3.3. **Review of work done on Methotrexate**

Laxmi et al.\(^\text{52}\) showed the preparation of niosomal MTX in chitosan gel and test the same of irritation and sensitization on healthy human volunteers and also comparing its efficacy with a marketed MTX gel in the treatment of localized psoriasis. Results showed that no significant irritation and sensitization is produced from niosomal MTX gel and is more efficacious than marketed MTX gel.

Sharma et al.\(^\text{53}\) has developed dermal drug delivery system that is required to localizes MTX in the synovial joint is needed to treat inflammation in RA. Developed vesicles were characterized for size, size distribution, shape, in vitro release, pH dependent, and storage stability. The MTX amount permeated through rat skin was three- to fourfold higher using oleic acid compared to those from plain drug solution or carbopol gel. At the end of the skin permeation assay using ufasomes, up to 50% of the administered dose was found in the skin.

Mohamed Ali et al.\(^\text{54}\) prepared MTX liposomes (LMTX) from DPPC, soy PC, egg yolk PC, and cholesterol. Gel formulations were applied daily, followed by irradiation with 80 joules from a 650-nm diode laser 3 times weekly for 12 weeks. Drug release increased as laser energy increased following Higuchi’s diffusion model and changed to zero order with energies ≥80 J; significantly more MTX was released at all time intervals. Gels showed zero order kinetic release and antipsoriatic activity. During 8 months’ follow-up, up to 60% of the patients treated with LMTX gel had no recurrence; this difference was statistically significant. So it is concluded that application of an 80-J diode laser to 0.25% LMTX hydrogel was beneficial for relieving psoriasis and did not exert systemic toxicity.

Hwang et al.\(^\text{55}\) in an attempt to develop an optimised MTX topical formulation containing pharmaceutically acceptable excipients, used a \(2^3\) factorial design to investigate the effects of a fatty alcohol, propylene glycol and ethanol on the in vitro skin permeation and uptake of MTX. The results of steady-state flux and skin uptake of MTX from these formulations ranged from 0.035 to 0.315 \(\mu g/cm^2/h\) and 1.146 to 7.929 \(\mu g/cm^2\), respectively. The results of the in vivo study demonstrated the localisation of MTX at the treated site for both formulations without significant uptake of MTX in the distant untreated epidermis and dermis. The levels of MTX in
the blood and liver following topical application of the optimised cream were significantly less than those of the gel formulation with 3% Azone.

**Chatterjee et al.**\(^{56}\) studied the effect of vehicles and enhancers on the in vitro and in vivo percutaneous absorption of MTX through hairless mouse skin. The group observed that MTX fluxes of 35 µg/cm²/h were achievable only with 1-15% (v/v) Azone in propylene glycol (PG). A clinically significant steady state in vivo blood concentration of MTX was achieved using delivery systems containing 2.5% Azone in PG. Area under the drug concentration--time curves for MTX were 2379 and 3534 ng h/ml from PG/2.5% Azone and PG/7.5% Azone systems, respectively.

**Nagle et al.**\(^ {57}\) developed Liposomal and niosomal MTX topical formulations indicated in the symptomatic control of severe, recalcitrant, and disabling psoriasis. The present work aimed at investigating the potential benefits of combining menthol with MTX in a vesicular gel base for not only improving the penetration and dermal availability of MTX, but also to render such a formulation more effective with greater patient acceptability. The results indicated that the vesicular gel containing menthol could cause maximum drug retention in the skin. The skin treated with menthol had a disrupted epidermis and microcavities. The in vivo studies also ascertained the effectiveness of the formulation in inducing a normal pattern of differentiation in the rat tail skin that initially showed parakeratosis, which is also characteristic of psoriatic epidermis. These results show the potential of vesicular gel containing MTX and menthol to improve penetration into the skin and cause drug retention in skin appendages.

**Misra et al.**\(^ {58}\) designed MTX loaded solid lipid nanoparticles (SLN), incorporate it in suitable gel base, and evaluate it *in-vitro* and clinically to justify the role of the developed gel in treatment of psoriasis. SLN were incorporated in Carbopol 934p (1% w/w) gel base. Type of lipid, drug:lipid molar ratio, and concentration of surfactant were found to be critical variables influencing particle size and PDE (P < 0.05). However, concentration of cosurfactant was observed as non-influencing parameter. The optimized MTX -SLN was smooth, spherical with average diameter about 123 nm, and PDE of 52.16%. *In vitro* skin deposition studies showed significantly higher (P < 0.05) deposition of MTX from MTX -SLN gel. Clinical studies have
demonstrated improvement in therapeutic response (P < 0.01) at all evaluation time points and reduction in local side effects.

Lu et al.\textsuperscript{59} found 13.59 ±2.9 µg/cm\textsuperscript{2} flux were obtained by using rabbit ear skin and gels containing 0.5-1.0% of MTX and 4% N, N-diethyl-m-toluamide (DEET). The relatively high levels of MTX reached in the skin on concomitant application of iontophoresis suggest that this technique may be appropriate for the treatment of psoriasis; however, this cannot be confirmed until the concentration of MTX required for therapeutic effect is established.

Czyżewska et al.\textsuperscript{60} checked the bi-directional transport of urea (U), uric acid (UA), inulin (I) and albumin (A) across rabbit peritoneum and its change under the influence of MTX. The transport coefficients increased with decreasing molecular mass of these compounds. MTX diminished the bi-directional transfer of U and UA by about 35% but no changes in I transport were found. Flux of A directed from the mesothelial to the interstitial side of the membrane under the influence of MTX was increased by about 35%, while the transport in the opposite direction remained unchanged. The obtained results confirm previous hypothesis concerning the membrane point of MTX. Suggestion of a complex mode of this action within peritoneal membrane was put forward.

Prasad et al.\textsuperscript{61} developed MTX loaded polyacrylamide-based hydrogel patch to see the effect of enhancers. Flux enhancement (161%) of MTX was achieved with ethyl acetate:menthol:ethanol (1:1:1) in combination with square-wave iontophoresis for 1 hour. Lower flux enhancement of 71%, 83%, and 93.5% was obtained in vitro with neat ethyl acetate, its binary composition with ethanol, and its ternary composition with ethanol and menthol, respectively, as compared to passive. In-vivo studies on mice shows plasma concentration approx 18.79 µg/ml with ternary mixture of ethyl acetate in combination with square wave. The reversibility studies conducted in vivo on mice demonstrated that the histological changes induced by the above-mentioned enhancers were transient and reversible in 48 hours. Study indicates that the above-mentioned enhancers are safe and well tolerated by the skin.

Kumar et al.\textsuperscript{62} prepared MTX hydrogel for Palmoplantar psoriasis (PPP) and studied the efficacy and safety of a recently marketed topical MTX (0.25%) preparation in a hydrogel base in patients with palmoplantar lesions. The response at the end of the
study was graded as minimal if there was up to 25% reduction in the EISF score, mild as 26-50% reduction, moderate improvement as 51-75% reduction in score, and marked improvement as >75% reduction in score. The scores at the end of the study were 3.5 ± 0.7 for palms and 4.8 ± 0.2 for the soles. MTX 0.25% in a hydrophilic gel is well tolerated but is not very effective in controlling the lesions of psoriasis on the palms and soles. A higher concentration in a different base with better penetration could possibly provide better results.

**Prasad et al.**\(^6\) studied iontophoretic method of enhancing the transdermal transport of MTX with 0.2mA/cm\(^2\) current density for permeation enhancement. This study describes the effect of physicochemical properties, like cross linking density of the hydrogel, copolymerisation, duration of electrical current and alcohol pre-treatment on the transport of MTX across the skin using hydrogel patches.

**Chaudhary et al.**\(^6\) developed and optimized mucoadhesive bilayered buccal devices comprising a drug MTX containing mucoadhesive layer and a drug free backing membrane. A combination of sodium alginate with carbopol-934 and glycerol as plasticizer gives promising results. The optimized patch exhibit an in vitro release of 82% through cellophane membrane and 70.78 % through buccal mucosa with satisfactory mucoadhesive strength and mucoadhesive time. The release kinetics through cellophane membrane was Higuchi while in buccal mucosa it is zero order. From Higuchi model we can say the mechanism of drug release is diffusion control. The ex vivo also fitted to Korsmayer-Peppas equation which characterize the release mechanism. The value of n is more than one so release was non Fickian i.e. not depends upon concentration gradient.

**Trotta et al.**\(^6\) developed and evaluated Deformable liposomes of MTX to investigate the effectiveness of dermal administration of MTX using soybean lecithin (PC) or hydrogenated lecithin (HPC) as phospholipid and dipotassium glycyrrhizinate (KG) as surfactant. The MTX amount permeated through pig skin were three- to four-fold higher using liposomes containing KG compared to those from water solution or normal liposomes. No significant differences were observed between PC-KG liposomes and HPC-KG liposomes. At the end of the skin permeation assay using deformable liposomes, up to 50% of the administered dose was found in the skin.
These results suggest that liposomes containing KG may be of value for the topical administration of MTX in the treatment of psoriasis.

Srisuka et al.\textsuperscript{66} investigated the physico-chemical characteristics and in vitro permeability of MTX-entrapped deformable liposomes prepared from phosphatidylcholine (PC) and oleic acid (OA), comparing with those of MTX-entrapped conventional liposomes prepared from PC and cholesterol (CH). All liposome formulations showed a narrow size distribution with the size range of 80–140 nm which is appropriate for the skin permeability. The percentage of MTX loading, entrapment efficiency and the stability of MTX-entrapped PC2:CH1 and PC9:CH1 liposomes were slightly higher than those of MTX-entrapped PC2.5:OA1 liposomes. However, the MTX-entrapped PC2.5:OA1 liposomes enhanced the skin permeability characterized by the higher concentration and flux of MTX diffused across or accumulated in the epidermis and dermis layers of porcine skin. This suggested that the PC2.5:OA1 deformable liposome was one of promising candidates to enhance the permeability of MTX for the treatment of psoriasis.

Rama et al.\textsuperscript{67} developed suitable matrix transdermal therapeutic system of MTX with different proportions of hydrophobic [Ethyl cellulose (EC)] and hydrophilic polymers [Hydroxy propyl methy cellulose E15 (HPMC)] by using a D-optimal mixture design. The in vitro release studies revealed that the drug release from the patches follows Higuchi kinetics (Correlation coefficient between 0.973 – 0.9949, \(p<0.001\)) and it shows the maximum release and flux (1212.88 ± 44.95 and 18.58 µg/hr/cm\(^2\) respectively). But, to reach the target flux (20.02 µg/hr/cm\(^2\)) physical and chemical enhancers are to be employed.
3.4. **Review of work done on Azathioprine**

**Bhaskar et al.** developed RP-HPLC method for the quantitative estimation of AZT in pure drug and its formulations. The detection wavelength was set at 280 nm and the linearity was found to be in the range of 30-90 μg/ml. The proposed method was found to be simple, precise, accurate, and reproducible for the estimation of AZT in pure drugs and its formulations.

**Epstein et al.** presents the first report of the use of topical AZT in the management of persistent symptomatic chronic oral graft-versus-host disease (GVHD). Topical AZT suspension was used as an oral rinse and was swallowed, maintaining the previously prescribed systemic dose of AZT, and resulted in improvement in a case of oral GVHD that was resistant to other approaches to management. Topical AZT may provide additional therapy in the management of immune-mediated oral mucosal disease. Clinical trials appear warranted based upon the results of topical AZT use.

**Gulati et al.** encapsulated AZT and 6-mercaptopurine (6-MP) (prodrug of AZT) into liposomes and investigated to find out the conditions for its optimal entrapment. AZT and also 6-MP show higher encapsulation efficiencies in MLVs as compared to LUVs. Variation in phospholipid composition does not seem to affect the loading capacity of either of the two drugs. The encapsulation efficiency of both the drugs improves upon addition of cholesterol in the bilayer, but the effect is seen only up to 30% cholesterol. AZT shows better incorporation in the positively charged liposomes as compared to those with neutral or negative charge. Entrapment efficiency for both the drugs markedly depends on the pH of the hydration medium, yielding better entrapment efficiencies at high pH values. The rise in solute concentration initially causes increase in the entrapment of the two drugs which is followed by a decreasing phase.

**Yalin et al.** prepared to optimize the formulation technique of AZT liposomes and evaluation the quality by a film-vibration technique by means of an orthogonal design test. The entrapment ratio of AZT liposomes was 49.19% and consisted of spherical multilamellar vesicles with 78.17% of particles in the liposomes smaller than 2.4 μm. From the results one can say that the formulation technique of AZT liposomes is rational and stable.
Moses et al.\textsuperscript{72} reported a case of cholestatic hepatitis developed one week after exposure to AZT. The subsequent prolonged cholestatic phase was followed by full clinical remission. Current knowledge on pathogenesis and epidemiology and the diagnostic challenges presented by this rare complication are discussed, followed by recommendations for monitoring and management.

Van Os et al.\textsuperscript{73} discussed bioavailability of 6-mercaptopurine after administration of azathioprine via three colonic delivery formulations. The bioavailabilities of 6-mercaptopurine after colonic azathioprine administration via delayed release oral, hydrophobic rectal foam, and hydrophilic rectal foam (7\%, 5\%, 1\%; respectively) were significantly lower than the bioavailability of 6-mercaptopurine after oral azathioprine administration (47\%) by Wilcoxon rank sum pairwise comparison. The therapeutic potential of these colonic delivery methods, which can potentially limit toxicity by local delivery of high doses of azathioprine, should be investigated in patients with inflammatory bowel disease.

Agrawal et al.\textsuperscript{74} discussed on letter to editor that low dose oral azathioprine therapy is an effective adjunctive treatment modality for controlling the severity of disease and reducing the frequency of exacerbation in patients with chronic hand eczema.

Sekar et al.\textsuperscript{75} formulated silver nanoparticles using green approach based on polysaccharides as reducing and stabilizing agent. In this study chitosan stabilized silver nanoparticles were prepared and azathioprine was conjugated with silver nanoparticles to treat the inflammation in rheumatoid arthritis. SEM images of Azathioprine Silver nanoparticles showed spherical particles in the range of 180nm to 220nm. An in vitro drug release study was carried out and percentage drug release was found to be 67.34\% at the end of 24 hours for formulation. In vitro toxicity of azathioprine loaded silver nanoparticles was studied in 3T3 NIH fibroblast cell line. The formulation plays a dual role, to target the diseased site and to release the drug in a controlled manner and produces synergetic effect to the inflammatory sites.

Lakshmi et al.\textsuperscript{76} investigated four simple and sensitive visible spectrophotometric methods (A-D) for the assay of AZT either in pure form or in pharmaceutical formulations. Methods A and B are based on the oxidation of AZT with excess N-bromosuccinimide (NBS) or chloramine-T (CAT) and determining the consumed NBS or CAT with a decrease in colour intensity of celestine blue (CB) (method A) or
galloccyanine (GC) (method B), respectively. Methods C and D are based on the
diazotisation of reduced AZT with excess nitrous acid and estimating either the
consumed nitrous acid (HNO(2)) with cresyl fast violet acetate (CFVA) (method C)
or by coupling reaction of the diazonium salt formed with N-1-naphthyl ethylene
diamine dihydrochloride (NED) (method D). All of the variables have been optimized
and the reactions presented. The concentration measurements are reproducible within
a relative standard deviation of 1.0%. Recoveries are 99.2-100.3%.

Harris et al.\textsuperscript{77} investigated that Azathioprine has been shown to reduce the steroid
requirements of patients with severe rheumatoid arthritis. 27 patients treated with
azathioprine have now been followed up for 30 months. At the end of this period only
10 were still taking the drug. Maximum steroid reduction occurred within the first 12
months of treatment. Some steroid-sparing effect seemed to persist after the drug was
stopped. There was no evidence that azathioprine prevented radiological deterioration.
No deaths occurred and toxic effects always reversed on stopping the drug.

Urowitz et al.\textsuperscript{78} determined efficacy and safety of azathioprine in 'high' and 'low' dose
regimens in rheumatoid arthritis (RA), both in short-term studies and in follow-up
over 40 months. The apparent increased risk of malignancy previously suggested by
others warrants further studies with larger populations and over a continuous longer
period.
3.5. **References**


