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Formulation and Evaluation of Microbubbles by Using Ultrasonication for Treatment of Hypertension

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Abstract: ***Microbubbles (MBs) are microscopic gas-filled bubbles with diameters ranging from 1 to 10µm, commonly used in medical applications such as drug delivery and ultrasound-based imaging. Their unique properties, including a large surface area and responsiveness to ultrasound, make them promising candidates for targeted therapeutic treatments, particularly in diseases such as cancer, hypertension, and heart conditions. This study investigates the preparation, characterization, and potential applications of microbubbles for the targeted delivery of Nicardipine, an antihypertensive drug used in the treatment of hypertension. The microbubbles are composed of a stabilizing albumin shell, encapsulating Nicardipine within the core. A series of techniques, including emulsification, sonication, and heat treatment, are employed to form and stabilize the microbubbles, which are then characterized using optical microscopy and optical spectroscopy for size distribution and concentration. The stability and behavior of the microbubbles under ultrasound exposure are also assessed to evaluate their effectiveness in drug delivery and imaging. The study aims to provide insights into the optimization of microbubble formulations for enhancing therapeutic efficacy while minimizing side effects in hypertension treatment. Additionally, the potential for using microbubbles in combination with ultrasound as a non-invasive method for monitoring blood pressure and improving drug absorption in targeted tissues is explored. These findings could contribute to the development of advanced drug delivery systems, offering a new approach to managing hypertension and related cardiovascular diseases.*** [5]

I. INTRODUCTION

A. Microbubbles

Microbubbles (MBs) are tiny gas bubbles with diameters in the micrometer range of 1–10 µm.
Microbubbles consist of three segments:

1) Inner gas phase
2) Outer liquid phase
3) Shell which separates Inner gas phase and outer liquid phase.

Microbubbles are easy to use in medical applications, particularly for drug delivery and imaging. With a gas-filled core and a stabilizing shell composed of biocompatible materials, microbubbles can be produced to enhance therapeutic efficacy and minimize adverse effects.

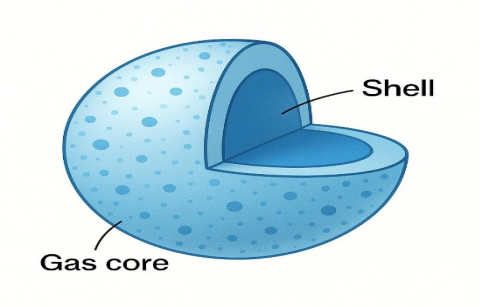


Fig no. 1 Microbubbles

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Their unique characteristics—such as their large surface area and ultrasound responsiveness. microbubbles are used in the treatment of cancer, diabetes, heart diseases.[17]

B. Hypertension

Hypertension, also known as high blood pressure, represents a patient elevation in arterial blood pressure and stands as a significant global health challenge. This condition affects a substantial portion of the adult population worldwide and is a major threat factor for a waterfall of cardiovascular conditions, including stroke, coronary roadway complaint, heart failure, and habitual order complaint. The impact of hypertension extends to increased morbidity and mortality, making its understanding and operation a critical area of medical exploration and clinical practice.[2]

In a Hypertension systolic blood pressure of 130 mm Hg or advanced or a diastolic blood pressure of 80 mm Hg or advanced, has led to a significant increase in the estimated frequence of hypertension within the population. This change emphasizes the significance of relating and managing individualities at earlier stages of blood pressure elevation to potentially ameliorate their cardiovascular health line. Despite the advancements in understanding and treating hypertension, a considerable number of grown-ups with the condition still have unbridled blood pressure. This highlights the complications involved in achieving optimal blood pressure control, which can be told by factors similar as patient adherence to treatment, the presence of other medical conditions, and the need for personalized remedial strategies.[1]

Normal blood pressure is defined as a systolic pressure below 120 mm Hg and a diastolic pressure below 80 mm Hg. Elevated blood pressure is characterized by a systolic pressure between120&29 mm Hg with a diastolic pressure below 80 mm Hg. Stage 1 hypertension is diagnosed when the systolic pressure ranges from 130 to 139 mm Hg or the diastolic pressure is between 80 &89 mm Hg. Stage 2 hypertension is defined by a systolic pressure of 140 mm Hg or advanced or a diastolic pressure of 90 mm Hg or advanced. A hypertensive extremity is indicated by blood pressure readings advanced than 180/120 mm Hg, taking immediate medical attention.[9]

II. SYMPTOMS

When blood pressure reaches very high levels, some individuals may experience the following symptoms:

- Severe headaches
- Blurred vision or other vision changes
- Chest pain
- Dizziness or lightheadedness
- Difficulty breathing or shortness of breath
- Nausea or vomiting
- Anxiety
- Confusion
- Buzzing in the ears
- Nosebleeds
- Abnormal heart rhythm (arrhythmia)
- Fatigue
- Heart palpitations

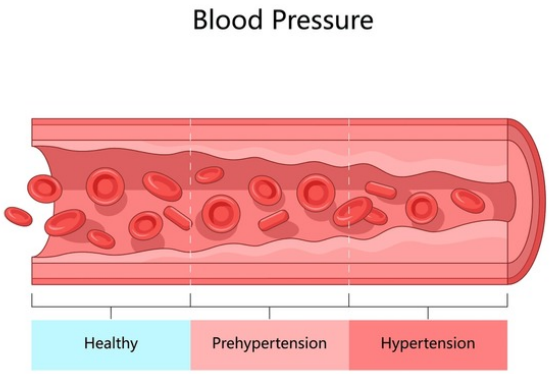


Fig no. 2 Hypertension stages

III. CAUSES

Hypertension, or high blood pressure, can arise from a complex interplay of various factors. It's often categorized into primary (essential) hypertension and secondary hypertension.

A. Primary Hypertension

This is the more common type, accounting for 90-95% of cases, and its exact cause isn't fully understood. It tends to develop gradually over many years due to a combination of:

1) Genetics: A family history of hypertension increases your risk. Multiple genes involved in blood pressure regulation, such as those in the renin-angiotensin-aldosterone system, have been implicated.

2) Age: The risk of hypertension increases with age as blood vessels naturally thicken and stiffen over time.
3) Lifestyle Factors:
   - Unhealthy Diet: High sodium intake, low potassium intake, and diets high in saturated and trans fats can contribute to hypertension.
   - Lack of Physical Activity
   - Obesity or Being Overweight
   - Excessive Alcohol Consumption
   - Stress

B. Secondary Hypertension

This type has an identifiable underlying cause and accounts for 2-10% of hypertension cases. Treating the underlying condition can often bring blood pressure back to normal. Some of the causes include:

1) Kidney Problems: Chronic kidney disease, polycystic kidney disease, and renal artery stenosis can affect fluid balance and hormone production, leading to hypertension.
2) Endocrine Disorders:
- Primary Hyperaldosteronism: Overproduction of aldosterone by the adrenal glands.
- Cushing's Syndrome: Excessive cortisol production.
- Pheochromocytoma: A rare tumor of the adrenal gland that produces excess hormones.
- Thyroid Disorders: Both hyperthyroidism and hypothyroidism can contribute.

3) Sleep Apnea: Repeated pauses in breathing during sleep can lead to increased blood pressure.
4) Congenital Blood Vessel Defects: For instance, coarctation of the aorta (narrowing of the main artery).
5) Medications: Certain drugs like oral contraceptives, decongestants, nonsteroidal anti-inflammatory drugs (NSAIDs), and some antidepressants can raise blood pressure.[15]

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V. MATERIALS

A. Drug

Nicardipine (Anti-hypertensive)

1) *Shells:* The shell can be made of proteins, lipids, surfactants, biocompatible polymers, or a combination of these substances. Some examples of shell materials include:

- Protein shell- microbubbles are used in ultrasound imaging and drug delivery because they can form the shell with a gas core, especially with air or perfluorocarbon gases. The most commonly used protein shell microbubble is human serum albumin.
- Phospholipid shell- microbubbles coated with lipids are used for biomedical imaging and drug delivery.
- A surfactant shell- as a coating can offer additional protection and prevent the microbubble from absorbing large molecules, for example, Polyethylene glycol (PEG).
- Polyelectrolyte multilayer (PEM)- shells represent a novel category of polymer-surfactant shell hybrids.[16]

2) *Gases:* The most commonly used is air because it can diffuse quickly in water-based environments,

- Air-filled microbubbles are often less stable, have a shorter half-life, and are less effective in vivo.
- Perfluorocarbon gases, such as perfluoro butane and perfluoro hexane, are frequently used because of their low blood solubility, which slows gas diffusion and improves microbubble stability.
- Sulfur Hexafluoride (SF6) is used due to its high molecular weight and poor solubility, which promote stability, SF6 has been used extensively in contrast-enhanced ultrasound imaging.
- Oxygen microbubbles filled with oxygen are being developed for potential applications in cancer treatment to address tumor hypoxia.
- Nitrogen has been incorporated into some formulations, but it tends to dissolve more quickly than perfluorocarbons, which reduces its stability compared to gases like sulfur hexafluoride and perfluoro butane.[19]

3) *Buffer:* Phosphate Buffer Saline (Help in maintaining pH)

VI. EQUIPMENTS

1) *Cyclomixer*

A vortex mixer, or Cyclomixer, is a simple device used commonly in laboratories to mix small vials of liquid. It consists of an electric motor with the drive shaft oriented vertically and attached to a cupped rubber piece mounted slightly off-center. As the motor runs the rubber piece oscillates rapidly in a circular motion. When a test tube or other appropriate container is pressed into the rubber cup (or touched to its edge) the motion is transmitted to the liquid inside and a vortex is created. Most vortex mixers are designed with 2 or 4-plate formats, have variable speed settings ranging from 100 to 3,200 rpm, and can be set to run continuously, or to run only when downward pressure is applied to the rubber piece.[11]



Fig no. 3 Cyclomixer

2) *Sonicator*

A bath sonicator, also known as an ultrasonic bath, is a type of sonic device that uses high-frequency sound waves to generate cavitation, which then cleans objects or disrupts cells. These devices typically involve a liquid-filled bath and a transducer that converts electrical energy into ultrasonic waves.

Working:The bath sonicator uses high-frequency sound waves, usually in the range of 20 kHz, to create tiny bubbles (cavitation) in a liquid. These bubbles rapidly expand and collapse, creating localized pressure and temperature changes. This cavitation action helps to dislodge and remove dirt, debris, or other contaminants from objects submerged in the bath.



Fig no. 4 Sonicator

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3) Centrifuge machine

A centrifuge is a device that uses centrifugal force to separate components of a mixture based on their density. It does this by rotating a container holding the mixture at high speed, causing denser components to move to the bottom and lighter components to move to the top.

Working:

The spinning motion creates a strong outward force, much stronger than gravity, which pushes the denser components to the outer edges of the rotating container.[22]



Fig no. 5 centrifuge

VII.METHODS

The common methods used for microbubble preparation:

A. Ultrasonication method

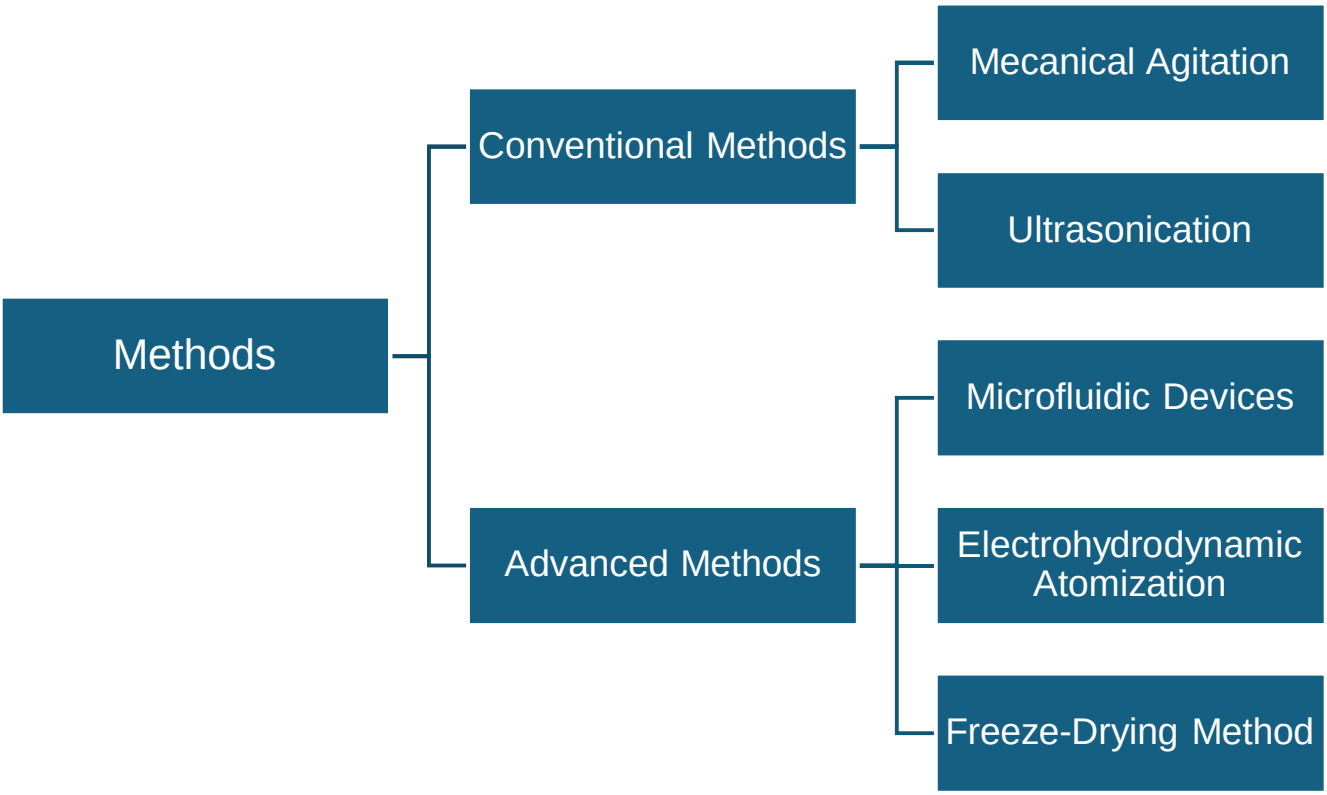


Chart no. 1 Methods of microbubbles preparation

Sonication is the primary technique for creating microbubbles, where high-intensity ultrasound is used to disperse gas or liquid in a coating material suspension. Two mechanisms are believed to be responsible for this process. Initially, the gas or liquid is mixed to create a suspension of small droplets/bubbles that automatically absorb a coating of protein or surfactant on their surfaces. Furthermore, the elevated temperatures and pressures produced by inertial cavitation in the suspension cause a chemical alteration of the surface layer, enhancing its stability. For protein coatings, the reason for changes is the cross-linking caused by superoxide generated during water sonolysis it has been observed that surfactant coatings undergo significant changes in structure.[26]

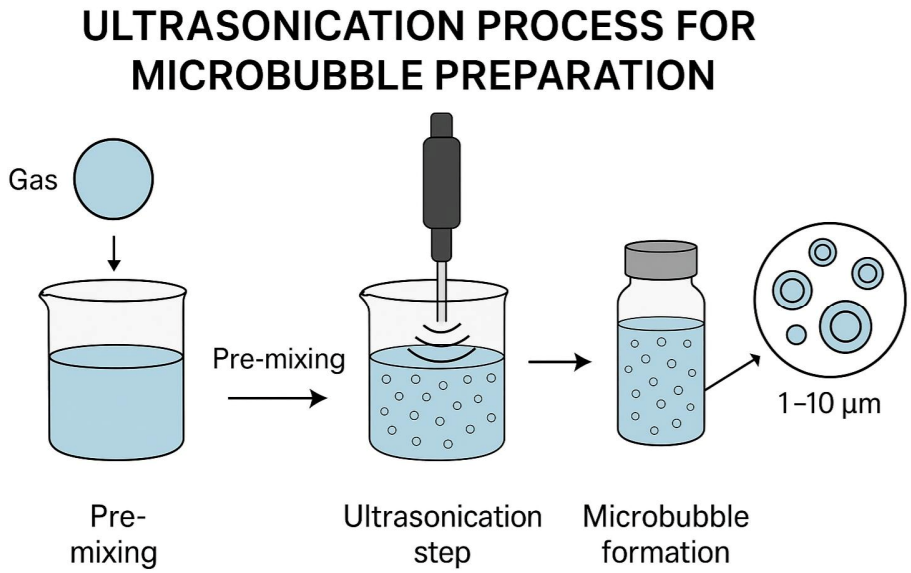


Fig no. 6 Ultrasonication process

B. Drug Profile

Nicardipine is used alone or together with other medicines to treat severe chest pain (angina) or high blood pressure (hypertension). High blood pressure adds to the workload of the heart and arteries. If it continues for a long time, the heart and arteries may not function properly. This can damage the blood vessels of the brain, heart, and kidneys, resulting in a stroke, heart failure, or kidney failure. High blood pressure may also increase the risk of heart attacks. These problems may be less likely to occur if blood pressure is controlled.

Nicardipine is a calcium channel blocker. It works by affecting the movement of calcium into the cells of the heart and blood vessels. As a result, nicardipine relaxes blood vessels and increases the supply of blood and oxygen to the heart while reducing its workload.This medicine is available only with your doctor's prescription.This product is available in the following dosage forms:

- Capsule, Liquid Filled
- Tablet, Extended Release

Side effects

- Allergies
- Kidney problems or
- Liver problems
- Aortic stenosis (narrowing of a valve in your heart) or
- Bowel blockage, severe or
- Congestive heart failure or
- Coronary artery disease or
- Heart attack or
- Hypotension (low blood pressure)
- Cardiogenic shock
- Galactose intolerance (rare hereditary problem) or
- Glucose-galactose malabsorption (rare hereditary problem) or
- lactase deficiency.[24]

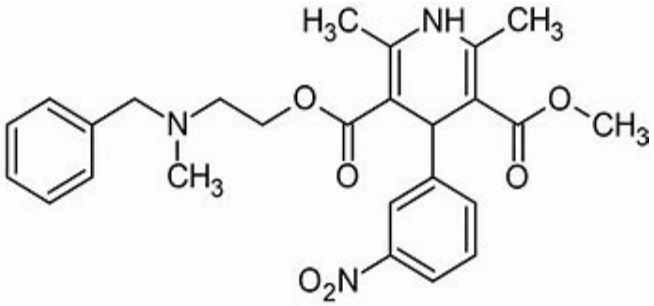


Fig no. 7 Nicardipine

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C. Physical Properties

- Appearance: Yellow powder
- Molecular Formula: $C_{26}H_{29}N_3O_6 \cdot HCL$
- Molar Mass: 515.99 g/mol
- Melting Point: Typically reported in the range of 179-182°C.
- Boiling Point: Approximately 603.4 °C.
- Density: Around 1.23 g/cm³.

D. Formulation

- Preparation of Albumin Solution: A 132 mg/mL human serum albumin (HSA) solution is prepared.
- Preparation of NicardipineSolution: A 0.30 mg/mL Nicardipinesolution is prepared.
- Mixing the Solutions: The albumin solution and the Nicardipinesolution are mixed together.
- Emulsification: This mixture is then subjected to an emulsification process. The image in Figure 1(A) depicts this using a high-speed homogenizer, suggesting mechanical agitation is used to create an emulsion.
- Sonication: Following emulsification, the emulsion is sonicated. The image shows a probe sonicator being immersed in the emulsion, indicating that ultrasound energy is applied to create the microbubbles.
- Heat Treatment/Stabilization: The microbubbles formed are then likely subjected to a heat treatment step. The text mentions that the vial containing the microbubbles is heated in a water bath at 90 °C for 10 minutes. This heat treatment is crucial for cross-linking the albumin and stabilizing the microbubble structure, entrapping the Nicardipine within the shell.
- Washing: After stabilization, the microbubbles are washed, likely through centrifugation and resuspension in a washing buffer (e.g., phosphate-buffered saline (PBS)), to remove any unbound Nicardipine.[3]

E. Evaluaion



Fig no. 8 Formulated Microbubbles

1) Microbubble Size and Distribution Analysis:

Optical Microscopy: Visual inspection of microbubbles under a microscope can reveal their size, shape, and count. Image analysis software can quantify these parameters.[12]

2) Microbubble Concentration Measurement:

Optical Spectroscopy: Measuring the optical density of a microbubble suspension can provide an accurate assessment of microbubble concentration.

3) Microbubble Stability and Performance:

Ultrasonography: In medical applications, microbubble testing involves injecting microbubbles into the bloodstream and then using ultrasound to visualize them. This allows for evaluation of their behavior and stability under ultrasound exposure.

Microbubble Detection Test:

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This technique uses ultrasound to detect microbubbles in the bloodstream, often used to confirm catheter placement or detect extravasation.

4) Other Evaluation Methods:

Core Permeability Testing: In oil and gas applications, microbubbles are used to improve fluid flow and recovery. Core permeability tests assess the effectiveness of microbubbles in restoring permeability after damage.

Microbubble Irrigation Systems: These systems use microbubbles to improve irrigation efficiency and root canal cleaning.[20]

VIII. RESULT AND DISCUSSIONS

Under a microscope the size of microbubbles was found in between 5- 10µm. Microbubbles were observed in spherical shape.

IX. CONCLUSION

In conclusion, microbubbles (MBs) represent a promising and innovative tool in the field of medical treatment, particularly for drug delivery and imaging. Their unique structure, consisting of a gas-filled core surrounded by a biocompatible stabilizing shell, enables efficient therapeutic targeting and enhances the effectiveness of ultrasound in both diagnostic and therapeutic applications. This study demonstrated that the combination of Nicardipine, a calcium channel blocker, with albumin-coated microbubbles offers a novel approach to treating hypertension. The preparation of these microbubbles through emulsification, sonication, and heat treatment successfully encapsulated Nicardipine within the microbubbles, preserving both the drug's activity and the stability of the microbubble formulation.

Characterization of the microbubbles revealed a consistent size distribution, suitable for circulation within the bloodstream, and their stability was maintained under ultrasound exposure, suggesting their potential for safe, non-invasive, and targeted drug delivery. Additionally, the ability of microbubbles to improve drug absorption and provide real-time imaging could revolutionize the treatment of hypertension and other cardiovascular diseases, offering greater precision in monitoring and controlling blood pressure.

This study underscores the importance of optimizing microbubble formulations for enhanced therapeutic outcomes and provides a foundation for future research exploring the broader applications of microbubbles in medical fields. By addressing critical issues such as drug stability, microbubble size, and ultrasound responsiveness, further development of microbubble-based drug delivery systems holds the potential to significantly improve the management of hypertension, reduce side effects, and enhance patient outcomes in cardiovascular therapy.

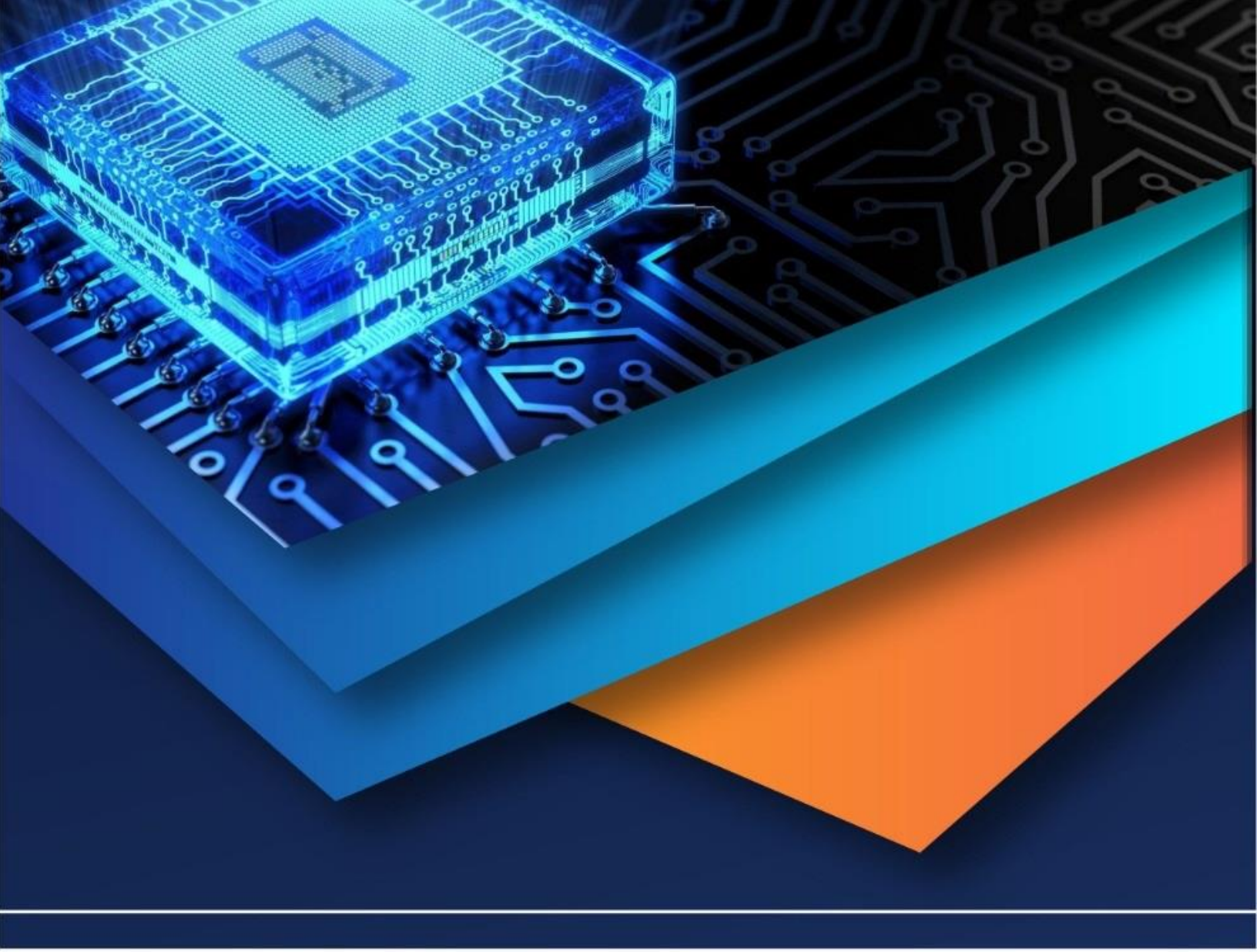
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