Design and Characterization of Biopolymer-Stabilized Nanopickering Emulsions Using Starch Nanoparticles

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ABSTRACT:

Pickering emulsions stabilized by solid particles have gained increasing interest as eco-friendly alternatives to conventional surfactant-based emulsions. Among various biopolymer-based stabilizers, starch nanoparticles (SNPs) offer distinct advantages owing to their natural origin, biocompatibility, biodegradability, and tunable amphiphilicity.Starch nanoparticles were prepared via nanoprecipitation and characterized for particle size, morphology, and surface charge. Oil-in-water nano-Pickering emulsions were formulated using SNPs at varying concentrations (0.5–2.0% w/v) and homogenized under high shear followed by ultrasonication. The emulsions were evaluated for droplet size distribution, polydispersity index (PDI), cloud point, and long-term stability under environmental stresses (pH, ionic strength, temperature). Morphological analysis was performed using optical and electron microscopy to confirm SNP adsorption at the oil-water interface.Theoretical standpoint, biopolymer-stabilized nano-Pickering emulsions bridge green chemistry with functional material design. The use of starch nanoparticles ensures biodegradability and sustainability, while the nano-scale dimensions enhance interfacial coverage and encapsulation efficiency of hydrophobic actives.This study aimed to design and characterize nano-Pickering emulsions stabilized with starch nanoparticles as a biopolymer-based stabilizer for potential food, pharmaceutical, and biomedical applications.

KEYWORDS:

Starch nanoparticles, Pickering emulsion, Biopolymer stabilizer, Nanocarrier, Encapsulation.

INTRODUCTION:

An emulsion is a heterogeneous system consisting of two immiscible liquids where one liquid (the dispersed phase) is dispersed in the form of small droplets throughout another liquid (the continuous phase). Because many natural and processed foods, including milk, salad dressing, ice cream, soft drinks, and cakes, are partially or entirely composed of emulsion. which enable them to give food systems crucial functions where stability, texture, taste, smell, appearance, and biological response can be efficiently adjusted to satisfy the desired standards for any given product. Emulsion is currently a common way to distribute functional substances like colour, flavour, preservatives, vitamins, minerals, and nutraceuticals due to its widespread use in food systems[(1)]. Nano Pickering emulsions' nanoscale droplet size, which is typically between 20 and 500 nm, offers several benefits for biological and pharmaceutical applications, particularly in the area of drug delivery systems based on nanocarriers[(2)]. More surface area, higher drug solubility, improved cellular uptake, extended circulation, and targeted distribution are all made possible by the smaller droplet size, especially for weakly water-soluble medications like carbamazepine, a model BCS Class II chemical [(3)]. For sensitive routes of administration such ophthalmic, parenteral, pulmonary, or mucosal drug delivery, this makes Nano Pickering emulsions especially appealing. In order to enable stimuli-responsive drug release, the solid particles can additionally be functionalised to react to environmental triggers (such as pH, temperature, and enzymes). A Pickering nanoemulsion's stability, bioavailability, and rate of dissolution are all improved when CBZ is added to the oil phase[(4)] . Carbamazepine (CBZ) is a widely prescribed anticonvulsant and mood - stabilizing agent used primarily for the treatment of epilepsy**,** trigeminal neuralgia**,** and bipolar affective disorder [(5)]. It acts by inhibiting voltage - gated sodium channels**,** thereby stabilizing hyperexcited nerve membranes and reducing synaptic transmission. Despite its therapeutic efficacy, CBZ presents significant biopharmaceutical challenges[(6)].

MATERIALS AND METHODS:

MATERIALS:

Starch nanoparticle was provided by Loba Chemie, Pvt, Ltd. Mumbai, and oil phase (olive oil) was contributed by R.S.Qube & Co, Chennai. The Drug Active Pharmaceutical Integredients the drug carbamazepine used as the antiepileptic efficacy was purchased by Yarrow Chem Products Mumbai. All other reagents and

Chemicals are used are of analytical grade and commercially available. The purified water obtained and prepared at Pharmaceutical lab was used throughout the experiment unless it is specified.

METHODS:

METHODOLOGY:

Preparation of starch Nanoparticles:

To prepare the 5g of starch nanoparticle using maize starch.

Materials needed:

- ✓ Starch -5g
- ✓ Distilled water
- ✓ Acetone (95-100%)-Antisolvent
- ✓ Centrifuge
- ✓ Hot air oven

Step:1 Starch gelatinization:

Weigh the 5 g of starch maize and dissolve in 100ml of water and heat the solution to 70-80ºc with constant stirring until the starch is fully gelatinized (clear, viscous solution).

Step:2 Nanoprecipitation:

Allow the starch solution to cool at room temperature and slowly add acetone dropwise (95-100%) into the starch solution under constant stirring .The starch nanoparticle will start precipitating as Acetone diffuses in water.

Step:3 pH Adjustment:

Adjust the pH to around to been acidic using Hcl because of smaller particles these step can be help for stability depending of starch source.

Step:4 Ultrasonicator:

Sonicate the solution for 10-15 minutes to reduce the particle size and prevent aggregation.

Step: 5 Centrifuge:

Centrifuge the suspension at 5000 rpm for 30 minutes and collect the nanoparticle pellets and discard the supernant further wash the pellets with distilled water atleast 2-3 times to remove the impurities and excess Acetone.

Step: 6 Drying:

Drying the Starch nanoparticle by using Hot air oven preheated with 40-45º to avoid starch degradation. Samples were dried at temperature for 12 hours until it gets dried the pellets are powder and stored in air tight container (Figure no.1:Starch Nanoparticles)[7].



Figure no.1:Starch Nanoparticles

Preparation of Aqueous phase:

To prepare the Aqueous phase using 0.5 g of Starch nanoparticle Powder make upto 35 ml of aqueous phase for 50 ml batch.

Materials needed:

- ✓ Starch pellets powder
- ✓ Distilled water
- ✓ Magenetic stirrer
- ✓ Probe sonicator
- ✓ Tween 80 (surfactant)

Step:1 starch nanoparticle powder:

Take a starch pellets powder 0.5 g and dissolve slowly in the distilled water completely it gets dissolved by using continuous stirring or magnetic stirrier.

Step :2 Dispersion:

The stirried Aqueous phase is kept in Sonicator for 30 minutes to break the Agglomerates and also reduces the particle size. After it use probe Sonicator for 30 to 60s for better dispersion.

Step:3 pH:

P^H is Neutral between (4-7) To Adjust the p^H by using 0.1N Hcl (or) 0.1 N NaoH p^H is important for particle size and O/W Interface (Figure no.2: Aqueous phase)[8].

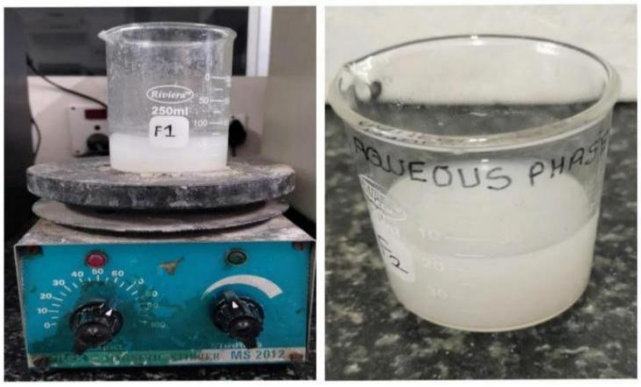

Figure no.2: Aqueous phase

Preparation of oil phase:

To prepare the oil phase using carbamazepine drug(API) of 0.5g make upto 15ml of aqueous phase for 50 ml batch.

Materials needed:

- ✓ Olive oil
- ✓ Drug (API) Carbamazepine
- ✓ Water bath
- ✓ Cosurfactant span 80

Step:1 oil phase:

Measure the oil accurately with 15ml and gently heat it place the oil in the cleanbeaker and add a span 80 for antioxidant property

Step:2 Drug mixture:

The drug carbamazepine is weigh accurately and slowly dissolve in the oil phase with continuous stirring or water bath to 35-45℃.Stir continuously with the magnetic stirrer until the drug is fully dissolved and the oil phase is clear and viscous.

Step:3 Homogeneity:

Continue the stirring for 5-10 minutes after dissolution ensure the uniform distribution of drug in oil phase.

Step:4 Cooling:

Cool the oil phase in the room temperature to the target emulsification temperature of aqueous phase typically 20-30℃. Ensure the temperuture difference between the two phases is minimal (±2-5℃) (Figure no.3: Oil Phase) [9].



Figure no.3: Oil Phase

Preparation of nanopickering emulsion:

Step:1 Preemulsification process:

Place the aqueous phase in the magnetic stirrer and slowly add the oil phase in with continuous stirring and forms the course emulsion with milky white colour indicates the nanopickering emulsion formation.

Step:2 Homogenization:

Place the nanopickering emulsion in the sonicator for 15 minutes to reduces the particle size and uniform mixing and then with probe sonicator for 30 minutes the sample in cool state and avoid foaming.

Step:3 Storage:

Stir gently for 1-2 minutes and tranfer the emulsion to amber or vials. Label the emulsion and store at under 4 ℃ with room temperature (Figure no.4: **N**anopickering Emulsion)[10].



Figure no.4: Nanopickering Emulsion

PHYSICOCHEMICAL CHARACTERISTIC OF NANOPICKERING EMUSLION:

pH :

- ✧ These emulsions typically have a neutral to slightly acidic pH range, approximately 5.5 to 7.0, when freshly prepared.
- ✧ Native starch nanoparticles possess hydroxyl groups on their surface.
- ✧ Surface modifications such as oxidation, acetylation, or octenyl succinate treatments can cause slight shifts in the (Figure no.5: pH of the Emulsion)[11].

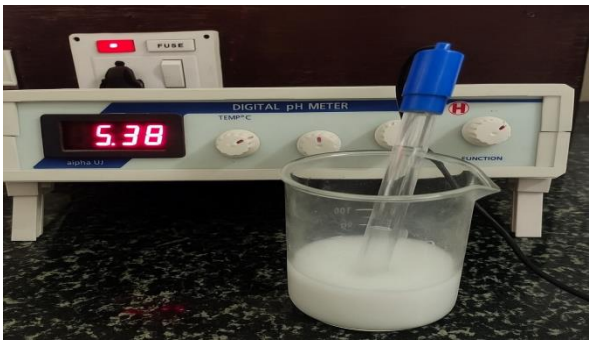

Figure no.5: pH of the Emulsion

VISCOSITY:

- ✧ Low oil concentration (5–10%) → 50–150 cP
- ✧ Moderate oil concentration (15–25%) → 150–500 cP

- ✧ High oil load (>30%) → 500–1500 cP (depending on starch NP size, surfactant use, and homogenization energy)
- ✧ Freshly prepared nanopickering emulsion (aqueous + oil phase stabilized by starch nanoparticles)[12].
- ✧ Temperature: Maintain at 25 ± 1 °C.
- ✧ Spindle: Select spindle according to sample viscosity (commonly spindle no. 62 or 64).
- ✧ Speed: Record readings at 10, 20, 50, and 100 rpm.
- ✧ Fill ~50 mL of emulsion into the sample beaker → lower spindle → allow equilibration → record viscosity in centipoise (cP or mPa·s)(Figure no.6:Viscocity) [13].

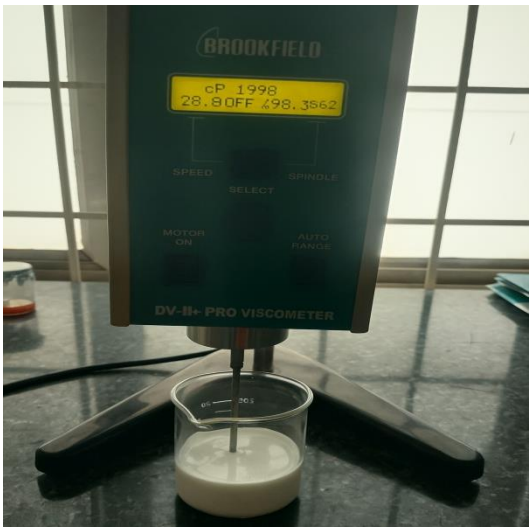

Figure no.6:Viscocity

PARTICLE SIZE:

Starch nanoparticles (stabilizer, before emulsification):

- ✧ Usually 50 - 300 nm, depending on preparation method (acid hydrolysis, nanoprecipitation, ultrasonication)[14].

Emulsion droplets (after emulsification):

- ✧ For nano - Pickering emulsion: 100 – 500 nm (well-sonicated, optimized stabilizer-to-oil ratio).
- ✧ For a micro -Pickering emulsion: up to 1-5 μm if stabilization is weaker or mixing is mild[15].

POLYDISPERSITY INDEX:

The Polydispersity Index (PDI) is a key indicator of the size distribution of droplets or particles in a nano-Pickering emulsion.

- ✧ Range: 0.0 → 1.0 (sometimes reported up to ~1.0+ for very polydisperse systems).
- ✧ PDI ≤ 0.1 → highly monodisperse (uniform particle size).
- ✧ 0.1 – 0.3 → moderately narrow size distribution (acceptable for nanoemulsions).
- ✧ 0.3 – 0.5 → broad distribution, less stable.
- ✧ >0.5 → very polydisperse, usually unstable (Table no.1: Polydispersity index)[16].

S.NO	Well Prepared System	LessOptimizedor Course Emulsion	Starch Nanoparticle Before Emulsification
1.	0.15 - 0.25	0.3 - 0.5	0.1- 0.2

Table no.1: Polydispersity index

EMULSIFICATION PROCESS:

Preparation of Aqueous Phase:

- ✧ Disperse starch nanoparticles (SNPs) in distilled water.
- ✧ Use magnetic stirrer and sonicator for uniform dispersion[14].

Preparation of Oil Phase:

- ✧ Select oil (e.g., olive oil) and dissolve API (if drug-loaded).
- ✧ Add co-surfactant (e.g., Span 80) if required.
- ✧ Maintain controlled temperature (35–45 °C)[17].

Primary Emulsification:

- ✧ Add oil phase slowly into aqueous phase under continuous stirring.
- ✧ Maintain oil-to-water ratio as per formulation design[18].

High-Energy Homogenization:

- ✧ Apply probe sonication or high-speed homogenizer.
- ✧ Time: 5–10 min (depending on desired droplet size).
- ✧ Result: formation of nano Pickering emulsion[19].

Characterization of Emulsion:

- ✧ Particle size & PDI (Dynamic Light Scattering).
- ✧ Viscosity (Rotational viscometer).
- ✧ pH measurement.
- ✧ Microscopy (CLSM/SEM) for interfacial adsorption[16].

Stability Studies:

- ✧ Storage at various temperatures.
- ✧ Monitor for creaming, coalescence, or phase separation.
- ✧ Long-term and accelerated stability testing (Figure no.7: Emulsification Process)[20].



Figure no.7: Emulsification Process

CLOUD POINT MEASUREMENT:

Sample preparation:

Take 5–10 mL of the nano-Pickering emulsion in a clean, transparent test tube.If necessary, dilute the emulsion 1:1 with deionized water to avoid saturation and facilitate cloud point observation.

Initial homogenization:

Mix gently using a magnetic stirrer or manual inversion to ensure uniform dispersion. Avoid vigorous shaking, which may cause foaming.

Heating protocol:

Place the test tube in a water bath set initially at room temperature (~25 °C).Gradually heat the sample at a controlled rate of 1 °C/min. Maintain uniform temperature distribution using a circulating water bath.Continuously observe the emulsion against a white background under ambient light.

Cloud point determination:

The cloud point is defined as the temperature at which the previously clear or slightly opalescent emulsion turns turbid or shows visible phase separation (onset of cloudiness).Record the temperature immediately when turbidity appears (Figure no.8: Cloud point measurement)[21].

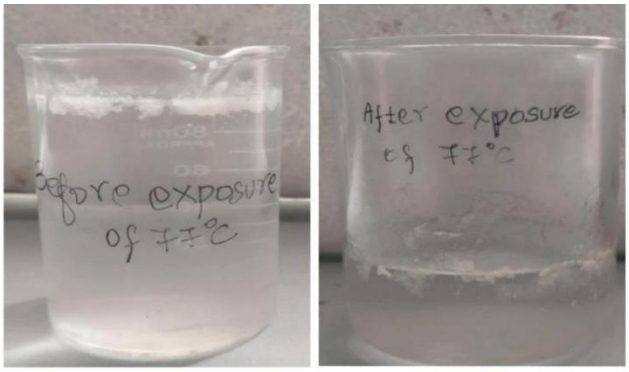

Figure no.8: Cloud point measurement

LITERATURE REVIEW:

1. **(Goodarzi F et al., 2019)**[22] A mixture with two or more liquid phases is called an emulsion. Emulsions are used in a variety of chemical, energy, and environmental industries, including the food, pharmaceutical, firefighting, and chemical synthesis sectors. When water and oil are combined during the oil

production process, together with asphaltene, a naturally occurring surfactant, water-in-oil emulsions are created on their own. Oil emulsions must be treated in order to recover both the water and oil phases for practical and financial reasons. A deeper comprehension of the variables influencing emulsion formation and stability is necessary to create more effective emulsion therapies. One significant factor affecting the stability and rheological properties of the emulsions is the change in droplet size.

2. **(Lu H et al., 2021),**[23] Because of its biodegradability, nontoxicity, tiny size, and huge specific surface area, nanostarch has garnered a lot of interest as a food-grade Pickering emulsion stabilizer. The creation, alteration, and use of Pickering emulsions containing nanostarch are explained in this review. Acid hydrolysis, acid hydrolysis in combination with other treatments, nanoprecipitation, ultrasonication, ball milling, and cross-linking are now the primary techniques for creating nanostarch. Hydrophobic modification greatly enhances nanostarch's emulsifying ability, making it a promising Pickering emulsion stabilizer. The stability of the emulsion is influenced by the hydrophobicity, charge, size, and content of the nanostarch.

3. **(Zhu F et al., 2019),**[24] Solid particles stabilize Pickering emulsions. Starch-based particles have drawn more at tention from researchers among the many emulsifiers. Starch is inexpensive, plentiful, non-allergic, and GRAS. Pickering emulsions based on starch are thought to produce more "natural" results. In Pickering emulsions, starch granules undergo modification to become effective emulsifiers. Octenyl succinic anhydride (OSA) modification, hydrolysis, grinding, non-solvent precipitation, ultrasound, and high pressure treatments are some of the starch modifications. The droplet size of the resulting Pickering emulsions ranges from around 1 to 100 μm, depending on the ionic strength, pH, starch type, and emulsion composition. Pickering emulsion polymerization, the encapsulation of bioactive ingredients for functional food systems, and the creation of emulsion-type products like mayonnaise are some of the various uses for Pickering emulsions.

4. **(Ling Tan S et al., 2015),**[25] To increase its absorption, carbamzepine (CBZ) was encapsulated in a parenteral oil-in-water nanoemulsion. Dynamic light scattering was used to quantify the zeta potential, polydispersity index, and particle size. Additional variables were also noted, including viscosity, pH, osmolality, drug loading efficiency, and entrapment efficiency. Emulsion droplets were found to be in the nano-range and nearly spherical in shape using transmission electron microscopy. Higuchi's equation provided the greatest description of the in vitro release profile.

5. **(Kumbhar SA et al., 2013),**[26] Carbamazepine is an anti-epileptic drug used from a long time in treatment of epilepsy but it has poor bioavailability when used as a conventional dosage form. The SMEDS of carbamazepine was prepared to enhance its bioavailability and its release rate was evaluated by its in-vitro release. The solubility of carbamazepine was determined in various oils, surfactants and co-surfactants. Pseudoternary phase diagrams were used to determine the microemulsion area of formulation. SMEDS of carbamazepine was evaluated for globule size, zeta potential, clarity, effect of centrifugation, assay, dilutability, refractive index, transmittance, and stability.

RESULTS AND DISCUSSION:

Using maize starch, starch nanoparticles (SNPs) were successfully created by nanoprecipitation and their size, shape, and dispersion stability were assessed. With an average diameter of 80-200 nm, the particles seemed well-dispersed, spherical, and smooth, indicating nanoscale creation appropriate for emulsion stabilization. A uniform milky white dispersion with droplet sizes ranging from 150 to 400 nm and a polydispersity index (PDI) of 0.15–0.25, suggesting a narrow size distribution, was created by the optimized nano-Pickering emulsions with olive oil as the dispersed phase and SNPs (0.5–2% w/v) as stabilizers. The emulsions' viscosity ranged from 120 to 480 cP and their pH ranged from 5.8 to 6.8, both of which are within the permissible range for cutaneous and oral formulations. After 30 days of storage, cloud point experiments showed stability up to 65 °C without phase separation.Whereas there was no discernible creaming or coalescence after 30 days of storage at 25 °C. These findings demonstrate that starch nanoparticles function as effective, biodegradable stabilizers that can create

stable oil-in-water nano-Pickering emulsions with desired physicochemical properties appropriate for use in food, medicine, and cosmetics.

PARAMETER	OBSERVED RANGE
Particle size (SNPs)	80–200 nm
Droplet size (Emulsion)	150–400 nm
PDI	0.15–0.25
pH	5.8–6.8
Viscosity	120–480 cP
Cloud point	65 °C
Stability	Stable for >30 days

DISCUSSION:

The viability of starch nanoparticles as natural stabilizers was confirmed by the biopolymer-based Pickering emulsions' outstanding physicochemical characteristics and stability. Their amphiphilic character and nanoscale size allowed for efficient interfacial covering, avoiding droplet coalescence and guaranteeing stability over time. These findings support the use of SNPs in the creation of long-lasting nano-carriers for hydrophobic medications like carbamazepine, with possible extensions to biomedical, cosmetic, and nutraceutical uses.

APPLICATION:

1. Food Industry Applications:

Starch nanoparticle (SNP)-stabilized Pickering emulsions have received extensive attention in food systems because starch is GRAS (Generally Recognized as Safe), biodegradable, renewable, and edible. SNPs, due to their amphiphilic surface

properties, adsorb irreversibly at the oil–water interface, forming a physical barrier that prevents coalescence and creaming[27].

- ✧ **Encapsulation of bioactives**: Hydrophobic nutraceuticals (e.g., curcumin, β-carotene, vitamin E, polyphenols) can be solubilized in oil droplets and protected against oxidation, degradation, and photolysis.
- ✧ **Controlled release in gastrointestinal tract**: SNPs enhance bioaccessibility by protecting lipophilic compounds until they reach the intestine, where enzymatic degradation of starch allows targeted release[28].
- ✧ **Low-fat and functional foods**: SNP emulsions are used to design low-fat mayonnaise, salad dressings, and beverages, improving mouthfeel and stability without synthetic emulsifiers.

- ✧ **Improved oxidative stability**: SNPs provide a steric barrier that delays lipid peroxidation compared to surfactant-based emulsions (Figure no.9: Food industry application)[29].

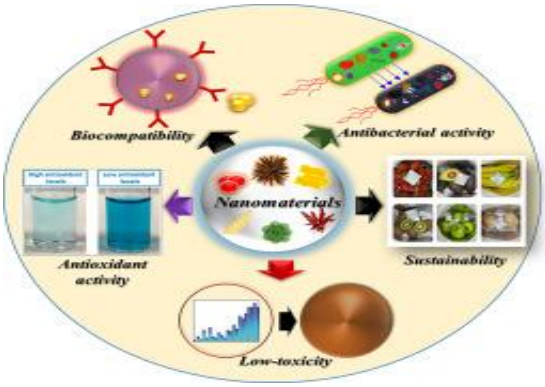

Figure no.9: Food industry application

2. Pharmaceutical and Drug Delivery Applications:

SNP-based Pickering emulsions are promising drug carriers due to their biocompatibility, biodegradability, and non-toxicity[30].

- ✧ **Poorly soluble drugs**: SNP emulsions enhance solubility and dissolution rate of hydrophobic APIs (e.g., anticancer agents, antifungals, antibiotics).
- ✧ **Controlled and sustained release**: SNPs adsorb at the droplet interface, retarding drug diffusion and allowing prolonged therapeutic effect.
- ✧ **Oral and parenteral delivery**: SNPs protect sensitive drugs from gastric degradation and can also be used in injectable emulsions due to starch's safety profile.

- ✧ **Nanocarriers for vaccines/biologics**: SNP emulsions have potential for delivering proteins, peptides, and antigens by providing protection and controlled immune activation (Figure no.10: Pharmaceutical and Drug Delivery Applications)[31].

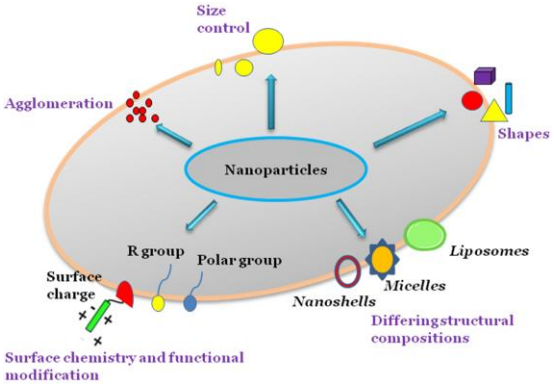

Figure no.10: Pharmaceutical and Drug Delivery Applications

3. Cosmetic and Personal Care Industry:

In cosmetic formulations, stability, skin compatibility, and natural ingredients are crucial. SNP emulsions replace conventional surfactants, which often cause irritation.

- ✧ **Creams and lotions**: SNP emulsions stabilize oil phases containing fragrances, vitamins, or UV-filters.
- ✧ **Sunscreen formulations**: Encapsulation of UV-filters in SNP emulsions provides sustained release and reduced skin penetration, lowering irritation risk.

Eco-friendly formulations: SNPs are biodegradable and align with consumer demand for "green cosmetics (Figure.11: Cosmetic and Personal Care Industry) [32].

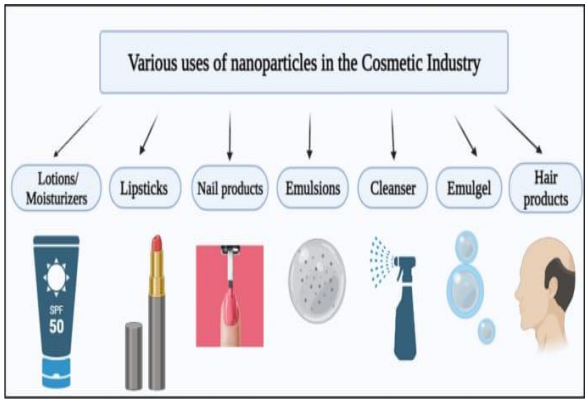

Figure.11: Cosmetic and Personal Care Industry

4. Agricultural and Environmental Applications:

Starch nanoparticle Pickering emulsions also play a role in sustainable agriculture and environmental remediation.

- ✧ **Agrochemical formulations**: Encapsulation of hydrophobic pesticides and herbicides increases their dispersion in water, reduces solvent use, and provides controlled release in the field[33].
- ✧ **Antimicrobial essential oils**: SNP emulsions enhance solubility of volatile essential oils (e.g., thymol, eugenol) used as bio-pesticides, extending their stability and effectiveness.

Oil spill remediation: SNP emulsions can be engineered to disperse hydrocarbons into nano-sized droplets, enhancing biodegradation and cleanup (Figure no.12: Agricultural and Environmental Applications)[34].

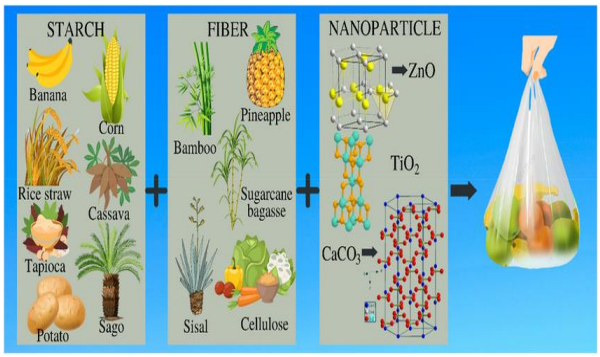

Figure no.12: Agricultural and Environmental Applications

5. **Biomedical Applications:**

Emerging research highlights SNP emulsions in therapeutics, tissue engineering, and bioimaging.

- ✧ **Antimicrobial systems**: SNP emulsions encapsulating antimicrobial oils (e.g., tea tree oil, oregano oil) show enhanced antibacterial and antifungal efficacy.
- ✧ **Wound healing**: SNP-stabilized emulsions loaded with bioactives accelerate tissue regeneration and reduce infection.
- ✧ **Nanovaccine carriers**: By encapsulating antigens or adjuvants, SNP emulsions prolong immune exposure and improve antigen stability.
- ✧ **Injectable gels and scaffolds**: SNP emulsions are being developed for 3D biomaterial scaffolds in regenerative medicine (Figure no.13: Biomedical Applications)[35].

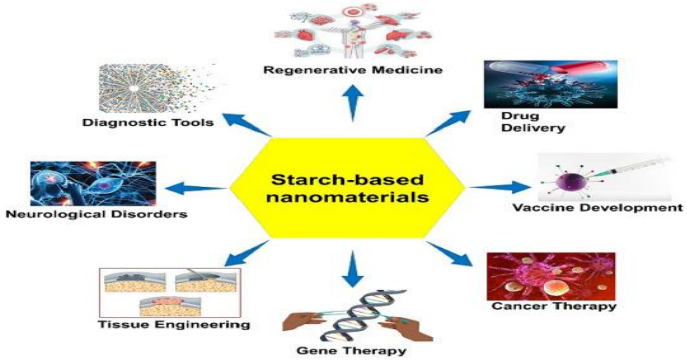

Figure no.13: Biomedical Applications

SUMMARY & CONCLUSION:

The present work highlights the potential of starch nanoparticles as sustainable and effective biopolymer stabilizers in the formulation of nano-Pickering emulsions. By exploiting the amphiphilic nature and interfacial activity of starch nanoparticles, emulsions with nanoscale droplet sizes, improved physical stability, and resistance to coalescence were successfully designed and characterized. The findings support the theoretical premise that irreversible nanoparticle adsorption at the oil–water interface reduces interfacial energy and provides a steric–electrostatic barrier, leading to enhanced emulsion stability compared with conventional surfactant systems.The results demonstrate that biopolymer-based Pickering emulsions not only address the demand for eco-friendly and biodegradable stabilizers but also provide versatile carriers for hydrophobic bioactives. Their favorable droplet characteristics,and cloud point stability suggest broad

applicability in food delivery systems, pharmaceutical drug carriers, cosmetic formulations, and biomedical platforms Over all, starch nanoparticle-stabilized nano-Pickering emulsions represent a promising step toward the development of next-generation, sustainable, and functional colloidal delivery systems, bridging the gap between green material science and applied product development..

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