‘Development of UV Stabilizing Biomedical Textile by LbL Coating of Tinuvin-622 Polymeric Nanoparticles’

MS in Biomedical Sciences

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‘Development of UV Stabilizing Biomedical Textile by LbL Coating of Tinuvin-622 Polymeric Nanoparticles’

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A thesis submitted in partial fulfillment of the requirements for the degree of

MS Biomedical Science

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Dedication

To my mother, Narmeen, and my sisters Hania, Sania and Zainab for their unconditional love and support.

Also, to every single person in the research phase; you might think that you are losing it but believe me you got this!

Declaration

I certify that this research work titled ***'Development of UV Stabilizing Biomedical Textile by LbL Coating of Tinuvin-622 Polymeric Nanoparticles'*** *is* my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources has been properly acknowledged / referred.

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MS Biomedical Science

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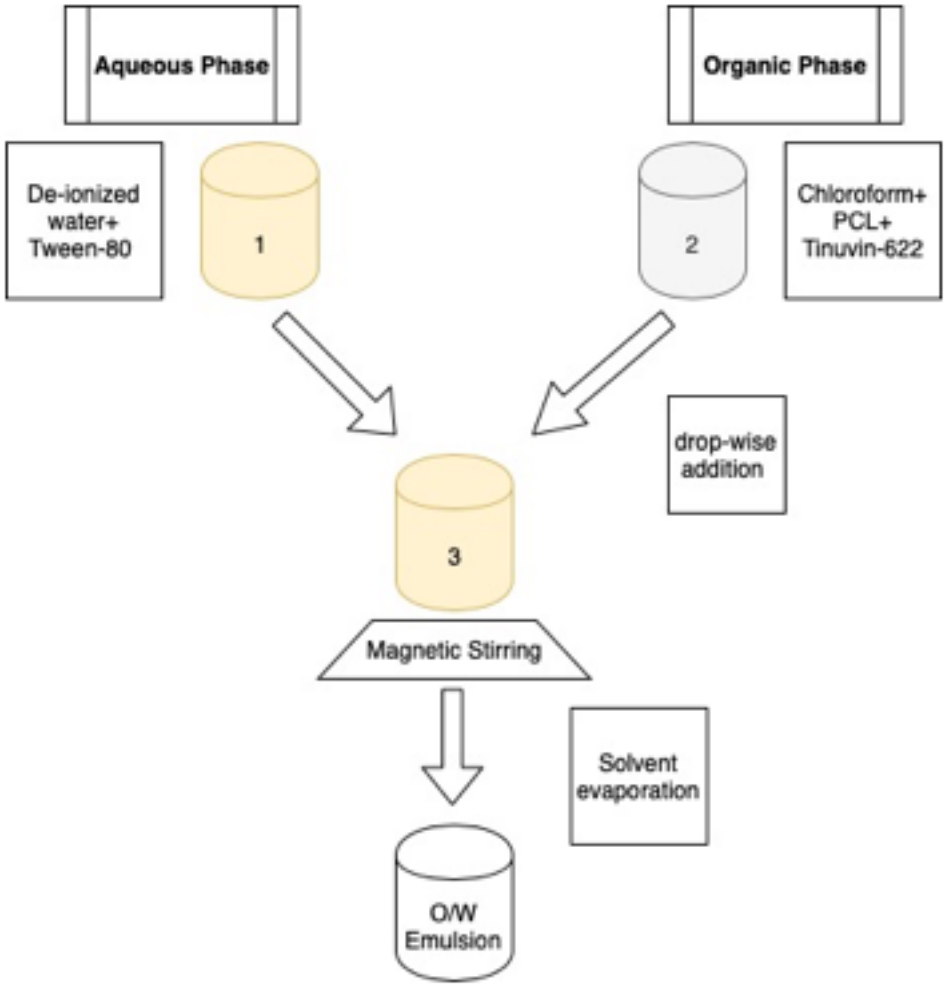
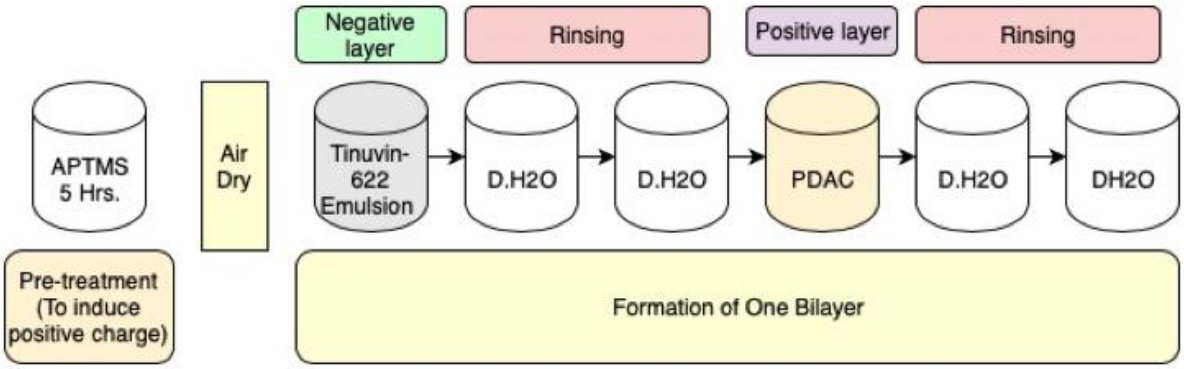
ABSTRACT

The purpose of this research was to formulate a protocol to develop a low cost, easily manufactured UV Stabilizing cotton textile by coating polymeric nanoparticles of PCL encapsulating a light stabilizer, HALS. HALS was coated with PCL to form nanoparticles which were suspended in a liquid formulation of a homogeneous solution of Tween-80 and Deionized water. Various parameters such as drug concentration, polymer concentration, surfactant concentration, organic solvent concentration, temperature, stirring speed and stirring time were varied to find the most stable and homogeneous drug loaded formulation. The best optimized formulation was then diluted and coated on industrially obtained cotton textile in combination with PDADMAC solution by using LbL coating method. Further characterization by Optical Microscopy, Scanning Electron Microscopy, Particle Size Analysis and Zeta Potential, UV Spectrophotometry, Fourier Transform Infra-Red Analysis, UV-Vis-NIR Spectrophotometry and UPF testing were performed on the emulsion as well as textile. The most stable emulsion showed a hazy appearance, with an average particle size of 200 nm, poly Dispersity Index of 0.13, zeta potential of -19. SEM results showed the formation of spherical nano-capsules and FTIR analyses showed the bonds found on the surface of the nanoparticles and the textile confirming the coating of polymer. UV spectrophotometric analysis at 215 nm showed an encapsulation efficiency of 84%. UPF testing showed a UPF value of 30.65 which is appropriate with respect to the application.

GRAPHICAL ABSTRACT



Emulsion Formation



Layer by Layer Coating

Introduction

1.1 Study Background

As the strength of ultraviolet radiations is increasing every year, it is important to develop efficient methods to block the UV rays in order to protect human skin from the detrimental effects of these radiations. Long-term exposure to UV rays can implement negative effects on human skin resulting in conditions like sun burn, early on-set of skin aging, photodermatosis, erythema (reddening of skin) and skin cancer (Gambichler, Altmeyer, & Hoffmann, 2002). Sun screen lotions are widely applied in medical and cosmetic industry as one of the most efficient solution to block UV rays, however, their temporary attachment to skin and adsorption into the skin pores with-in a short interval of time rules out the possibility of real impact.

The development in nanotechnology research has immensely benefitted the pharmacology. As textiles play a very important role in providing protection to skin, hence many researches have been carried out to utilize nanoparticles to impart special properties on the textiles. A number of techniques have been studied to introduce UV protection to fabrics by applying certain semi-conductor metal oxides. (Hossain & Rahman, 2015) Many organic UV absorbers, particularly Hindered Amine Light Stabilizers (HALS) have been used to protect surfaces from the effects of UV rays (Sekar, 2000).

1.2 Detrimental Effects of UV rays:

The detrimental effects of ultraviolet rays are having a progressively enhanced effect on our environment and society.

The wavelength range of 40 to 400 nm comprise the Ultraviolet radiations rendering a considerably high energy to cause a number of detrimental effects to organic materials. Sun is the primary source of UV rays in our surroundings. Although, most part of the UV rays is screened by the stratospheric ozone layer and only the UV rays ranging in long wavelengths, i.e., UVB (290 -320 nm) and UVA (320-400 nm) manage to touch the Earth's surface. Still these rays cause various damages not just to our bodies but also to other organic materials around us. For instance UVA and UVB rays are the cause for development of pathologies, such as premature aging of the skin, cataracts, skin cancer, Alzheimer's disease, immune system suppression and inflammatory disorders (Ansel, Mountz, Steinberg, DeFabo, & Green, 1985). UV

radiations also damage other organic substances such as plastics, textiles, timber and paints in different forms like chalking and discoloration. (Perenich, 1998)

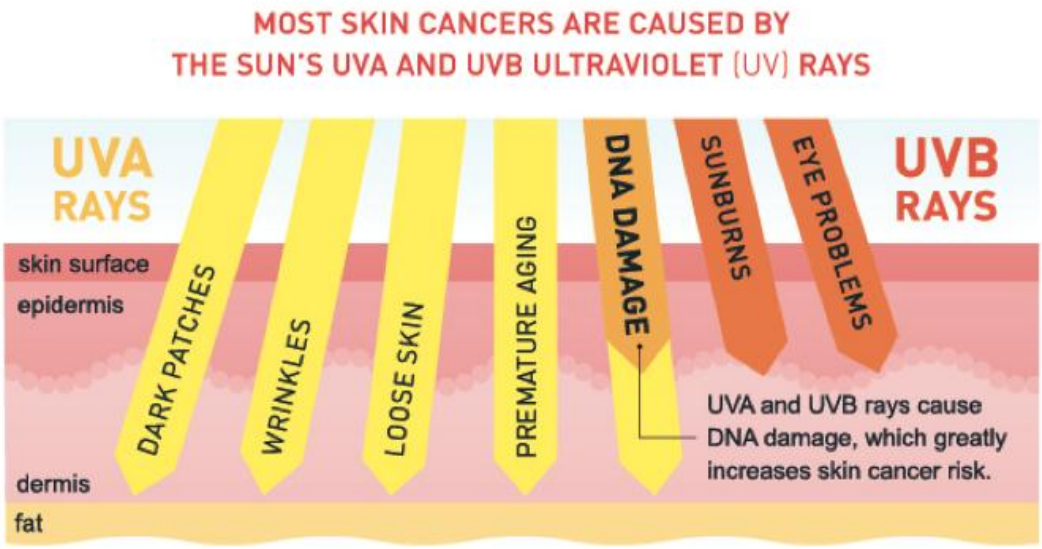


Figure 1 Detrimental Effects of UV Radiations

1.3 Protection against UV Radiations:

A number of different measures are being taken now a days to stay protected from the negative effects of UV rays. Many forms of sun blocks and sunscreen lotions with a range of Sun Protection Factor (SPF) are being formulated and sold in medical and cosmetic stores. A number of preventive measures are also being advised such as minimal exposure to sun rays, reduce the use of tanning beds etc.

Recently, researchers have developed the modification of textile fabrics by imparting special UV resistant features on them in order to provide a better protection against UV rays.

1.4 Role of Nanotechnology in Combating UV Radiations:

Nanotechnology revolves around the idea of designing as well as producing and applying nano-sized materials for the betterment of humans. Over recent few years, the field of nanotechnology has proven to be promising technology to create innovative products and improve functionalization of a material which was not deemed possible earlier. Recently, the use of nanotechnology has been incorporated in providing UV finishing for providing protection against harmful radiations. Two main types of UV blocking nanoparticles have

been studied, organic UV blocker and inorganic UV blocker. Nanoparticles has made it possible to achieve high level transparency while maintaining good UV-absorption or blocking properties. Another advantage of nanoscale dimension is that it promotes a uniform distribution of the particles in host material which enhances the effectiveness of blocking harmful UV radiations.

1.5 Polymeric Nanoparticles:

Polymeric Nanoparticles (PNPs) are made from biocompatible and biodegradable polymers having a diameter range of 1-1000 nanometers. They are used as drug carriers in pharmaceutical preparations. The drug can be entrapped or encapsulated in the polymeric nanoparticle or it can be attached to the matrix of nanoparticle resulting in the formation of nano-capsules or nanospheres. These can be distinguished on the basis of how the drug is carried in. In nanospheres, drug is uniformly distributed in the matrix system while in nano-capsules, it is found in the inner core inside the membrane of polymeric covering. The PNPs are broadly used in a number of fields including sensors, photonics, medicine, environmental technology, conducting materials, medicine, electronics and pollution control. (Geckeler & Stirn, 1993)

Synthetic Polymers	Natural polymers
1. Poly anhydrides	1. Pectins
2. Polyamides	2. Carrageenan
3. Polyvinyl alcohol	3. Starch
4. Poly lactic acid	4. Cellulose
5. Poly acrylic acid	5. Alginates
6. Poly cyano acrylates	6. Chitosan
7. Poly ethylene oxide	7. Xantham Gum
8. Poly caprolactone	8. Gellan Gum

Table 1 List of Natural and Synthetic Polymers

1.5.1 Use of Nanoparticles in Imparting UV Resistance Features on Biomedical Textiles:

Nanoparticles containing functional molecules having UV absorption or blocking properties have been developed in recent years. Various studies have been conducted on the

development of UV resistant textiles in order to establish protection against skin diseases and skin cancer. Both organic and inorganic reagents have been used to develop UV resistant coating for textiles to impart special properties. Coatings like these have been employed in many commercial products such as sunscreen lotions used for topical application, coating for cars, paints etc. (Spanhel, 2006). Nanoparticles are advantageous because of their high surface area and energy as it provides a better affinity to surface of fabrics consequently enhancing the durability of the coatings (Riva, Algaba, & Pepió, 2007).

The past few years have witnessed a rapid development of methods of surface modification to enhance the UV-protection treatment of textiles by using nanotechnology. In most cases, ZnO or TiO2 nanoparticles are used as functional coating to enhance the UV blocking properties. However, a number of UV absorbers have also been tried to achieve the same purpose. Being relatively cheap and generally transparent, they are being used in colored fabrics and textiles (Perenich, 1998) (Riva et al., 2007).

1.5.2 Layer by Layer Coating:

Layer by layer (LbL) coating/deposition technique involves the alternative deposition of oppositely charged colloids. It was first recommended by R. Iler in 1966. In early 1990s, LbL assembly was rediscovered by Decher and colleagues to prepare a polyelectrolyte multilayer thin film (Tian et al., 2016).

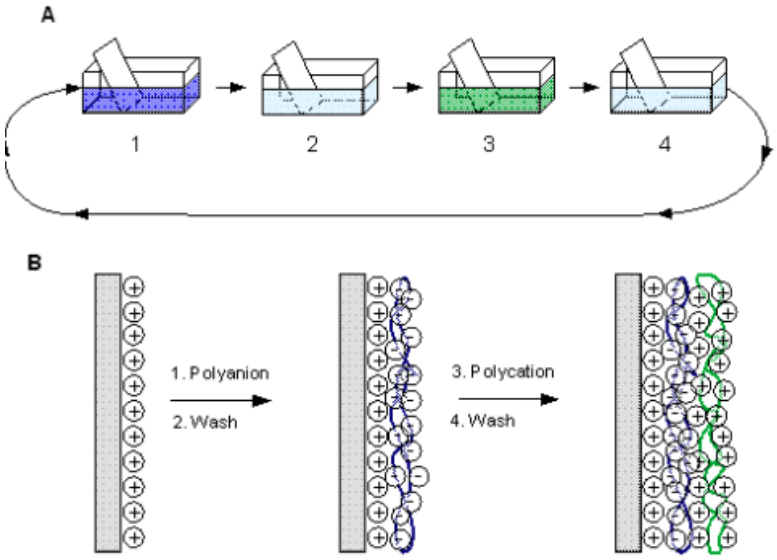


Figure 2 Schematic Illustration of Layer by Layer Coating Method

1.6 Ultra Violet Protection Factor (UPF):

Ultra Violet Protection Factor (UPF) is a numerical representation of the capability of textile fabrics to block the harmful UV rays and protect the skin. It is defined as:
"Ratio of time required to show burning symptoms such as redness on the skin with and without protection, when exposed to sunlight." (Perenich, 1998)

Mathematically, it is given and calculated as:

$$\text{UPF} = \frac{\text{MED protected skin}}{\text{MED unprotected skin}}$$

Where, MED is the Minimal Erythemal Dose or simply the amount of energy required to cause the first pigmentation of skin after 20 – 24 hours of continuous exposure to the sun.
(The International Commission on Non-Ionizing Radiation Protection,* 2004)

1.6 Aim of the Study:

The aim of the study is to create a UV resistant textile that can be used in healthcare as well as everyday use in order to provide an efficient protection against the UV rays.
As the use of sunscreen lotions and sun blocks for topical applications are not deemed as an efficiently long-term solution to provide protection against the harmful UV radiations, it is required to find other solutions. In this project one such solution has been proposed by modifying the surface of industrially available cotton fabric and turn it into a UV resistant biomedical textile. The surface modification is done by coating the textile with an anti UV emulsion carrying polymeric nanoparticle encapsulating the anti UV agent.
The polymer chosen in this study is Poly ε-caprolactone (PCL) owing to its prior use in a vast range of biomedical applications and a slow degradation rate. The anti UV agent being tested here is HALS that is a non-toxic, non-volatile Hindered Amine Light Stabilzer already being used in a number of surface coating applications for protection against UV radiations. The anti UV agent will be encapsulated in PCL in organic phase and mixed with an aqueous phase containing distilled water and a suitable surfactant to form an emulsion which will be studies for stability. It will be then diluted and coated on the textile in combination with Poly diallyl-dimethyl ammonium chloride (PDADMAC) by Layer by Layer technique. The emulsion and textile will be characterized by SEM, FTIR, Zeta Potential Measurements and

DLS, Optical Microscopy, Optical Profilometry, UV Spectrophotometry and Ultraviolet Protection Factor Measurement. The textile will be washed several times using a house hold detergent and again its UPF will be measured in order to understand the effectivity of UV resistivity after several wash cycles.

LITERATURE REVIEW

2.1 Human Skin:

Human skin, being the largest and the most complex organ possesses excellent characteristics of providing a physical barrier between external environment and the human body. It provides an effective foremost line of defense against harmful external physical and chemical factors and aids in regulating the homeostasis of human body. (Boer, Duchnik, Maleszka, & Marchlewicz, 2016). Along with playing physical protection roles, human skin acts as a thermoregulatory and resorptive organ, plays its role in metabolism, takes parts in immunological processes and provides protection against pathogenic microorganisms (Zhai & Maibach, 2004)

2.1.1 Structure of Human Skin:

The skin is structurally assembled into two primary layers; epidermis and dermis.
These two layers are formed by a combination of physiologically different components including epithelial, glandular, neurovascular and mesenchymal components.

- **Epidermis:** The outermost layer, epidermis is of ectodermal origin and acts as the point where body connects with the external environment. The biological and physical characteristics of epidermal layer makes it capable of playing a significant role in resisting the stressors of external environment, for example chemical agents, harmful UV radiations and infectious pathogens (Mark Elwood & Jopson, 1997) (Lowe, 2006) (Slominski & Wortsman, 2000). The most numerous cells present in epidermis are the Keratinocytes. Keratinocytes functions to express the cytokeratin and build up the desmosomes. The second most plentiful cells in the epidermal of skin are melanocytes. They are derived from the neural crest and are involved in the synthesis of melanin (Nordlund, 2007)(Slominski, Tobin, Shibahara, & Wortsman, 2004). Melanin is stored in the keratinocytes present in epidermal layer and acts as the only source of pigmentation in skin as well as a natural sunscreen, blocking the harmful UV radiations from penetrating into the skin effects including regulation of epidermal homeostatic regulation, acting as free radical scavengers in order to provide

protection against free oxidative species and antimicrobial activity (Slominski et al., 2004). A basement membrane separates the epidermis and dermis.

- **Dermis:** The dermis lies under the epidermis and is derived from mesoderm. It forms the various structures of skin such as glands including sweat and sebaceous, hair follicles and nerve endings. The dermis contains fibroblasts and immune cells which actively play their role in responding physiologically towards any stimuli. (Slominski & Wortsman, 2000).

Human skin is affected by a number of biological and physical stressors present in the environment. One of the most common physical stressors abundantly occurring in our surroundings is the Ultra Violet radiations. The protection of human skin from ultraviolet radiations is of utmost importance as the imbalances result in early onset of ageing, skin cancer, wrinkles, dermatitis and other sorts of disorders of immune system. (Wilson, Moon, & Armstrong, 2012). UVR also significantly alters the mechanical characteristics of the stratum corneum resulting in a lessened cell to cell cohesion thus effecting mechanical integrity.(Biniek, Levi, & Dauskardt, 2012). They can cause cellular damage which results in photoaging, DNA damage by triggering the formation of pyrimidine dimers, oxidative stress, genetic mutations inflammatory responses and suppression of immune system (Meeran, Punathil, & Katiyar, 2008)(Das, 2010).

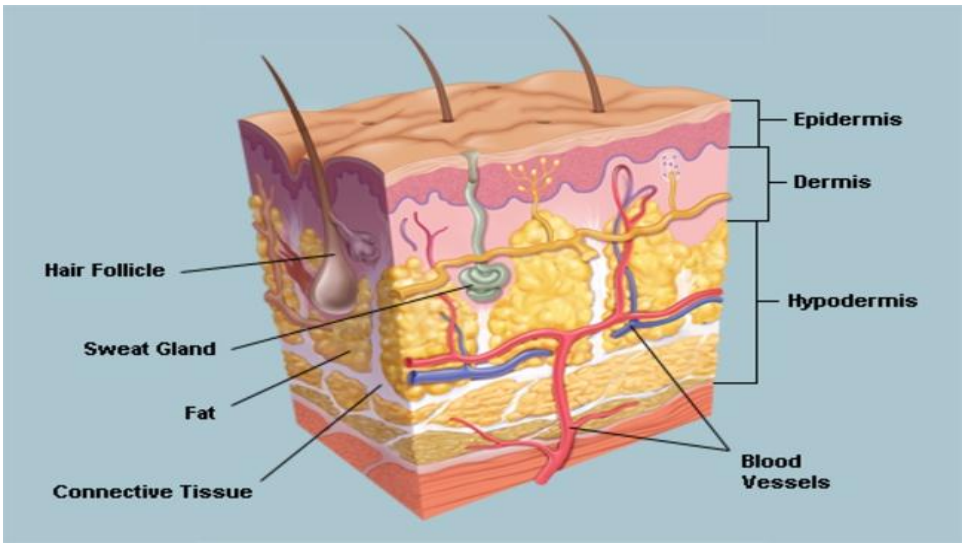


Figure 3 Structural Layers of Human Skin

2.1.2 Ultra Violet Radiations:

Electromagnetic rays can be described by two main theories: a) waves b) quantum or corpuscular (Gorenšek & Sluga, 2004) According to quantum theory, lights exists in the form of small energy packets known as photons. Further, a smaller wavelength has higher frequency and high levels of energy. Ultraviolet radiations, constituting the shorter wavelengths are energetically very high. The spectrum of day light reaching to us through earth's atmosphere is 290 – 3000 nm and radiations between 290 – 400 nm are referred to as Ultraviolet radiations (Zohdy, B. El Hossamy, El-Naggar, I. Fathalla, & M. Ali, 2009) (Lee, 2009).

The ultraviolet radiations are categorized on the basis of their wavelength ranges. UV-A falls in a range of 315 to 400 nm, UV-B from 290-315 nm and UV-C lies from 100-290 nm. Ozone layer absorbs the highest energy UV-C while Ultra violet rays A and B manage to reach the earth's surface and become a cause of serious health issues (Abidi, Hequet, Tarimala, & Dai, 2007).

UV radiations can be classified as the most ubiquitous and prominent naturally and abundantly occurring physical carcinogen in our surroundings (De Gruijl, 1999). As the UV radiations possess the properties of both, tumor initiation and tumor promotion, they are established as "complete carcinogen". UV radiations also function as a mutagen and being classified as a non-specific damaging agent they are known to be considerate risk factor for skin diseases that occur due to environmental effects such as skin cancer. (D'Orazio, Jarrett, Amaro-Ortiz, & Scott, 2013).

2.1.3 UVR Penetration through Human Skin:

As the ozone layer is depleting and its effectiveness is suffering a major threat due to the use of a particular gas known as Chloro-Flouro-Carbons (CFCs), it allows the UV-A and UV-B rays to pass through it and reach the atmosphere. (Davis, Capjack, Kerr, & Fedosejevs, 1997) These UV rays tend to penetrate the human skin in a wavelength-dependent fashion. UV-A, the longer wavelength, deeply penetrates into the dermis and reaches well inside where it efficiently generates free radicals or ROS (Reactive Oxygen Species) that result in reactions of indirect photosensitization that can cause DNA damage. On the other hand, all of the UV-B radiations are almost absorbed by the epidermal layer with a very little amount of it reaching the dermis.

They are absorbed by the DNA directly and cause modifications. These modifications include the molecular rearrangement in pyrimidines of the DNA causing the formation of specific photoproducts for example cyclo-butane dimers and 6–4 photoproducts. These modifications can result in a number of genetic mutations and skin cancer.

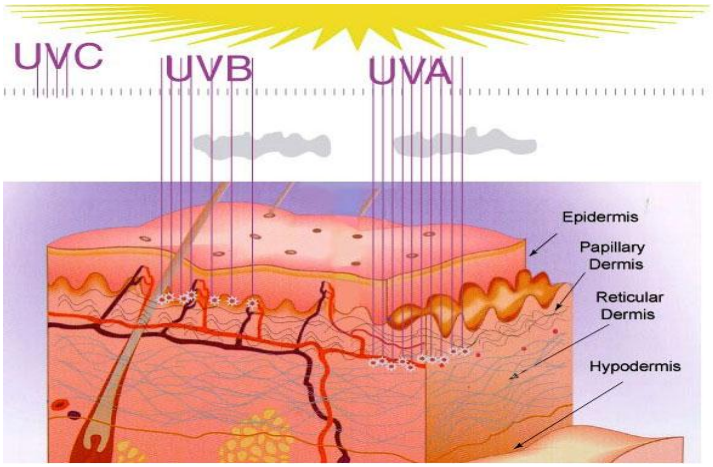


Figure 4 Penetration of UV Radiations into Human Skin

2.1.4 Common Diseases Caused by UVR:

Ultra violet rays, being abundant in the atmosphere, effects human skin physiology in many ways. Some of the consequences occur immediately or acutely while the onset of others is delayed in the lifetime. (D'Orazio et al., 2013)

Common skin maladies that are caused by UV rays include sunburn, inflammation, degenerative aging and cancer. (Mark Elwood & Jopson, 1997)

- **Sunburn:** UV-B rays activate the cytokines that begin a cascade reaction along with the neuroactive and vasoactive mediators of the skin and this results in the onset of "Sunburn". (J Clydesdale, W Dandie, & Konrad Muller, 2002). If the UV exposure crosses a certain extent, it leads to an apoptotic death of keratinocytes. These keratinocytes, known as sunburn cells, are identified by the presence of a pyknotic nuclei.(Bayerl, Taake, Moll, & Jung, 1995)

- **Hyperkeratosis:** UV rays also cause hyperkeratosis that is the increase in thickness of epidermis. A constant exposure to UV rays' initiates damage response pathways in keratinocytes activating damage signals such as p53 that alters the physiology of keratinocytes, facilitates cell cycle arrest, activate DNA repair and if the damage is

significant it also initiates apoptosis. After several hours of exposure, the damage response signals are reduced, and the epidermal keratinocytes robustly proliferate facilitated by different epidermal growth factors. An enhanced cell proliferation of keratinocytes UV exposure results in the of epidermal keratinocytes which in turn increases epidermal thickness.(Coelho et al., 2009) (Scott et al., 2012)

- **Photoaging:** Photoaging is a UV induced dermal condition characterized by premature aging of skin, rough and wrinkled skin, uneven skin tone and mottled pigmentation with the histological alterations occurring in the connective tissues (Kang, Fisher, & Voorhees, 2001) It is a degenerative and cumulative problem involving skin and skin support and depends mainly on the extent of sun exposure. The risk of developing benign and malignant neoplasm increases in photoaged skin. The UV radiations trigger a complicated sequence of molecular responses that result in damaging the connective tissues of skin hence causing the early onset of aging(Sjerobabski Masnec & Poduje, 2008)

- **Photodermatoses:** Photodermatoses are a group of disorders caused by an unusual reaction to UV component of the sunlight. Sunlight. They are classified into primary and secondary types. Former includes solar urticaria, chronic actinic dermatitis etc. and occurs due to a photosensitizer such as photo-contact allergy, phototoxic reaction etc. Secondary photodermatoses includes conditions like xeroderma pigmentosum, dermatomyositis, porphyria etc. and they involve a genetic change such as disorders of DNA repair or metabolic disorders in porphyria. Both types of dermatoses are a result of UV induction (Lehmann & Schwarz, 2011)

- **Skin Cancer** Being the most commonly diagnosed cancer type (Armstrong & Kricker, 2001) skin cancer has great influences on the lifestyle and it can be even deadly.(Burdon-Jones, Thomas, & Baker, 2010). It is a persuasive fact that the main trigger of skin cancer is contact with UV radiations. (Saladi & Persaud, 2005)(De Gruijl, 1999). UV exposure stimulates the melanocytes to release more melanin, resulting in sunburn or skin tanning, both of which damages the skin, skin cells, and the genetic material within the cells. (D'Orazio et al., 2013)

There are two main types of skin cancer on the basis of cell of origin: Basal and, Squamous Cell Carcinoma (BCC & SCC), both of them fall under Nonmelanoma Skin Cancers (NMSCs) and Melanoma (Agelli, Clegg, Becker, & Rollison, 2010)

The risk of skin cancer incidence can be reduced by reducing UV radiation exposure. According to studies, almost 90% of melanomas are caused by UV exposure. (Agelli et al., 2010)(Armstrong & Kricker, 1993).

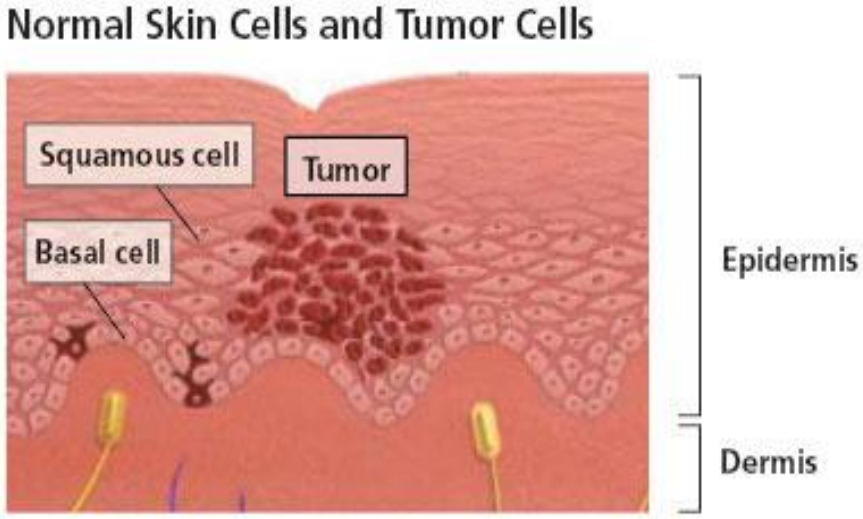


Figure 5 Effects of Tumor on Skin Cells

Skin cancer incidence is heavily affected by two main factors, skin pigmentation and UV exposure. (Lucas, McMichael, Armstrong, & Smith, 2008)

2.1.5 Protection against Ultra Violet Radiations:

For the past many years, sunscreens in different forms such as lotions, sunblock etc for topical topical applications with inorganic and organic UV filters have been in use. They offer absorption in different spectrum ranges and either filter or scatter UV radiations (Lucas et al., 2008). In the last few decades the use of sunscreens has proved to more popular and important as people are more aware of the harms caused by UV radiations, but we still find inconsistency regarding their effectiveness. Both organic and inorganic components are being used in the sunscreen formulations. Inorganic, such as *zinc oxide* and *titanium oxide,* have a broad range of spectral activity as compared to organic components but organic surpasses the cosmetic acceptability (T. Maier & Korting, 2005).

Textiles also play an important role in providing protection against UV rays. The UV protection properties of textile depend on the fiber content, weave, dye, finishing process and should fall between 40 – 50+ in order to be categorized as excellent UV protection (Abidi et al., 2007).

2.1.5.1 Topical Application of Sunscreens:

Sunscreens have become since more than 40 years the most popular means of protection against UV radiation (UVR) in Western countries. Organic and inorganic filters with different absorption spectrum exist. They filter or scatter UVR. Protection from UVB is quantified as a minimal erythema dose-based sun protection factor. UVA protection testing is less standardized: Persistent pigment darkening and critical wavelength are currently used methods. But their temporary attachment to skin and adsorption into the skin pores within a short interval of time rules out the possibility of real impact. Also, Sunscreens indeed impair vitamin D synthesis if they are used in the recommended amount of 2 mg/cm2.

2.1.5.2 Biomedical Textiles for UV Protection:

Nowadays, skin cancer is one of the most prevalent cancers in the world, so surface modification of textiles against UV radiation especially for child wear, sportswear, workwear, and leisurewear becomes more important. UV protection effect of textiles depends on nature of fibers, fabric construction factors, dyeing, and finishing agents.

UV protection properties often make use of a transparent layer of UV-absorbent materials on the surface of the fabric. In other words, UV absorbers are organic or inorganic materials with strong absorption in the UV range of 290–360 nm, applied on textile fabrics to improve UV protection factor (UPF) and sun protection factor (SPF). Among them, nanostructures and natural materials are more active and effective because of their small size and more safety, respectively (Bashari, Shakeri, & Shirvan, 2019)

2.2 Biomedical Textiles:

With the development in polymer technology, a new wave of diversity is seen in the medical textile industry in terms of design and material. It has facilitated a diverse range of applications of biomedical textiles, cosmetic textiles and implantable medical textile devices (Dattilo, King, Cassill, & Leung, 2002). According to M.W. King, bio textiles are special functionalized structures that are made from textile fiber to be used in particular biological environment. The interaction of these structures with biological cells and fluids with

reference to their biostability and biocompatibility determine their performance has. King has coined a definition for biomedical textiles which is stated as: “Bio textiles are non-living, long-lasting or short term, fibrous textile structures made out of either artificial or organic components that are made use of in in-vitro, in-vivo and ex-vivo scientific atmosphere to be incorporated in healthcare system to aid in protection, therapy or analysis of and damage or illness, and as such, provide to enhance the health, healthcare problem, convenience and health and fitness of the ill” (King, 2001).

2.2.1 Structures of Biomedical Textile:

The biomedical textiles come in various structures as given below:

- Knitted
- Braided
- Non-woven
- Woven

2.2.1.1 Knitted:

In these designs, the wool is interlocked horizontally with each other in and the content of stitching is straight. This makes the smooth, diversely used and very conformable, and have more preeminent managing features as compared to weaved components (Francis & Sparkes, 2011).

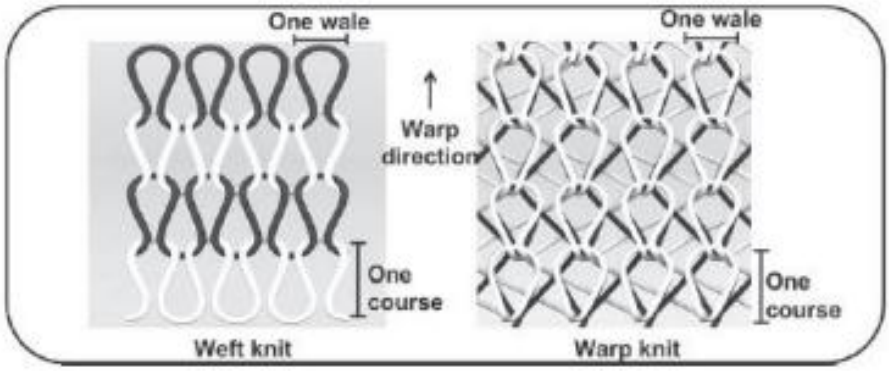


Figure 6 Knitted Design of Textile

2.2.1.2 Braided:

The braided textile has junctions that are symmetrical having different yarns which are tangled at various frequencies and angles. The braid is constructed of four variables: the horizontal repeat distance (line, l), vertical repeat distance (stitch,s) , yarn width (w), and braid angle between the yarn and machine directions **(**θ) (Murgo et al., 1998**).**

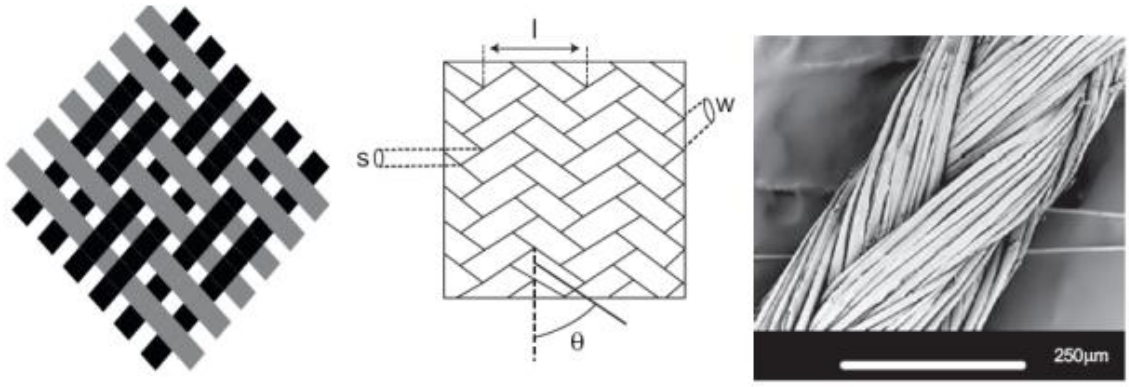


Figure 7 Braided Design of Textile

2.2.1.3 Non-Woven:

This type of textile is created from materials without the progressed venture of assembling strings first. A set of long fibers or short staples short staple is braced together by a mechanical technique. (Mao & Russell, 2004).

2.2.1.4 Woven:

The term weaved encompasses the idea of a fabric setting where the main architectural wools are focused at right angle to each other. In light of the orthogonal association between the high and weft yarns, weaved parts show low prolongation and high part toughness in both rules. There are variety of weaved designs such as simply, twill, silk, and leno patterns that are widely used in dental ribbon fabrications and vascular graft (Gandhi, 2012).

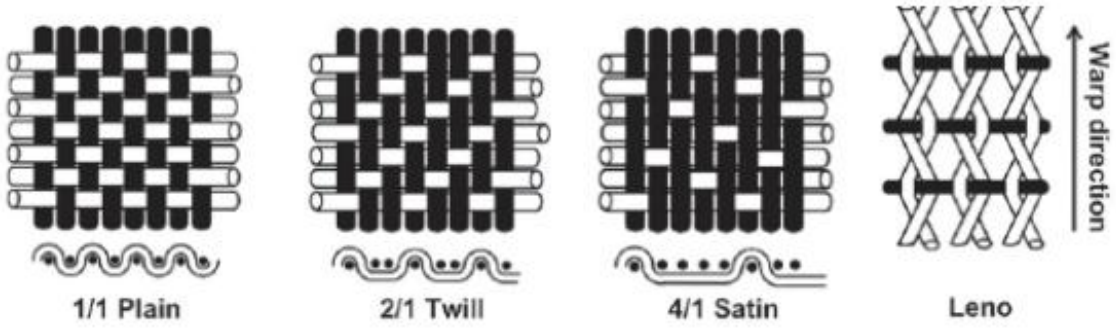


Figure 8 Woven Design of Textile

2.2.2 Materials Used in Making Biomedical Textiles:

Following fiber varieties are being used to construct biomedical textiles:

- Monofilament: single yarn, extruded
- Multifilament Yarn: yarn comprising of numerous strands that can be handled or bent together
- Staple fiber: short lengths of multifilament yarn used for non-woven and other custom applications.

2.2.3 Modifications of Biomedical Textile by Coating Systems:

Polymers are widely used on fabrics to induce novel features on them (Tahir Ansari, Farheen, M. Saquib, M. Niyaz Hoda4, & Amit Kumar, 2012). They are applied in order to increase quality and sustainability of textile or to produce specialized features in the for instance anti-microbial properties, anti-UV properties, temperature sensing feature (Kunzelman, Chung, Mather, & Weder, 2008), fragrance generating, self-washing, fire proof, super hydrophobic, electronically conductive and many other extra ordinary functional features using stimulating elements sensitive polymers (Hu & Lu, 2014)(Institute of Physics (Great Britain), Meng, Li, & Ibekwe, n.d.)

2.2.4 Layer by Layer Coating:

Layer by layer (LbL) coating/deposition method implicates the alternative accumulation of positively and negatively charged colloids. It was first recommended by R. Iler in 1966 and in early 1990s, LbL assembly was rediscovered by Decher and colleagues to prepare a polyelectrolyte multilayer thin films. (Ai, Jones, & Lvov, 2003)(Tang, Wang, Podsiadlo, & Kotov, 2006), and is a replacement to Langmuir Blodgett accumulation and SAMs.

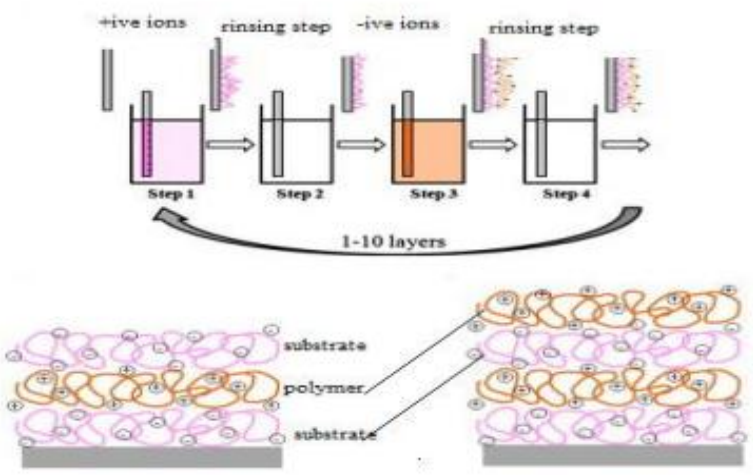


Figure 9 Schematic Illustration of LbL Coating Method

Layer by Layer assembly technique was initially developed to fabricate composite thin film coatings on surfaces to enhance features or induce new features. Polyanions and polycations are sequentially deposited on surfaces leading to a build-up of multilayer films of polyelectrolytes. The alternate deposition of sequential multilayer can be performed by submersing the substrate into the cationic and anionic solutions, alternately. After depositing each layer, the substrate is rinsed by immersing into a washing solution such as distilled water. These adsorption steps are repeated sequentially in order to deposit multilayers on the surface of a substrate. A diverse variety of functionalized molecules can be amalgamated in the thin films, such as nanoparticles carrying drugs or functional agents, dyes, proteins and a few types of supramolecular species. (G. Decher, 1997)(Bertrand, Jonas, Laschewsky, & Legras, 2000)(Schmitt et al., 1993a). A number of biomedical systems are incorporating the use of polyelectrolyte multilayered, for example, carriers for drug delivery (Jana, Gearheart, & Murphy, 2001), biosensors, regenerative neurobiology (Binks & Furlong, 1999)or antibacterial/anti infection protection (Boulmedais et al., 2004)

By using the Layer by Layer technique polyelectrolyte microcapsules have been produces not

just for different subjects of studies (Boulmedais et al., 2004) (Veronika Kozlovskaya et al., 2003) but also for practical use such as vehicles able to deliver different bio-molecules (G. Decher, Hong, & Schmitt, 1992) enabling diagnostic assays and permitting to the study of content release and allowing their use in various biomedical applications (Jewell & Lynn, 2008)(Gero. Decher & Schlenoff, 2012).

2.2.5 Textile Modifications Using Layer by Layer Coatings:

In previous studies, various coatings employing LbL technique have been applied to textiles to impart flame retardant, UV blocking, anti-wetting, electrical conductance and antibacterial properties. (Schmitt et al., 1993b) (Pan & Zhao, 2018)(Xiao et al., 2017) Dubas et. al., incorporated nanoparticles of silver to impart antimicrobial properties on fabric (Dubas, Kumlangdudsana, & Potiyaraj, 2006). Fang et al., imparted antibacterial and flame-retardant properties to the cotton fabrics using layer by layer method. They employed aqueous solutions of potassium alginate and polyhexamethylene guanidine phosphate (PHMGP) as anionic and cationic deposition solutions respectively. The coating induced antibacterial and flame retardant properties to the cotton fabrics and antibacterial activity increased with increasing the number of bilayers (Fang et al., 2016). Gomes et al., imparted antibacterial properties to the cotton fabric through LbL technique using sodium alginate and chitosan (Gomes, Mano, Queiroz, & Gouveia, 2013). In another study Chen et al., flame retarding, electrical conductivity and antibacterial properties were imparted to cotton fabrics through LbL assembly technique using potassium alginate carbon nanotubes and PHMGP. Antibacterial activity was evaluated by Kirby-Bauer test and was shown to increase with increase in number of bilayers. ZOI of 12.1mm was recorded for 5 bilayers coated sample against E. coli (at 3x106 cfu/ml) (Chen et al., 2016)

2.3 Polymeric Nanoparticles:

Polymeric Nanoparticles (PNPs) are made from biocompatible and biodegradable polymers having a diameter range of 1-1000 nanometers. They are used as drug carriers in pharmaceutical preparations. The drug can be entrapped or encapsulated in the polymeric nanoparticle or it can be attached to the matrix of nanoparticle resulting in the formation of nano-capsules or nanospheres. These can be distinguished on the basis of how the drug is carried in.

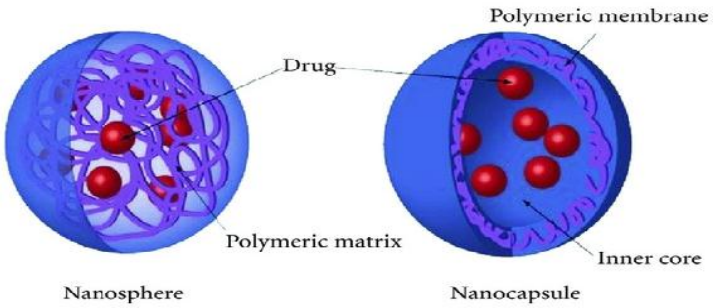


Figure 10 Types of Polymeric Nanoparticles

In nanospheres, drug is uniformly distributed in the matrix system while in nano-capsules, it is found in the inner core inside the membrane of polymeric covering.

The PNPs are broadly used in a number of fields including sensors, photonics, medicine, environmental technology, conducting materials, medicine, electronics and pollution control. They serve as an effective carrier for proteins, drugs or DNA to specific organs or cells. Their small size in nanometers helps to effectively cross cell membrane barriers and can easily flow through blood stream. These nanoparticles can be easily modified to form different nano-structure for use in medical applications.(Geckeler & Stirn, 1993)

2.3.1 Advantages of Polymeric Nanoparticles in drug delivery:

PNPs are known to be very stable hence they can be used to incorporate volatile pharmaceutical substances inside their core resulting in the increased stability of substance. They are simple and very cost effective to fabricate. They can be prepared in large quantities through a number of techniques They provide the capacity to deliver a large amount of drug to a target site. The ability to evolve drug release and a vast choice of polymers has made PNPs a model candidate for cancer therapy, vaccine delivery, contraception, topical application and functional coating systems for textiles.

2.3.2 Techniques for the Formation of Polymeric Nanoparticles:

Polymeric Nanoparticles can be prepared by two methods: firstly, by using a pre-existing polymer and secondly, by polymerization or polyreactions of monomers. (Geckeler & Stirn, 1993). There are various methods used for the formation of polymeric nanoparticles, depending on the following factors:

1. Type of polymeric system
2. Area of application od polymeric nanoparticles
3. Required size of polymeric nanoparticles

Given below is a list of most popular techniques used for the preparation of polymeric nanoparticles:

- Nanoprecipitation
- Solvent evaporation
- Salting out
- Dialysis
- Supercritical Fluid Technology (SCF)(Geckeler & Stirn, 1993)

2.3.4 Nanoprecipitation:

Nanoprecipitation is the most common technique used for the formation of polymeric nanoparticles (Lboutounne, Faivre, Falson, & Pirot, 2004) It's a very simple and efficient method and can be used easily to produce small nanoparticles with large drug loading range and a uniform size distribution (Budhian, Siegel, & Winey, 2007) Nanoprecipitation is relatively simpler technique, requires less energy and resources. It is based on the interfacial deposition because of the displacement of a solven t by a non-solvent. It is formed in the presence of two phases that are partially or completely miscible with each other.

- **Organic Phase/ Oil Phase:** It consists of an organic solvent in which the polymer and the drug are dissolved. A surfactant is sometimes added into the organic phase to prevent the aggregation of carrier molecules.
- **Aqueous Phase:** It consists of water along with a suitable surfactant. The surfactant prevents the aggregation of particles. Aqueous phase can also contain polymer to form coating or if the drug is hydrophilic in nature it can also be added into the aqueous phase.

The formation of nanoparticles through nanoprecipitation technique comprises of three phases.

1. Nucleation
2. Growth
3. Aggregation (Lince, Marchisio, & Barresi, 2008)

2.4 UV Absorbers:

Whether organic or inorganic, UV absorber compounds with no color that exhibit significant absorption in the Ultra Violet range of spectra. UV absorbers function in following ways:

- Convert electronic excitation energy into heat that dissipates in the surroundings
- Act as singlet oxygen quenchers
- Function as free radical scavengers

2.4.2 Functionalized Anti UV Coatings Incorporating Nanoparticles:

Various studies have been conducted on the development of UV resistant textiles in order to establish protection against skin diseases and skin cancer. Both organic and inorganic reagents have been used to develop UV resistant coating for textiles to impart special properties.

2.4.2.1 Metallic UV Absorbers:

ZnO nanoparticles have considerate UV absorptivity and antimicrobial properties, hence they've been used in Anti UV finishing of textiles. Yadav et. al., did a study to compare the effectiveness of ZnO in bulk and ZnO nanoparticles that were pad-dried on textile giving a 75% higher UV protection than bulk ZnO (Yadav et al., 2016). Vigneshwaran et. al., prepared nanocomposites of ZnO and soluble starch and incorporated it on cotton fabric by a technique "pad-dry-cure" using acrylic binder. The coated textile fibers exhibited an 80% efficiency of UV-protection and antibacterial activity even after 25 washes (Vigneshwaran, Kumar, Kathe, Varadarajan, & Prasad, 2006).

TiO2 nanoparticles, in the same way, show a significant photocatalytic activity and several methods have been investigates to study the use of TiO2 nanoparticles as external coating on surfaces. Li et. al., formed nano TiO2 sol and coated it on polyester fabrics via sol-gel technique using a precursor, tetrabutyl titanate and ethanol. (Li, Deng, & Zhao, 2009). Nanocrystalline TiO2 was used to coat on wool fabric by Montazer et. al., to increase the UV protection exhibited by the said fabric. They showed that by increasing the amount of Ti2, the protection increased (Montazer & Pakdel, 2010).

Many other nanoparticle applications have been done for the same purpose. Shateri-Khalilabad produced a simple method by using Ag nanoparticles having high density

deposition on the cotton fabric surface which was later combined with octyltriethoxysilane to form fabric having lower surface energy. UV blocking activity of AgNPs having average size of 500 nm was tested via AATCC where it showed Ultraviolet Protection Factor of 266.01. Studies also show the use of CeO2 nanoparticles to enhance the UV blocking capability of textiles(Shateri-Khalilabad & Yazdanshenas, 2013). Duan et. al. prepared a CeO2 sol and coated it on cotton fabrics by dip-pad-cure method and then later treated with dodecaflouroheptyl-propyl-trimethoxylsilane (DMTMS) in ethanol. They observed a considerate lowering of transmittance in UV region. The transmission at wavelengths below 350 nm was reduced to almost zero which indicates a promising protection from UV radiations (Duan et al., 2011).

2.4.2.2 Organic UV Absorbers:

Organic UV absorbers are relatively cheap (Riva et al., 2007) and generally transparent or colorless hence giving them an advantage to be used in colored textiles (Perenich, 1998).
Organic UV absorbers majorly include the o-hydroxyl benzophenones, o-hydroxy phenyl triazines and o-hydroxy phenyl hydrazines (Saravanan, 2007). The ortho-hydroxyl group is deemed to be important for the proper utilization of anti-UV agent and to make the it soluble in alkaline solutions. Organic products are used to protect and cushion materials to provide a broad security against UV radiations (SM, HM, AN, R, & HM, 2016). An accurate mixture of UV absorbers along with anti-oxidants can result in very enhanced outcomes. (Entezari, Tohidi Nejad, Hafezi, & Afrasiabi, n.d.). Ortho-hydroxy phenyl and diphenyl triazine having an excellent self-dispersing formulation and sublimation fastness are fit to be used in high temperature dyeing in pad-baths. (Sekar, 2000).

2.5 HALS:

HALS is light stabilizer of choice used in many applications owing to its oligomeric structure and high molecular weight. It belongs to the class of organic compounds known as HALS (Hindered Amine Light Stabilizers) which contain an amine functional group. They stabilize the ultra violet radiations by reacting with radical oxygen species being formed.

2.5.1 Mechanism of Action of Hindered Amine Light Stabilizers:

Unlike the traditional UV absorbers that absorb harmful UV rays from the sun and convert them into heat energy that is later dissipated into surroundings, HALS work in a different fashion. HALS tend to neutralize the photochemically produced free radicals and hence

provide protection against the harmful UV radiations. During the neutralizing process, HALS regenerate themselves hence providing a very long-term protection.

The process of HALS scavenging the free radicals and their regeneration is termed as Denisov cycle after Evguenii T. Denisov (Denisov, 1991) and is very complex (Hodgson & Coote, 2010).

The HALS undergoes oxidation resulting in the formation of a nitroxy radical. It reacts with another other free radical such polypropylene and leads to the formation of alkoxy amine. Alkoxy amines in turn react with peroxy radicals and reproduce the original HALS molecule that restarts the same cycle (C. Maier & Calafut, 1998).

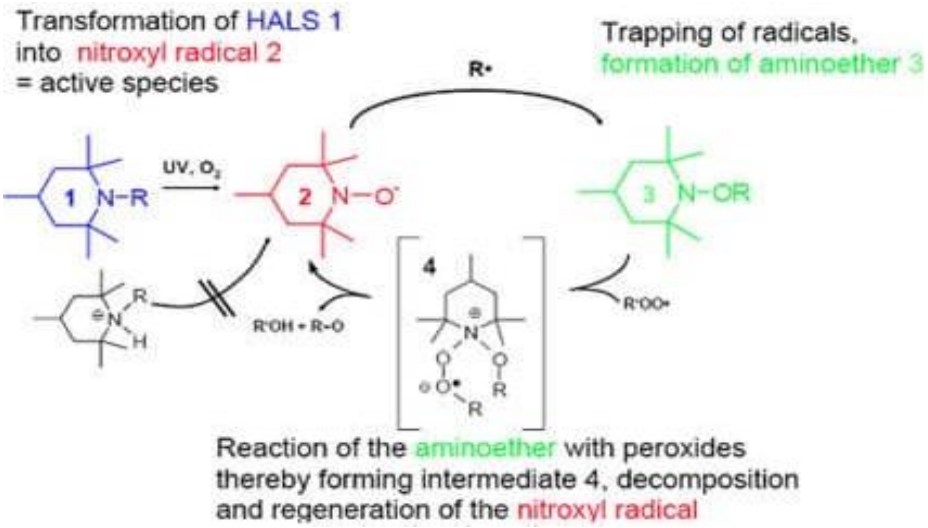


Figure 11 Mechanism of Action of HALS

2.6 Ultra Violet Protection Factor:

Ultra Violet Protection Factor (UPF) is a numerical representation of the capability of textile fabrics to block the harmful UV rays from reaching the epidermis. It is expressedas:

"Ratio of time required to show burning symptoms such as redness on the skin with and without protection, when exposed to sunlight." (Perenich, 1998)

Mathematically, it is given and calculated as:

$$UPF = \frac{\text{MED protected skin}}{\text{MED unprotected skin}}$$

Where, MED is the Minimal Erythemal Dose or simply the amount of energy required to cause the first pigmentation of skin after 20 – 24 hours of continuous exposure to the sun. The table given below displays the UPF ratings of various categories.
(The International Commission on Non-Ionizing Radiation Protection, 2004)

UPF Range	Protection Category	Effective UV-R transmission (%)	UPF Rating
15-24	Good	6.7-4.2	15-20
25-39	Very Good	4.1-2.6	25,30,35
40-50, 50+	Excellent	Less than 2.5	40,45,50,50+

Table 2 UPF Rating and Protection Categories

2.6.1 *In Vitro* Measurement of UPF:

In *in vitro* measurements of UPF, both direct and diffuse transmittance are considered to determine the UV protective capabilities of a fabric. The figure below represents the behavior of ultra violet light on a fabric in various forms such as reflection, absorption, scattering and also direct transmission.

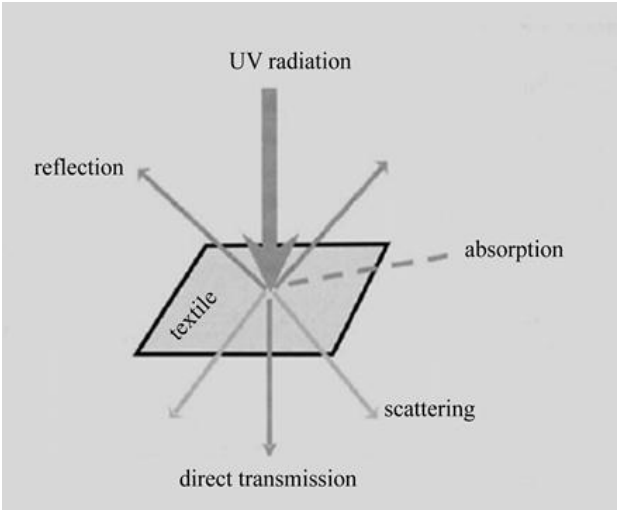


Figure 12 UV Absorption, Reflection and Transmission in Textiles

Spectrophotometers and spectroradiometers are the two devices used to measure the spectral radiation. They are capable of recording the scattered and transmittance radiation with the aid of an integrated sphere that is located behind the sample. (Gambichler et al., 2002). To determine the in vitro UPF, the spectral irradiance (both source and transmitted spectrum) is weighted against the erythemal action spectrum and the UPF is calculated as below:

$$\mathrm{UPF} = \frac{\int E_\lambda S_\lambda T_\lambda}{\int E_\lambda S_\lambda T_\lambda \mathrm{d}_\lambda}$$

Where,

Eλ: erythemal effectiveness of ultra violet radiation

Sλ: solar spectral irradiance in watts per meter squared

Tλ: spectral transmission of the sample

Dλ: bandwidth in nanometers

λ: wavelength in nanometers

Materials and Methods

3.1 Materials

3.1.1 Chemicals

a) HALS (ordered from BASF) was used as UV stabilizing agent
b) Ultra-Pure Water was used as solvent for aqueous phase and for making dilutions of emulsion
c) Poly epsilon Capro Lactone (PCL) (ordered from MERK & Co.) was used as polymer
d) Tween 80 (1.08g/ml) (ordered from Sigma Aldrich) was used as surfactant
e) Chloroform (ordered from Sigma Aldrich) was used as organic solvent
f) Raw cotton fabric (obtained from industry) was used as a substrate for coating
g) 3-Aminopropyl) trimethoxysilane (APTMS) (ordered from Sigma Aldrich) was used for pre- treatment of cotton fabric
h) Poly-diallyl-dimethyl-ammonium chloride (PDAC) was used for formation of bilayers
i) Safranin dye (courtesy of ASAB, NUST) was used for staining purposes during optical microscopy

3.1.2 Apparatus used for Experimentation

a) 50 ml beakers for preparing aqueous phase
b) 10 ml screw vials for preparing organic phase
c) 100 ml beakers for preparing emulsions
d) 10ml and 5ml pipettes for measuring liquids
e) Pipette pumps
f) Magnetic stirring bars for stirring the solution
g) Glass slides for optical microscopy and profilometery
h) Droppers
i) Double end spatula

3.1.3 Equipment used for Experimentation and Characterization

a) Electronic Weighing Balance (SCHIMADZU)

b) Hotplate (WiseStir)
c) Sonicator (Cole Parmer)
d) Rotary evaporator (Buchi Rotavapor R-210)
e) Vacuum oven (FISTREEN OVA031)
f) Optical Light Microscopy (Amscope)
g) Optical Profilometer
h) Centrifuge Machine (Siemensstr .25, HERMILE Labortechink GmbH)
i) FTIR machine ALPHA Platinum-ATR (BRUKER)
j) Analytical Scanning Electron Microscope (JSM-6490 A) and Ion Sputtering Device (automatic gold coater JFC-1500)
k) X-Ray Diffraction Analysis Machine
l) Atomic Force Microscope

3.2 Methods:

3.2.1 Preparation of Emulsion

3.2.1.1 Preparation of Blank Formulation:

Aqueous Phase:

Appropriate amount of deionized water was measured using a volumetric cylinder and added into a beaker. The beaker was placed on a hot plate and a temperature of 50-60°C. Few milliliters of Tween 80 were measured using a pipette into the beaker and stirred using a magnetic stirrer keeping the speed at high speed until a miscible pale-yellow solution of Tween 80 was obtained.

Organic Phase:

Suitable amount of poly ε-caprolactone (PCL) was weighed on an electronic weighing balance and added to a glass screw vile. Chloroform was taken into the vile. The glass vile was screwed tight and placed into a water bath sonicator until the PCL was completely dissolved in chloroform and a clear solution was obtained.

Emulsion Formation:

Tween-80 solution was added in to 50 mL glass beaker and placed on a magnetic stirrer with a desirable speed. Previously prepared organic phase was added into the aqueous phase with a suitable ratio of organic to aqueous phase The solution was left to be stirred for several minutes to ensure the complete mixing of both phases. Chloroform was evaporated from the formulation.

3.2.1.2 Optimization of Blank Formulations:

The formulations were optimized by varying different parameters including concentration of surfactant (tween 80), concentration of PCL, ratio of organic to aqueous phase, stirring speed and stirring time.

For optimizing each parameter numerous formulations were made by keeping all other factors constant and varying the parameter being optimized. Thus, optimum value of each parameter was obtained, and final emulsion was made using optimized parameters.

While optimizing each parameter, all formulations were checked visibly for their stability. Those formulations that showed phase separation, precipitations or clumping were rejected.

The formulations with no or least settled down and clumped precipitates were considered as the stable ones. For each variable most stable formulation among all the prepared nanoprecipitations was selected for further optimization.

3.2.1.3 Preparation of Drug Loaded Formulation:

Aqueous Phase:

Suitable amount of deionized water was measured using a volumetric cylinder and added into a beaker. The beaker was placed on a hot plate and a desirable temperature was maintained. Few mL of Tween 80 were measured using a pipette and added into the beaker and stirred using a magnetic stirrer keeping at high speed until we obtained a miscible pale-yellow solution of Tween 80.

Organic Phase:

Required amount of poly ε-caprolactone was weighed on an electronic weighing balance and added to a glass screw vile. Chloroform was taken into the vile in order to obtain a suitable polymer concentration. The glass vile was screwed tight and placed into a water bath sonicator until the PCL was completely dissolved in chloroform and a clear transparent solution was obtained.

This was followed by taking HALS and adding them in a glass screw vile. Chloroform was taken using a pipette and added into the vile in order to obtain required concentration of HALS solution. The glass vile was screwed tight and placed into a water bath sonicator at 30 degrees until the HALS was completely dissolved in chloroform and a clear transparent solution was obtained.

Both the PCL solution and the HALS solution were added into separate glass viles and sonicated again in order to obtain organic phase.

Emulsion Formation:

Tween-80 solution was added in to a glass beaker and placed on magnetic stirrer at a high speed. Organic phase was added into the aqueous phase keeping a suitable ratio of organic to aqueous phase. The solution was left to be stirred to ensure the complete mixing of both phases. Chloroform was evaporated from the formulation.

3.2.1.4 Optimization of Drug Loaded Formulations:

All the optimized parameters of blank formulation were kept constant while varying the concentration of HALS in order to find the most suitable concentration. The formulation that showed no phase separation, least clumping and least precipitate formation was selected for further characterization.

3.2.2 Characterization of Emulsion:

3.2.2.1 Stability Studies

The developed formulations were physically observed with naked eye for a period of 60 days. They were kept oblivious in a dark box at room temperature to analyze for phase separation or precipitate formation.

3.2.2.2 Optical Microscopy

Emulsion Formulations were checked under optical microscope (Optika B-600 MET) at different magnifications (5x, 10x, 20x, 50x) to visualize the precipitates. A drop of emulsion was put onto glass slide and observed under microscope. For drug loaded emulsion a hydrophilic dye safranin was used to stain the water phase and see the precipitates clearly.

3.2.2.3 Scanning Electron Microscopy

SEM analysis was performed to analyze the morphology and particle size of nanoparticles in the emulsion. For preparing sample for SEM analysis, a drop of emulsion was diluted in 5ml of ultra-pure water and drop of resulting solution was put onto a glass slide and dried in vacuum oven at 40 degrees for 7 hours. Afterwards it was sputter coated with gold and microscopic images were taken at 20k volts by JSM 6490LA, JEOL microscope, Japan.

3.2.2.4 Energy Dispersive X-ray Spectroscopy

The EDS analysis was conducted to perform an elemental analysis of blank and drug loaded formulations. It was conducted along with SEM. It depends on the principle that different atoms have unique atomic structure thus they respond differently to X-rays and hence form different peaks on their electromagnetic emission spectrum.

3.2.2.5 Particle Size Analysis (DLS) and Zeta Potential Analysis

Dynamic light scattering was performed to obtain the zeta potential and size range of nanoparticles (NPs) in the prepared emulsions that is the measurement of diameter of the particle. Samples were diluted with water and analyzed using zeta sizer nano-zsp (malvern). Zeta sizing works on the principle of Dynamic Light Scattering determining the Brownian motion of particles to find their size. The larger the particles the slower will be their movement. A Zeta sizer takes two pictures after a short interval of 100 microseconds to see the movement of particles.

3.2.2.6 Fourier Transform InfraRed Spectroscopy:

FTIR machine ALPHA Platinum-ATR (BRUKER) was used to conduct FTIR analysis. The ATR machine allows us to use the sample in its original form without the formation of kbr pellets as required in conventional FTIR machines. FTIR analysis was conducted on HALS, PCL, Tween-80, blank formulation and drug formulation. A tiny quantity of sample was dropped at the scanning point made of diamond and the head of ATR is touched over it. The results can be viewed on attached screen using particular softwares.

3.2.2.7 Ultra-Violet Spectrophotometry

UV analysis was conducted on drug loaded formulations and the blank formulation was initially run as the blank. For this purpose, 4 mL of diluted blank formulation was initially run as blank in the spectrophotometer keeping the wavelength range from 200 to 400 nm. Then, 4 mL the diluted drug loaded formulation was run to determine the UV spectra of HALS.

3.2.2.8 Entrapment Efficiency

In order to find out the amount of drug successfully encapsulated in the nanoparticles suspended in the formulation, entrapment efficiency was calculated. For this purpose, blank and drug loaded samples were first centrifuged at appropriate processing conditions. This resulted in the formation of a pellet that consisted of un-entrapped particles is obtained and discarded and the supernatant is then undergone UV spectrophotometry by running distilled water as blank.

For the designing of calibration curve, serial dilutions of HALS were prepared. A stock solution of HALS in acetone and distilled water was initially prepared.

For making serial dilutions, 50mL of stock solution was taken in a separate beaker and 50mL distilled water was added into it keeping the total volume 100 mL and resulting in the formation of first dilution. For second dilution, 50 mL of volume from the first dilution was taken in a separate beaker and 50 mL distilled water was added into it keeping the total volume 100 ml. A total of 7 dilutions were prepared in the same way.

Spectrophotometric analysis was conducted twice on the dilutions in a random order and the mean absorption of each dilution at 215 nm (the wavelength of HALS) was recorded. A curve of mean absorption and concentration was plotted, and encapsulation efficiency was calculated by eliminating the amount of free drug found in the supernatant from the initial amount of the drug loaded. Following formula was used for this purpose:

Entrapment Efficiency (%) = [(Total drug – Free drug) / Total drug] * 100

3.2.3 Layer by Layer Coating on Textile:

For this purpose, we obtained a 100 % cotton fabric from industry. The fabric was cut into swatches of 75 mm x 35 mm.

Pretreatment: The fabric swatches were pretreated and dried in air.

Layer by Layer Coating: PDAC solution and HALS emulsion were taken in 100 mL beakers for coatings. A simple assembly for LbL coating was arranged. Six 100 mL beakers were set in a line where the first beaker contained HALS emulsion, second and third beaker contained deionized water, fourth beaker contained PDAC solution and fifth and sixth beaker again contained deionized water.

The pretreated fabrics were dipped in emulsion solution for several minutes and then rinsed in deionized water twice. They were then dipped in PDAC solution and again rinsed twice in subsequent beakers.

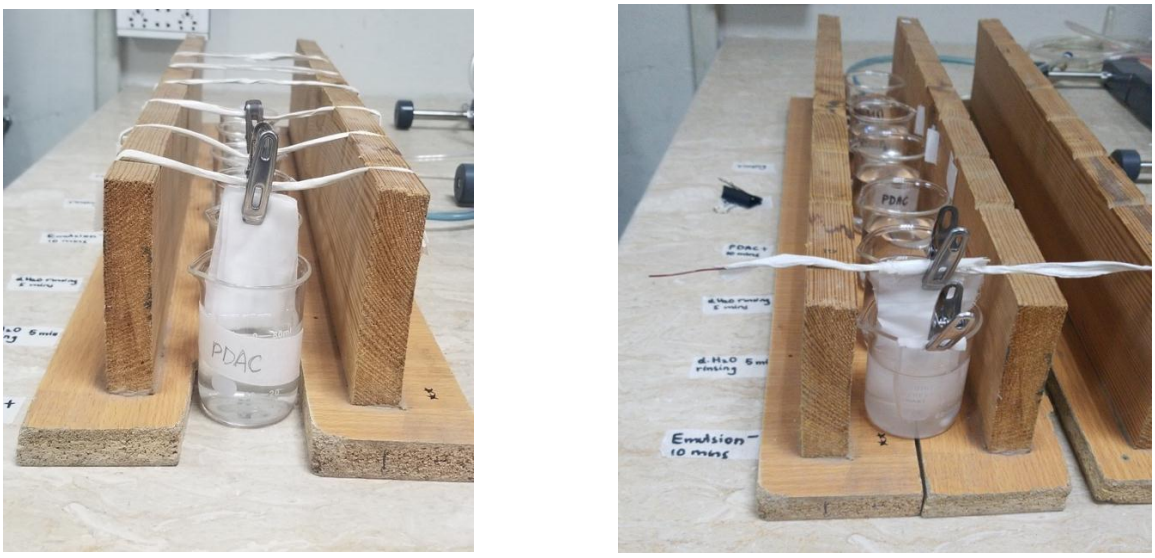


Figure 13 Setting for LbL Coating

The emulsion imparted a negative charge on the fabrics and the PDAC solution imparted a negative charge. Hence, the layers were coated on the principle of opposite charges electrostatically attracting each other. This process leads to the deposition of 1 bilayer. The same procedure was repeated to get 5, 10 and 15 bilayers. The fabrics were then dried and stored in petri dishes to avoid moisture.

3.2.4 Characterization of Textile:

3.2.4.1 Optical Microscopy

Coated fabric samples were checked under optical microscope (Optika B-600 MET) at different magnifications (5x, 10x, 20x and 50x) to notice any differences found between uncoated and coated samples and between the samples having different number of bilayers.

3.2.4.2 Optical Profilometry

Optical Profilometry (OP) was performed to calculate the average roughness (nm) of the uncoated and coated samples. Fabric swatches were fixed onto glass slides using paper clips and analyzed using Nanovea PS-50.

3.2.4.3 Scanning Electron Microscopy

For SEM analysis fabric swatches were fixed onto glass slides using a double tape. Afterwards it was sputter coated with gold and microscopic images were taken by JSM 6490LA, JEOL microscope, Japan.

3.2.4.4 Energy Dispersive X-ray Spectroscopy

The EDS analysis was conducted to perform an elemental analysis of blank and drug loaded formulations. It was conducted along with SEM. It depends on the principle that different atoms have unique atomic structure thus they respond differently to X-rays and hence form different peaks on their electromagnetic emission spectrum.

3.2.4.5 Fourier Transform InfraRed Spectroscopy

FTIR machine ALPHA Platinum-ATR (BRUKER) was used to conduct FTIR analysis. The ATR machine allows us to use the sample in its original form without the formation of kbr pellets as required in conventional FTIR machines. FTIR analysis was conducted on HALS, PCL, uncoated and coated fabrics to match the peaks. Small pieces or fabric were directly placed on the scanning point of ATR and spectra was viewed on the monitor attached.

3.2.4.6Ultra Violet Protection Factor (UPF Testing)

The UV hindrance attributes of textile under UV presentation was assessed by computing the Ultraviolet Protection Factor (UPF). It was calculated using the AATCC Test Method 1833 developed in 1998 by AATCC Committee RA106 using UV/vis spectrophotometer Camspec M550 UV Penetration and Protection Measurement System using the following formula:

$$\mathbf{UPF} = \frac{\sum_{\lambda=290}^{490} E_\lambda \text{x } S_\lambda \text{ x } \Delta_\lambda}{\sum_{\lambda=290}^{490} E_\lambda \text{x } S_\lambda \text{ x } \Delta_\lambda \text{ x} T_\lambda}$$

The textile patches with 1,5,10 and 15 Bilayers were properly dried, sealed in plastic bags and labeled. The textile patch with 15 Bilayers was washed 20 times with a household detergent, dried, sealed and labeled. Altogether, six samples (uncoated, 1 BL, 5 BL, 10 BL, 15 BL and 15 BL washed) were analyzed for UPF Ratings.

RESULTS AND DISCUSSION

4.1 Results and Discussion of HALS Emulsion

4.1.2 Optimization of Formulation

To formulate the optimized emulsion, the technique of nano-precipitation was used in which HALS was chosen as a light stabilizing agent and its solubility in different organic solvents and chloroform was found to offer the best solubility of more than 40g/100g solution. It was planned to be encapsulated in a layer of a polymer. For this purpose, we required a polymer which was compatible to be used with the drug in the same organic solvent. After reviewing literature, it was decided to use poly epsilon-caprolactone as it offered a very good solubility in chloroform. From further lab testing it was found that HALS and PCL together dissolve freely in chloroform. Other choices were ethanol and acetone, but the drug and polymer did not show a very good solubility together in any other organic solvent.

For the aqueous phase water was chosen as an aqueous medium and a surfactant had to be added to this medium to ensure the formation of nanoparticles once the organic phase was added to the aqueous phase drop by drop. The surfactant also prevents the nanoparticles from aggregation thus maintains their shape. From the literature, Tween 80 was found to be the most suitable surfactant as it has an HLB no. (Hydrophilic Lypophilic Balance No.) of 15.0 hence, manifests in both organic and aqueous phases. It is miscible in water and formed a pale solution with water. A hit and trial method were devised to establish the quantities of all the substances needed to prepare the optimized formulations and different parameters were also varied to find the best formulation.

During these studies, a few physical parameters were set to determine whether the formulation was to be accepted for further studies or not.

These parameters were as follows:

1. **Phase Separation:** Any formulation that showed a phase separation was rejected
2. **Precipitation:** Any formulation that showed the formation of precipitates at the bottom was rejected.
3. **Formation of clumps or aggregates:** Any formulation that showed the formation of clumps or aggregates was rejected.

Therefore, a number of formulations were made to vary and compare each factor mentioned in the table above. Most of the formulations showed milky white color that

turned into translucent upon evaporation of chloroform. The best formulation that showed homogeneity, no phase separation, no precipitation and no clumping, stayed stable for a period of 60 days was selected for further characterization and testing. The chosen formulation had the following parameters:

The optimized parameters were replicated several times and same results were obtained

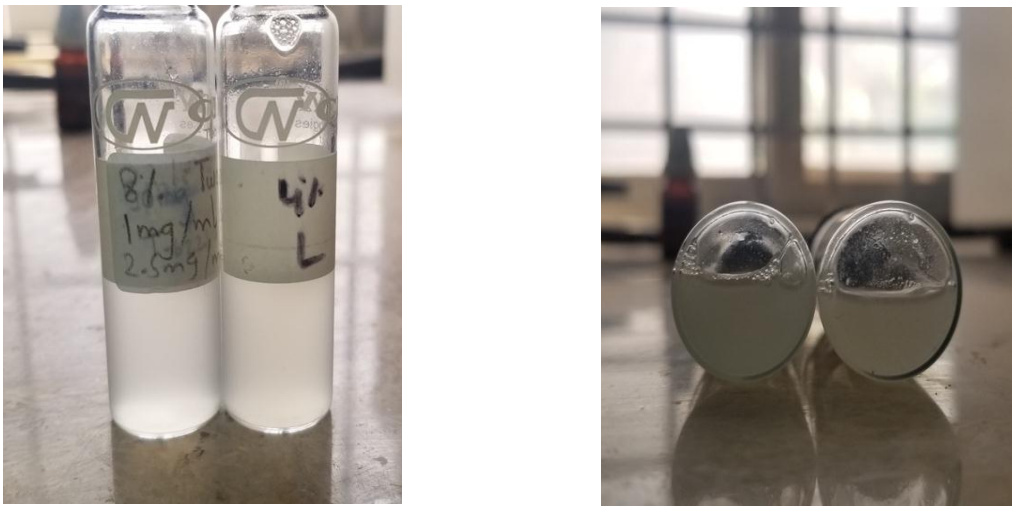


Figure 14 Optimized Emulsion

4.1.3 Stability Studies

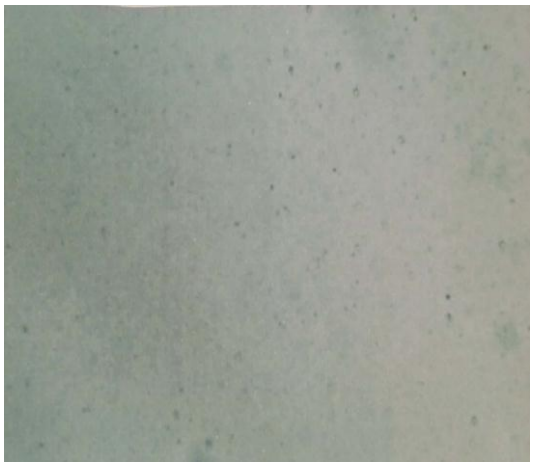
The chosen formulation was stored for 60 days at room temperature in a dark box and observed for phase separation, formation of precipitates and formation of aggregates. The formulation was replicated and seen for any change in color or state as mentioned above. No change in color or phase separation was observed. No new precipitates than the initial state if any were formed.

4.1.4 Optical Microscopy

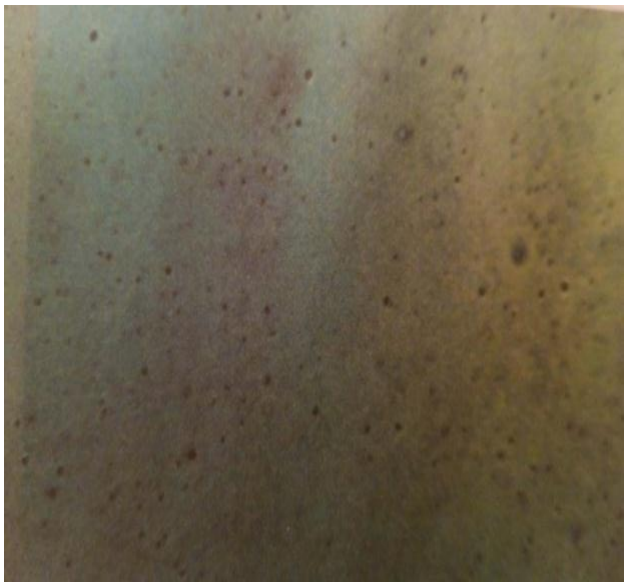
Optimized blank and loaded formulations were visualized under optical microscope to observe the dispersion and morphology of the precipitates in the water phase. Precipitates appeared to be well dispersed in the water phase and spherical in shape verifying the formation of nano capsules. Observations were made at 5x, 10x and 50x.

Images of blank formulation are given below:

Blank emulsion at 5x



Blank emulsion at 10x



Blank emulsion at 50x

Figure 15 Images of Blank Formulation under Optical Microscope

Images of drug loaded formulation dyed with safranin are given below:

Drug loaded emulsion at 5x

Drug Loaded emulsion at 10x

Drug loaded emulsion at 50x

Figure 16 Images of Drug Loaded Formulation under Optical Microscope

4.1.5 Scanning Electron Microscopy

SEM was performed in order to study the specific external morphology of nanoparticles formed in blank and drug loaded emulsions. The figures given below illustrate the SEM images of blank emulsion at a magnification of 30,000x keeping the area in view at 2 um and 500 nm. The figures show a well dispersed system of nanoparticles with a size range of 60 nm to 120 nm.

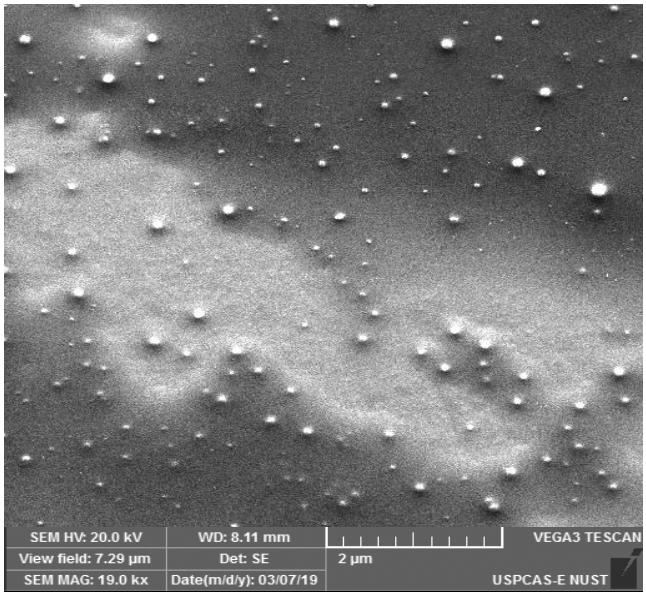


Figure 17 SEM Imaged of Blank Formulation at 2um

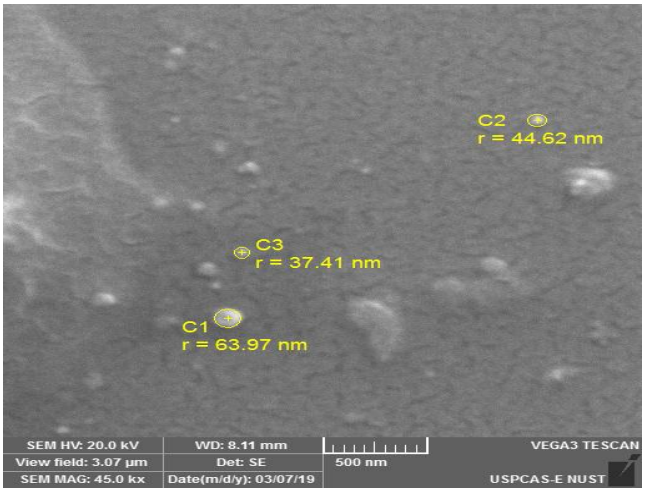


Figure 18 SEM Imaged of Blank Formulation at 500 nm

The figures given below illustrate the SEM images of blank emulsion at a magnification of 30,000x keeping the area in view at 2 um and 500 nm. The figures show a well dispersed

system of nanoparticles with a size range of 60 nm to 120 nm. The shape of the particles was found to be spherical.

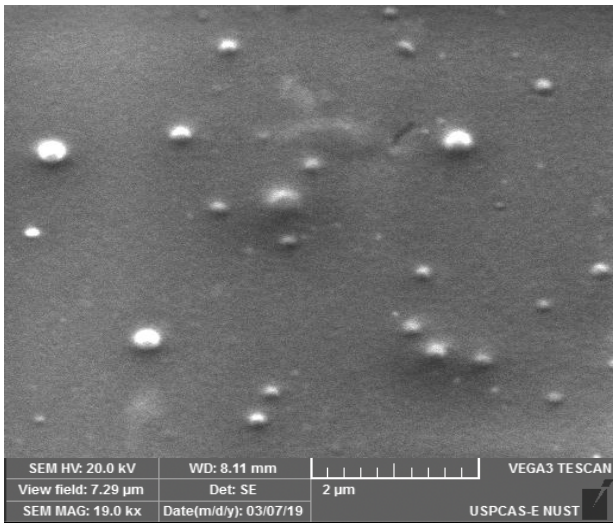


Figure 19 SEM Imaged of Drug Loaded Formulation at 2 um

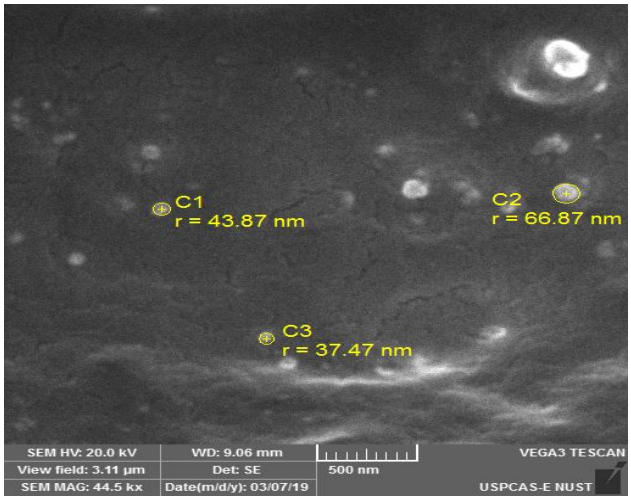


Figure 20 SEM Imaged of Drug Loaded Formulation at 500 nm

4.1.6 Energy Dispersive Spectroscopy

Energy Dispersive Spectroscopy was performed to analyze the elemental composition of emulsions. It was performed along with SEM and the results are given below:

The figures below show the elemental analysis of blank emulsion and the spectrum region of analysis. Carbon formed 73% of weight and oxygen formed 27% of weight of the blank

emulsion. Carbon and Oxygen found in the elemental analysis indicates the presence of polymer PCL.

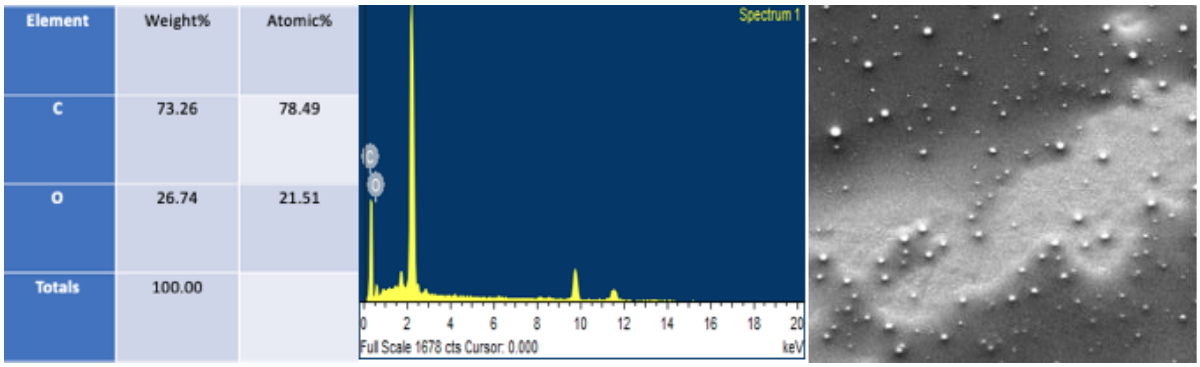


Figure 21 EDS of Blank Emulsion

In the following figures, the elemental analysis of drug loaded emulsions is illustrated.

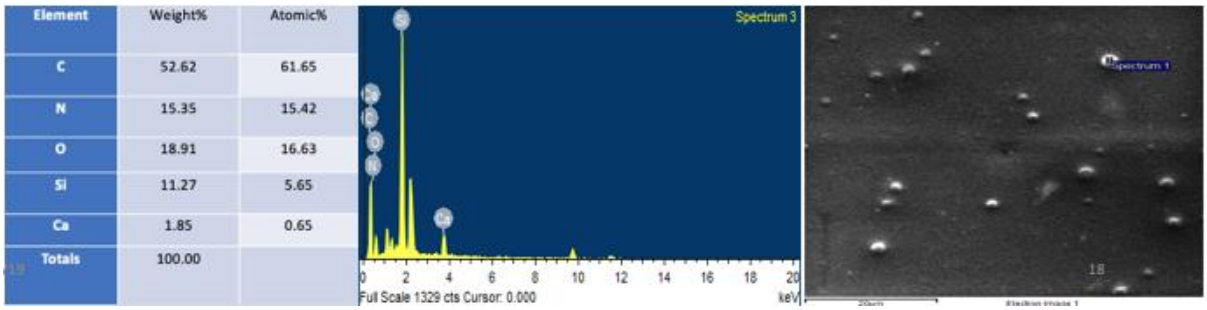


Figure 22 EDS of Drug Loaded Emulsion

In this figure, Carbon is found to be 52%, oxygen 18% and nitrogen 15% by weight. Carbon and oxygen here are the components of Poly ε-caprolactone while Nitrogen found in the drug loaded emulsion belongs to the piperidine group hence indicates the presence of HALS which shows that our drug has been successfully loaded. The 11% silicon weight is due to the glass slide on which the sample was loaded. Almost negligible percentage of Calcium might be due to the presence of impurities.

4.1.7 Fourier Transform InfraRed Spectroscopy

The FTIR Analysis of pure HALS, pure PCL, blank emulsion and drug loaded emulsion along with structures of HALS, PCL and water are plotted as below:

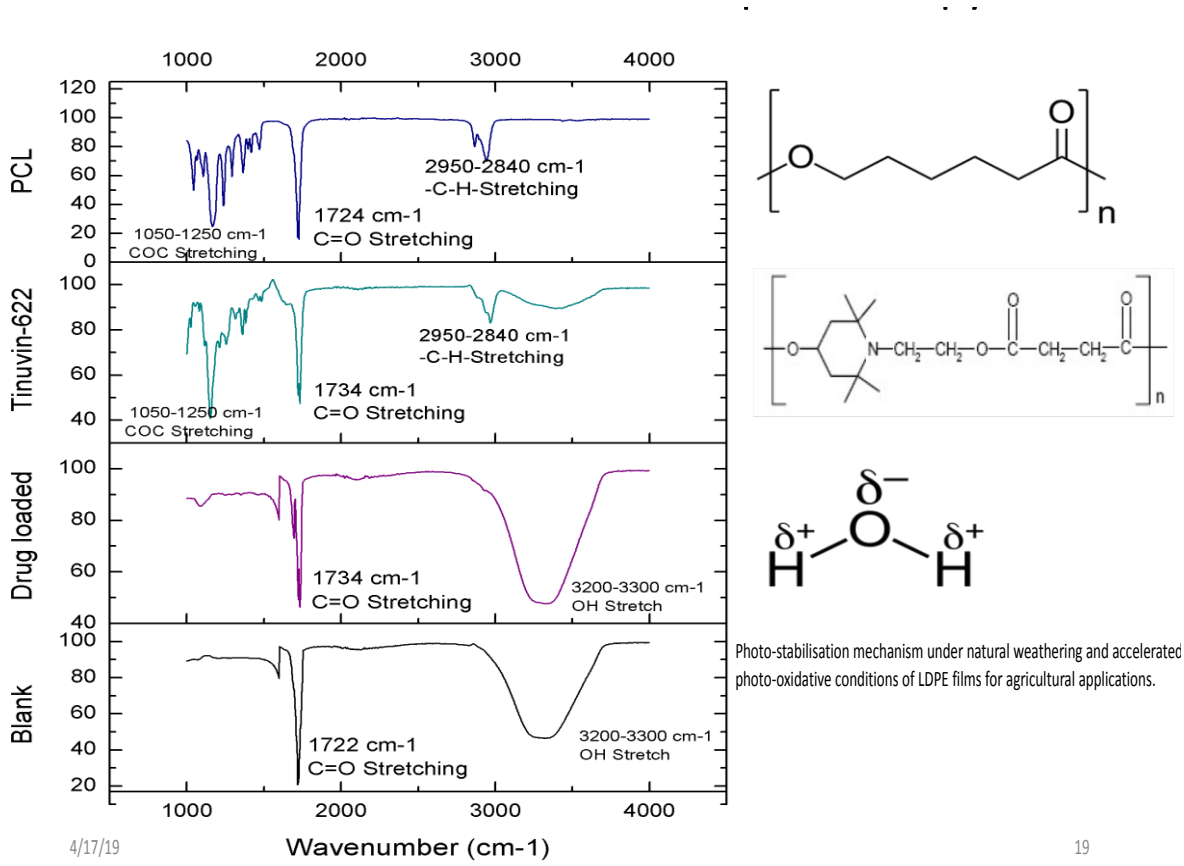


Figure 23 FTIR of Components of Emulsion

The presence of both HALS and PCL are characterized by the formation of peaks at a range of 1720 – 1750 cm-1 indicating the carbonyl double bonds (-C=O). A peak 1732 cm-1 can be clearly in that confirms the presence of HALS loaded Poly Caprolactone nanoparticles. The peak at 3200-3300 cm-1 in both loaded and blank emulsion is the stretch of hydroxyl group (-OH) of water. It is noticeable that the spectra of blank emulsion also show a peak indicating the presence of empty Poly Caprolactone nanoparticles.

4.1.8 Particle Size Analysis (DLS) and Zeta Potential Analysis:

The data obtained from DLS and Zeta sizing is illustrated below. It shows that the polydispersity index and zeta potential for the formulation is good. The drug loaded

formulation has a PDI of 0.13 which shows that the particles are dispersed fairly uniformly in terms of their size as the values of PDI lower than 0.2 indicate good colloidal stability according to Mohnaraj & Chen (Mohanraj & Chen, 2006). The PDI decreased from 0.17 to 0.13 upon addition of HALS. This trend of decrease in PDI upon addition of drug was observed earlier as well by Dias et al., (Dias et al., 2016). The average particle size of drug loaded nanoparticles is 203 nm.

Formulation	Hydrodynamic radius (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
Blank	200	0.17	-19
Drug Loaded	203	0.13	-19.5

Table 3 Comparison of Hydrodynamic Radius, PDI and Zeta Potential of Blank and Drug loaded Emulsion

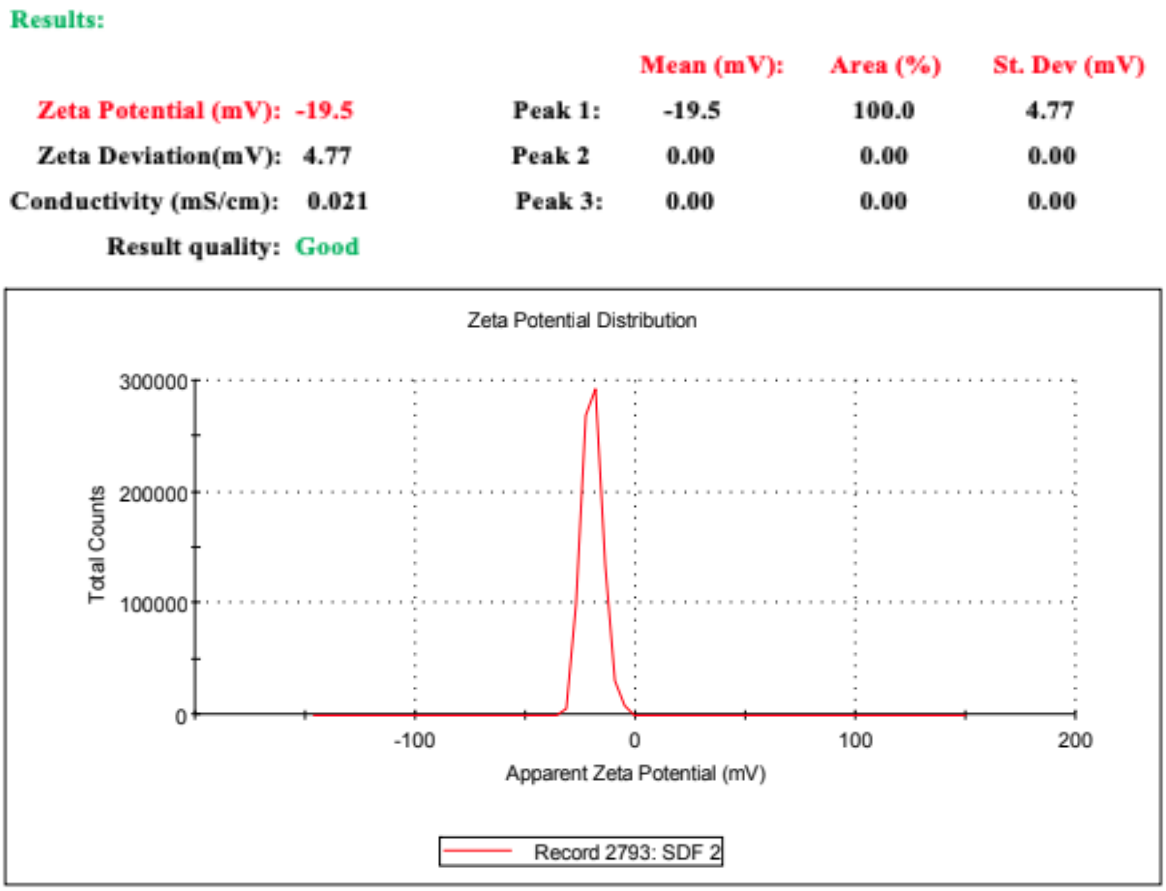


Figure 24 Zeta Sizer Data for Zeta Potential of Drug Loaded Emulsion

Results:

		Size (d.nm):	%Intensity:	St. Dev (d.n..
Z-Average (d. nm): 202	Peak 1:	234.1	100.0	85.40
PdI: 0.139	Peak 2:	0.000	0.0	0.000
Intercept: 0.948	Peak 3:	0.000	0.0	0.000
Result quality: Good				

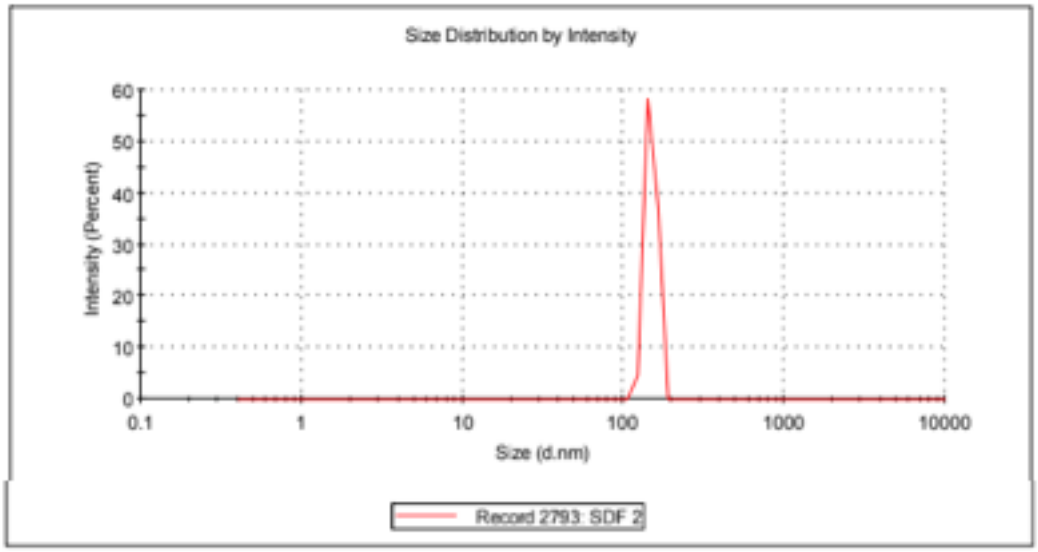


Figure 25 Zeta Sizer Data for Size of Nanoparticles of Drug Loaded Emulsion

4.1.9 UV-Spectrophotometry

The UV spectrophotometric analysis was performed to find out the wavelength on which HALS shows a maximum observation. The absorbance values were recorded three times and a mean absorbance value was than recorded. The maximum absorbance of 2.67 was observed at the wavelength of 215 nm.

The same values for UV spectrophotometric analysis were found by Farajzadeh when he observed the spectra of HALS before and after saponification (Farajzadeh, Nasserzadeh, Ranji, & Feyz, 2007).

The figure given below shows the profile of HALS as analyzed through UV spectrophotometry:

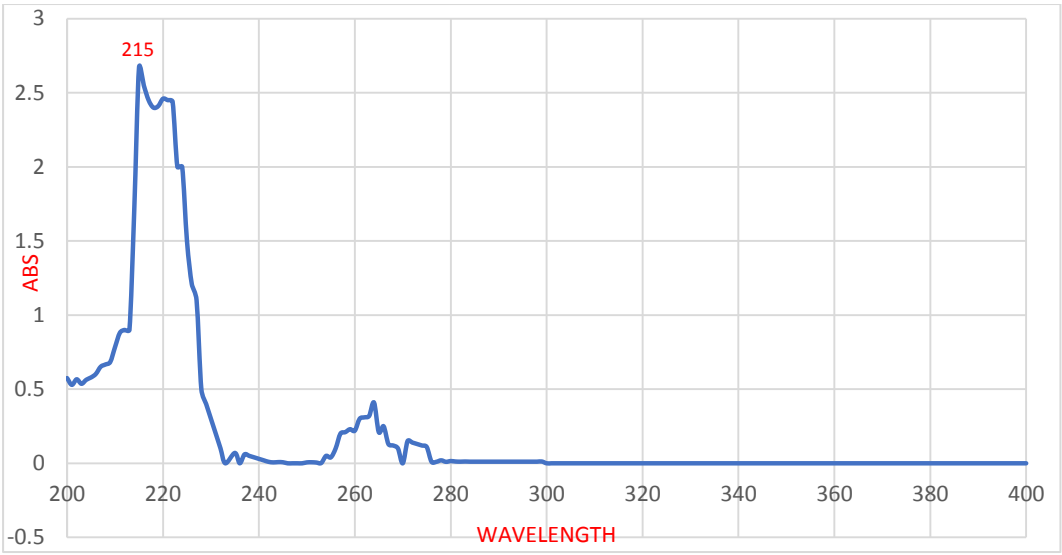
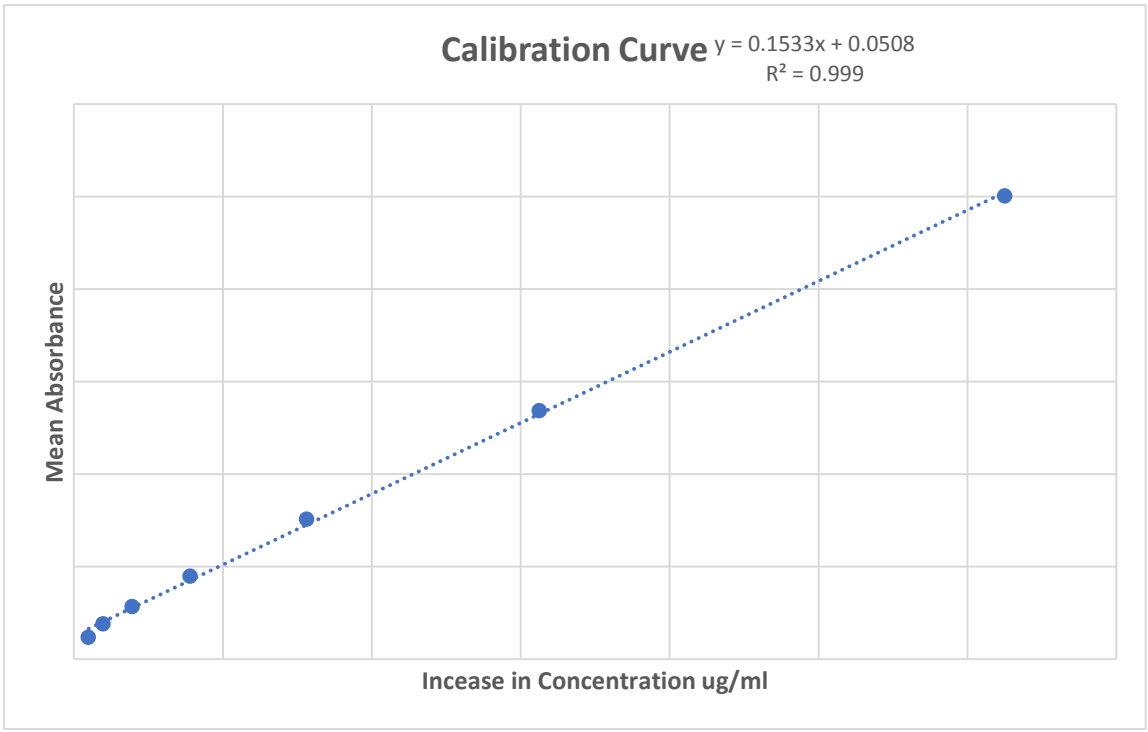


Figure 26 Spectrophotometric Analysis of HALS

4.1.10 Entrapment Efficiency

The table given below shows the results of calibration curve of HALS by making serial dilutions

The following calibration curve was obtained:



Drug Content:

The free drug content was calculated using the linear equation obtained from calibration curve:

$$\mathbf{y = 0.1533x + 0.0508}$$

Where, y = mean absorbance (the absorbance of unknown sample (supernatant) at 215 nm)
x = drug content

Thus,

$$\mathbf{x = \frac{y - 0.0508}{0.1533}}$$

The absorbance of supernatant at 215 nm was found to be 0.0984

Thus,

$$\mathbf{x = \frac{0.0984 - 0.0508}{0.1533}}$$

$$\mathbf{x = 0.3107\ mg}$$

Now, calculating the Entrapment Efficiency,

Entrapment Efficiency (%) = **[(Total drug – Free drug) / Total drug] * 100**

Entrapment Efficiency (%) = **[(2mg/mL – 0.3107 mg/mL) / 2mg/mL] * 100**

= 84.46 %

4.2 Textile Results:

4.2.1 Optical Microscopy:

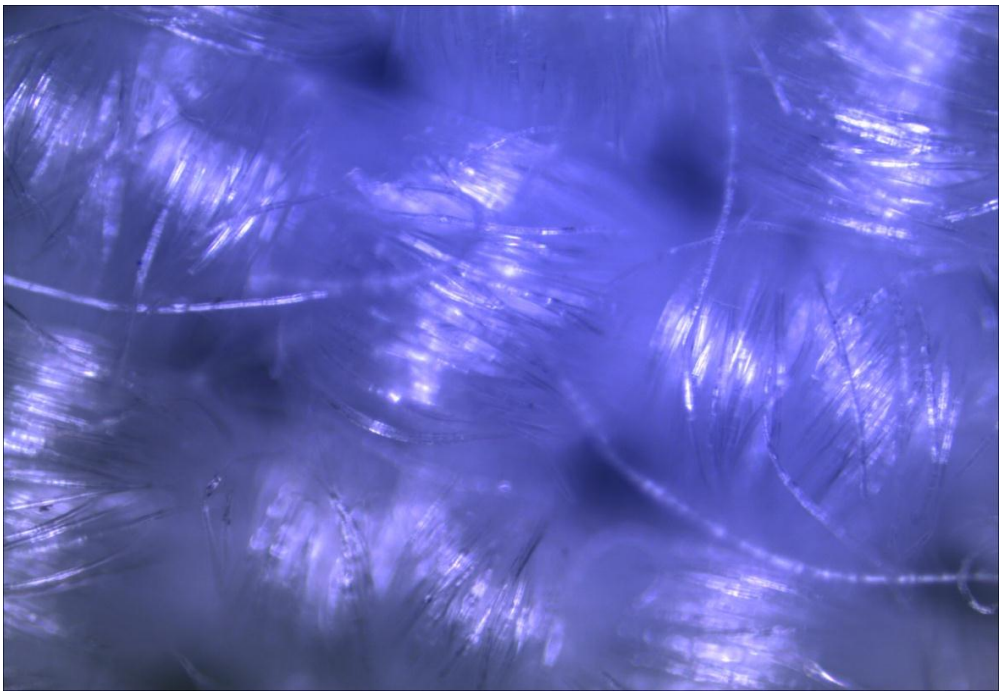
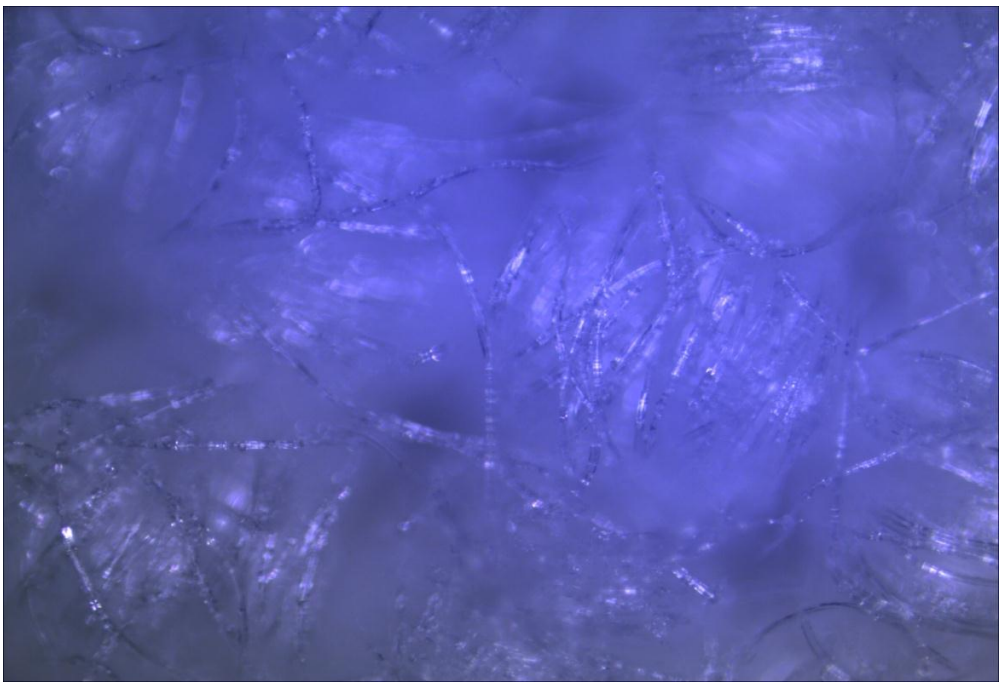
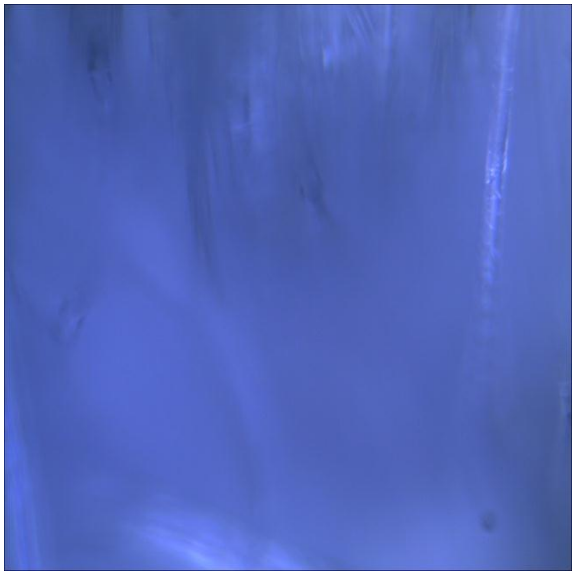


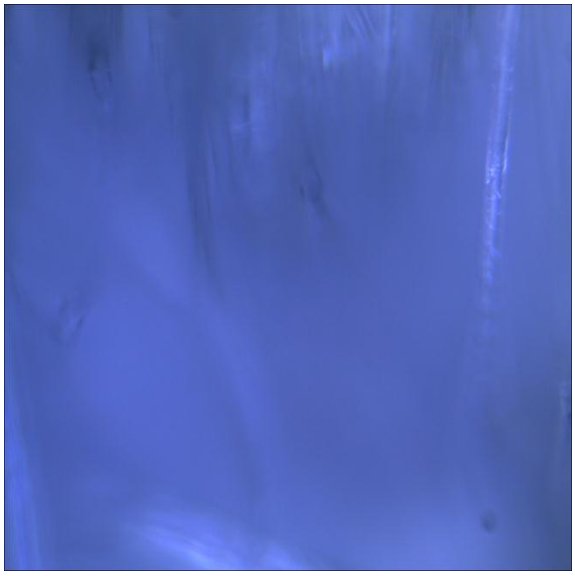
Figure 0-27 Optical Microscopy Results of Uncoated Textile



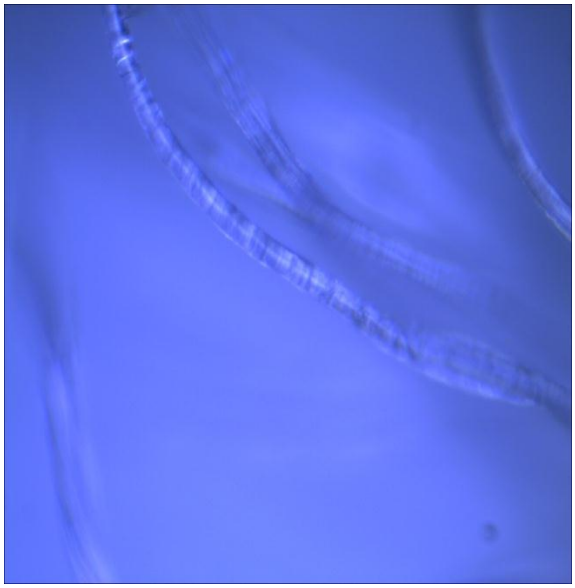
Coated Textile at 10x



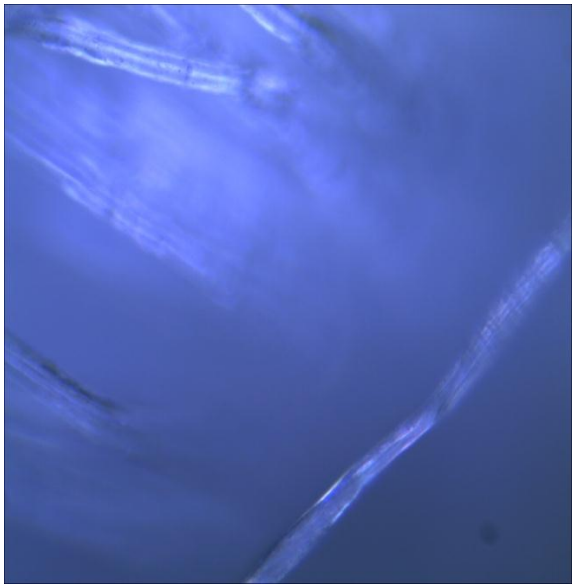
1 Bilayer Coated Textile at 10x



5 Bilayer Coated Textile at 10x



10 Bilayer Coated Textile at 10x



15 Bilayer Coated Textile at 10x

Figure 28 Optical Microscopy Results of Coated Textile

4.2.2 Optical Profilometry:

Optical Profilometry was performed to analyze the surface changes with increasing the number of coatings in the form of roughness in nanometers. The average roughness (nm) of 25-50 mm length of uncoated and fabric samples coated with varied number of bilayers was observed and it was found that average roughness decreased with increasing the number of

coatings. The rough fabric surface was smoothened by the coating as it filled in the pores or spaces in the fabric. The figure given below illustrates the trend of decreasing average roughness (nm) and its value for each sample:

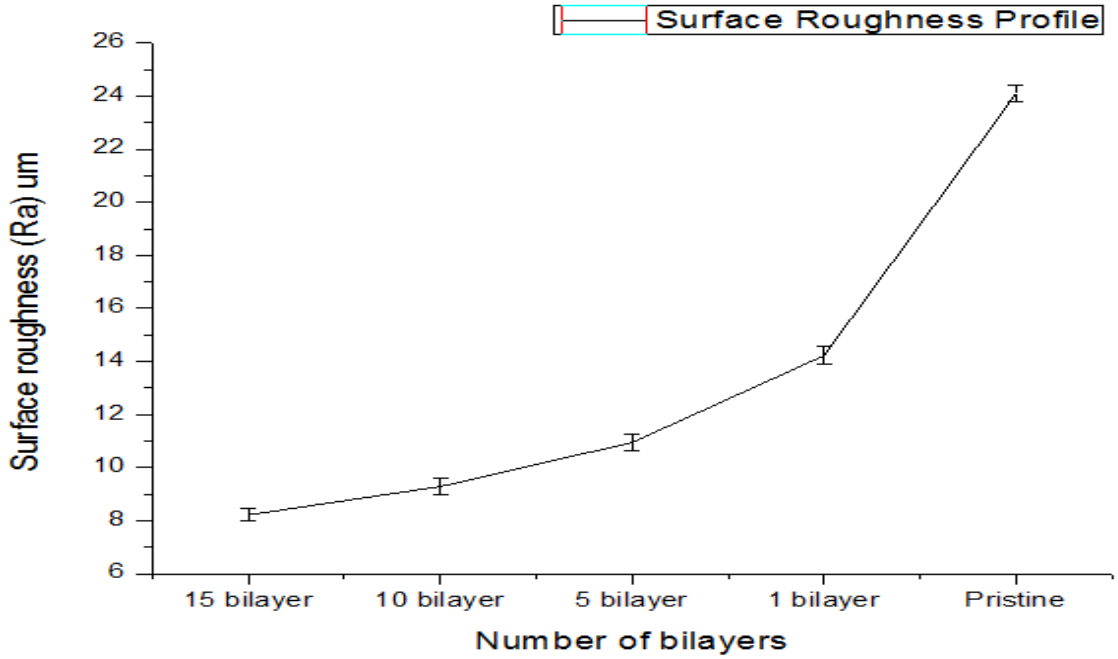


Figure 29 Trend of Average Roughness (nm) with increasing number of bilayers

4.2.3 Scanning Electron Microscopy:

SEM was performed at a magnification of 30,000x keeping the area in view at 50um, 20um, 2um and 500 nm. Given below are the SEM images of uncoated textile. We can clearly see the fibers of textile showing a pristine surface.

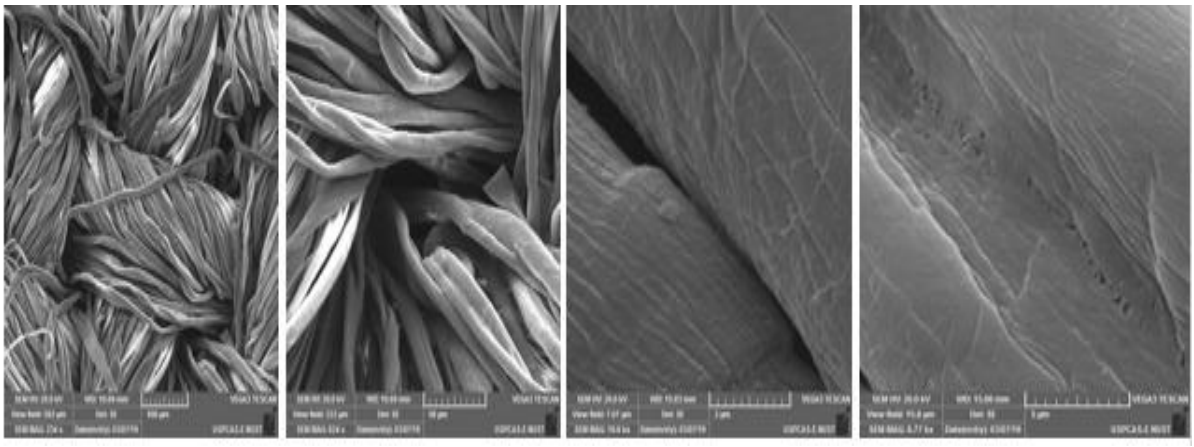


Figure 30 SEM Images of Uncoated Textile

The figures given below illustrate the 15-bilayer coated textile at a magnification of 30,000 keeping the area in view at 50 um, 20 um, 2 um and 500 nm. These figures clearly show the morphological and surface changes in the textile after coating. The coating is visible, and the particles can be seen. The average particle size in the coated textile was found to be in a range of 80 nm to 130 nm.

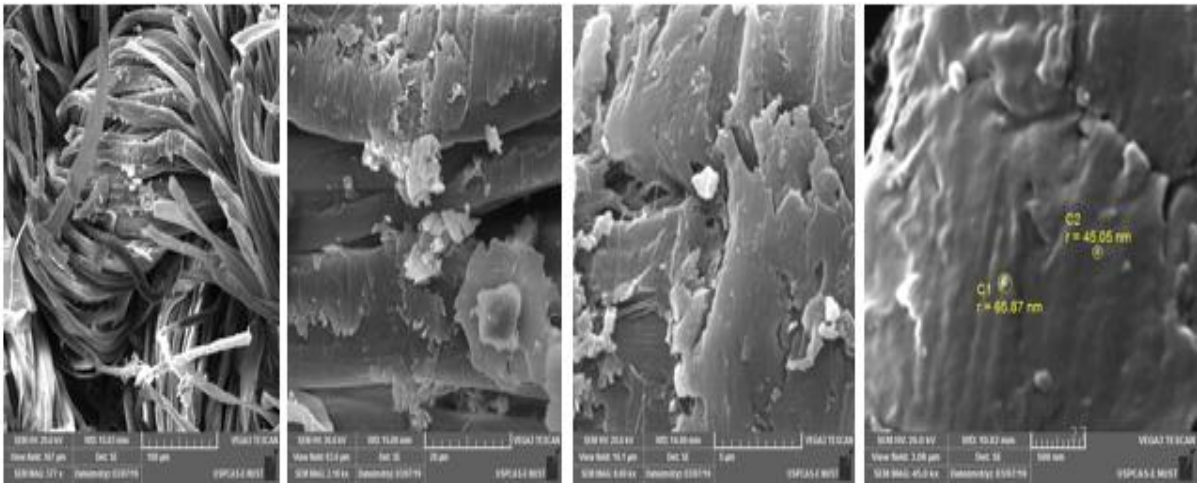
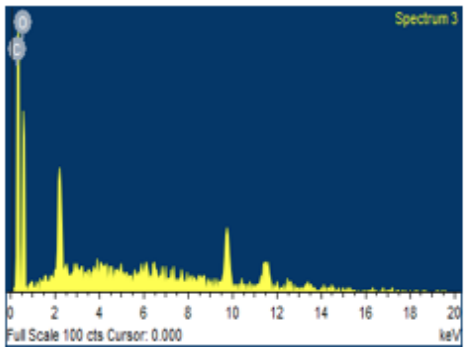


Figure 31 SEM Images of 15-Bilayers Coated Textile

4.2.4 Energy Dispersive Spectroscopy:

Energy Dispersive Spectroscopy was performed to analyze the elemental composition of both uncoated and coated textiles. The figures below show the elemental analysis of uncoated textile and the spectrum region of analysis. Carbon formed 52% of weight and oxygen formed 47% of weight in the sample. Carbon and Oxygen found in the elemental analysis indicates the presence of polymer PCL as well as cellulose of the cotton fabric.

Element	Weight%	Atomic%
C	52.31	59.37
O	47.69	40.63
Totals	100.00	



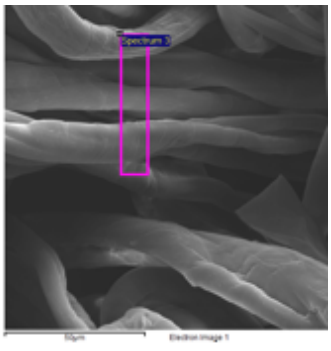
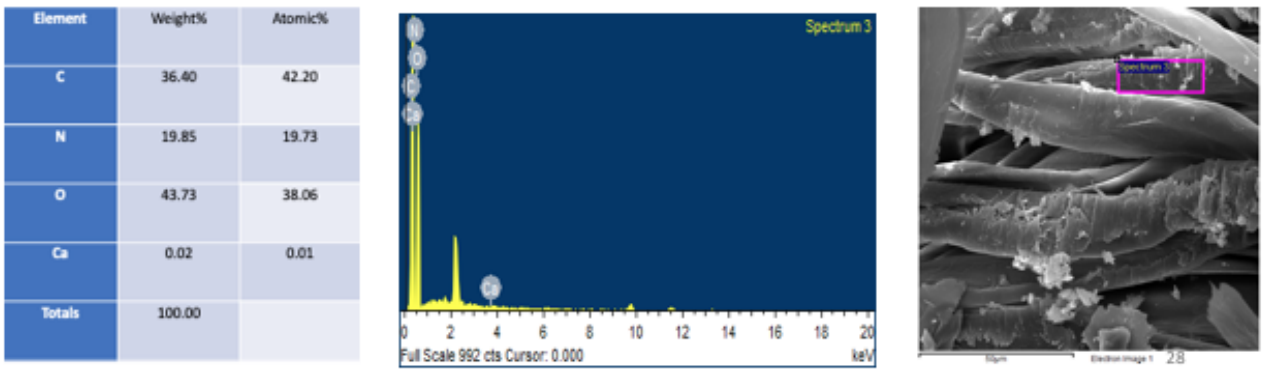


Figure 32 Energy Dispersive Spectroscopy of Uncoated Textile

In the figure given below, Carbon is found to be 36%, oxygen 43% and nitrogen 19% by weight. Carbon and oxygen here are the components of Poly ε-caprolactone as well as of cellulose forming the cotton fabric, while Nitrogen found in the 15-bilayer coated textile belongs to the piperidine group hence indicates the presence of HALS which shows that our drug has been successfully loaded and HALS loaded nanoparticles have been successfully coated on the textile. Almost negligible percentage of Calcium might be due to the presence of impurities.



Element	Weight%	Atomic%
C	36.40	42.20
N	19.85	19.73
O	43.73	38.06
Ca	0.02	0.01
Totals	100.00	

Figure 33 Energy Dispersive Spectroscopy of 15-bilayer Coated Textile

4.2.5 Fourier Transform Infrared Spectroscopy

The figure given below shows the FTIR analysis of uncoated textile, coated textile, pure HALS and pure PCL along with the structures of PCL, HALS and cellulose.

The presence of both HALS and PCL are characterized by the formation of peaks at a range of 1720 – 1750 cm-1 indicating the carbonyl double bonds (-C=O). A peak 1732 cm-1 can be clearly in that confirms the coating of HALS loaded Poly Caprolactone nanoparticles. The peak at 3200-3300 cm-1 in both coated and pristine textiles is the stretch of hydroxyl group (-OH) of cellulose.

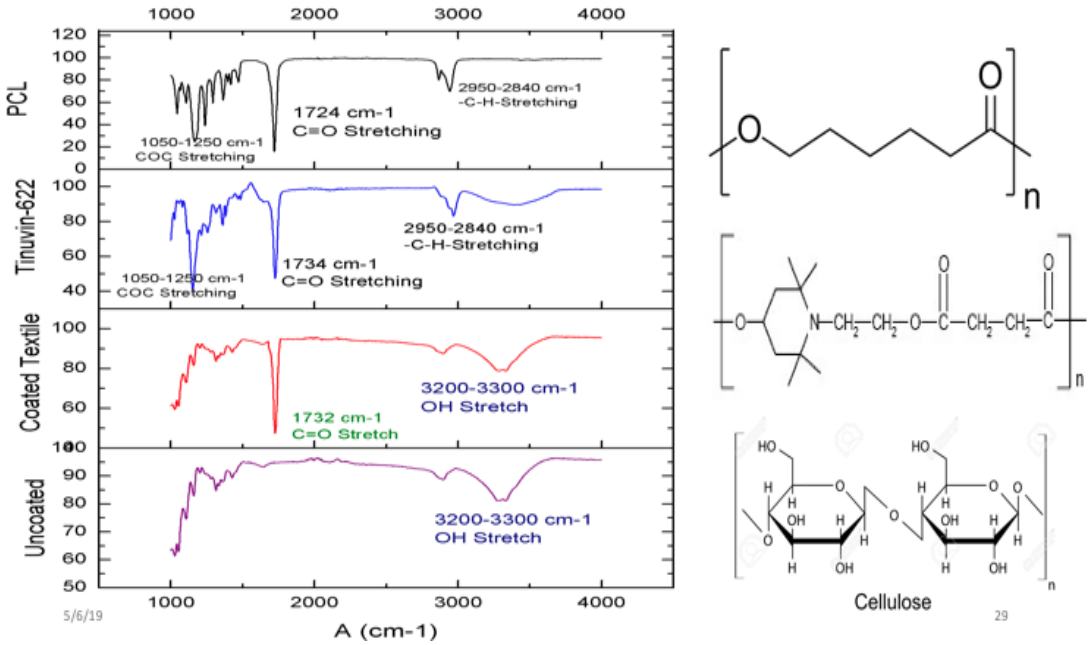


Figure 34 FTIR Analysis of Component of Textiles

4.2.6 Ultra Violet Protection Factor:

UPF can directly evaluate the UV-blocking activity of the as prepared products. UPF ratings indicate how much the material reduces UV exposure. For example, a rating of 20 indicates that 1/20th of the hazardous UV rays falling on the surface will pass through and that the skin's exposure will thus be reduced by a factor of 20. UPF is based on an in-vitro measurement and is a ranking of the sun-protective abilities of a textile. The UPF rating of 15-24 is classified as good, 24-39 as very good and 40-50+ as excellent UV blocking properties for fabrics. The results given below show average UPF values of uncoated and coated fabrics.

Type of Textile	Average UPF Value	UPF Rating	Protection Category
Uncoated	6.31	Below 15	Poor
Coated w/ 1 BL	27.95	25-39	Very Good
Coated w/ 5 BL	29.02	25-39	Very Good
Coated w/ 10 BL	30.62	25-39	Very Good
Coated w/ 15 BL	31	25-39	Very Good

Table 4 UPF Ratings of Uncoated & Textile Coated with 1,5,10,15 BLs

The graph given below shows the average UPF values of uncoated and coated fabric along with the trend with increase in number of bilayers.

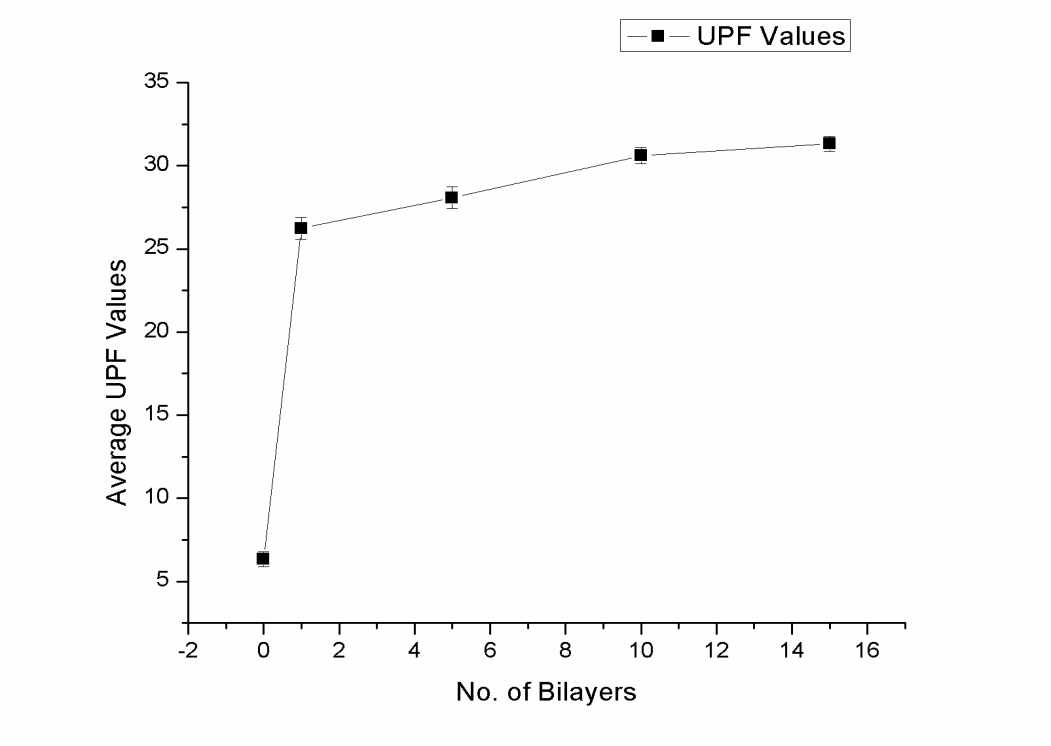


Figure 35 Mean UFP Values with Increasing no. of Bilayers

The table given below shows the % UVA and UVB Blocking with increasing the numbers of Bilayers.

Type of Textile	% UVA Blocking	% UVB Blocking
Uncoated	74.2	75.5
Textile coated w/ 1 BL	82.5	88.2
Textile coated w/ 5 BL	86	91
Textile coated w/ 10 BL	88.1	96.7
Textile coated w/ 15 BL	89.5	97.2

Table 5 % UVA and UVB Blocking with Increasing the Number of Bilayers

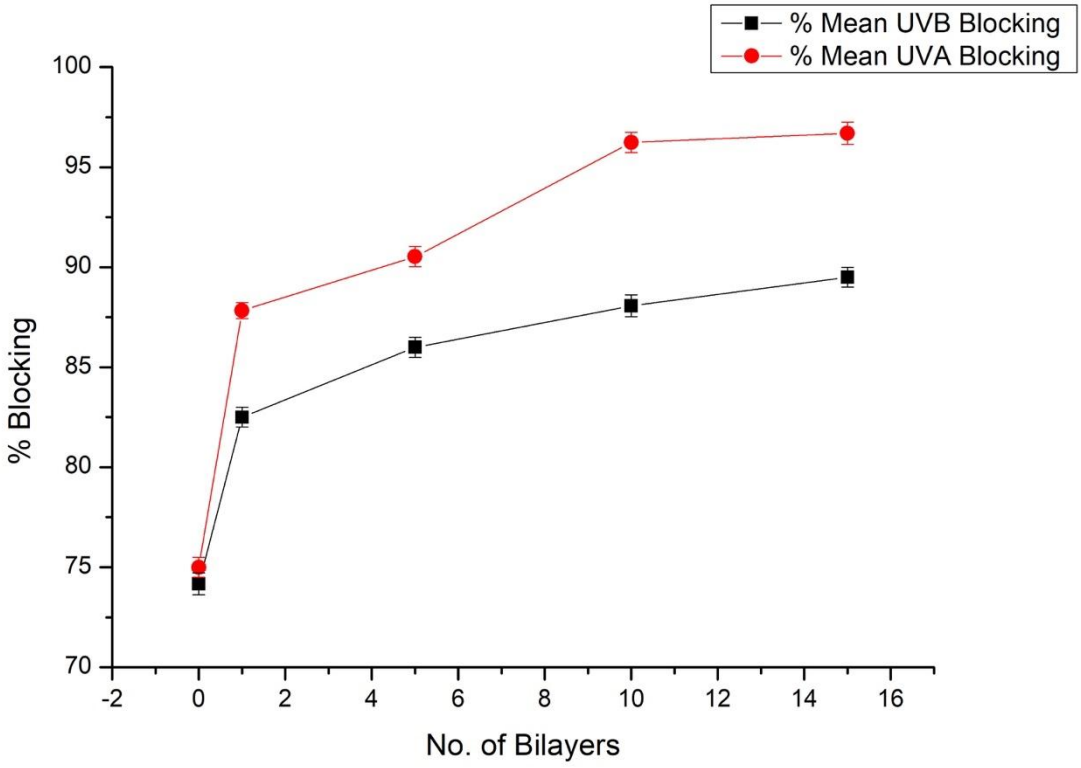


Figure 36 Mean UVA and UVB Blocking with Increasing no. of Bilayers

The textile patch with 15 bilayers that was washed several times with household detergent showed a slight decrease in the UPF value. The 20 wash cycles stimulated the lifecycle of textile and it was assumed to provide the least UV protection as compared to the rest of stages of its lifetime. The table given below compares the UPF values of 15 BL textiles and 15 BL textile washed.

Type of Textile	Average UPF value	UPF Rating	Protection Category
Textile coated w/ 15 BLs	31	25-39	Very Good
Textile coated w/ 15 BLs washed	24	15-24	Good

Table 6 Comparison of UPF Ratings of Textile Coated with15 BL Unwashed and Washed

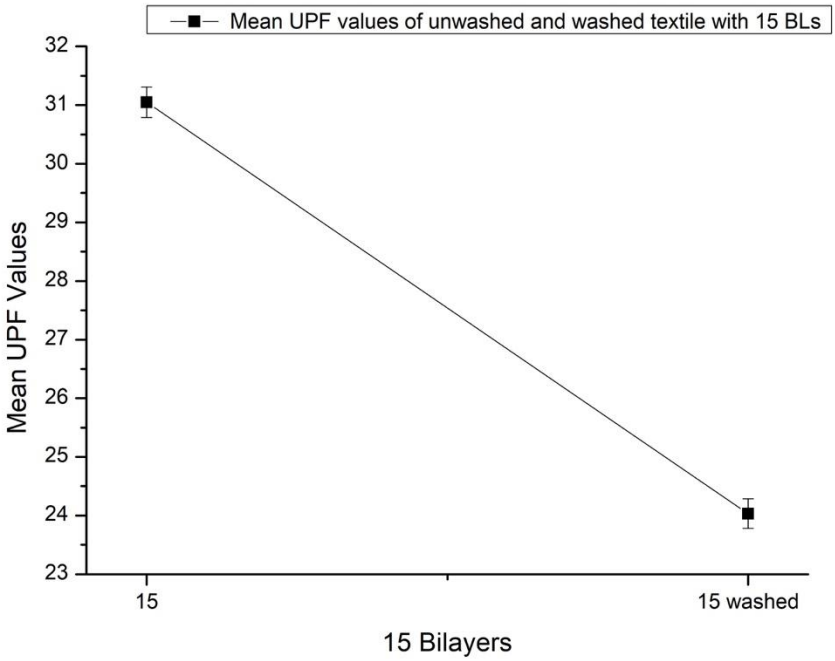


Figure 37 Comparison of Mean UPF Values of unwashed and washed textile with 15 BLs

Type of Textile	**% UVA Blocking**	**% UVB Blocking**
Textile coated w/ 15 BLs	89.5	96.8
Textile coated w/ 15 BLs washed	78.5	85

Table 7 Comparison of % UVA and UVB Blocking by unwashed and washed textiles w/ 15 BLs

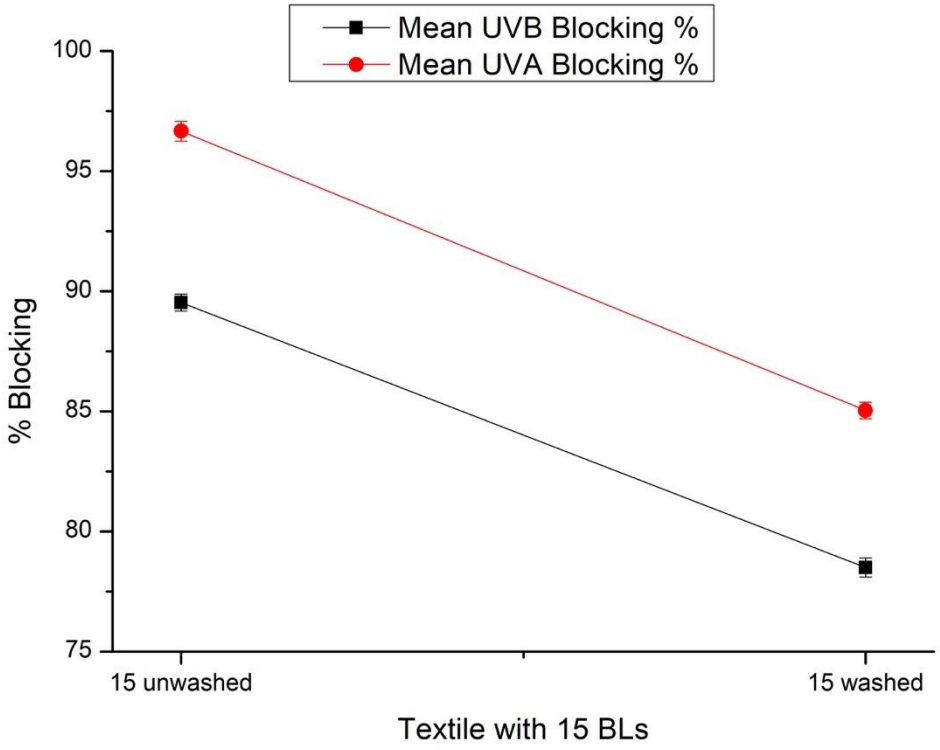


Figure 38 Comparison of % UVA and UVB Blocking by unwashed and washed textile with 15 BLs

Conclusions and Recommendations

The study focused on the formation of a formulation that could be used to modify the surface of textile in order to enhance its properties of UV protection. HALS was chosen as a light stabilizer to undergo the process of Nanoprecipitation to form Nano capsules of the light stabilizing agent. The nanoparticles comprised of an inner core of the agent an outer core of the polymer, PCL, suspended in an aqueous suspension of water and surfactant, Tween 80. The formulation was optimized by varying parameters such as stirring time and speed, concentration of polymer, drug and surfactant and temperature. The formulations with least to no precipitation and homogeneity were hazy and stayed stable for a period of 8 weeks. The most stable ones were chosen for further characterization through SEM, FTIR, EDS, DLS and Zeta Potential and Spectrophotometric analysis. Further in vitro testing was conducted in the form of calculation of entrapment efficiency of the anti UV agent. The most stable formulation was further diluted and coated on textile swatches in combination with a positively charged polymer, PDADMAC using Layer by Layer coating technique. The coated textile was further characterized through SEM, EDS, FTIR, Optical Profilometry, XRD and in vitro measurement of UPF. The textile was subjected to 20 wash cycles and again characterized through vitro measurement of UPF. The textile showed a very good protection category with a rating of 25-39 even after washing.

For future recommendations for this study it is suggested to work along the established protocols. The particle size of nanoparticles can be further reduced by varying parameters such as stirring time and speed, concentration of polymer, drug and surfactant and temperature or by using techniques other than nanoprecipitation. A combination of two or more compatible Hindered Amine Light Stabilizer can be used to enhance the UPF further and increase the durability of UV resistance. The emulsion itself can be further taken and tested on skin cell lines or mice skin order to learn in vivo anti UV activity of the emulsion in the form of a sun block.

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