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Thiolated Chitosan Synthesis And Its Permeability Effect On Drug From Transdermal Patches Of Meloxicam

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Abstract
The purpose of the present study was to improve the permeability of chitosan by covalent attachment of thiol group. Amide bond was formed between carboxylic group of thioglycolic acid and primary amino group of chitosan. Matrix type transdermal patches of Meloxicam were prepared by solvent casting evaporating method and the prepared patches were evaluated for physicochemical properties. Rabbit skin was used for in vitro study of Meloxicam from patches by using Franz diffusion cell. The study shows that as the concentration of the thiolated chitosan increases the permeation of the drug also increased. It was also found the release of drug was non-Fickian.

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I. Introduction

Chitosan is biodegradable polymer which is prepared by removing acetyl group from chitin in alkaline condition and in nature it is found as polysaccharide. The -NH2 group of chitosan is very much important and is responsible for preparation of different types of chitosan conjugates with acids [1]. Due to good properties of chitosan it is used in various pharmaceutical formulations including local (ocular), peroral (tablets), parentral (dispersion system) and gene delivery [2,3,4].

Thiomers (thiolated chitosan) prepared have several advantages as compared to parent molecule such as higher mucoadhesion, solubility and permeation property[5]. Meloxicam is non steroid anti inflammatory drug used for the treatment of rheumatoid arthritis, pain and ankylosing spondylitis. But it has certain side effects associated with gastric ulcer. To overcome the side effects caused by COX-2 inhibitors, including Meloxicam transdermal applications introduce an alternative route. Inflammatory area can be treated with a transdermal patch that also exhibit systemic effects. The transdermal patches allow penetration of the drug through the skin into the application site such as the chest, inner forearm, and the ear by adding penetration enhancers to the formulations. These systems have good patient adhesion compliance to their easy application and finishing application whenever necessary. Medications can be protected against first-pass metabolism as a very important benefit.

The purpose of the present study was to investigate the penetration effect of thiolated chitosan by formulating matrix type transdermal patches of Meloxicam by using different proportion of chitosan and thiolated chitosan.

II. Materials and Methods

Meloxicam (Leads Pharma Islamabad, Pakistan), Chitosan (Sigma Aldrich, Germany), thioglycolic acid (Sigma Aldrich, Germany), Di-n-butylphthalate (Sigma Aldrich, Germany), Potassium dihydrogen phosphate (Merck, Germany), (Ethanol (Merk, Germany), (NaOH (Merk, Germany), PH Meter (Denver Model No. 215, USA), Weighing Balance (Model No. AX-200, Shimadzu, Japan), Pharma Test Dissolution Apparatus (PTWS 11/P, Hamburg, Germany), Analytical Balance (Schimadzu AX 200, Shimadzu, Japan), Franz Diffusion UV/Visible Spectrophotometer (Shimadzu 1800, Japan), Magnetic stirrer (Germany), Water Distillation Apparatus (IRMECO GmbH, IM-100, Germany), Sonicator (Elma D78224, Germany), Shaking Water Bath (Shel Lab, 1217-2E, USA), Vernier caliper (Germany) and all other chemicals of analytical grade.

Synthesis of Thiolated Chitosan

According to the method of Bhatia and Ahuja, 2013 thiolated chitosan was prepared by developing ester linkage between chitosan and thioglycoloic acid (TGA) in which thioglycolic acid provide thiol group. This

process was carried out in the presence of HCL. Chitosan and TGA were used in ratio of 1:2for reaction and placed on magnetic stirrer for mixing at room temperature. Few drops of HCL were added at 70 ^{o}C for 90 minutes. Methanol was added to the reaction mixture to form precipitate of thiolated chitosan. Unreacted TGA was removed by adding methanol. Furthermore thiolated chitosan precipitate was kept at -80 ^{o}C for 4 h, after that lyophilized at -50 ^{o}C with 0.0016 mbar pressure to obtain the dry mass of thiolated chitosan.

Fabrication of Transdermal Patches

Formulation of Patches with chitosan and thiolated chitosan

Meloxicam matrix type patches were develop by using solvent evaporation techniques. Different concentrations of polymer were used. First of all backing membrane was prepared by using PVA 4% (w/v) solution was synthesized by dissolving 4g of PVA in distilled water. More distilled water was added to make the volume up to 100ml in conical flask. The mixtures were heated up to 80 ^{o}C by using magnetic stirrer after that solution was cooled at room temperature. Then the solution was placed on sonicator to remove entrapped air bubbles. After that 12ml of solution was cost on petri dishes and dried at room temperature in open air. The drug polymer matrix solution was prepared by using of magnetic stirrer. When the drug and polymer are mixed properly then (15% of polymer) plasticizer was added. The petri dishes were allowed to cool by using funnel to control the evaporation. When the solution is complete dried than the film is removed and cut into 1.7 cm^2 areas. Dissolution study was performed by using different formulation with different ration of polymers.

Table 1: Composition of Meloxicam with different ratio Chitosan and Thiolated Chitosan

Formulation Code	Drug quantity	Polymer		Di-n-butyle Phthalate	Ethanol
		Chitosan	Thiolated Chitosan		
FMLXTI	120	1:1		15% of polymer	10 ml
FMLXT2	120	1.4:0.6		15% of polymer	10 ml
FMLXT3	120	1.7:0.3		15% of polymer	10 ml
FMLXT4	120	2:0		15% of polymer	10 ml
FMLXT5	120	0.6:1.4		15% of polymer	10 ml
FMLXT6	120	0.3:1.7		15% of polymer	10 ml

Table 2: Meloxicam formulation with different amount of thiolated chitosan for permeation enhancer

Formulation code	Drug Quantity	Polymer		Plasticizer Di-n-butyle phthalate	Ethanol
		Chitosan	Thiolated Chitosan		
FMLXT1	120	600	600	15% of polymer	10 ml
FMLXT2	120	840	360	15% of polymer	10 ml
FMLXT3	120	1020	180	15% of polymer	10 ml
FMLXT4	120	1200	0.00	15% of polymer	10 ml
FMLXT5	120	360	840	15% of polymer	10 ml
FMLXT6	120	180	1020	15% of polymer	10 ml

Drug Polymer Compatibility study

Differential Scaning Calorimetry Study

DSC study was carried out for analysis of drug and polymer physicochemical chemical incompatibilities. For the evaluation of drug polymer interaction DSC instruments (Metter Toledo DSC 822e, Greifensee Switzarland) equipped with star computer programme was used. Drug sample having weight of 4-8 mg of was placed in aluminium pan, sealed with punched lid. The analysis was carried out at a rate of 50 to 350^{o}C and the heating rate was 10^{o}C/min for maintaining temperature. The nitrogen gas flow was 20ml/min.

Fourier Transform Infra-red spectroscopy

Due to close contact in any formulation there is a possibility of drug polymer interaction and this interaction study was carried out by using FT-IR spectroscopy. The drugs are alone and in combination with polymers were checked through FT-IR to check the interaction between the drugs and polymers. The FT-IR used was (Perkin Elmer, UK) ranging from 650-4000 cm. Sample of weight 8 mg was placed on the stage of machine. After that a sharp peaks of reasonable intensities were obtained.

Physicochemical evaluation of patches

All the patches formulations were evaluated for following

Thickness

The patches prepared were evaluated for thickness and uniformity. Microscrew gauge was used for thickness of patches [8].

Weight Uniformity

For determination of weight uniformity 10 patches were randomly collected from the formulation and weight individually. Digital electronic balance was used for the weighting of patches. Average weight of 10 patches were calculated and confirmed to single patch weights.

Folding Endurance

To evaluate the efficiency of patches, folding endurance test was performed. The folding endurance value of patch was performed by rapidly folding a patch at the same point broke [14]. When the patch is folded for a number of times at the same time point without breaking will define the value of the folding endurance.

% Moisture uptake

From each formulation 3 patches were selected randomly to determine the moisture uptake and weighed accurately. At room temperature these patches were placed on desiccator along with saturated solution of aluminum chloride to maintain humid condition. After 3 days the patches are removed from desiccator and weighed again. The individual % moisture uptake was calculated from the difference between the final and initial weight as the percentage of initial weight. After that average % moisture uptake was calculated.

%Moisture loss

From each of the formulation 3 patches were selected randomly and weighed accurately. These patches were placed in desiccator at 37 ^{o}C along with dry condition maintain by the anhydrous calcium chloride. After 3 days the patches were removed from desiccator, weighed individually and the moisture loss was determine by difference b/w the initial weight and final weight as % age of initial weight [18].

Moisture content

The formulation to be test were marked properly and kept in desiccators for the analysis of moisture content by using silica gel maintained at room temperature for about 24 hours. The formulation were removed from desiccator and weighed individually again and again till they show a content weight. % Age of moisture content was calculated by evaluation [24].

Drug Content

Drug content test was performed for the formulated patches, by placing the patch in 20ml of volumetric flask, sonicated for 8 hours. The sonicated solution then filtered and drug content were determined by using spectrophotometric at their respective wavelength of the drug.

Stability study

The patch selected for the study were kept for six month in incubator maintained at 37 ± 0.05 ^{o}C and 75±5 % RH. After the interval of six month patches were removed from incubator and evaluated for physical appearance and the drug content.

***In vitro* drug release studies of prepared patch:**

Pharma test dissolution apparatus were used for the dissolution study of prepared patches and also for evaluation of *in vitro* drug release according to the method described in The USP. The patches were placed in the bottom of the each vessels of dissolution medium. These studies were carried out at 32± 0.5 ^{o}C at 50 rpm. Vessels were covered with lids at specific time interval, 0, 0.05, 1, 1.5, 2, 4, 6, 8, 12, 20 and 24 h. Samples of 5 ml were collected from dissolution medium and were simultaneously replaced with an equal volume of fresh dissolution medium. Spectrophotometer was used to analyze the drug in the dissolution medium at their respective wavelength using phosphate buffer PH 7.4 as a blank [7].

Drug release Kinetics

Following kinetics models were applied according to the nature of data obtained from different formulation, to study release kinetics.

Zero order kinetics

For constant release of drug zero order release may be applied [22, 12]. Zero order release kinetic model may also be for those active pharmaceutical ingredients which do not disintegrate in dosage form [1]. It is represented by following equation.

$W=k_1t$

Where

w= drug release at time = t

K_1= rate constant for zero order release

T = Time

First order kinetics equation

It was proposed by [17] Wagner this model is used for absorption and release or elimination of drug from biological system. This model may be used in those conditions in which skin condition exist, and it is represented by following equation.

$In (100-W) = In100-k_2t$

Where

w= drug release at time = t

K_1= rate constant for first order release

T = time

Higuchi Square of time evaluation

Higuchi model describe non eroding matrix for example ointment base. Modified drug delivery system may also follow Higuchi model [6]. The Higuchi model equation is as follow.

$W= k3\ t^{1/2}$

W= drug release at time =t

K3= Higuchi dissolution rate constant.

t= time

Korsmeyer Peppas equation for mechanism of drug release

This model is semi empirical and produces relationship among release drug and elapsed time with an exponential function.

Mathematically it is represented by following equation.

Korsmeyer-Peppas: $M_t / M_\infty = kt^n$

M_t / M_∞ = Function of released drug

K4 = Kinetic constant that represent structural and geometrical characteristics of device'

n = drug release diffusion exponent [15]. To represent different drug release mechanism or n values this model has been used. Drug release mechanism follow Fickian diffusion when n is equal 0.45 when the value of n is greater than 0.45 then it is non-Fickian. When the n value is equal to 0.89 then it follow typical zero order release or case II transport. When the n value is greater than 0.89 then it may follow super case II transport [1].

***In vitro* drug permeation study of Meloxicam**

Franz diffusion cell was used to study permeation of selected drug Meloxicam across the rabbit skin. The already prepared skin was placed b/w donor compartment and receptor compartment in such a way that the stratum corneum of the skin facing donor compartments [21]. The prepared patch was placed on the skin having drug releasing surface. The receptor compartment have phosphate buffer of PH 7.4 and at temperature 32 ± 0.05 ^oC the receptor fluids was maintain water Jackets and maintain the required temperature around the receptor compartments. Magnetics beads were also used to stirrer the receptor fluids. From the receptor compartments 2ml of fluid were taken at regular interval of 0, 0.5, 1, 1.5, 2, 8, 12, 16, 20, and 24 h. To maintain sink condition same amount of water was added to receptor compartment which was drawn for sampling. The samples drawn from the receptors compartment were analyzed spectrophotometrically against their respective were wavelength. The drug permeated from the patches were calculated and plotted against time. The flux was calculated from drug permeated per cm^2/h [7].

III. Result and Discussion

The thickness and weight of the patches are shown in table 3. The thickness of patches was measured with the help of micrometer screw gauge. The thickness range was from .20mm to .24mm. The weight of patches also range from 220mg to 235mg .The low value of standard deviation shows that the patches have approximately uniform weight and thickness. Folding endurance is also shown in table 3 which shows that the patch would

maintain their integrity and would not break. Percentage moisture absorbance and moisture loss are shown in table 3. The percentage of moisture absorbance was greater in formulation MLXT5 and MLXT6 as compared to other formulations because it contains greater amount of thiolated chitosan. The greater moisture absorbance was due to removal of amino group from chitosan and addition of thiol group to chitosan which opens the polymeric network and absorb moisture.

Tab 3: Meloxicam transdermal patch physical parameters and drug contents flaxseed oil as permeation enhancer.

Formulation Code	Weight (mg±SD)*	Drug contents (%)	Thickness (mm±SD)*	Folding Endurance (times)	% Moisture Absorbance	% Moisture Loss	Hardness	Flatness (%)
MLXT1	220.23±.006	98.12±.034	.20±.002	180	08.32±1.3	7.3±1.6	213±1.3	100
MLXT2	225.67±.003	99.34±.034	.21±.003	188	8.34±1.6	7.5±2.1	227±1.8	99.98
MLXT3	228.12±.005	98.87±.023	.22±.005	198	8.7± 1.3	8.2±2.4	233±2.1	99.96
MLXT4	232.23±.014	99.64±.031	.21±.007	206	8.1±1.9	7.8±1.9	240±1.9	99.98
MLXT5	235.89±.018	98.37±.121	.23±.004	216	9.2±1.4	8.8±1.7	237±1.6	100
MLXT6	230.34±.018	97.25±.023	.24±.007	205	9.4±1.7	8.6±2.4	242±1.8	99.88

SD= Standard deviation, *Mean (n=3)

To study the release mechanism of drug from polymer different kinetics models are used. These models are zero order, first order, Higuchi, Hixon Crowell and Korsmeyer- pepas which are shown in table 4. The *in vitro* drug permeation was found to be first order kinetics and release mechanism was found to be (nonFickian).

Table 4: Controlled Release Patches of Meloxicam Containing Different ratio Chitosan: Thiolated Chitosan.

Formulation Code	Zero Order	First order	Higuchi Order	Hixon Crowell	Korsmeyer		Release Mechanism
	R^2	R^2	R^2	R^2	R^2	n	Non Fickian
MLXTI	0.589	0.573	0.812	0.876	0.843	0.643	Non Fickian
MLXT2	0.754	0.843	0.921	0.935	0.935	0.687	Non Fickian
MLXT3	0.718	0.921	0.943	0.987	0.945	0.612	Non Fickian
MLXT4	0.763	0.953	0.963	0.923	0.923	0.687	Non Fickian
MLXT5	0.823	0.994	0.953	0.978	0.976	0.654	Non Fickian
MLXT6	0.801	0.942	0.972	0.943	0.943	0.612	Non Fickian

Stability Study of the Patches

Stability Study of the Patches is shown in table 5. At the beginning, during and at the end the accelerated stability shows that the tested patches have approximately uniform drug contents, good flexibility and elastic properties, thus ensuring the stability of prepared patches. The patches kept for stability were found to be smooth, flexible and no change in the physical appreance of the patches.

Tab 5: Physical stability characteristics of Meloxicam

Evaluation Parameter	F. Code	1st month	2nd month	3rd month	5th month	6th month
Drug contents	MLXT5	99.24	98.26	98.23	98.12	98.32
	MLXT6	99.53	98.97	97.98	97.23	97.12
Appearance	MLXT5	No change	No change	No change	No change	No change
	MLXT6	No change	No change	No change	No change	No change

***In vitro* Permeation study of Meloxicam**

The effect of permeation depends on the penetration enhancer used. In the present study thioaled chitosan was used both as controlled release and also increase the permeation of drug without addition of permeation enhancer. *In vitro* was found to be more in those formulations which have high proportion of thiolated chitosan such as formulation MLXT6 as shown in table 6. Maximum flux, permeability coefficient, enhancement ratio and low lag time value represent that it had increase the permeation of Meloxicam. The increase permeability of thiolated chitosan is due to thiol group attchement to the chitosan and also causes the opening of polymer chain which makes the polymer matrix to absorbed moisture and swells up. When the polymer swells up then it can not resist the drug diffusion which make it possible to rapid release of drug. The patches containing high proportion of thiolated chitosan have maximum permeability across the rabbit skin as shown in fig 1.

Tab 5: Transdermal patches of Meloxicam by using thiolated chitosan as permeation enhancer through rabbit skin, values are mean and standard deviation of 3 determinations.

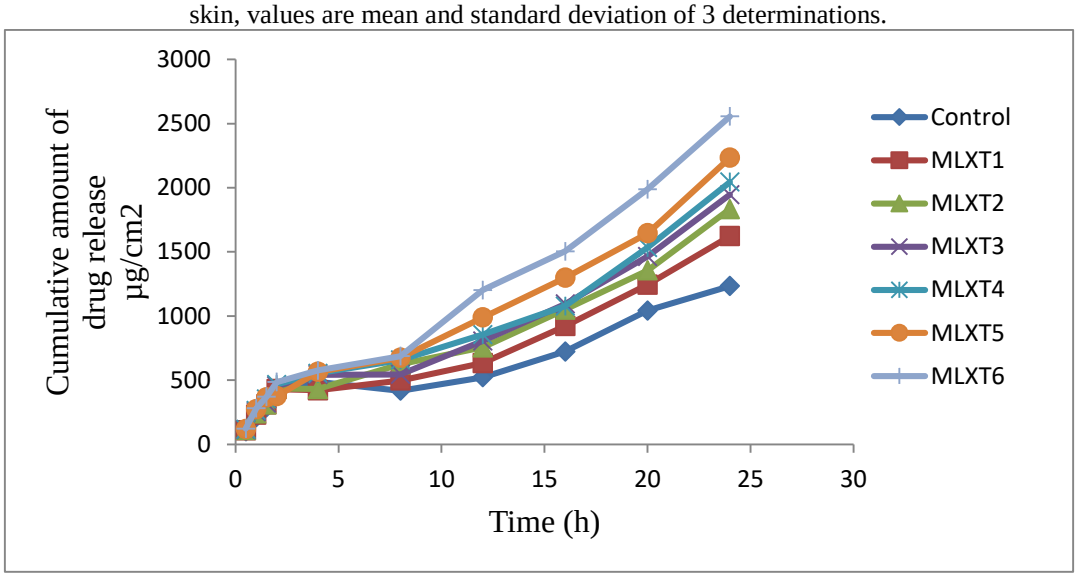

Fig 1: Cumulative amount of Meloxicam permeated by using different concentration of Thiolated chitosan as permeation enhancer through rabbit skin.

Rabbit skin				
Formulation code	**Flux (µg/cm^2.h) ± SD**	**Kp (cm/h) ± SD**	**ER**	**T_{lag}(h) ± SD**
Control	**21.53 ± 1.72**	**0.689 ± 0.002**	**1.00**	**3.87 ± 0.006**
MLXT1	21.21± 2.12	0.310 ± 0.006	1.34	3.08 ±0.004
MLXT2	35.23 ± 2.72	0.611 ± 0.003	3.32	3.35 ± 0.003
MLXT3	60.34 ± 1.93	0.987 ± 0.023*	7.19	2.75 ±0.006
MLXT4	87.23 ± 3.54	1.113 ± 0.034*	12.48	2.24 ± 0.008
MLXT5	112.34 ±3.22	3.112 ± 0.052*	15.85	2.13 ± 0.011
MLXT6	139.45 ± 4.23	4.431 ± 0.004*	17.84	1.43 ± 0.012

IV. Conclusion

In the present study thiolated chitosan was prepared by covalent attachment of thiol group to the chitosan and further different concentration with chitosan was used for permeability of drug Meloxicam. Matrix type transdermal patches were prepared by chitosan and thiolated chitosan in different ratio. Stability study shows that there was no significant changes were found. It was found that the formulation having high amount of thiolated chitosan have maximum permeability. The release mechanism of Meloxicam from polymers chitosan and thiolated chitosan shows that the patches follow non-Fickian and follow zero order release kinetics.

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