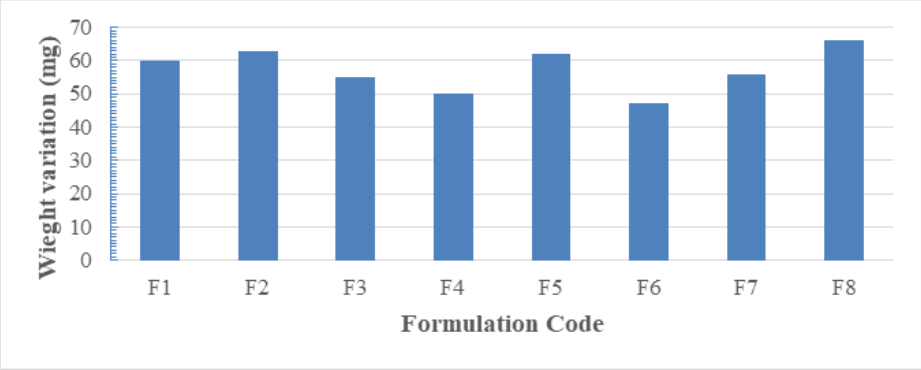


**Figure 15: Weight variation of different formulations of prepared transdermal films**

**Drug content uniformity test:** This test was done to be sure that drug distributed uniformly in each formulated films. The average drug content of prepared medicated films were found to contain between 90.13% ± 0.90% (F1) and 102.10% ± 0.54% (F3) of the labeled amount of drug per film as presented on Figure 16 and Table 7. The average percentage deviation of all formulations was found to be within the limit (± 10%), and hence all the formulations passed the test for content uniformity as per official requirements (B.p, 2009). From the obtained results, it was clear that there was proper evenly distribution of drug throughout the films regardless of the polymer type and proportions. Hence it was concluded that drug was uniformly distributed in all the formulation, with acceptable deviation. Low values of standard deviation (SD) in drug content and previous data reflected that there was no clear change within each batch. The drug content analysis of prepared formulations showed that the process employed to prepared films was capable of giving uniform drug content, with minimum batch variability.

**Table 7: Drug content, moisture content, moisture uptake and bioadhesion of films**

| Formulation Code | Drug content (%) | Moisture content (%) | Moisture uptake (%) | Bioadhesion (cm) |
|---|---|---|---|---|
| F1 | 90 | 38 | 212 | 15 |
| F2 | 95 | 123 | 168 | 32 |
| F3 | 102 | 104 | 108 | 27 |
| F4 | 96 | 57 | 157 | 16 |
| F5 | 98 | 91 | 150 | 35 |
| F6 | 102 | 66 | 111 | 50 |
| F7 | 97 | 101 | 109 | 42 |
| F8 | 100 | 98 | 143 | 14 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5 | 27 | 27 | 31 | 56 | 49 | 23 | 30 | 88 |
| 6 | 34 | 39 | 39 | 76 | 52 | 33 | 40 | 95 |
| 7 | 54 | 50 | 42 | 82 | 62 | 38 | 54 | 100 |
| 8 | 65 | 55 | 45 | 90 | 73 | 47 | 67 | 100 |
| 9 | 71 | 62 | 51 | 99 | 88 | 55 | 80 | 100 |
| 10 | 82 | 67 | 57 | 100 | 94 | 59 | 83 | 100 |
| 11 | 88 | 71 | 62 | 100 | 100 | 64 | 89 | 100 |
| 12 | 93 | 77 | 68 | 100 | 100 | 68 | 94 | 100 |

It was apparent from the plots, that the drug release could be sustained and varied with respect to the proportion of polymers. It can be seen that the rate of drug release could be modified in a predictable manner by varying the type and proportion of the polymers used (Figure 20).

**Figure 20: *In vitro* release profiles of transdermal salbutamol films**

In general, it was noticed that, the descending order of drug release was as follow: F8, F4, F1, F5, F7, F2, F3 and F6 respectively. So, salbutamol release was slower from films F3 and F6 that contain HPMC and low amount of sodium alginate with small amount of PG due to the high viscosity of HPMC compared to amount of bioadhesive polymer sodium alginate. This might be returned to the large hydrophobicity, and the consequent lower dissolution and slower erosion of anise oil that prevent the free and deep water penetration into the film.

Regarding the formulation of sustained release, the minimum of 60% of drug release over a 12 h period was desired for the purpose of this study for transdermal delivery. On the one hand, formulations F3 and F6 were the optimum releases that only release less than 70% of drug during 12 hours that means is ideal for sustaining the drug release. The other formulations were failed to sustain the drug release. The final percentages of different formulations at the end of 12 hours were as follow (F1=93, F2=77, F4=100, F5=100, F7=94 and F8=100). In the case of formulations, F3 (T50%=8.73 hr) and F6 (T50%=8.83 hr).

**Kinetic analysis of *in vitro* release data:** After exclusion of the bad films, the satisfactory physicochemical formulated films were exposed to in-vitro dissolution studies. Salbutamol release from films was sustained and controlled over 12 hr. To study the mechanism of this release, various kinetic models were applied. Analysis of the release data according to Higuchi model, zero-order and first-order kinetic models and the release parameters were shown in Table 9. The correlation coefficients (r2) of different relations were found to be in the range of 0.921-0.969 for Higuchi diffusion, 0.853-0.994 for zero order and 0.779-0.966 for first order model. The data of

[15] Peppas NA. *Pharm Acta Helv*. **1985**; 60, 110-1.

[16] Sanap GS, Dama GY, Hande AS, et al. *Int J Green Pharm*. **2008**; 2, 129-133.

[17] Shah SS, Joshi R, Praphu P. *Asian J Pharm.* **2010**; 4, 79-86.