**Background:** The present work focuses on the process development of a suitable topical formulation incorporated with piperine indented for Vitiligo therapy. The objectives were further alienated into formulation development of preliminary conventional formulations, later novel drug developments and further extension of nanotechnology products. The intention of the work was to develop a formulation which can retain the piperine in deep dermis of the skin where melanocytes are present and facilitates repigmentation by stimulating melanocytic proliferation and formation of melanocytic dendrites. Thus product development of conventional topical dosage forms to novel drug delivery systems including nanotechnology was employed.

**Scope:** It is reported that as many as 65 million people suffer from Vitiligo worldwide and India hosts 0.46 - 8.8% vitiligo patients against 0.5-1% of the world Vitiligo population. Conventional treatment of Vitiligo involves corticosteroids and UV radiation which has unwanted but unavoidable side effects like Cushing’s syndrome, skin carcinoma etc. When it was understood that conventional treatments using chemicals cause undesired side effects, a team of scientist discovered piperine, a biological alkaloid can stimulates the replication of melanocytes as well as induces the formation of melanocytic dendrites in vitro. The need for developing a suitable formulation was identified.

**Experimental:** By appreciating the scope, suitable formulations were developed. In the process, under preliminary conventional topical formulations, ointment and cream was considered in the preliminary stage. Novel products like phytosomes and vesicular systems like transferosomes, otherwise known as ultradeformable vesicular drug delivery systems and nano sized cubosomes were taken into account. Fusion method and emulsification was employed to prepare ointment and cream. In the preparation of phytosomes the drug-phospholipid were refluxed and sovent evaporated under reduced pressure. Film hydration technique by rota evaporator was deputed in the preparation of transferosomes using phosphatidyl choline. Cubosomal nano particles were prepared by a fusion of top down approach were glyceryl mono oleate and polaxamer 407 and bottom top approach was sonicated dispersion was employed. In all the cases sonicated piperine was used. Modified
diffusion cell and consistency tester were indigenously fabricated. Tape stripping method was employed to quantify the drug in the tissue.

**Results:** X-Ray diffraction studies were performed for piperine powder before and after sonication. The peak height was found to be reduced in piperine powder after sonication indicating that crystallinity has reduced increasing the solubility. The size of the particles also was brought down from 59.8 µm to 13.77 µm after sonication. By performing the partition coefficient, the drug concentration of piperine found in organic phase (n-hexane) and aqueous phase (phosphate buffer pH 6.6) was found to be 0.972mg/ml and 0.02mg/ml respectively. The partition coefficient value of piperine was found to be 1.68. Piperine was found to be soluble in the order-chloroform > ethanol > acetone but insoluble in water.

Based on the tissue bio availability, cream was optimised in conventional dosage forms. Minimum, maximum and average globule size of cream was found to be 4.72µ 52.75µ and 28.75 µ respectively. The globules retained its size and no coalescence was found indicating that the formulation is stable. Pharmacological studies revealed that the group subjected to the topical cream therapy found to commence repigmentation within 59 days and the group subjected to cream and UV therapy was found to repigment by three weeks but repigmentation was spotted scatter (patchy) type whereas in the former group it was found to be homogenous. Almost complete pigmentation was achieved for the first and second group towards 59 and 50 days respectively.

Surface morphology of phospholipid complex (phytosomes) as examined by SEM appeared as brick like cubical crystals. Short term accelerated studies showed that the shelf life of the prepared piperine dispersion complex emulsion system was found to be 17.65 months if stored at 27 °C.

In transfersomes, the size of the vesicles were almost same before (66.97 µ) and after extrudation (and 68.92 µ) through a membrane whose pore size (12µ) is smaller than that of the vesicles. Dermal drug retention was observed more with span 80 incorporated transfersomes which also showed more entrapment efficiency compared to its comparable tween 80 formulation.

Entrapment efficiency of cubosomes C1, C2 and C3 were found to be 46.82%, 62.54% and 76.25% respectively. TEM pictures showed the size of optimised cubosomes in the range of 40.31 nm. **In-ovo** studies have shown normal blood vessel proliferency, no atypical growth
and regular embryonic movements were observed throughout the biocompatible study period of 24h.

The group which was subjected to topical piperine-cubosomal therapy (G3) was found to commence slight repigmentation within 15 days, but not homogenous while G1 (control) and G2 group (only cubosomal therapy) of animals were found to have little effect at this stage. Towards one month of therapy, G2 also showed slight homogenous repigmentation while in G3 the rate of repigmentation was found to augment and almost homogenous. Observations in couple of months revealed repigmentation process was significant in both G2 and G3 while G1 remained the same. In three months period (90 days) of experimental therapy we found almost complete repigmentation while G1 showed no change from albino hairs. In G3 repigmentation was spotted scattery (patchy) type whereas in G2 it was found to be homogenous. While comparing with the previous work of antivitiligo study with brown rabbits, G3 group was shown better homogenous recovery. Black rabbits took almost double the period to depigment, but recovery comparatively was found to be quite faster.

Periodical Confocal experiments suggest that as time proceeds drug-cubosomal concentration in the stratum corneum decreases and dermal region exceeds. DSC investigation of the skin treated by optimised formulation showed a decreased endothermic peak at 52.4°C while untreated skin exhibited the transition at 68.5°C.

Comprehensively when all the optimised formulations were compared for their tissue drug tailoring, drug diffused into the buffer and left over the dorsal surface, the following results could generate. When urea was incorporated in ointment the drug retention within the tissue was enhanced by 0.6%. It was found that the tissue drug bioavailability for each optimized formulations, drug tailored in the dermal region was in the descending order: ointment (22.5%) > cream (69.06%) > phytosomes (72.07%) > transfersomes (75.25%) > cubosomes (88.03%). Drug quantification from each optimized formulations diffused into the buffer was phytosomes (22.60%) > transfersomes (20.75%) > cream (18.62%) > ointment (16.2%) > cubosomes (11.01%). Drug left over the dorsal skin surface of each optimized formulation in the descending order was ointment (59.99%) > cream (8.02%) > phytosomes (5.32%) > cubosomes (0.79%) > transfersomes (0.38%).

**Conclusion:** Out of all formulations, cubosomes had shown the maximum dermal drug tailoring when compared to transfersomes, phytosomes, cream and ointment. It can be
concluded that the kinetics, efficiency and the drug transport can be tailored for trans-epidermal, deep tissues and systemic depending on the formulation composition, dose and form. In sum, it has to be said when cubosomes at drug: GMO ratio of 1:1 and Poloxamer 407, 9% were fixed, the drug piperine was able to tailor to the dermis at its maxima, and so this formulation is the optimized one which was proven by the pharmacological experimental data.