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ANTIMICROBIAL ACTIVITY OF COTTON AND SILK FABRIC TREATED WITH HERBAL EXTRACT BY MICROENCAPSULATION TECHNIQUE

A PROJECT REPORT

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BONAFIDE CERTIFICATE

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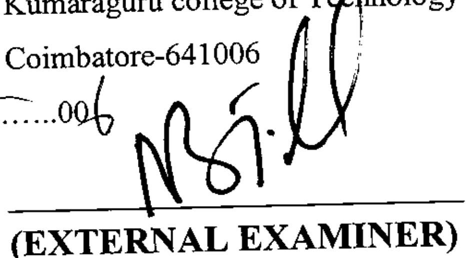

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ABSTRACT

The Project is carried out on antimicrobial activity of microcapsules which is Encapsulated with mixture of herbs like neem, tulsi and turmeric. The extract preparation process has been standardized. The microcapsules were prepared from the mixture of herbs by plain diffusion method, a natural encapsulation technique with yeast and applied on cotton and silk fabric by pad-dry-cure method. The microcapsules were fixed on cotton and silk fabric using the binder UF Silpure FBR-5 (PA) B at 120°C. The antimicrobial activities of the finished fabric were assessed by using three types of bacteria such as Staphylococcus aures, Escherichia coli and Pseudomonas, which are commonly, present in textile environment.

The results of antimicrobial activity from the test such as parallel streak method and disc diffusion method showed that activity of the mixture of herbs found very effective among the three types of bacteria selected, the antimicrobial activity of prepared microcapsule against pseudomonas was formed very good. Hence the herbal microcapsule treated fabric could be applied in the field of medicine.

The SEM photographs ensured the fixing of the microcapsules firmly in the yarn structure of plain woven cotton and silk fabric.

Key words: Antimicrobial activity, herbs, microencapsulation, ultra-fresh silpure

சாராம்சம்

இந்த ஆய்வு அறிக்கையானது, வேப்பிலை துளசி, மஞ்சள் முதலிய மூலிகைகளை உள்ளடக்கிய உறையப்பட்ட நுண்ணிய குடுவை மருந்துகளின் (Microcapsules) நுண்ணுயிர் எதிர்ப்பு செயல்பாடு (Antimicobial activity) பற்றியதாகும். இந்த நுண் குடுவை மருந்துகள், பல்வேறு மூலிகைகளின் மருத்துவ குணத்தை ஊடு பரவுதல் உள்ளிட்ட பௌதிக முறைகளின் மூலும் இறுதி வடிவமாக்கி, ஈஸ்ட் (Yeast) என்னும் இயற்கை வேதிப்பொருளால் உறையிட்டு, தயாரிக்கப்படுவதாகும். இந்த நுண் மாத்திரைகளை நீரில் கரைத்து நீர்த்த மருந்துக் கரைசலாக்கி (Diffusion Method) யு.எ.ஃ.ப் சில்ப்புயூர் (UF silpure FBR (PA) B எ.ஃ.ப்.பி. ஆர் - 5 என்ற வேதிப்பொருளை அதனோடு சேர்த்து பட்டு மட்டும் பருத்தியியால் ஆன துணியில் இயந்திரத்தின் உதவியோடு (Pad – Dry – cure Method) சரி சமமாகப் பரப்பி, பின் 120°C வெப்ப நிலையில் உலரவைத்து எடுப்பதே இந்த ஆய்வின் செயல்பாட்டு நிலை ஆகும். பின் ஸ்டைஃபிலோகாக்கஸ் ஆரியஸ் (Staphylococcus) எஸ்செரிசியா கோலி (E.coli) மற்றும் சூடோமோனஸ் (Pseudomonas) போன்ற நுண்ணுயிர்கள் மருத்துவமனைச் சூழலிலும், துணிவகைகள் உபயோகப்படும் இடத்திலும் பொதுவாகக் காணப்படுபவை ஆகும். எங்கள் ஆய்வின் இயற்கை வேதிப் பொருளை பயன்படுத்தித் தயாரிகக்ப்பட்ட நுண்ணுயிர் எதிர்மறை செயல்பாடு கொண்ட துணியானது இந்த மூன்று நுண்ணுயிர் செயல்பாட்டினை அழித்து, சுகாதாரமான சூழலை உருவாக்குவதில் வெற்றியை எட்டியுள்ளது. எங்களின் இறுதி சோதனையில் இந்த நுண்ணுயிர் எதிர்ப்பு செயல்பாடு பல்வேறு வேதியியல் சோதனைகளால் பரிசோதிக்கப்பட்டு மிகவும் உறுதியான, வெற்றிகரமான ஆய்வாக வெளிவந்துள்ளது. இந்த ஆய்வின் சாராம்சத்தோடு நுண்ணுயிர் ஆய்வு புகைப்படக்கருவி (SEM) மூலம் எடுத்த புகைப்படங்களை இணைத்துள்ளோம். இந்த நுண்ணுயிர் எதிர்ப்புத்திறன் கொண்ட மூலிகைத் துணியானது மிகச் சிறந்த பலனை அளிக்கக்கூடியது.

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1. INTRODUCTION

Microencapsulation may be defined as the micro packaging technique, where in an active core materials encapsulated in a polymer shell of limited permeability. The objective of this technology is either to protect the active core material from the external environment till required or to affect the controlled release of the active core to active desired delay until the right stimulus is encountered. This process is initially developed for the carbonless copy industry. However has now attracted the attention of wide range of industries including pharmaceutical, agriculture, chemical, food processing, cosmetics, and also textiles.

Antimicrobial fabrics gained significant importance in the recent years due to its wide acceptance as surgical apparels, baby clothing, undergarments etc. in this present investigation, herbal plant extracts are used as antimicrobial finishing agents. The control of bacterial, fungi and dust mites can be achieved using normal textile finishing process to create a value added product with a strong appeal for consumers.

In a liquid mixture, molecules can either be in solution, or suspension, or they will precipitate (settle out and usually to the bottom or top). Molecules (in our case, phytochemicals) that have been extracted by a solvent from the plant material, will precipitate out if the solvent is removed. We remove solvent, and so to prevent precipitation, we encapsulate (surround) the solutes with other molecules to, in a sense, isolate them from the remaining solvent deficient liquid. The microsphere is called a micelle and they range from 1 to 100 microns in size. Regular tinctures or fluid extracts are usually at saturation or below when newly made, and suffer precipitation due to agglomerization of solutes as they age since their solvents (a mixture of alcohol and water) are left in. Where the manufacturers try to remove their solvents through evaporation, they get precipitation of the phytochemicals, which they filter off if their final product is a liquid. The mixture can still be at "saturation" (meaning its holding all the molecules in solution that it can) however; part of the herb has been lost (filtered or decanted away). It is no longer "holistic" at that point. If all the liquid is evaporated, then everything left is a precipitate and the extract is known as a concrete. It may, however, be un-absorbable by the body at this point.

2.LITERATURE VIEW

2.1 MICROENCAPSULATION

Broadly speaking, microencapsulation may be defined as a micro packaging technique, wherein an active core material is encapsulated in a polymer shell of limited permeability. The objective of this technology is either to protect the active core material from the external environment till required or to affect the controlled release of the active core to achieve desired delay until the right stimulus is encountered. Interestingly, this process initially was developed for the carbonless copy industry, however, has now attracted the attention of a wide number of industries including pharmaceutical, agricultural, bulk chemical, food processing, cosmetics as also textiles. The textile industry has been late and slow to react to the possible opportunities this technique could offer. Efforts are still seen more at the research and development stage. Interesting possibilities do exist in medical and technical textiles where this technology could be used for applying finishes and for imparting desirable properties that either are not possible through conventional application procedures or are not cost-effective. Some other examples include, antimicrobial, moth repellency, flame retardancy etc. Use of phase change materials in the core, has also been suggested for thermal regulation functions in clothing for sports and extreme weather. Although there is a great potential of the microencapsulation technology to succeed in textile arena, most applications, as of now are very specific and therefore have had limited commercial success.

The following characteristics of microcapsules do interest the researchers:

a) Size and size distribution - low size increases the mechanical strength as also ease of application,

b) Loading fraction - This is the weight ratio of core to wall of the microcapsule, the higher is this ratio the better is the production efficiency but poorer would be the stability,

c) Release properties- Rate of release from microcapsules depends largely on the structure of the polymer wall, which in turn is influenced by the conditions employed in the preparation. Wall characteristics like crystallinity, cross-link density and porosity play a big role in determining the release rate. As the crystallinity and cross-link density of the wall increases, the release rate reduces substantially. The other important factor is the outside

environment; if it is of the same type as that of the core material, the rate of release will be high.[2] The core ingredient may be released by, i) Mechanical stimulus, ii) Chemical stimulus, or iii) Thermal stimulus. The resultant release rate can normally be expressed as a first order rate process, i.e., $-dc/dt = kc$, where k is the diffusion constant and c is the concentration gradient.

d) Thermal stability – it is very important when field of application is at a high temperature.

2.2 MICROENCAPSULATION TECHNIQUES

2.2.1 Controlled Release Mechanism

Microcapsules: the active agent forms a core surrounded by an inert diffusion barrier.

Microspheres: the active agent is dispersed or dissolved in an inert polymer.

Cyclic Molecules: the active agent is entrapped in the cavity of cyclic molecules (e.g.cyclodextrines).[11]

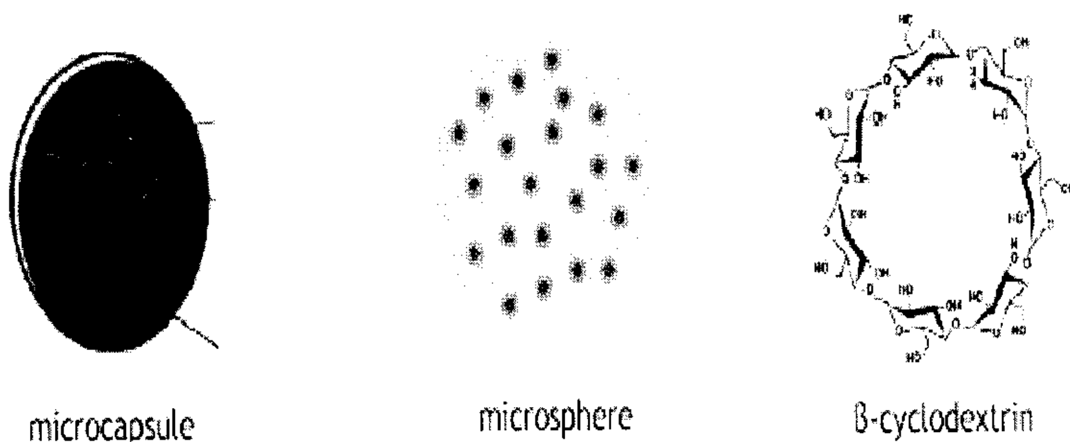

Fig2.1: controlled release mechanism

Institute scientists develop microcapsules that provide a variety of release mechanisms, including:

1. Mechanical rupture
2. Thermal release
3. Permeation
4. Dissolution
5. Delayed and targeted release
6. pH and osmotic release
7. Photolytic release
8. Biodegradation
9. Triggered release

2.2.1.1: Advantages of controlled delivery systems:

- Protection of the active core material from the external environment.
- Controlled-release of the active core by:
- Diffusion through polymer structure
- Rupture of polymer wall
- Bioerosion of the polymer

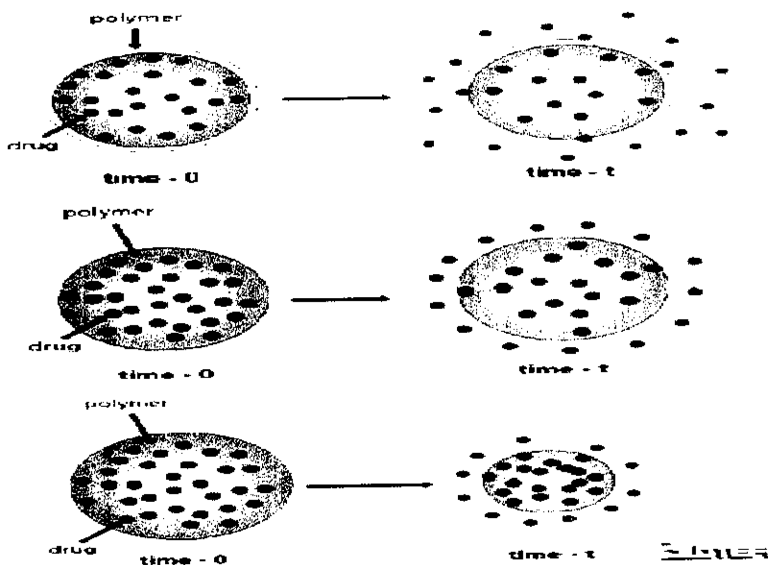

Fig2.2: Control delivery system

2.2.1.2 Disadvantages of controlled delivery systems:

- Diffusion is activated after polymer relaxation.
- Microorganisms adapt to the presence of the antimicrobial.

2.2.2 PREPARATION METHODS OF MICROCAPSULES

Many different methods have been proposed for the production of microcapsules (over 200 methods have been identified in the patent literature), depending on core and wall-polymer solubility, particle size, wall thickness and wall permeability, physical properties and over-all economics of manufacture. They can, however, be classified into seven groups according to the coating method:

- Phase separation
- Interfacial and in situ polymerization
- Spray drying
- Centrifugal extrusion process
- Air suspension coating
- Pan coating
- Emulsion hardening process.

Nelson has given a good review of microencapsulation processes. However here we give a brief description highlighting the above methods.

2.2.3 PHASE SEPARATION METHOD

In phase separation, the core material is first suspended in a solution of the wall material. The wall polymer is induced to separate as a viscous liquid phase (not as a precipitate) by several different methods (e.g. by adding a non-solvent for the polymer, by lowering the temperature, or by adding a second polymer with higher solubility in the core material). This separation process is known as coacervation. Coacervation is recognized by the appearance of actual separation of liquid layers.[6]

Coacervation can be simple or complex. Simple coacervation involves only a single colloidal solute, while complex more than one colloid. In simple coacervation, addition of a water miscible non- solvent (e.g. ethanol) to an aqueous polymer solution causes formation

of a separate polymer-rich phase due to partial miscibility effect. The system is, however, difficult to control.

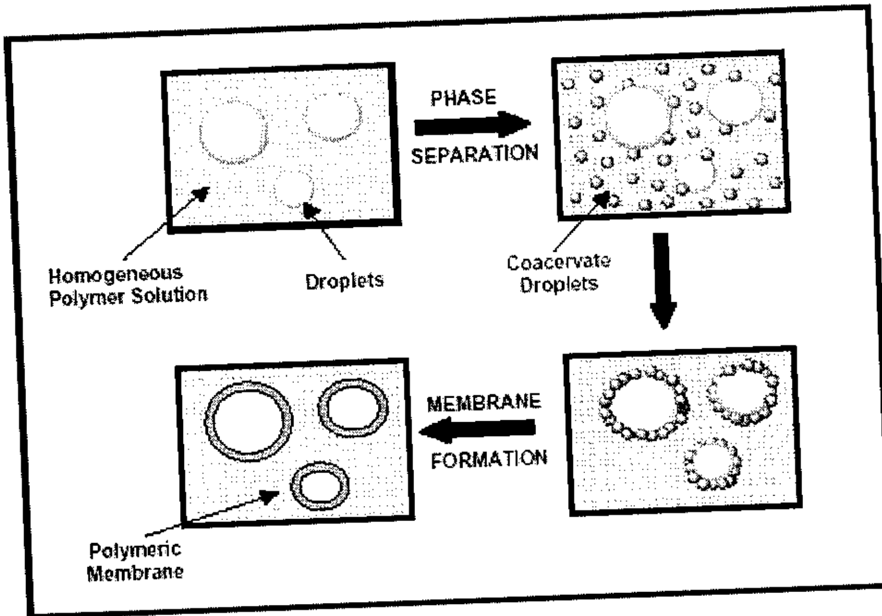

Fig2.3: phase separation method

Complex coacervation results from the mutual neutralization of two oppositely charged colloids in aqueous solution. Microencapsulation by complex coacervation consists of three stages (Fig2.4: Spray capsules).

- Dispersion of active component to be encapsulated into an aqueous solution of a polyelectrolyte.
- Deposition around the core material of an aqueous solution of a second electrolyte of opposite charge; and
- Gelation of the coacervate.

2.2.4 INTERFACIAL AND IN SITU POLYMERIZATION

The principle involved here is the classical interfacial polycondensation polymerization, which is widely used to produce synthetic fibers and films such as polyester, nylon and polyurethane. Pennwalt Corporation used this for the production of pesticide formulations for agricultural applications. Microencapsulation by interfacial

polycondensation involves the dispersion of a pesticide and an organic diacid chloride by mechanical agitation in water. Once the

Appropriate droplet size is achieved, an aqueous solution of Damien is added which penetrates the capsule wall until all the diacid chloride has been utilized, forming stable Microcapsules.

A process developed by 3M for the microencapsulation of fragrances uses the principle of interfacial polymerization, and has been used for advertising purpose.

Methods based on in situ polymerization have been employed to prepare microcapsules for carbon-less copying paper systems. Condensation polymerization systems are used to form the microcapsule wall polymers that contain the colour systems.

In a nutshell, the first step in all interfacial polymerization process is to form an emulsion. This is followed by initiation of a polymerization process to produce a capsule wall. Most commercial products based on interfacial or in situ polymerization process employ water-immiscible liquids. This technology is not designed for solids, although it can be used to encapsulate solids dispersed in a liquid.

2.2.5 SPRAY DRYING

The earliest commercial encapsulation process was spray drying used in 1927 to produce flavors entrapped in gum Arabic carrier. In the widely used spray drying process, the dried solid is formed by spraying (atomizing) an aqueous solution of the core material and the film-forming wall material as fine droplets into hot air. The water then evaporates and the dried solid is separated usually by air separation. Several process variables can be important in achieving the desired properties, including the core: wall material ratio, and the concentration, viscosity and temperature of the starting solution of spray dryer is also important [1]

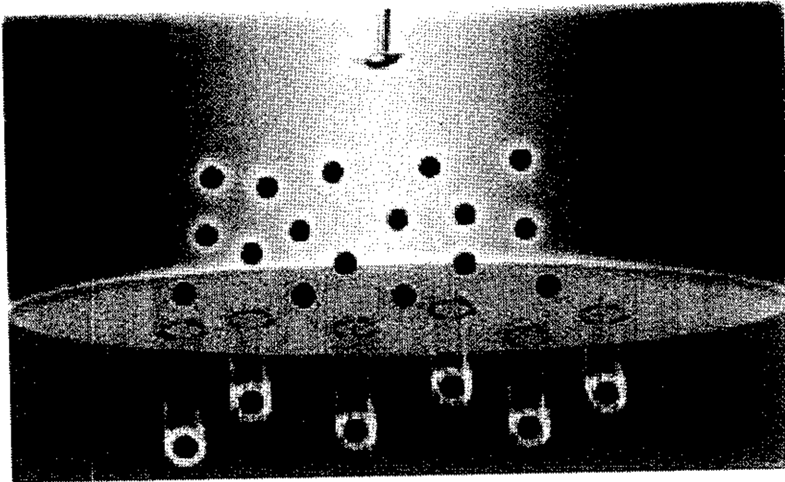

Fig2.4: Spray capsules

Spray chilling, colling and congealing are variations of conventional of spray-drying technique. In spray chilling, the process is carried out by mixing the core material with a molten wax or fat. The emulsion or suspension is then chilled below its melting point to form particles (Fig2.4: Spray capsules).

2.2.6 CENTRIFUGAL EXTRUSION PROCESS

In centrifugal extrusion processes, liquids are encapsulated by using a rotating extrusion head with concentric nozzles. The fluid core material is pumped through a central tube while liquefied wall material is pumped through a surrounding annular space.A membrane of wall material is formed across a circular orifice at the end of the nozzle and the core material flows into the membrane, causing the extrusion of a rod of material. Droplets break away from the rod and hardening takes places on passage through a heat exchanger solid capsules are removed by filtration or other mechanical means, and the immiscible carrier fluid, after passing through the filter, is reheated and recycled

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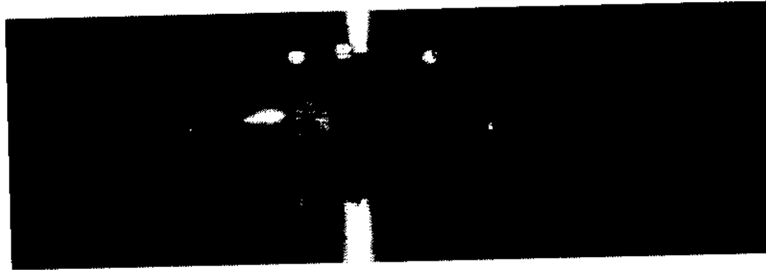

Fig.2.5: Centrifugal Extrusion process

In this system, a biliquid column is used, which also forms droplets upon breaking up of the jet. The system can produce several million core-shell microcapsules per minute (Fig.2.5: Centrifugal Extrusion process).

2.2.7 PAN COATING METHODS

This method has been used in the pharmaceutical industry for many years to produce small-coated particles or tablets. Solid particles are tumbled in rotating pan, whilst suitable coating material is slowly added, with a controlled temperature profile. Additional coatings of film forming polymers may be added in successive stages. Rohm GubH offers suitable coating polymers under the general trade name Eudragit. Pan coating is used in the pharmaceutical industry to produce a controlled-release product (Fig2.6: coating pan).[1]

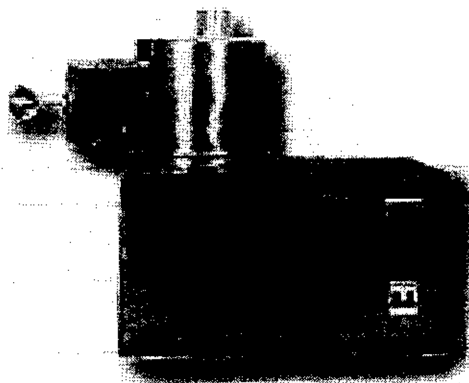

Fig2.6: coating pan

2.2.8 EMULSION HARDENING PROCESS

Microencapsluation processes based on emulsion hardening can be used when the core compound (usually a drug) is highly soluble in the polymer solution. Initially, the drug-polymer solution is emulsified in an immiscible liquid, then solvent is removed (e.g. by evaporation) and the core compound solidifies inside the polymer solution droplet. These methods are used for the production of microcapsules with a coating of poly (lactic) acid as a biodegradable polymer film for use in injects able particle systems.

2.2.9 NATURAL MICROCAPSULES

Microorganisms offer certain advantages over conventional microencapsluation processes, as the microcapsules are preformed. Microorganisms were first used to encapsulate only fat-soluble materials. Dunlop has found that it is possible to Encapsulate core material such as a dye into yeast with a more natural fat content (i.e. less than 40%). Its development team employed lipid-ex-tending substances that are taken into the yeast cell. If a substance is soluble or freely dispersible within this lipid extending substance, then the yeast also absorbs the core material. The Dunlop work has been further refined byAD2, Birmingham, to such an extent that yeast containing low levels of fat (less than 10%) can now be used as microcapsules without so-called lipid extending substances.[1]

The list of core material for yeast encapsulation process include flavors and fragrances, pheromones, insecticides, dyes, vitamins, drugs, detergents, rodenticides, nematocides, fungicides, molluscicides, insect and plant growth regulators, food colorants, etc. how ever, to date this method has only been exploited in the agrochemical field.

2.2.10 AIR SUSPENSION COATING

In air suspension techniques, particles are coated by dissolved or molten polymers that are suspended in upward-moving air stream in an unstable equilibrium. Equipment for this (and other) types of coating is made by several firms. The most widely used device is the wurster coating chamber. The wurster process is used to encapsulate tablets, granules, crystals and powers. It is not used with liquids unless they are absorbed on a porous solid.

The solid particles to be encapsulated are placed in a coating chamber, where they are suspended in an air stream. Which causes cyclic flow of the particles past a nozzle at the chamber bottom.

The nozzle sprays a liquid coating phase onto the particles. The freshly coated particles are carried away from the nozzle by air stream and up into the coating chamber. The coating solidifies because of solvent evaporation or cooling of a melt. At the top of the spout, the particles settle and fall back to the bottom of the chamber, where they once again are carried by the air stream past the spray nozzle and up into the coating has been applied in the desired thickness.

The wurster process, including hyrocolloids, solvent soluble polymers, sugars, waxes and fats, can apply a wide range of coating materials. These materials may be applied as solution, dispersions or hot melts. The solution may also contain the particles being encapsulated and can be sprayed in the form of a thin film. Increasingly, restrictive solvent emission standards have prompted extensive efforts to develop aqueous based coating systems, e.g. aqueous dispersions of ethyl cellulose and acrylic resin.

2.3 APPLICATIONS OF MICROENCAPSULATION IN TEXTILES

Microcapsule, due to its unique characters, has been widely used in a number of industrial sectors including agriculture, medicine, cosmetic and space science. The textile industry, although initially slow to exploit the technology of microencapsulation, is now into this field aggressively. Microencapsulation technology has now been applied in colorization and finishing. The Japanese industry was the first to apply the principle for dyeing and printing of textiles. In Europe and USA, various patents in this field have been continuously on the rise. The technology of microencapsulation is gaining popularity in textile applications.

2.3.1 DYEING AND PRINTING OF TEXTILES

There has been variety of problems faced by the textile dyer and printer. Microencapsulation technology can be help to combat some of them effectively. Aggregation of dyes has been a menace to textile dyers, as it results in specks. However the

textiles, thereby getting novel patterns and designs. Different dyes have different dyeing characteristics and hence will cause great difficulty in getting such arranged specks. Microencapsulation of dyes can effectively fulfill the requirements of such effects on textiles. The capsule wall must enable the desired mixing and concentration appropriate dye to be achieved.[4]

The matsui shikiso Chemical Company found in the early 1970s that disperse dyes were most suited to the microencapsulation process. Matsui has put a number of microencapsulated dyes under the trade name fine colour N Type. The capsules contain disperse dyes in high concentration for dyeing polyester. The same company has claimed a coloring method, where a continuous length of textile product is serially treated with an aqueous liquid containing a thermoplastic resin and thermoplastic resin and thermo ghromic and/or photo chromatic microcapsules having a volume based median diameter of 0.5-1.5μm by padding. Synthetic- fiber textiles are dyed or printed to have a spotted pattern by spraying, impregnation, coating or printing with a paste which contains water- insoluble particles of dye (disperse dyes mostly) of particle size 10 to 200 and comprising dye and resin or wax, which is insoluble in water. The material is then thermo fixed and washed. These dyes are the microencapsulated dyes used in speck dyeing.

The Hayashi Chemical Co. has also developed microencapsulated dyestuff. Multi-colored effects are achieved using its technology on polyester, cotton, acrylics, polyamide and wool. The microencapsulate walls are generally made with high molecular mass hydrophilic polymers such as gelatin, pectin, agar, methylcellulose, acrylic or maleic acid. The Hayashi products. MCP HP dyestuff, are microencapsulated disperse dyes, which on contact with textile fibers and after appropriate treatment, produce colored specks 50-3000μ in diameter. The MCP dyestuffs have also been used to rotary screen-printing. Hayashyi has further developed its microencapsulated printing technique to form duplex multi-colored fabric. Interest in double-surface multi-colored dye as grown, as there is an increasing market for textile fabric with dual surfaces of different color tones for use as garments, interior decorations and other applications.

Sandoz developed a process for electrostatic dyeing with microencapsules containing dyes in liquids of high dielectric constant. In this process of textile dyeing or

printing, use is made of dyeing preparations in the form of particles consisting of highly resistive high-molecular weight polymer as carrier, in which are encapsulated a dye and a liquid with a high dielectric constant, in which the (carrier) polymer is virtually free from swelling and, optionally, known assistants, dispersing agents and solvents. The preparations are applied to the textile substrate with the aid of an electrostatic field and then fixed.

There has been constant improvement in the microencapsulating technology. Roting-Werke Riepe KG, Germany, designed a micro encapsulation process that is not affected by the nature colorant. On application to the textile, the colorant is released by melting the microcapsule wall. This technique ensures good dyeing characteristics
On different fibers with little migration of colorant, producing a finish that is fast to washing and rubbing.

Micro encapsulation technique has also been applied to the printing of textile material. The Fuji Photo Film Co. has combated the migration of dye in textile
Printing processes by mixing microcapsules containing dye with other containing a fastness-improving agent.

The 3M company has developed a micro system, in which colorant can be transferred to a textile simply by rubbing the capsule against the target surface. Up to 50% of the colorant is bound to the exterior surface of the microcapsule, which contains a wetting agent; this enables a small time delay between rupturing of the capsules and dyeing. Without this mechanism, colorant is often lost on the mechanical capsule-rupturing device.

2.3.2 TEXTILE FINISHING

Textile finishing covers a large area. Apart from the conventional finishing operations, the area now extend to specialty finishes for imparting specific properties like antimicrobial, moth repellency, flame retardanc and the like. Thus the developments of industrial textiles (e.g. medical textiles) are the consequence of the special finishes on the textile substrate. Microencapsulation technique has been applied in many finishing operations.

An interesting application is the encapsulation of fragrances and a variety of other ingredients on to non-oven fabrics by the classical coacervation processes. The fragrance is released upon application of pressure on the fabric but the capsules resist breaking under the normal conditions. Eurant, Italy was developed fragrance containing microcapsules by the cocacervation technique. Fragrances[1] such as lavender oil or pine oil have been encapsulated in gum Arabic and gelatin capsules. The possible end uses of these products include ribbons, handkerchiefs, curtains, ties and furnishing.

Microencapculation has also found use in aromatherapy. Aromatherapy is the practice of applying and inhaling essential oils from plants as a physical and emotional boost to the body. Kanebo Ltd., Japan, has a great interest in high-quality cosmetics and has developed a number of innovative applications using the microencapsulation technology. An aromatherapy product manufactured by Kanebo is marketed under the name Esprit de Fleurs and was initially incorporated into stockings and tights. However, microcapsules containing specific aroma therapeutic essential oils have now been impregnated onto sweaters, ties, T-shirts and number of other products. The fragrance with in the microcapsules, which are tightly lodged in tiny cavities of a porous acrylic material on textile, persists even after hand washing of the fabric up to ten times. Fragrance fibrous Kanebo that consist of perfumes has also produced material bound to a variety of fibres using a low temperature reactive organopolysiloxane resin. As silicon binders are used, no unpleasant odors of binders interferes with the desired fragrance.

Microencapsulation technique has also been used to get an effective deodorizer. An e.g. is the herb armur cork (phellodendron amurense). Products like insect repellant ladies underwear are also been produced with the microencapsulations technique. Procter and Gamble has prepared microcapsules using urea formaldehyde as the shell material, containing perfumes or deodorants material.

Yeast cells containing biocide or insecticide have been applied to 100% cotton fabric, cotton wool and wool fabric. Fabrics with biocide-filled yeast have been successfully tested as per AATCC test method 100, a microbial challenge test. Mothproof agents have also been encapsulated in yeast and applied to wool fabric. This product, developed for the wool carpet industry, enables the industry to reduce the level of mothproofing agents in wastewater.

Akzo has manufactured and marketed a product range in Europe called Diolen Bactekiller, an antibacterial fibre developed by Kanebo, Japan. An active ingredients based on zeplite with the addition of an antibacterial metal ion is encapsulated within a polyester fibre using a special spinning process. This fibre has excellent bactericidal and deodorizing properties.

Over the last few years, encapsulation of enzymes and other bio-molecules have gained attention. Their use in bioreactors had been widely investigated. These processes enabled the production of high-value products, which otherwise are difficult by conventional processes. Focher describes the method of chemically anchoring enzymes in synthetic matrices and reference is made to physical anchorage by encapsulation in membranes of fibres. Workers at Queen University, Belfast, have combined textile knowledge with biotechnological skill to produce an apparatus for encapsulating and immobilizing cells, enzymes and other biological materials.

Microencapsulation techniques have been applied to a number of other special applications. The process has found use in enhancing the thermal properties of fabric or fibers. This consists of a coating of a polymer binder containing microcapsules, which contain temperature-stabilizing means selected from phase change materials and plastic crystals. Pause describes the possibility to enhance thermal storage capacity of fabric and consequently its thermal insulation through the application of a microencapsulation phase change material (micro PCM). The technique of microencapsulation has been applied to a process of preparing colored thermo-sensitive fibrous structure. The process involves addition of a fibrous substrate comprised mainly of cellulosic fibres; microcapsule contains a thermo chromic material, a cationic surfactant and non-ionic surfactant to a dye bath and then adding an amino modified polyoganosiloxane exhaustion agent to the bath at a pH not more than 7. Microcapsules encapsulating a substance for improving the physiological conditions of human skin are also reported.

Another area has been the preparation of microcapsules using ethyl cellulose as the wall material, containing resin, cross linking agents and/or catalysts for the improvement of durable-press or wash-and-wear characteristics of textile fabrics. E.g. natural or regenerated cellulose.

Fire hazards have led to the incorporation of flame-retardants in various fabrics' upholstery materials. Workers at BTTG have investigated the use of addictive in textiles that facilitates the detection of an unseen fire hazard. Microencapsulation technique has been investigated as a means of protecting the sensory early warning agent during processing. Some success has been achieved using the concept swelling starch granules or short cellolosic fibre in liquid ammonia containing dissolved sensory early warning agent precursor.

2.3.4 USES IN TEXTILES

A microencapsulation technology is being used in the production of products ranging from insect repellents to antimicrobial agents in medical textiles, according to the latest issue of Performance Apparel Markets. Microencapsulation is already used to impart a wide range of features, including thermochromic and photochromic dyes, used to make garments which change color when exposed to temperature changes or sunlight; anti-microbial and deodorizing finishes; insect-repellent and insect-resist treatments; flame retardant finishes, with improved durability of the treatment to bleaching, domestic washing and dry cleaning; enhanced chemical protection, for the military and other users, particularly for chemical decontamination of hazardous chemicals and chemical warfare agents; and cosmeto-textile, capable of imparting skin care benefits, combating aging, and promoting a feeling of wellness or well-being.

Table2.1 Core materials for microencapsulation

S.No	MATERIAL TYPE	Examples
1	Solvents	Benzen, toluene, cyclohexane, chlorinated phenyls, paraffins, esters, ethers, alcohols, and water.
2	Plasticizer	Phthalate, adipate and phosphate-type, silicones, and chlorinated hydrocarbons.
3	Acids & Bases	Boric acid, caustic alkali, and amines.
4	Catalysts	Curing agents, oxidants, free radical initiators and reducing agents.
5	Colorants	Pigments and dyes, especially leuco dyes for carbonless copying papers.
6	Adhesives	Polysufides, cyanoacrylates, isocyanates, epoxresins, thermally sensitive compositions.
7	Fragrances	Menthol, essences, and speciality compositions.
8	Foods	Oils, fats, spices and flavors.
9	Agricultural Chemicals	Herbicides, insecticides and pesticides.
10	Pharmaceuticals	Aspirin, vitamins and amino acids.
11	terial	Reprographic toners, coupling agents, developers, silver halides, fixing agents, photo chromatic compound, and liquid crystals.
12	Rust inhibitors	Zinc chromate and other compounds.
13	Others	Detergents, bleaches and fire retardant,

Table2.2 Wall materials for microencapsulation

S.No	MATERIAL TYPE	Examples
1	Natural	Gum acacia (gum Arabic), agar, agarose, maltodextrins, sodium alginate, calcium alginate, dextran, fats and fatty acids, cetyl alcohol, milk solids, molasses, gelatin, gluten, albumin, shellac, starches, casemates, stearms, sucrose, and waxes (e.g. beeswax, carnauba, and spermaceti).
2	Semi-synthetic	Cellulose acetate, cellulose acetate butyrates, cellulose acetate phthalate, cellulose nitrate (collodion), ethyl cellulose, hydroxypropylcellulose,Hydroxypropyl-methylcellulose,Hydroxypropyl-methyl-cellulose phthalate, methyl cellulose, Sodiumcarboxy methylcellulose, hydrogenated tallow, myristylalcohol (1-tetradecanol), glycerylmono-or dipalmitate, hydrogenated easter oil, glycerylmono-di-or tristearate, and 12-hydrooxystearyl alcohol.
3	Synthetic	Acrylic polymers and copolymers e.g. polyacrylamide, poly (alky cyanoacrylate) and poly (ethylene vinyl acetate), aluminium monostearate, carboxvinyl polymers (carbopol) polyamides, poly (methyl vinyl ether maleic anhydride), poly (adiply-L-lysine), polycarbonates, polyterephthalamide, polyvinyl acetate phthalatePoly (Terephthaloyl-L-lysine), polyaryl-sulfones, poly (methylmethacrylate), poly (c-caprolactone),polyvinlylpyrrolidone,polydimethlosiloxae, polyoxyethylenes,polyesters,polyglycolic acid, polylactic acid and copolymers,polyglutamic acid, polylysine, poly-styrene, poly (styrene acrylonitrile),and polyimides.

2.4 DRUG PROFILE

2.4.1 TURMERIC

BOTANICAL: Curcuma longa (linn.) FAM ILY: Zingiberaceae

Turmeric is a mild aromatic stimulant seldom used in medicine except as a coloring. It was once a cure for jaundice. Its chief use is in the manufacture of curry powders. It is also used as an adulterant of mustard and a substitute for it and forms one of the ingredients of many cattle condiments. Tincture of Turmeric is used as a colouring

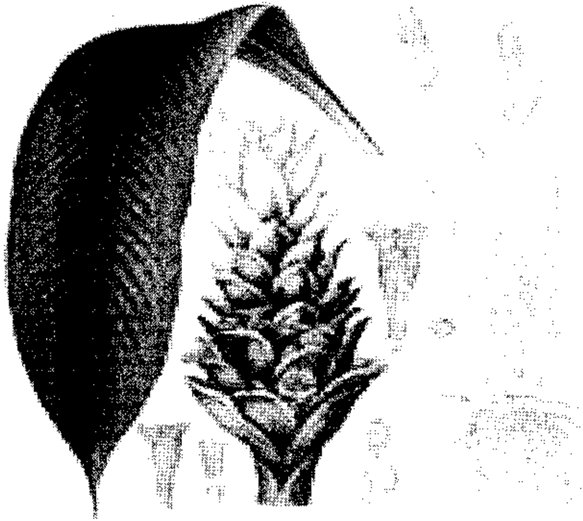

Fig 2.2: Turmeric

agent, but the odour is fugitive. It dyes a rich yellow. Turmeric paper is prepared by soaking unglazed white paper in the tincture and then drying. Used as a test for alkaloids and boric acid (Fig 2.2: Turmeric).

2.4.2 NEEM

BOTANICAL: Melia Azadirachta: **FAMILY**: Meliaceae

The oil obtained from the fruit is used for burning, that from the bark is used medicinally and is anthelmintic and emetic; it is applied externally for rheumatism. The decoction of Azadirachta is said to be cathartic and in large doses slightly narcotic; it is also supposed to have febrifuge properties, it is used as a remedy for hysteria. The Hindu considers it a stomachic and taps it for toddy. The name Bead Tree is derived from the hard nuts, which are used for making rosaries. An ointment to destroy lice is made from the pulp and is also used for scald head and other skin diseases. The oil from the nuts is useful for cramps, obstinate ulcers, etc (Fig2.3: Neem).

Fig2.3: Neem

2.4.3 TULSI

BOTANICAL: Ocimum sanctum

Renowned as a general tonic and vitalizer."The Elixir of Life", Tulsi has been traditionally employed in hundreds of different formulations for the treatment of a wide range of disorders, including those of the mouth and throat, lungs, heart, blood, liver, kidney, and the digestive, metabolic, reproductive and nervous systems. Tulsi is commonly

used to treat coughs, colds and flu, head and ear aches, rheumatism and arthritis, malaria, fever, allergies, and various skin diseases, to reduce the toxicity of various poisons, including insect and reptile bites, to expel intestinal parasites, repel insectsand purify the air (Fig.2.4: Tulsi).

Fig.2.4: Tulsi

2.4.3.1 Tulsi's Chemistry

The chemical composition of Tulsi is highly complex, containing many nutrients and other biologically active compounds, the proportions of which may vary considerably between strains and even among plants within the same field. Furthermore, the quantity of many of these constituents is significantly affected by differing growing, harvesting, processing and storage conditions that are not yet well understood. The nutritional and pharmacological properties of the whole herb in its natural form, as it has been traditionally used, result from synergistic interactions of many different active photochemical. Consequently, the overall effects of Tulsi cannot be fully duplicated with isolated compounds or extracts. Because of its inherent botanical and biochemical complexity, Tulsi standardization has, so far, eluded modern science. Perhaps best known of the many active compounds that have been identified and extracted are eugenol (an essential oil) and ursolic acid. Although Tulsi is known as a general vitalizer and increases physical endurance, it contains no caffeine or other stimulants. Tulsi is traditionally taken in a variety of forms, including cold and hot fresh or dried leaf tea infusions (herbal teas), fresh green leaf, leaf juice, ground or powdered leaf, alcohol tinctures, poultices and oil (ghee) preparations, as well as seed, root and stem formulations, both internally and topically. In addition, modern

human clinical studies and animal experiments also employ various extracts and isolated compounds, sometimes administered by injection. . The differing Tulsi preparations likely vary to a considerable degree, both chemically and in their overall pharmacological effects.

2.4.5 YEAST

Yeast is unicellular fungi. The precise classification is a field that uses the characteristics of the cell, ascospore and colony. Physiological characteristics are also used to identify species. One of the better-known characteristics is the ability to ferment sugars for the production of ethanol. Budding yeasts are true fungi of the phylum Ascomycetes, class Hemiascomycetes. The true yeasts are separated into one main order Saccharomycetales.

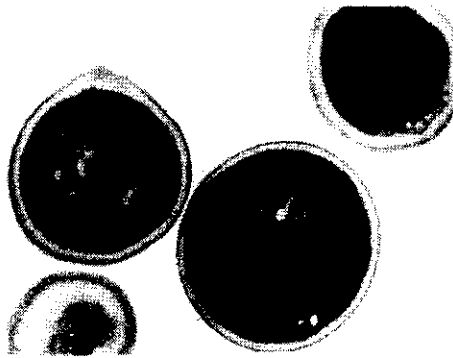

Fig.2.5: Yeast

Yeasts are characterized by a wide dispersion of natural habitats. Common on plant leaves and flowers, soil and salt water. Yeasts are also found on the skin surfaces and in the intestinal tracts of warm-blooded animals, where they may live symbiotically or as parasites. The common "yeast infection" is typically Candidiasis is caused by the yeast-like fungus Candida albicans. In addition to being the causative agent in vaginal yeast infections Candida is also a cause of diaper rash and thrush of the mouth and throat.

Yeasts multiply as single cells that divide by budding (e.g. Saccharomyces) or direct division (fission, e.g. Schizosaccharomyces), or they may grow as simple irregular filaments (mycelium). In sexual reproduction most yeasts form asci, which contain up to eight haploid ascospores. These ascospores may fuse with adjoining nuclei and multiply through vegetative division or, as with certain yeasts, fuse with other ascospores.[8]

The nvasio power of yeast genetics is partially due to the ability to quickly map a phenotype-producing gene to a region of the S. cerevisiae genome. For the past two decades S. cerevisiae has been the model system for much of molecular genetic research because the basic cellular mechanics of replication, recombination, cell division and metabolism are generally conserved between yeast and larger eukaryotes, including mammals (Fig.2.5: Yeast).

The most well-known and commercially significant yeasts are the related species and strains of Saccharomyces cerevisiae. These organisms have long been utilized to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic beverages and in the baking industry to expand, or raise, dough. Saccharomyces cerevisiae is commonly used as baker's yeast and for some types of fermentation. Yeast is often taken as a vitamin supplement because it is 50 percent protein and is a rich source of B vitamins, niacin, and acid.

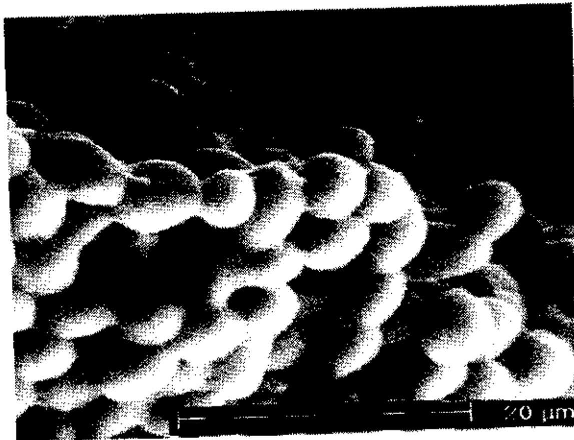

Fig2.6: Fresh yeast – Electron micrograph

In brewing, Saccharomyces carlsbergensis, named after the Carlsberg Brewery in Copenhagen, where it was first isolated in pure culture by Dr. Emil Christian Hansen (1842-1909) in 1883 is used in the production of several types of beers including lagers. S. Carlsbergensis is used for bottom fermentation. S. Cerevisiae used for the production of ales and conducts top fermentation, in which the yeast rise to the surface of the brewing vessel. In modern brewing many of the original top fermentation strains have been

modified to be bottom fermenters. Currently the (Fig2.6: Fresh yeast) S. carlsbergensis designation is not used, the S. cerevisiae classification is used instead.

The yeast's function in baking is to ferment sugars present in the flour or added to the dough. This fermentation gives off carbon dioxide and ethanol. The carbon dioxide is trapped within tiny bubbles and results in the dough expanding, or rising. Sourdough bread is not produced with baker's yeast, rather a combination of wild yeast (often Candida milleri) and an acid-generating bacteria (Lactobacillus sanfrancisco sp. Nov). It has been reported that the ratio of wild yeast to bacteria in San Francisco sourdough cultures is about 1:100. The C. milleri strengthens the gluten and the L. sanfrancisco ferments the maltose.

The fermentation of wine is initiated by naturally occurring yeasts present in the vineyards. Many wineries still use nature strains, however many use modern methods of strain maintenance and isolation. The bubble in sparkling wines is trapped carbon dioxide, the result of yeast fermenting sugars in the grape juice. One yeast cell can ferment approximately its own weight of glucose per hour. Under optimal conditions S. cerevisiae can produce up to 18 percent, by volume, ethanol with 15 to 16 percent being the norm. The sulfur dioxide present in commercially produced wine is actually added just after the grapes are crushed to kill the naturally present bacteria, molds, and yeasts.

The yeast like fungus, Candida albicans, is commonly found in the mouth, vagina, and intestinal tract. Candida is a normal inhabitant of humans and normally causes no ill effects. However, among infants and individuals with other illness a variety of conditions can occur. Candidiasis of the mucous membranes of the mouth is known as thrush. Candidiasis of the vagina is called vaginitis. Candida also causes severe disease in persons with AIDS and chemotherapy patients.

2.4.6 Ultra-Fresh Silpure

The first topically applicable silver antimicrobial for textiles. The product consists of a very fine colloidal suspension of silver, which is mixed with a large volume of a second liquid before being applied by pad to fabrics.

The key to the technology is the ability to use nanotechnology to produce an extremely fine dispersion of the silver. The result is an aqueous dispersion with a mean particle size of 180nm that is compatible with normal textile processes. The method of

production allows for a product that has a much more reasonable cost than previous silver treatments.

This combination of fine particle size and controlled release provides for effective and unchanging antibacterial activity over a wide range of washes, at levels well below those necessary for existing organic antimicrobials, but without the potential for discoloration seen with other silver products.

2.5 BACTERIA

2.5.1 STAPHYLOCOCCUS AUREUS

Kingdom – Bacteria
Phylum – Firmicutes
Class – Bacilli
Order – bacillales
Family – Staphylococcaceae
Genus – staphylococcus
Species – s. aureus

The most important genus of the Micrococcaceae family is Staphylococcus. The Staphylococcus genus is classified into two major groups: aureus and non-aureus. S. aureus is a leading cause of soft tissue infections, as well as toxic shock syndrome (TSS) and scalded skin syndrome. It can be distinguished from other species of Staph by a positive result in a coagulase test (all other species are negative).

The pathogenic effects of Staph are mainly ` nvasions` with the toxins it produces. Most of these toxins are produced in the stationary phase of the bacterial growth curve. In fact, it is not uncommon for an infected site to contain no viable Staph cells. The S. aureus enterotoxin causes quick onset food poisoning, which can lead to cramps and severe vomiting. Infection can be traced to contaminated meats, which have not been fully cooked. These microbes also secrete leukocidin, a toxin which destroys white blood cells and leads to the formation of pus and acne. Particularly, S. aureus has been found to be the causative agent in such ailments as pneumonia, meningitis, boils, arthritis, and osteomyelitis (chronic bone infection). Most S. aureus are penicillin resistant, but vancomycin and nafcillin are known to be effective against most strains.

Of the non-aureus species, S. epidermis is the most clinically significant. This bacterium is an opportunistic pathogen, which is a normal resident of human skin. Those susceptible to infection by the bacterium are IV drug users, newborns, elderly, and those using catheters or other artificial appliances. Infection is easily treatable with vancomycin or rifampin.

2.5.2 LABORATORY INDICATIONS

- Anaerobic glucose fermentation with acid production
- Catalase +
- Nitrate +
- Coagulase +

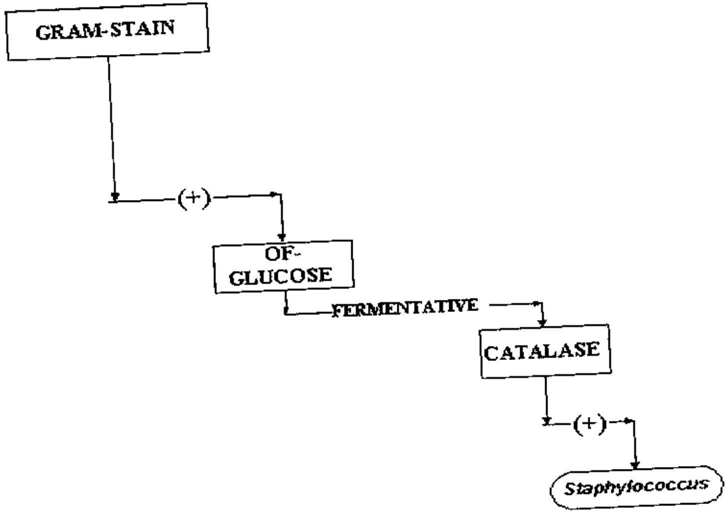

Fig.2.7: Laboratory indications

S.aureus expresses many potential virulence factors: (1) surface proteins that promote colonization of host tissues; (2) nvasions that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase); (3) surface factors that inhibit phagocytic engulfment

(capsule, Protein A); (4) biochemical properties that enhance their survival in phagocytes (carotenoids, catalase)

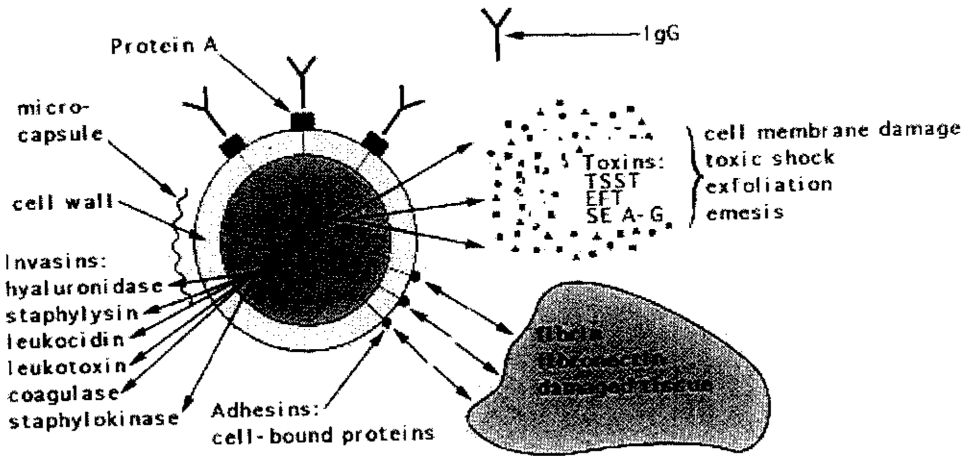

Fig.2.8: Virulence determinants of staphylococcus aureus

production); (5) immunological disguises (Protein A, coagulase, clotting factor); and (6) membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin; (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease (SEA-G, TSST, ET (8) inherent and acquired resistance to antimicrobial agents.

2.5.3 RESISTANCE OF STAPHYLOCOCCI TO ANTIMICROBIAL DRUGS

Hospital strains of S.aureus are usually resistant to a variety of different antibiotics. A few strains are resistant to all clinically useful antibiotics except vancomycin, and vancomycin-resistant strains are increasingly-reported. The term MRSA refers to Methicillin resistant staphylococcus aureus. Methicillin resistance is widespread and most methicillin-resistant strains are also multiply resistant. A plasmid associated with vancomycin resistance has been detected in Enterococcusfaecalis which can be transferred to S.aureus in the laboratory, and it is speculated that this transfer may occur naturally (e.g. in the gastrointestinal tract). In addition, S.aureus exhibits resistance to antiseptics and disinfectants, such as quaternary ammonium compounds, which may aid its survival in the hospital environment.

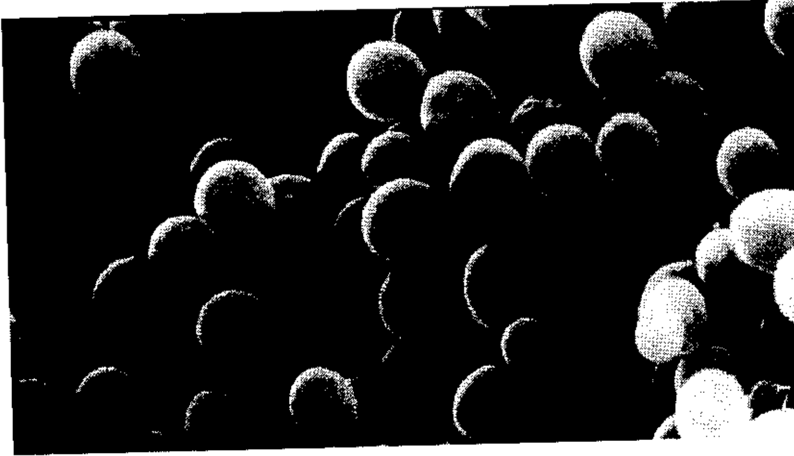

Fig.2.9: Staphylococcus

Staphylococcal disease has been a perennial problem in the hospital environment since the beginning of the antibiotic era. During the 1950's and early1960's, staphylococcal infection was synonymous with nosocomial infection. Gram-negative bacilli (e.g. E. coli and Pseudomonas aeruginosa) have replaced the staphylococci as the most frequent causes of nosocomial infections, although the staphylococci have remained a problem, especially in surgical wounds. S.aureus responded to the introduction of antibiotics by the usual bacterial means to develop drug resistance: (1) mutation in chromosomal genes followed by selection of resistant strains and (2) acquisition of resistance genes as extrachromosomal plasmids, transducing particles, transposons, or other types of DNA inserts. S. aureus expresses its resistance to drugs and antibiotics through a variety of mechanisms. [9]

Beginning with the use of the penicillin in the 1940's, drug resistance has developed in the staphylococci within a very short time after introduction of an antibiotic into clinical use. Some strains are now resistant to most conventional antibiotics, and there is concern that new antibiotics have not been forthcoming. New strategies in the pharmaceutical industry to find antimicrobial drugs involve identifying potential molecular targets in cells (such the active sites of enzymes involved in cell division), then developing inhibitors of the specific target molecule. Hopefully, this approach will turn up new antimicrobial agents for the battle against staphylococcal infections. In fact, in the past two years alternatives to vancomycin have been approved with the increase in VRSA (vancomycin resistant S.aureus) isolates.

2.5.4 ESCHERICHIA COLI

Domain – Bacteria

Phylum – Proteobacteria

Class – Gamma proteobacteria

Order – Enterobacteriales

Family – Enterobacteriaceae

Genus – Escherichia

Species – Escherichia coli

Escherichia coli, usually abbreviated to E. coli, discovered by Theodor Escherich a pediatrician and bacteriologist, is one of the main species of bacteria that live in the lower intestines of warm-blooded animals, including birds and mammals. They are necessary for the proper digestion of food and are part of the intestinal flora. Its presence in groundwater is a common indicator of fecal contamination. The name comes from its discoverer, Theodor Escherich . It belongs among the Enterobacteriaceae, and is commonly used as a model organism for bacteria in general. One of the root words of their family's scientific name, "enteric", refers to the intestine, hence "gastroenteritis" (from 'gastro-', stomach, 'entero-' intestine, '-itis', inflammation). "Fecal" is the adjective for organisms that live in feces, so it is often used synonymously with "enteric".

The number of individual E. coli bacteria in the feces that one human passes in one day averages between 100 billion and 10 trillion. All the different kinds of fecal coli bacteria and all the very similar bacteria that live in the ground (in soil or decaying plants, of which the most common is Enterobacter aerogenes are grouped together under the name coliform bacteria. Technically, the "coliform group" is defined to be all the aerobic and facultative anaerobic, non-spore-forming, Gram-negative, rod-shaped bacteria that ferment lactose with the production of gas within 48 hours at 35°C (95°F). In the body, this gas is released as flatulence).

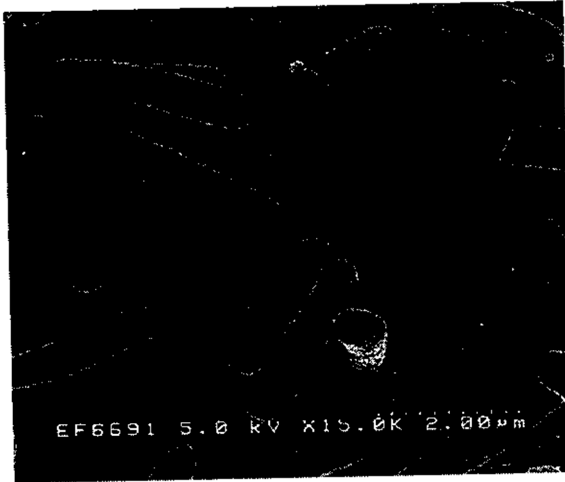

Fig.2.10: E.coli

2.5.4.1 Role in microbiology

Because of its ubiquity, E.coli is frequently studied in microbiology and is the current "workhorse" in molecular biology. Its structure is clear, and it makes for an excellent target for novice and intermediate students of the life sciences.

E.coli plays an important role in modern biological engineering. Researchers often use the bacteria as "factories" to produce large amounts of DNA and/or protein. One of the first useful applications of recombinant DNA technology was the manipulation of E.coli to produce human insulin for patients with diabetes.

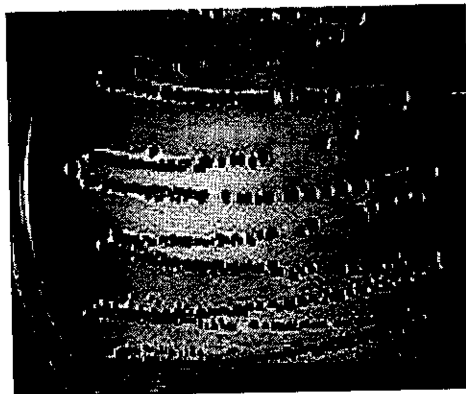

Fig.2.11: E.coli studied in microbiology

2.5.4.2 Strain

A "strain" of E. coli is a family with some particular characteristics that make it recognizable from other E. coli strains, the way poodles are recognizable from other strains (or "breeds") of dogs, and different strains of E. coli live in different kinds of animals, so it is possible to tell whether fecal material in water came from humans or from birds, for example. New strains of E. coli arise all the time from the natural biological process of mutation, and some of those strains have characteristics that can be harmful to a host animal. Although in most healthy adult humans such a strain would probably cause no more than a bout of diarrhea, and might produce no symptom at all, in young children, or in people who are or have recently been sick, or in people takingcertain medications, an unfamiliar strain can cause serious illness and even death.A particularly virulent example of such a strain of E.coli is E.coli O157:H7.

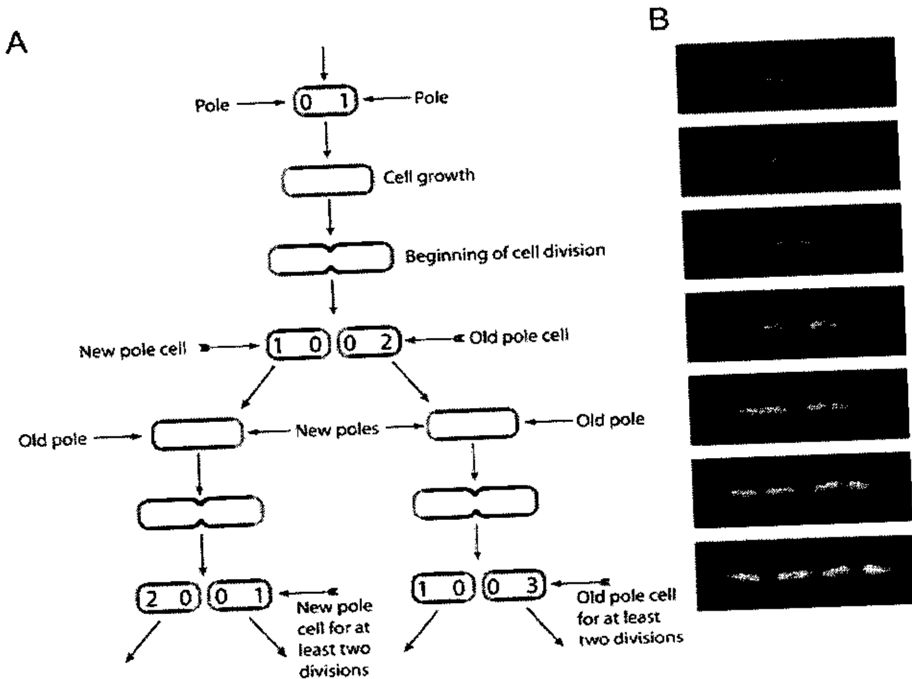

Fig.2.12: Formation of E.coli

2.5.5 PSEUDOMONAS

Kingdom – Bacteria

Phylum – Probacteria

Class – Gamma Proteobacteria

Order – Pseudomonadales

Family – Pseudomonadaceae

Genus – pseudomonas

Species – Pseudomonas aeruginosa

2.5.5.1 General characteristics of the genus

Pseudomonas-Gram-negative which is rod-shaped, of size 0.5-0.8 um x 1-3 um is Strictly aerobic; the only anaerobic activities may be denitrification and arginine1degradation ornithine. The E.coli is motile by polar flagella; some strains also produce lateral flagella. Oxidative, chemoorganotrophic metabolism takes place, Catalase-positive. Usually oxidase-positive and No organic growth factors are required. Diffusible and/or insoluble pigments may be produced GC content of the DNA: 58-68 mol%.

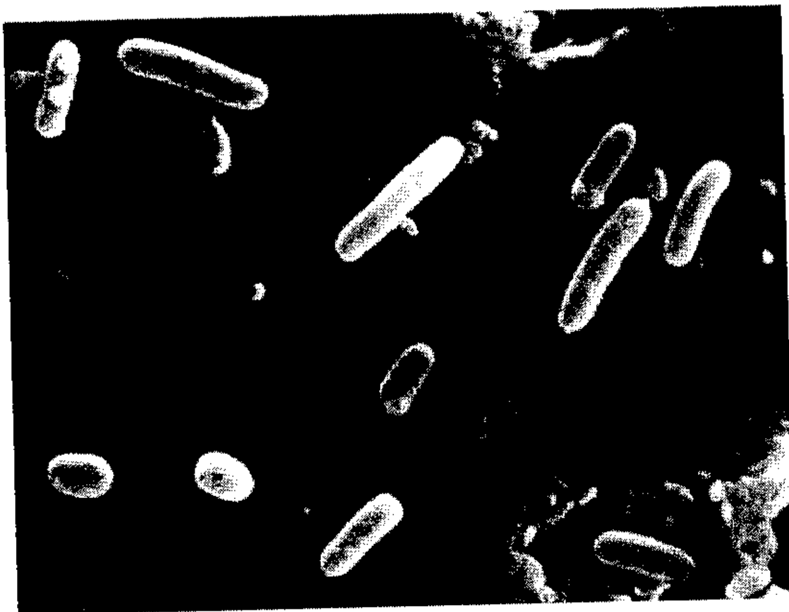

Fig.2.13: Pseudomonas aeruginosa

Pseudomonas aeruginosa is the most frequent isolated non-fermenter in the laboratory. It has several features that distinguishes it from the other species

- It can grow at 42 degrees celsius
- Produces a bluish pigment (pyocyanin) and a greenish pigment
- Characteristic fruity odor

The basis of this organism's pathogenicity involves several toxins and chemicals which the bacterium secretes upon infection. The lipopolysaccharide layer helps the cell adhere to host tissues and prevents leukocytes from ingesting and lysing the organism. Lipases and exotoxins then procede to destroy host cell tissue which then leads to the complications associated with infection. P. aeruginosa prefers to inhabit moist environments but it can survive in a medium as deficient as distilled water. It will also grow on just about any laboratory medium and is beta-hemolytic on blood agar. Treatment of Pseudomonoas infection consists of a combination of two antibiotics: for example an anti-pseudomonal penicillin and an aminoglycoside. The best way to reduce the spread of P. aeruginosa in the hospital is to use good aseptic technique on hospital instruments and when in contact with patients.

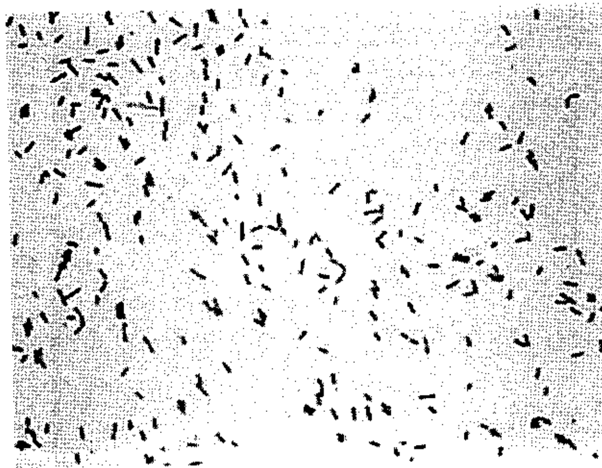

Fig 2.14: Gram stain of Pseudomonoas cells

Pseudomonoas aeruginosa is a Gram-negative, aerobic rod belonging to the bacterial family Pseudomonadaceae. The family includes other genera, which, together with certain

other organisms, constitute the bacteria informally known as pseudomonads. These bacteria are common inhabitants of soil and water. They occur regularly on the surfaces of plants and occassionally on the surfaces of animals. The pseudomonads are well known to plant microbiologists because they are one of the few groups of bacteria that are true pathogens of plants. In fact, Pseudomonoas aeruginosa is occasionally a pathogen of plants. But Pseudomonoas aeruginosa and two former Pseudomonoas species (now reclassified as Burkholderia) are pathogens of humans. A general treatment of the pseudomonads is presented in the Genus Pseudomonoas .

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2.5.5.2 Characteristics

Pseudomonoas aeruginosa is a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. Almost all strains are motile by means of a single polar flagellum. The bacterium is ubiquitous in soil and water, and on surfaces in contact with soil or water. Its metabolism is respiratory and never fermentative, but it will grow in the absence of O_2 if NO_3 is available as a respiratory electron acceptor.

The typical Pseudomonoas bacterium in nature might be found in a **biofilm**, attached to some surface or substrate, or in a **planktonic form**, as a unicellular organism, actively swimming by means of its flagellum. Pseudomonoas is one of the most vigorous, fast-swimming bacteria seen in hay infusions and pond water samples.

In its natural habitat Pseudomonoas aeruginosa is not particularly distinctive as a pseudomonad, but it does have a combination of physiological traits that are noteworthy and may relate to its pathogenesis.

Pseudomonoas aeruginosa has very simple nutritional requirements. It is often observed "growing in distilled water" which is evidence of its minimal nutritional needs.

In the laboratory, the simplest medium for growth of Pseudomonoas aeruginosa consists of acetate for carbon and ammonium sulfate for nitrogen.

Pseudomonoas. aeruginosa possesses the metabolic versatility for which pseudomonads are so renowned. Organic growth factors are not required, and it can use more than seventy-five organic compounds for growth.

Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as high as 42 degrees.

It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics.

Pseudomonoas aeruginosa has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water.

These natural properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominance as a nosocomial pathogen.

Pseudomonoas aeruginosa isolates may produce three **colony types**. Natural isolates from soil or water typically produce a small, **rough** colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance which is large, **smooth**, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a **mucoid** appearance, which is attributed to the production of **alginate slime**. The smooth and mucoid colonies are presumed to play a role in colonization and virulence.

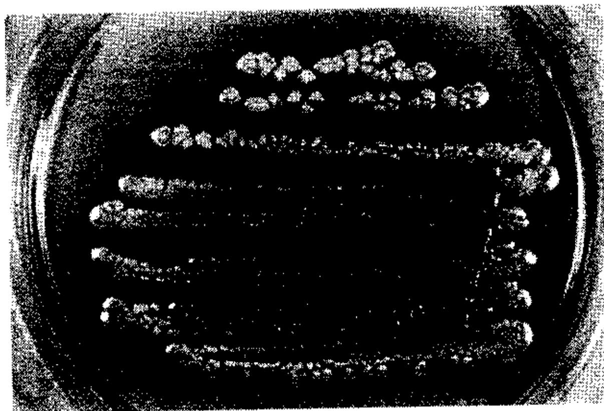

Fig.2.15: .Pseudomonoas aeruginosa colonies on agar

Laboratry indication:

- Oxidase +
- Beta-hemolytic
- Characteristic odor and color
- Motile

Pseudomonoas aeruginosa is a Gram-negative bacterium that is noted for its environmental versatility, ability to cause disease in particular susceptible individuals, and its resistance to antibiotics. The most serious complication of cystic fibrosis is respiratory tract infection by the ubiquitous bacterium Pseudomonoas aeruginosa. Cancer and burn patients also commonly suffer serious infections by this organism, as do certain other individuals with immune systems deficiencies. Unlike many environmental bacteria, Pseudomonoas aeruginosa has a remarkable capacity to cause disease in susceptible hosts. It has the ability to adapt to and thrive in many ecological niches, from water and soil to plant and animal tissues. The bacterium is capable of utilizing a wide range of organic compounds as food sources, thus giving it an exceptional ability to colonize ecological niches where nutrients are limited. Pseudomonoas aeruginosa can produce a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These proteins range from potent toxins that enter and kill host cells at or near the site of colonization to degradative enzymes that permanently disrupt the cell membranes and connective tissues in various organs. This bacterium is also noted for its resistance to many antibiotics.

2.6 FABRIC

2.6.1 COTTON

2.6.1.1 Chemical Structure of Cotton

Cellulose is a polymer of **β-D-Glucose**, which in contrast to starch, is oriented with **$-CH_2OH$** groups alternating above and below the plane of the cellulose molecule thus producing long, unbranched chains. The absence of side chains allows cellulose molecules to lie close together and form rigid structures. Cellulose is the major structural material of plants. Wood is largely cellulose, and cotton is almost pure cellulose. Cellulose can be hydrolyzed to its constituent glucose units by microorganisms that inhabit the digestive tract of termites and ruminants. Cellulose may be modified in the laboratory by treating it with nitric acid (**HNO_3**) to replace all the hydroxyl groups with nitrate groups (**$-ONO_2$**) to produce cellulose nitrate (**nitrocellulose** or **guncotton**) which is an explosive component of smokeless powder. Partially nitrated cellulose, known as **pyroxylin**, is used in the manufacture of collodion, plastics, lacquers, and nail polish. [12]

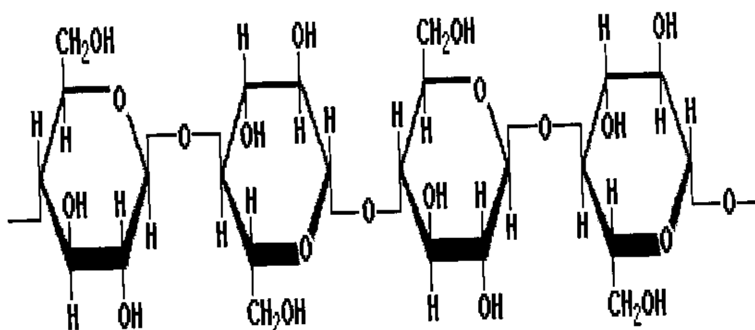

Fig2.16: Chemical Structure of Cotton

2.6.2 SILK

2.6.2.1 Structure of Silk:

The secondary structure of silk is an example of the **beta pleated sheet**. In this structure, individual protein chains are aligned side-by-side with every other protein chain aligned in an opposite direction. The protein chains are held together by intermolecular hydrogen bonding, that is hydrogen bonding between amide groups of two separate chains. This intermolecular hydrogen bonding in the beta-pleated sheet is in contrast to the intramolecular hydrogen bonding in the alpha-helix.[14]

The hydrogen on the amide of one protein chain is hydrogen bonded to the amide oxygen of the neighboring protein chain. The pleated sheet effect arises form the fact that the amide structure is planar while the "bends" occur at the carbon containing the side chain.

Fortunately, the "side" chain R groups in silk are not very bulky. The basic primary structure of silk consists of a six amino acid unit that repeats itself. The sequence where every other unit is glycine in silk is: -gly-ala-gly-ala-gly-ala-. Although glycine and alanine make up 75-80% of the amino acids in silk, another 10-15% is serine and the final 10 % contain bulky side chains such as in tyr, arg, val, asp, and glu.

These amino acids with bulky side chains disrupt the regular patterns set by the gly-ala-ser. Different species of silkworms produce different portions of ordered and disordered regions. The disordered regions provide a small amount of elasticity since the ordered beta-pleated sheet is already fully extended and cannot stretch further without breaking.[13]

The beta pleated sheet motif is found in many proteins along with the alpha helix structure. The chains may run parallel (all N terminals on one end) or anti-parallel (N terminal and C terminal ends alternate).

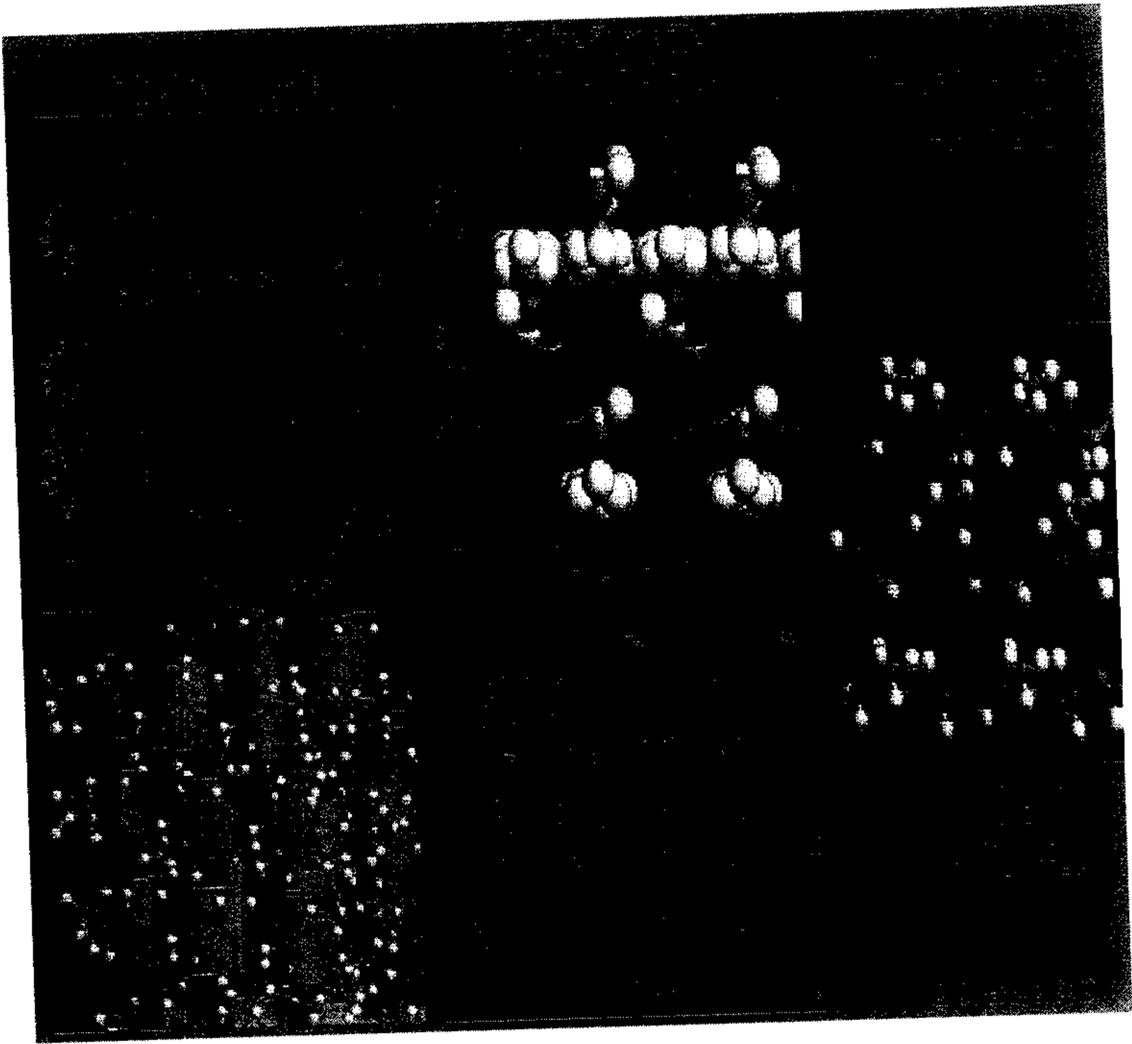

Fig2.17: Silk Structure

3. MATERIALS AND METHODS

3.1 INTRODUTION

This chapter explains the objective, materials and methods in the study of antimicrobial activity.

3.2 OBJECTIVE

- Application of herbal extract for protein and cellulosic fabric in the form of microcapsules,
- Assessment of its antimicrobial activity

The core material was defined as the specified material to be coated and it can be either solid or liquid in nature. The core material of herabal extract mixture is from neem, turmeric and tulsi, which is in semi solid form.

Table 3.1 List of Medicinal Plants Extracted

Sl.no	Medicinal Plants		Material type used
	Comman name	**Botanical name**	
1	Neem	Azadirachta indica	Leaves
2	Tulasi	Ocimum sanctum	Leaves
3	Turmeric	Curcuma longa	Rizhomes

Fresh yeast was used as the wall material, which is having good capsulating capacity compare with other natural products. UF silpure FBR-5 (PA) was taken as the binder to have a good adherence of the finish on materials.

Woven fabric of Cotton and silk were indnilially soured and bleached plain weave. Cotton (60s count, EPI of 120,PPI of 92) and Silk (60s count, EPI of 124,PPI of 94) were taken for the study. The microcapsules were applied by pad-dry-cure method

3.3 PREPARATION OF HERBAL EXTRACT

- One kilogram of each core material (neem, tulsi & turmeric) is taken separately and distilled water is added to the float level.
- 1% of chloroform is added to each of the core material in a distill bath and allowed to dissolve for 24 hours.
- The extracts are filtered and kept in the water bath, heated until a semisolid form is obtained.
- The extracts are kept for drying in the sunlight till the standard form is obtained (given below) which would take 3 to 5 days.
- Form of core material obtained:
  1. Neem – semi liquid residue
  2. Tulsi – semi solid residue
  3. Turmeric – dry coarse powder

3.4 THE IMPORTANCE OF CELL VIABILITY ON ENCAPSULATION OF HERBAL DRUG IN YEAST CELLS

- To assess the importance of cell viability on the encapsulation process cells were pre-treated prior to the encapsulation process.
- The suspension of fresh yeast (50 grams) was treated overnight with 2 grams of sodium azide – a respiratory inhibitor used to prevent the cell from performing energy dependent processes. The solution is kept for an overnight.
- The cells were then taken and sterilized in autoclave at 120°celcius for 20 minutes prior to encapsulation.
- Sterilization by autoclaving is a thermally destructive process denaturing any carrier protein molecules likely to be involved in facilitated diffusional process, which may be responsible for encapsulation process.

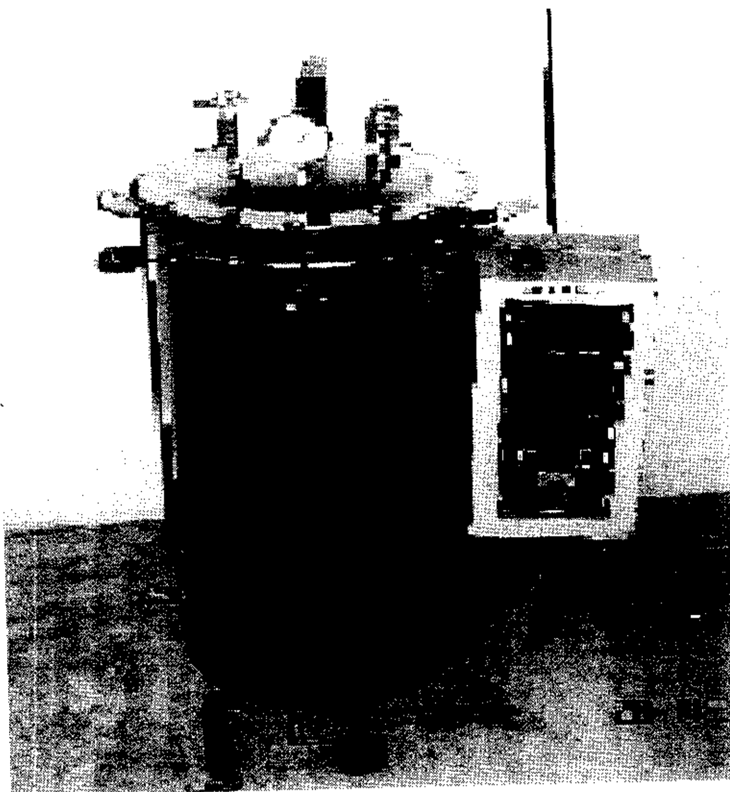

Fig.3.1: Autoclave

3.5 PREPARATION OF MICROCAPSULES

- The herbal extract (drug) 45 grams, yeast 180ml and distilled water 540ml is taken followed by standard volumetric ratio of 1:4:8 respectively.
- The concoction is agitated in a gallenkamp rotary incubator at 100rpm of 40°c for 4 hours.
- The cells were centrifuged for 10 minutes then wash is given for five cycles with distilled water and freeze dried which will take about 2-4 days.

Fig3.2: Microcapsule

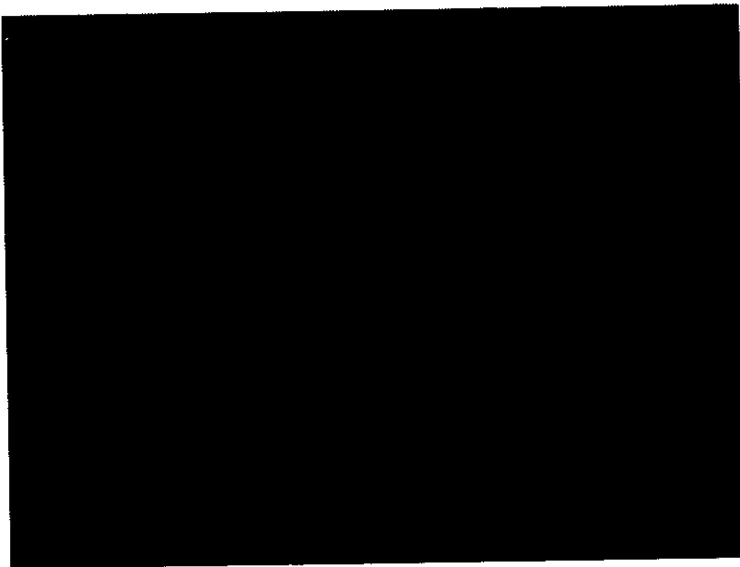

Fig 3.3 Microcapsules

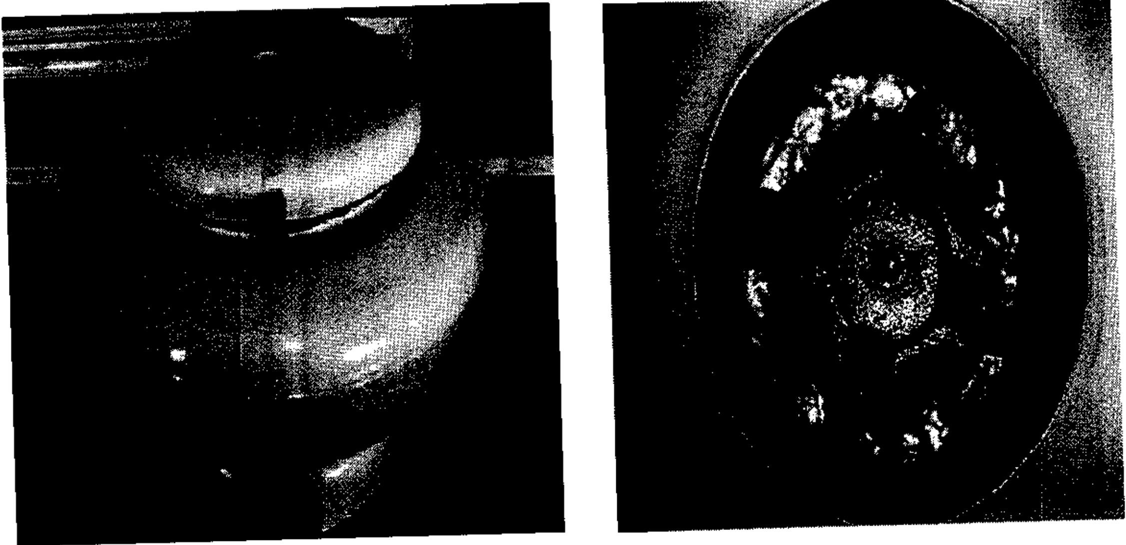

Fig3.4: Centrifuge

3.6 APPLICATION OF MICROCAPSULES ON COTTON AND SILK MATERIAL USING THE PADDING MANGLE

- The microcapsules are fixed on cotton and silk fabric with a binder by pad-dry method.
- The microcapsules were diluted in water at the concentration of 6gm/liter along with binder (ultrafresh-silpure) 1.5 ml.
- The microcapsules were applied on fabric by pad-dry cure method with material: liquor ratio of 1:10 and pH of 5.5-6.
- The curing was carried out at 120c for 2 min.
- The following table illustrates the fabrication details.

Table 3.2 Process Specifications

Padding	**Process Specification**
M: l	1:10
Binder (ultrafresh-silpure)	1.5ml
Capsules	6grams
Fabric (weighed)	30.27 Gms
pH	5.5-6
Drying	
Temperature	120°c
Time	1 min 30 sec
Speed	80 volts
Fabric	
Cotton (bleached)	100*40cm
Silk (bleached)	100*40cm

3.6.1 MACHINE SPECIFICATION

3.6.1.1 Padding mangle

- Capacity: 600 ml
- Temperature:0°c to25°c
- Speed range: up to 13m/min in step of 1
- Air pressure: 7kg/cm2 pressure for pneumatics
- Bowl size: 120mm dia*500 mm wide
- Hardness: 65/70 shores.

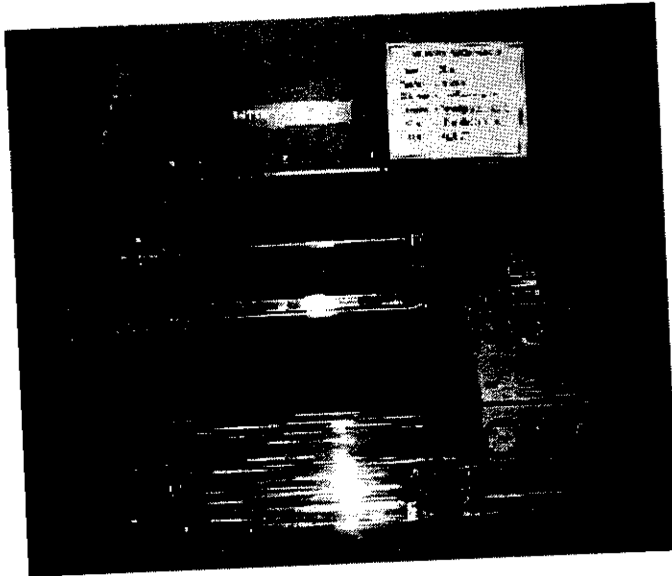

Fig.3.5: Padding Mangle

3.6.1.2 Drying setting and curing chamber

- Speed control: 0 to 270 volts
- Heating control: 0 °c to 100°c
- Heating range: 8KW to 24 KW
- Temperature: 0°c to1000°c

Fig.3.6: Curing Chamber

3.6 TESTING

- The testing is carried out by the disc diffusion method for microcapsule and parallel streak method for the cotton and silk fabric that is treated.
- The bacteria that are tested for the staphylococcus, pseudomonas, e.coli.

3.6.1 ANTIBACTERIAL ACTIVITY ASSESSMENT OF TEXTILE MATERIALS

3.6.1.1 Parallel Streak Method (AATCC 143 1993)

The Parallel streak method has filled a needed for a relatively quick and easily executed qualitative method to determine antibacterial activity of diffusible antimicrobial agents on treated textile materials. In parallel streak method, the agar surface`e is inoculated making it easier to distinguish between the test organism and the contaminated organisms, which may present on the unspecialized specimen. The parallel streak method has proven effective over a number of years of use in providing evidence in antimicrobial activity against both gram positive and gram-negative bacteria.

Principle

Specimen of test material, including corresponding untreated controls of the same material, are placed in intimate contact with nutrient agar which has been previously streaked with an inoculums of a test bacterium. After incubation, a clear area of interrupted growth underneath and along the sides of the test material indicates antibacterial activity of the specimen. The standard strain of bacteria is used which is specific to the requirements of the material under test.

Terminology

- **Activity** of an antibacterial agent, a measure of effectiveness of the agent.
- **Antibacterial agent** in the textiles, any chemical, which kills bacteria, interferes with the multiplication, growth or activity of bacteria.
- **Zone of Inhibition**, clear area of no growth of a microorganism, cultered on to the surface of an agar growth medium, in proximity to the borders of a specimen placed in direct contact with this agar surface. A zone of inhibition occurs as a result of the diffusion of antimicrobial agent from the specimen.

Procedure

- Dispense sterilized nutrient (or appropriate medium) agar [cooled to 47± 2°c(117±4°F)] by pouring 15 ± 2 mL into each standard (15*100mm) flat-bottomed petri dish. Allow agar to get firmly before inoculating.
- Prepare inoculum by transferring 1.0 ± 0.1mL of a 24 hour broth culture into 9.0 ± 0.1 of sterile distilled water contained in a test tube or small flask. Mix well using appropriate agitation.
- Using a 4 mm inoculating loop, load one loopful of the diluted inoculum agar plate by making five streaks approximately 60 mm in length, spaced 10 mm apart covering the central area of a standard petri dish without refilling the loop. Take care not to break the surface of the agar while making the streaks.
- Gently press the test specimen transversal across the five incoulum streaks to ensure intimate contact with the agar surface. This may be accomplished more easily by pressing the specimen to the agar surface with a biological section lifter or with a spatula, which has been sterilized by flaming and then air, cooled immediately before use.
- If the specimen curls, preventing intimate contact with the inoculated surface, place sterile glass slides on the ends of the specimen to hold it in place.
- Incubate at 37 ± 2°c (99±4°F) for 18-24 h.

Evaluation

Examine the incubated plates for interruption of growth along the streaks of inoculum beneath the specimen and for a clear zone of inhibition beyond its edge. The average width of a zone of inhibition along a streak on either side of the test specimen may be calculated using the following equation:

$$W=(T-D)/2$$

where,

W= width of clear zone of inhibition in mm

T= total diameter of test specimen and clear zone in mm

D= diameter of the test specimen in mm.

3.6.1.2 Disc Diffusion methods

Generally diffusion method is preferred to serial dilution methods because of the ease with which quantitative results can be obtained. They cannot be used when, because of absorption by, or incompatibility with, medium the medicament does not diffuse freely.

A geometric series of dilutions is prepared for the antibiotic under test and for the standard preparation. Plates are seeded with the test organism and the medium is allowed to set on a perfectly horizontal surface so that the agar is constant in depth throughout the dish. The organism may be mixed with agar before pouring or applied to the surface of the medium after it has set. The plates may be petri dishes or large flat dishes up to 0.5 m square.

The solutions are contained in

- Cups cut in the medium using a sterile cork borer about 10mm in diameter, the agar disc being removed by vacuum device or a splayed-out steel pen nib.
- Cylinders of plain steel, glazed porcelain, Pyrex glass or sterilisable plastic having an external diameter of about 8mm and a height of about 10mm.These are usually warmed so that they sink slightly, to a constant depth, when placed on the agar.
- Filter paper or cellulose discs, which absorb a fixed volume of solution.
- Standard ceramic insulation beads (fish spine beads), which attract a fixed volume when touched on the surface of the solution. The surface of the agar must be dry if this method is used.
- The test and standard solutions are placed in the container in random order, to prevent the bias that could be caused by a regular order of plating.
- The volume is critical for the cup method but is not significant when cylinders are used provided they are at least two-thirds full. Care is taken not to seal the tops of the cylinders with the lid pf the plate.
- The plates are left at room temperature for two hours to allow diffusion of the antibiotic to get ahead of growth of organisms. Then they are incubated at the appropriate temperature, usually for about 16 hours.
- After incubation, inhibition of the growth can be seen as a clear zone around each container. The diameter of this is proportional to the log of the concentration of the antibiotic. As soon as possible each diameter is measured and this is best done using an

optical system that projects the image of the plate on to a large grid. Two diameters at the right angles are used as a check on ellipticity of the zone.

The result can be processed in two ways:

1.A graph is plotted of log concentration of standard against zone diameter and the results for the test preparation are plotted on the same graph provided the two lines are parallel, the relative potencies of the standard and test are represented by the horizontal distance between the lines.

2. Parallelism between the lines can be confirmed mathematically and the potency of the test obtained by calculation.

As with all bioassays the validity of the method should be confirmed and the error of the result indicated. Although it may be difficult, attempt should be made to use test and standard solutions of approximately equal strength, because the most accurate result is obtained under these circumstances.

3.6.1.3 Air permeability tester

Field of applications

This equipment is designed for measuring the permeability of fabrics to air and is applicable to industrial fabrics that are permeable to air. However this standard is not suitable for parachute fabric.

Principle

This method is based on the measurement of the rate of flow of air through a given area of fabric by a given pressure drop across the fabrics.

Related standard:

Is: 11056-1984

Sampling

Samples are drawn in accordance with the procedure laid down in material specification for the fabric or as agreed to between the parties concerned shall be held to be representative of the buyer.

Atmospheric conditions for conditioning and testing

Prior to test the samples shall be conditioned to moisture equilibrium in the standard atmosphere of 65+/-2 percent relative humidity and 27+/-2 degree temperature from dry side as laid down in Is: 6359-1971 the test shall be carried out in the standard atmosphere.

Apparatus:

The apparatus is consist of the following arrangements

- Means for drawing or forcing air through the fabric of known area.
- Circular orifice of definite area.
- Provision to hold the fabric so that there is no peripheral leakage of air.
- Means for adjusting the pressure drop across the fabric to a known amount.
- Means for measuring the calibration of air flow meter.

Procedure

Take the conditioned specimen and mount a portion between the clamp and circular orifice with sufficient tension to eliminate wrinkles if any taking care to see that the fabric is not distorted in its own place.

Start the suction fan or other means to force the air through the fabric and adjust the rate of flow air till pressure drop of one-centimeter water head across. Note the rate of flow of air in cm^3/s.repeat the test at different places. In all at least 5 test shall be carried out.

Calculation

Calculate the rate of flow of air per cm^2 of fabric in cm^3/s by the following formula:

$R = r/a$

Where

R= rate of flow of air/cm^3 of fabric in cm^2

r = mean rate of flow of air in cm^3 /s, and

a= area cm^2 of fabric under test in cm^2

4. RESULTS AND DISCUSSION

This chapter deals with the antimicrobial assessment of the Microcapsules and treated fabric. The comparison of Airpermeability for control to the treated fabric in cotton and silk is done. The morphological analysis of the treated fabric and microcapsule is given in the photographs using the optical microscope and QUANTA SEM.

4.1 MICROENCAPSULATION TESTING

4.1.1 Antimicrobial activity of Microcapsules- Disc diffusion method

Fig.4.1 shows the disc diffusion result of mixture of microcapsules containing Neem, Tulsi and Turmeric against Staphylococcus, E.coli and Pseudomonas. It was found that the microcapsule from the table 4.1 shows higher zone of inhibition against the Pseudomonas is 12.5 mm compared to the Staphylococcus and E.coli.

Table 4.1 Disc diffusion method

S.NO	Types of bacteria	Capsule size (mg)	D (mm)	T (mm)	W =(T-D)/2 (mm)
1	Pseudomonas	5	3	13	5
		10	4	20	8
		15	5	30	12.5
2	Staphylococcus	5	3	11	4
		10	4	18	7
		15	5	27	11
3	E.coli	5	3	9	3
		10	4	12	4
		15	5	15	5

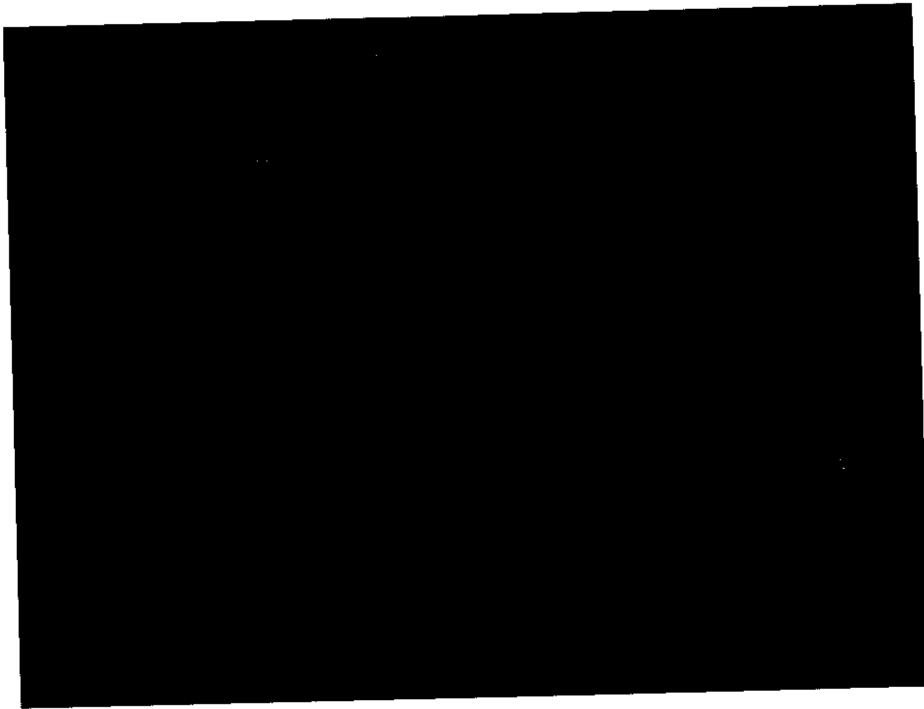

Fig 4.1: Antimicrobial activity of Microcapsules

4.2 FABRIC TESTING

4.2.1 Antimicrobial activity of fabrics treated with microcapsules-Parallel Streak method (AATCC 147 1993):

Fig 4.2 and 4.3 shows the parallel streak results of the treated cotton and silk fabric against Staphylococcus, E.coli and Pseudomonas. It was fond that the Pseudomonas showed higher zone of inhibition against Staphylococcus and E.coli.

Fig 4.2: Antimicrobial activity of Cotton fabric

(1- Pseudomonas activity, 2- S.aureaus activity, 3- E.coli activity)

(A-control cotton fabric, B-Treated cotton fabric)

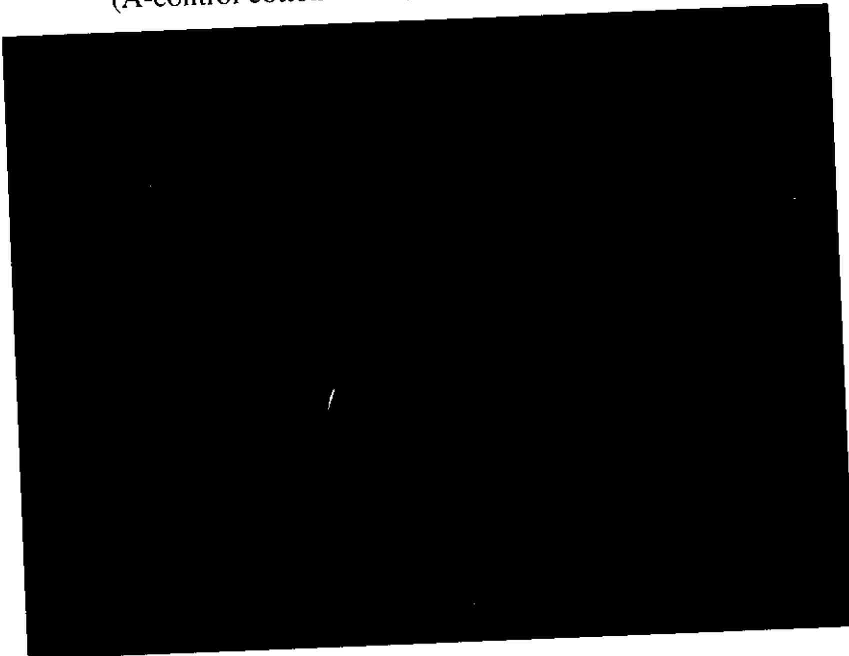

Fig 4.3: Antimicrobial activity of Silk fabric

(1- Pseudomonas activity, 2- S.aureaus activity, 3- E.coli activity)

(A-control silk fabric, B-Treated silk fabric)

4.3 AIR PERMEABILITY TEST

The comparison of Airpermeability for control to the treated fabric in cotton is analyzed. The Airpermeability is found to be less in the treated fabric than in the control fabric in case of cotton. This may be due to the (10.32% reduction) less cover factor of cotton than silk which showed no change in air permeability after the treatment. The results are shown in Table 4.2 and 4.3.

Table 4.2 Control cotton fabric

S.No	1000(lph)	100(lph)	10(lph)	Total	Air permeability ($cm^3/cm^2/sec$)
1	2500	475	58	3033	210.62
2	2500	475	55	3030	210.41
3	1500	175	40	1715	119.09
4	1400	150	38	1588	110.27
5	2400	350	40	2790	193.75
Average					163.82

Table 4.3 Treated cotton fabric

	1000(lph)	100(lph)	10(lph)	Total	Air permeability ($cm^3/cm^2/sec$)
1	2050	390	35	2475	171.87
2	2000	220	37	2257	156.73
3	2050	225	38	2313	160.62
4	1500	250	35	1785	123.95
5	1500	210	38	1748	121.38
Average					146.91

Percentage of change = (163.82-146.91) / 163.82 * 100 = 10.32%

The comparison of Air permeability for control to the treated fabric in silk is analyzed. The Air permeability is found to be same for both the treated fabric and the control fabric in case of silk. Table 4.4and 4.5 shows the result.

Table 4.4 Control silk fabric

	1000(lph)	**100(lph)**	**10(lph)**	**Total**	**Air permeability ($cm^3/cm^2/sec$)**
1	800	175	19	994	69.03
2	1000	150	19	1169	81.18
3	1050	150	18	1218	84.58
4	1000	150	18	1168	81.11
5	950	150	19	1119	77.70
Average					78.72

Table 4.5 Treated silk fabric

	1000(lph)	**100(lph)**	**10(lph)**	**Total**	**Air permeability ($cm^3/cm^2/sec$)**
1	980	110	21	1111	77.15
2	850	100	20	970	67.36
3	950	125	19	1094	75.97
4	1100	150	19	1269	88.12
5	1050	155	19	1224	85.00
Average					78.72

The Air permeability for both the treated and control silk fabric remain the same.

4.4 EVALVATION OF MICROCAPSULE TREATED FABRIC UNDER SACNING ELECTRON MICROSCOPE (SEM)

The prepared microcapsules were applied on the cotton and silk fabric by pad-dry-cure method and the presents of microcapsules were examined at the magnification level of 1000x and 2000x for cotton and silk fabrics respectively under SEM. Fig 4.4 and fig 4.5 shows the SEM photographs of the treated samples.

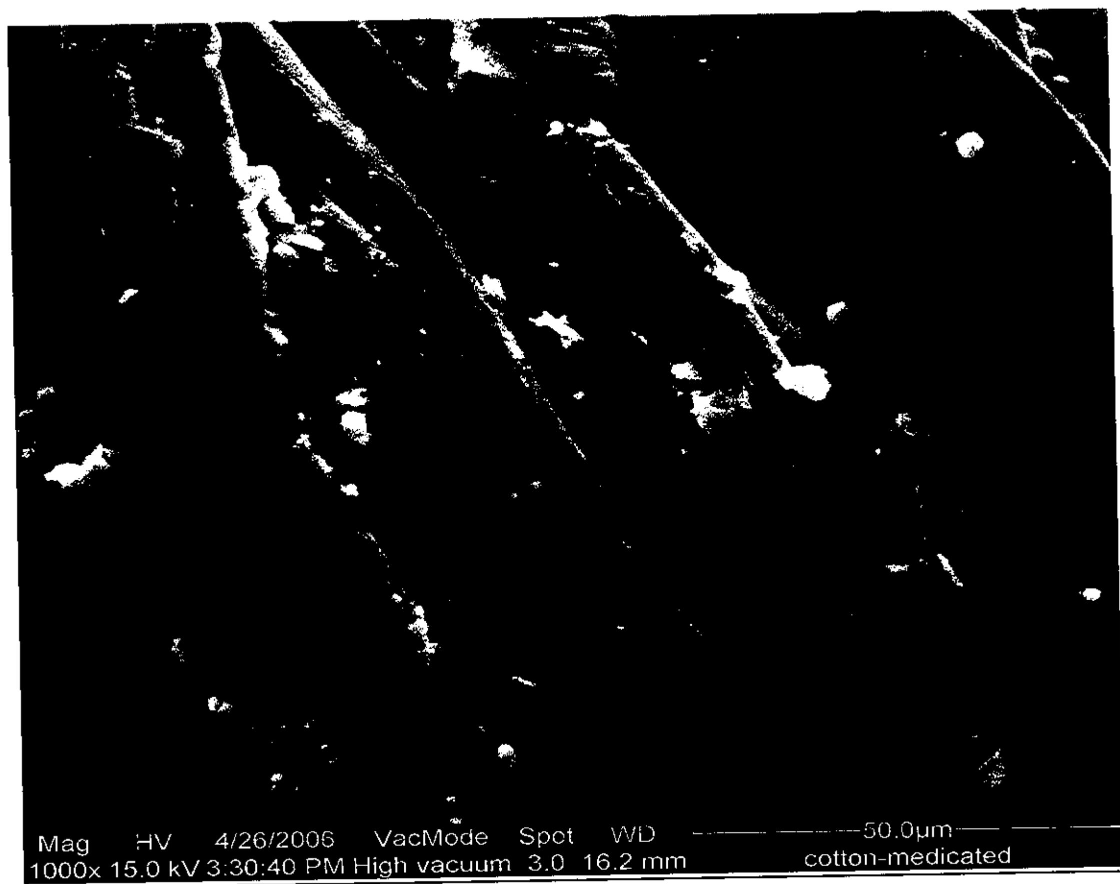

Fig 4.4 SEM photo of treated cotton

From the SEM photos it is inferred that the microcapsules were entrapped between the yarn structures in a very strong condition.

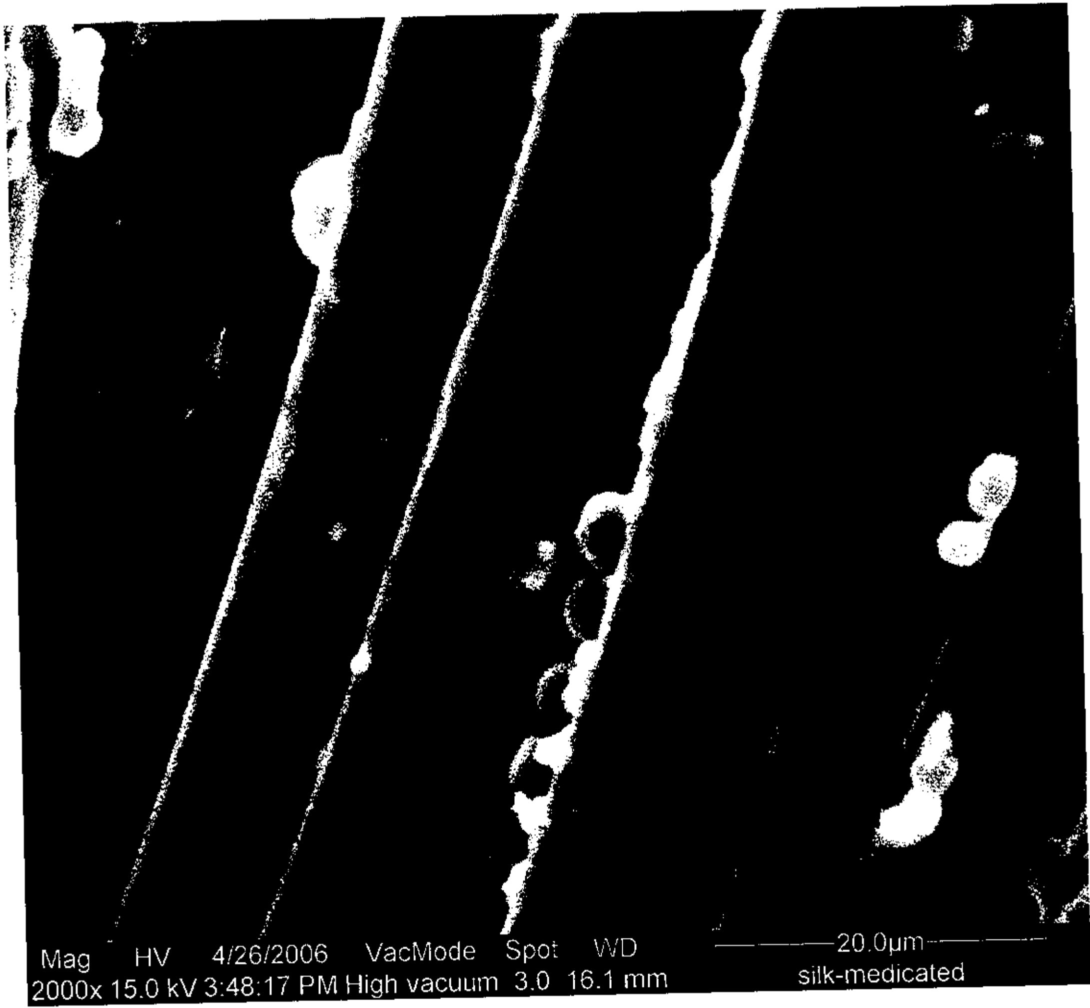

Fig 4.5 SEM photo of treated silk

5. CONCLUSION

1. A microcapsule of mixture of herbs like neem, turmeric and tulsi has been developed by natural encapsulation technique using yeast.
2. The treated fabric showed effective antimicrobial activity.
3. The antimicrobial activity of treated fabric and confirmed that if can be applied for medicinal fabric.
4. The presence of microcapsules in the finished sample was visualized by a SEM (Scanning Electron Microscope) and photographs were taken at various magnification level.

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