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In-Vitro Drug Release Profile & Stability Studies of Transdermal Patch of Allopurinol

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ABSTRACT

AIM- The aim of the present investigation is to study *In-Vitro* Drug Release Profile Studies of Transdermal Patch of Allopurinol. **MATERIAL & METHODS-** The matrix type transdermal patches of allopurinol were prepared by solvent evaporation technique by using different ratio of ethylcellulose (EC) and polyvinylpyrrolidone K-30 (PVP) polymers. The polymers EC and PVP were weighed and mixed in different ratios by keeping the total polymers weight at 1.6 g added in a chloroform solvent using magnetic stirrer. On the basis of preliminary studies, the optimized polymers ratio 3:2 (EC:PVP) were mixed with the different permeation enhancers like DMSO, Tween-80, eucalyptus oil and olive oil. The rate of evaporation was controlled by inverting a funnel over the petridish and the solvent was allowed to evaporate for 24 h at room temperature. After 24 h, the films were collected and a wax paper was applied on other side of the films as a release liner to complete the formulation. The dissolution studies were performed by using dissolution rate test apparatus (USP-II) for the assessment of the release of the drug from the transdermal patches (3.14 cm^2). The apparatus was equilibrated to 32 ± 0.5^0C and the dissolution medium was 20% methanol in PBS pH 7.4. The paddle speed was kept constant at 50 rpm. Stability studies of formulation was conducted according to ICH guidelines by storing at 40 °C and 75 % RH for 3 months. **RESULTS-** The cummulative amount of drug release from control formulations (without enhancer) A1, A2, A3, A4 and A5 were found to be 40.70, 46.68, 52.38, 59.66 and 50.61% respectively in 24 h. The highest percentage of drug release (59.66%) was observed from formulation A4 (EC/PVP, 3:2) which was significantly *($p<0.05$)* greater than the lowest value 40.70% obtained from the formulation A1 (EC/PVP, 4.5:0.5). The percentage of drug release order was as follows: A4>A3>A5>A2>A1. The drug

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content of the patch was found 97.11, 96.91 and 96.84% after 30, 60 and 90 days respectively, indicated that no significant *(p >0.05)* change after 3 months. **CONCLUSION-** The results of Allopurinol transdermal matrix patch showed that the most promising formulation was AT1 (formulation containing EC: PVP, 3:2; Allopurinol 20%; dibutylpthalate 30% and 2% tween-80 all in %w/w).

KEYWORDS-

In-Vitro, Drug Release Profile, Stability Studies, Transdermal Patch, Allopurinol, Dissolution Studies

INTRODUCTION

The transdermal route of drug delivery has many advantages for the administration of drugs in local and systemic therapy. But, skin is widely recognized for its effective barrier properties as compared to other biological membranes due to impermeable nature of stratum corneum. The low permeability of the skin makes it a minor port of entry for drugs. Various drug delivery modules are available for increasing the permeation of molecules such as active and passive tools, which includes iontophoresis, sonophoresis, penetration enhancers and other drug delivery modules. The vesicular drug delivery is potentially beneficial as vesicles, which tend to fuse and adhere to the cell surface and this is believed to increase the thermodynamic activity of the drug at the stratum corneum interface of skin leading to enhanced permeation. These vesicles act as drug reservoirs. Modification of surface charge over the vesicles can alter the drug release rate at the target site. Drug delivery systems are being renovated by the ceaseless efforts made by the formulators, which have been achieved with minimal side effects, low dosing and reduction in dosing frequency of drug to improve patient compliance (Karadzovska *et al*., 2013 and Jain *et al*., 2002).

The diffusion process controls the transportation of active substances across the skin. The permeation of various active substances such as hydrophilic and lipophilic is governed by Ficks first law. Several factors affect the permeation profiles of active substance through the skin such as solubility, charge, partition coefficient (Log P), molecular weight (molecular weight not more than 500 Daltons) and concentration gradient. The permeation efficiency

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depends upon the solubility and partition coefficient of the drug (Naik *et al*., 2000, Hadgraft *et al*., 2000 and Gerber *et al*., 2008).

In our previous study, formulation and its evaluation parameters were established for the transdermal patch of Allopurinol for low dose maintenance therapy to reduce the risk of potential side effects in gout patients. However, here in present work an attempt was made for the *In-Vitro* Drug Release profile and stability studies of different developed transdermal patches.

MATERIAL & METHODS

Formulation of Transdermal Patches by Optimized Formula

The matrix type transdermal patches of allopurinol were prepared by solvent evaporation technique by using different ratio of ethylcellulose (EC) and polyvinylpyrrolidone K-30 (PVP) polymers. The polymers EC and PVP were weighed and mixed in different ratios by keeping the total polymers weight at 1.6 g added in a chloroform solvent using magnetic stirrer. The dibutyl phthalate 30% w/w of polymer was incorporated as plasticizer. Drug 20 % w/w of polymer weight was added slowly to the polymers solution and mixed thoroughly by continuous stirring for 30 minutes to obtain a homogenous solution. The five formulations were prepared by using same drug and different polymers ratio without permeation enhancer in order to determine the optimum combination of drug and polymers. On the basis of preliminary studies, the optimized polymers ratio 3:2 (EC:PVP) were mixed with the different permeation enhancers like DMSO, Tween-80, eucalyptus oil and olive oil. The permeation enhancers were added in three different concentrations *i.e.* 2%, 5% and 10% w/w of total polymers weight for each. The resulting drug-polymers solution was poured in petridish of 64 cm^2. The aluminum foil was uniformly spread on petridish on which drug-polymers solution was poured. The rate of evaporation was controlled by inverting a funnel over the petridish and the solvent was allowed to evaporate for 24 h at room temperature. After 24 h, the films were collected and a wax paper was applied on other side of the films as a release liner to complete the formulation (Arora and Mukherjee, 2002; Verma and Chandak, 2009).

EVALUATION OF PATCHES

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In-Vitro Drug Release

The dissolution studies were performed by using dissolution rate test apparatus (USP-II) for the assessment of the release of the drug from the transdermal patches (3.14 cm^2). The commercially available water impermeable adhesive backing membrane was placed over the patch and it was further fixed on glass slide (2.3x2.3 cm) using cyanoacrylate adhesive. Then the transdermal patch was covered with a dialysis membrane and placed at the bottom of dissolution vessels with the release surface facing upward. The apparatus was equilibrated to 32 ± 0.5^0C and the dissolution medium was 20% methanol in PBS pH 7.4. The paddle speed was kept constant at 50 rpm. The samples were withdrawn at appropriate time intervals upto 24 h and analyzed by UV spectrophotometer at 260 nm using 20% methanol in PBS pH 7.4 solution as a blank. After each sampling, an equal volume of fresh dissolution fluid was added to the dissolution vessel to maintain a sink condition (Garala *et al.*, 2009; Shah *et al.*, 1986).

Stability Studies

Stability studies of formulation was conducted according to ICH guidelines by storing at 40°C and 75 % RH for 3 months. The samples were withdrawn at 30, 60 and 90 days and evaluated for physical appearance and drug contents (Aggarwal *et al.*, 2011).

Statistical Analysis

The formulation parameters were statistical evaluated by Graph pad prism 5 using one-way analysis of variance (ANOVA), followed by Dennett test multiple comparison tests and unpaired t-test. The obtained results were expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

In-vitro drug release studies of patches

The modified paddle over disc assembly using 20% methanol in PBS pH 7.4 as a dissolution medium at 32 ± 0.5^0C was used to conduct dissolution studies. The result of *in vitro* dissolution studies of prepared transdermal patches are presented in tables. The cummulative amount of drug release from control formulations (without enhancer) A1, A2, A3, A4 and A5 were found to be 40.70, 46.68, 52.38, 59.66 and 50.61% respectively in 24

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h. The highest percentage of drug release (59.66%) was observed from formulation A4 (EC/PVP, 3:2) which was significantly *(p<0.05)* greater than the lowest value 40.70% obtained from the formulation A1 (EC/PVP, 4.5:0.5). The percentage of drug release order was as follows: A4>A3>A5>A2>A1.

It was observed that increase in the concentration of hydrophilic polymer PVP, the rate of drug release increased, except for formulation A5 (Morrow *et al.,* 2007). The addition of hydrophilic PVP to insoluble ethyl cellulose tends to enhance its release rate constant. This outcome can be attributed to the leaching of the soluble fraction which leads to formation of pores. Thus, decrease mean diffusion path length of drug molecule into the diffusion medium and increase in the external film area exposed to the dissolution medium, increase internal porosity and decrease the tortuosity (Mayorga *et al.,* 1996). The initial burst release effect was observed in the all formulations. This may be because of the higher percentage of PVP in these formulations and PVP hydrophilic layer might need very little "time lag" to establish a concentration profile in patches resulting in a burst release in the dissolution studies. Similar finding have also been reported by others (Murthy and Hiremath, 2001).

The formulation A5 was showed increase in the concentration of hydrophilic polymer, the rate of drug release decreased. This may be attributed to the previous finding that higher concentration of PVP K-30 may decrease the crystalline drug in patch and thus deceased drug releases (Gobel *et al.*, 2003).

The highest cummulative percentage of drug release *i.e.* 59.66% was observed from formulation A4 (EC/PVP, 3:2) in 24 h. Therefore, formulation A4 was selected for incorporation of permeation enhancers in three different concentrations *i.e.* 2%, 5%, and 10% in order to enhance permeation.

Table No.1: *In-vitro* dissolution profile of Allopurinol from transdermal patches containing EC/PVP in different proportion

Time (h)	Cummulative % drug release				
	A1	**A2**	**A3**	**A4**	**A5**
1	0.69 ± 0.11	1.81 ± 0.11	2.29 ± 0.11	0.826 ± 0.22	3.15 ± 0.33

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2	3.81 ± 0.23	3.36 ± 0.45	4.32 ± 0.53	5.68 ± 1.55	6.55 ± 0.44
4	5.23 ± 0.25	7.63 ± 1.78	9.76 ± 1.22	11.52 ± 0.68	11.25 ± 1.11
6	12.50 ± 1.33	13.80 ± 1.55	17.52 ± 3.35	18.06 ± 1.11	19.32 ± 2.67
8	20.81 ± 2.45	18.49 ± 2.78	24.41 ± 4.56	28.72 ± 3.34	25.51 ± 1.63
10	23.89 ± 1.22	25.56 ± 4.66	33.87 ± 1.88	36.55 ± 1.65	31.85 ± 2.11
12	27.66 ± 0.45	32.34 ± 1.77	39.67 ± 3.11	43.37 ± 3.66	37.02 ± 2.78
18	35.15 ± 2.44	40.76 ± 3.88	47.16 ± 1.56	52.61 ± 2.98	43.96 ± 2.20
24	40.70 ± 0.56	46.68 ± 1.99	52.38 ± 0.61	59.66 ± 0.81	50.61 ± 2.11

Table No. 2: *In-vitro* dissolution profile of Allopurinol from transdermal patches containing EC/PVP (3:2) and different proportion of DMSO 2%

Time (h)	**Cummulative % drug release**		
	AD1	**AD2**	**AD3**
1	1.55 ± 0.11	1.99 ± 0.12	6.67 ± 1.11
2	12.32 ± 2.56	6.16 ± 2.67	17.74 ± 2.22
4	18.13 ± 3.34	11.34 ± 3.66	31.46 ± 3.47
6	25.86 ± 3.16	20.82 ± 2.89	42.67 ± 3.89
8	30.92 ± 3.78	37.65 ± 3.11	50.87 ± 2.10
10	37.72 ± 3.88	44.51 ± 3.67	59.35 ± 3.34
12	44.17 ± 2.99	52.17 ± 4.25	65.15 ± 3.46
18	51.08 ± 2.22	61.88 ± 2.64	72.25 ± 2.28
24	64.58 ± 2.67	70.49 ± 3.66	78.29 ± 3.66

Table No. 3: *In-vitro* dissolution profile of transdermal patches containing EC/PVP (3:2) and different proportion of tween-80 2% (AT1), 5% (AT2) and 10% (AT3)

Time (h)	**Cummulative % of drug release**		
	AT1	**AT2**	**AT3**
1	2.49 ± 0.21	7.31 ± 1.17	5.76 ± 0.33

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2	6.70 ± 1.45	13.82 ± 1.22	9.43 ± 1.56
4	14.97 ± 1.22	19.39 ± 2.67	12.34 ± 2.55
6	27.38 ± 2.56	25.87 ± 2.23	19.47 ± 2.17
8	38.94 ± 2.11	34.60 ± 3.56	23.83 ± 1.67
10	53.74 ± 4.45	40.61 ± 4.22	30.51 ± 2.44
12	59.63 ± 2.22	48.26 ± 3.33	42.16 ± 4.66
18	72.61 ± 3.56	60.51 ± 4.45	54.76 ± 4.89
24	88.72 ± 2.59	77.32 ± 3.55	70.38 ± 3.33

Table No. 4: *In-vitro* dissolution profile of transdermal patches containing EC/PVP (3:2) and different proportion of eucalyptus oil 2% (AE1), 5% (AE2) and 10% (AE3)

Time (h)	**Cummulative % of drug release**		
	AE1	**AE2**	**AE3**
1	3.65 ± 0.22	1.94 ± 0.55	3.11 ± 0.44
2	9.23 ± 1.34	5.45 ± 1.24	10.40 ± 1.66
4	13.29 ± 2.56	9.33 ± 2.66	24.10 ± 2.78
6	21.41 ± 2.33	14.74 ± 2.46	31.22 ± 1.11
8	25.18 ± 2.67	32.58 ± 3.57	40.70 ± 3.67
10	30.58 ± 1.11	39.68 ± 4.22	47.10 ± 2.89
12	38.40 ± 3.78	47.66 ± 3.78	53.40 ± 1.67
18	52.28 ± 4.88	60.28 ± 3.89	64.95 ± 3.77
24	68.58 ± 3.88	76.83 ± 3.22	83.52 ± 3.11

Table No. 5: *In-vitro* dissolution profile of transdermal patches containing EC/PVP (3:2) and different proportion olive oil 2% (AO1), 5% (AO2) and 10% (AO3)

Time (h)	**Cummulative % of drug release**		
	AO1	**AO2**	**AO3**

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1	1.72 ± 0.12	0.756 ± 0.10	1.31 ± 0.22
2	6.54 ± 0.67	6.51 ± 1.91	3.14 ± 2.56
4	16.18 ± 2.89	14.43 ± 2.82	8.95 ± 1.71
6	27.29 ± 3.91	21.71 ± 1.71	14.47 ± 2.98
8	32.37 ± 2.82	26.22 ± 2.72	20.49 ± 2.82
10	36.89 ± 2.85	35.15 ± 3.67	28.92 ± 3.73
12	41.17 ± 3.74	46.90 ± 2.63	40.37 ± 3.63
18	49.18 ± 2.64	64.47 ± 3.51	53.49 ± 3.69
24	64.50 ± 3.53	73.08 ± 1.47	67.93 ± 3.39

Stability Studies of Patch (AT1)

The stability study of optimized formulation (AT1) was conduct according to ICH Guidelines; the formulation was stored at 40 °C and 75 % relative humidity for 3 months. The result indicated that no change in physical appearance was observed after 90 days. The drug content of the patch was found 97.11, 96.91 and 96.84% after 30, 60 and 90 days respectively, indicated that no significant *(p >0.05)* change after 3 months. The results of in vitro permeation studies of fresh batch and 3 month old batch, also confirm that no significant change in drug release after 3 months. So on the basis of results, the optimized fluoxetine transdermal patch (AT1) was found stable enough.

CONCLUSION

The results of Allopurinol transdermal matrix patch showed that the most promising formulation was AT1 (formulation containing EC: PVP, 3:2; Allopurinol 20%; dibutylpthalate 30% and 2% tween-80 all in %w/w). These promising results showed the feasibility of delivering Allopurinol through transdermal matrix patch. The developed transdermal patches of Allopurinol may prove to be a better alternative to conventional dosage forms in reatment of gout.

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