“A Review on Formulation and Evaluation Methods for Preparation of Herbal Antimicrobial Gel”

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Abstract: The increasing prevalence of microbial infections, especially those associated with impaired wound healing and biomedical implant failure has spurred the development of new materials having antimicrobial activity. Hydrogels are a class of highly hydrated material finding use in diverse medical applications such as drug delivery, tissue engineering, as wound fillers and as implant coatings, to name a few. The biocompatible nature of many gels makes them a convenient starting platform to develop selectively active antimicrobial materials. Hydrogels with antimicrobial properties can be obtained through the encapsulation or covalent immobilization of known antimicrobial agents, or the material itself can be designed to possess inherent antimicrobial activity. Antimicrobial hydro gels are extremely attractive materials for use as wound dressings and fillers. Due to their high water content, gels provide a moist, heavily hydrated environment to the wound area, facilitating cellular immunological activity essential to the wound healing process. However, this same hydrated environment can also facilitate microbial infection. Thus, gels capable of imparting antimicrobial action in addition to serving their primary functional role (e.g. wound healing, drug delivery, etc...) are desirable.

Keywords: Hydrogel, encapsulation, glutaraldehyde, cross-linking, gelling agent, antimicrobial

1. INTRODUCTION:
Antimicrobial exertion is the capability of a substance to inhibit or kill microbial cells. Different types of antibiotics are being used in the treatment of one form of complaint or the other. Ultimate of these antibiotics were firstly deduced from microorganisms or shops. Still, presently these antibiotics are attained by colorful synthetic processes\textsuperscript{[1]}. Herbal drug, also called botanical drug or phytomedicine, refers to the use of any factory's seeds, berries, roots, leaves, dinghy, or flowers for medicinal purposes. Long rehearsed outside of conventional drug, herbalism is getting farther mainstream as over- to-date analysis and exploration show their value in the treatment of complaint. Traditional drug is an important source of potentially useful new composites for the development of chemotherapeutic agents. The first step towards this thing is the network of shops used in popular drug. therefore, antimicrobial exploration is geared towards the discovery and development of new antibacterial and antifungal agents. Plant medicines are constantly considered to be less poisonous and freer from side goods than the synthetic bones\textsuperscript{[2]}. In an earlier study, medicinal shops have been reported to be truly salutary in crack care, promoting the rate of crack mending with minimum pain, discomfort, and scarring to the case.

1.1. Gel
A gel is a circumfluous system of at least two ingredients, conforming of a condensed mass enclosing and transfused by a liquid. Gels and jellies are composed of small number of solids dispersed in fairly large quantum of liquid, yet they retain more solid- suchlike than liquid- suchlike character. The specific of gel and jelly is the presence of some form of cutaneous structure, which provides solid- suchlike parcels. A gel is a semi-solid that can have parcels ranging from soft and weak to hard and tough. Gels are defined as a mainly dilute cross-linked system, which exhibits no inflow when in the steady-state, although the liquid phase may still diffuse through this system.

Fig.1.1 gel

1.1.2. Types of gels:
- Hydrogels- A hydrogel is a network of polymer chains that are hydrophilic, sometimes set up as a colloidal gel in which water is the dispersion medium. 3-dimensional solid results from the hydrophilic polymer chains being held
1.2. Human skin

The skin is the outer covering of the body. It is the largest organ of the integumentary system. The skin has multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments and internal organs. Human skin is similar to that of most other mammals, except that it is not protected by a pelt. Though nearly all human skin is covered with hair follicles, it appears hairless. There are two general types of skin, hairy and globose skin. The adjective cutaneous literally means “of the skin”. Skin plays a key role in protecting (the body) against pathogens and excessive water loss. Its other functions are insulation, temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B folates. Severely damaged skin will try to heal by forming scar tissue.

Fig. 1.2. inner layer of skin

2. FORMULATION METHODS FOR GEL PREPARATION:

Gels are transparent semisolid preparation meant for external application to the skin or mucous membrane. Gels are semisolid systems consisting of either suspension made up of small inorganic particles or large organic molecules in a liquid vehicle that appear jelly-like by the addition of a gelling agent. These are organic hydrocolloids or hydrophilic inorganic substances. They contain Tragacanth, Sodium Alginate, Pectin, Starch, Gelatin, Cellulose Derivatives, Carbomer, and PolyVinyl Alcohol Clays. These are numerous gelling agents varying in gelling ability.

Fig. 2. preparation of hydrogel
2.1. Heating/cooling a polymer solution--- Physically cross-linked gels are formed when cooling hot solutions of gelatine or carrageenan. The gel formation is due to helix-formation, association of the helices, and forming junction zone [3]. Carrageenan in hot solution above the melting transition temperature is present as random coil conformation. Upon cooling it transforms to rigid helical rods. In presence of salt (K+, Na+, etc.), due to screening of repulsion of sulphonic group (SO3–), double helices further aggregate to form stable gels. In some cases, hydrogel can also be obtained by simply warming the polymer solutions that causes the block copolymerisation. Some of the examples are polyethylene oxide-polypropylene oxide [4], polyethylene glycol-polylactic acid hydrogel [5].

2.2. Ionic interaction--- Ionic polymers can be cross-linked by the addition of di- or tri-valent counterions. This method underlies the principle of gelling a polyelectrolyte solution (e.g. Na+ alginate) with a multivalent ion of opposite charges (e.g. Ca2+ + 2Cl–). Some other examples are chitosan-polylysine [6], chitosan-glycerol phosphate salt [7], chitosan-dextran hydro gels [5].

2.3. Complex coacervation--- Complex coacervate gels can be formed by mixing of a polyanion with a polycation. The underlying principle of this method is that polymers with opposite charges stick together and form soluble and insoluble complexes depending on the concentration and pH of the respective solutions. One such example is coacervating polyanionic xanthan with polycationic chitosan [8]. Proteins below its isoelectric point are positively charged and likely to associate with anionic hydrocolloids and form poly ion complex hydrogel (complex coacervate) [9].

2.4. H-bonding--- H-bonded hydrogel can be obtained by lowering the pH of aqueous solution of polymers carrying carboxyl groups. Examples of such hydrogel are hydrogren-bound CMC (carboxymethyl cellulose) network formed by dispersing CMC into 0.1M HCl [10]. The mechanism involves replacing the sodium in CMC with hydrogen in the acid solution to promote hydrogen bonding. The hydrogen bonds induce a decrease of CMC solubility in water and result in the formation of an elastic hydrogel. Carboxymethylated chitosan (CM-chitosan) hydro gels can also be prepared by cross-linking in the presence of acids or polyfunctional monomer. Another example is polyacrylic acid and polyethylene oxide (PEO-PAAce) based hydrogel prepared by lowering the pH to form H-bonded gel in their aqueous solution [4]. In case of xanthan-alginate mixed system molecular interaction of xanthan and alginate causes the change in matrix structure due to intermolecular hydrogen bonding between them resulting in formation of insoluble hydrogel network.

2.5. Freeze-thawing--- Physical cross-linking of a polymer to form its hydrogel can also be achieved by using freeze-thaw cycles. The mechanism involves the formation of microcrystals in the structure due to freeze-thawing. Examples of this type of gelation are freeze-thawed gels of polyvinyl alcohol and xanthan [4, 11].

2.6. Chemical cross-linking--- Chemical cross-linking covered here involves grafting of monomers on the backbone of the polymers or the use of a cross-linking agent to link two polymer chains. The cross-linking of natural and synthetic polymers can be achieved through the reaction of their functional groups (such as OH, COOH, and NH2) with cross-linkers such as aldehyde (e.g. glutaraldehyde, adipic acid dihydrazide). There are a number of methods reported in literature to obtain chemically cross-linked permanent hydro gels. Among other chemical cross-linking methods, IPN (polymerise a monomer within another solid polymer to form interpenetrating network structure and hydrophobic interactions [4](incorporating a polar hydrophilic group by hydrolysis or oxidation followed by covalent cross-linking) are also used to obtain chemically cross-linked permanent hydro gels. The following section reviews the major chemical methods (i.e. cross-linker, grafting, and radiation in solid and/or aqueous state) used to produce hydro gels from a range of natural polymers.

2.7. Chemical cross-linkers--- Cross-linkers such as glutaraldehyde, epichlorohydrin, etc have been widely used to obtain the cross-linked hydrogel network of various synthetic and natural polymers. The technique mainly involves the introduction of new molecules between the polymeric chains to produce cross-linked chains. One such example is hydrogel prepared by cross-linking of corn starch and polyvinyl alcohol using glutaraldehyde as a cross-linker. The prepared hydrogel membrane could be used as artificial skin and at the same time various nutrients/healing factors and medicaments can be delivered to the site of action.CMC chains can also be cross-linked by incorporating 1, 3-diaminopropane to produce CMC-hydrogel suitable for drug delivery through the pores. Hydrogels composite based on xanthan and polyvinyl alcohol cross-linked with epichlorohydrin in another example. k-carrageenan and acrylic acid can be cross-linked using 2-acrylamido-2-methylpropanesulfonic acid leading to the development of biodegradable hydro gels with proposed use for novel drug delivery systems [12]. Carrageenan hydro gels are also promising for industrial immobilisation of enzymes [13]. Hydrogels can also be synthesized from cellulose in NaOH/urea aqueous solutions by using epichlorohydrin as cross-linker and by heating and freezing methods [14, 15].
3. EVALUATION OF GEL:

3.1. **Physical properties:** In this parameter evaluate to morphology of gel such as colour, odour, taste and physical appearance through direct visualization.

3.2. **Rheology test:** In this parameter determine the rheology properties of gel such as viscosity and consistency of gel. **Viscosity** determinations of the prepared in situ gels as well as sols were carried out on a cone and plate geometry viscometer (Brookfield, Massachusetts, USA), using spindle No 40. Viscosity of in situ gelling solutions was measured at different angular velocities at a temperature of 37°. A typical run comprised changing of the angular velocity from 0.0 to 100 rpm. The averages of two readings were used to calculate the viscosity.

3.3. **Determination of gel strength:** The experiment was done by placing the gels in standard beaker below the probe. In this an analytical probe is then immersed into the sample. The Texture Analyzer was set to the ‘gelling strength test’ mode or compression mode with a test-speed of 1.0 mm/s. An acquisition rate of 50 points per seconds and a trigger force of 5 g were selected. An aluminium probe of 7.6 cm diameter was used for all the samples. The study was carried out at room temperature. The force required to penetrate the gel was measured as gel strength in terms of g.

3.4. **Determination of pH:** pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter.

3.5. **Spreadability Test:** For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1kg weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability.

\[ S = \frac{M \times L}{T} \]

- M: Weight tied to the upper slide
- L: Length moved on the glass.
- T: Time Taken

3.6. **Compatibility test and Content uniformity:** Drug content of gel was determined by dissolving accurately weighed 1gm of gels in Methanol. After suitable dilution absorbance was recorded by using UV-visible spectrophotometer at 222 nm. Drug content was determined using slope of standard curve.

3.7. **Skin Irritation test:** Skin irritation test can be determined by ether small portion of gel applied direct to skin or invitro test on mice model method.

3.8. **Test for stability:** The stability study for the topical herbal gel formulation was done as per ICH guidelines in a stability chamber for a period of 6 months. The selected topical herbal gel formulation was loaded in a humidity chamber (Floor standing model. 3 units in one with individual humidity and temperature controller at 25°C ± 2°C/60% RH ± 5% RH, 32°C ± 2°C/60% RH ± 5% RH and 40°C ± 2°C/75% RH ± 5% RH. Samples were withdrawn at an initial, first, second, third and sixth months and evaluated for change in color, odor, homogeneity, pH, viscosity.

3.9. **Antibacterial activity test:** variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods.

**a) Agar diffusion well-variant.** The bacterial inoculums was uniformly spread using sterile cotton swab on a sterile Petri dish agar. Nine serial dilutions yielded concentrations of 100, 80, 60, 20, 10, 5, 2.5, and 1.25 mg/mL for extracts and fractions and four serial dilutions yielded concentrations of 20, 15, 10 e 5 mg/mL for pure substances, 50 µL of natural products were added to each of the 5 wells (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24 h at 36°C ± 1°C, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm.

**b) Agar diffusion disc-variant.** Natural products were dissolved and diluted with solvents as mentioned previously. Same number of subsequent dilutions was performed as described above. However, natural products serial dilutions were performed out of initial concentrations 2.5 greater than the ones performed for well-variant method (i.e. 250 mg/mL for extracts and fractions and 50 mg/mL for pure substances); 7 mm filter paper discs (Whatman, no. 3) were impregnated with 20 mL of each of the different dilutions. The discs were allowed to remain at room temperature until complete diluents evaporation and kept under refrigeration until ready to be used. Discs loaded with natural products were placed onto the surface of the agar. Commercial chloramphenicol discs (30 mg) and paper discs impregnated with 20 mL of diluents used to dilute natural products were used as control. Tests were performed in duplicate.

**c) Bioautographic method direct-variant (chromatogram layer).** Direct variant of the bio autographic method carried out in this work is outlined as follows: (1) preparation and application of natural products on thin layer chromatography plates (TLC) (silica gel G60 F254, Merck); (2) preparation and application of the bacterial inoculums to TLC plates; (3) incubation; and (4) growth detection by colorimetric assay (INT) and measurement of growth inhibition diameters.

**d) Bioautographic method indirect-variant (agar diffusion).** In this procedure, first step corresponded to bio autographic variant-direct step 1. In step 2, TLC plates were covered with Mueller-Hinton agar layer (9 mL of the medium on 81 cm2 Petri plate area). However, contact between bacterial suspension and natural products were performed by two distinct procedures: mixing with agar (100 mL test-bacterial suspensions were mixed with 9 mL of agar and carefully poured on TLC plate) and swabbing with a cotton swab (inoculum was spread on the agar surface as described previously). Only the extracts evaluated in this procedure were tested with two types of bacterial inoculums and all tests were carried out in duplicate. In order to compare between the two variants of bioautigraphic method, results obtained with the use of “pour plate” technique (bacterial suspension mixed with agar) were validate.
e) Minimum inhibitory concentration (MIC) determination. The antibacterial activity of natural products was studied by employing a microdilution method, using two different culture media: Mueller-Hinton broth and Luria Bertania (LB). The inoculums were prepared as described previously. Natural products were dissolved in DMSO (10% of the final volume) and diluted with culture broth to a concentration of 2 mg/mL. Further 1:2 serial dilutions were performed by addition of culture broth to reach concentrations ranging from 2 to 0.0156 mg/mL; 100 µL of each dilution were distributed in 96-well plates, as well as a sterility control and a growth control (containing culture broth plus DMSO, without antimicrobial substance). Each test and growth control well was inoculated with 5 µL of a bacterial suspension (108 CFU/mL or 105 CFU/well) the trays were again incubated at 36ºC for 30 min, and in those wells, where bacterial growth occurred, INT changed from yellow to purple. MIC values were defined as the lowest concentration of each natural product, which completely inhibited microbial growth.

f) Statistical evaluation: Statistical differences between the two variants of diffusion method and two variants of bio autographic method were detected by analysis of variance (ANOVA) followed by Duncan test when required. The Student’s T-test was used to compare results between the two assays: the direct variant bio autographic method (performed with 24 and 48 h S. aureus grown cultures) and indirect variant of bio autographic method (performed with both type of inoculate). P values lower than 0.05 (p < 0.05) were considered significant.

4. CONCLUSION:
Gels are a convenient method of topical administration, it is inflammable, easy to apply self medication dosage form. Gels are used to achieve optimal cutaneous and percutaneous drug delivery. They can avoid gastrointestinal drug absorption difficulties caused by gastrointestinal pH. They avoid systemic and portal circulation following gastrointestinal absorption. There are various herbal gels present in market which shows antimicrobial properties, such as, Aloe vera, Neem, etc. This Review paper will be helpful for future formulation work as will provide various methods of formulating gels and also their evaluation parameters at the same place.

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