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Artistic routes in green chemistry for the preparation of Iron Nanoparticles useful for Cancer Therapy

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ABSTRACT

Green synthesis of iron nanoparticles, which has been proposed as a cost-effective and an environmentally friendly option. Synthesis of nano particles using plant is one type of green chemistry which is a combination of nanotechnology and plant biotechnology. Synthesis of iron nanoparticles has been demonstrated using plant extract, by reducing $FeCl_3$.By going through the process we encountered that the FeNPs can be characterised by UV-Vis Spectrometer and other methods such as MTT, Hoechst, AO-EB, Comet, FTIR, FESEM, Phase contrast, EDX, XRD. The different types of antioxidants present in the plant of sample juice reduce Fe metal ions, according to its structure and function. The process will be simple, and the reactions for formation of nanoparticles will be fast as well as stable. And the outcome of this process will be a value-added product and checked on various Cell lines by using the concept of magnetism.Thus this proves that using of plant material is beneficial for synthesizing of FeNPs by the process of green chemistry. And its property of differentiating between marked or radiolabelled cells, drug designing, the Radiofrequency method of hyperthermia and MRI The current scenario of this research area the findings of the mechanism and enzymatic behaviour of NPs synthesis as well as detection and characterisation of biomolecules in plants such as proteins, amino acids, polysaccharides, alkaloids, vitamins, involved in the production of NPs.These super magnetic nanoparticles have been broadly developed, and it has no side effects of conventional chemotherapy and not only that, but it also plays a leading role in many technological applications such as microelectronics biology and medicine. Upcoming projects and its applications are also discussed.

KEYWORDS: Nanotechnology, FeNPs, MRI, FTIR, XRD, SEM,TEM.

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INTRODUCTION

Nanotechnology is the utilisation of science at a molecular level [1].First-rate growth in Nanotechnology has set a path for many branches such as Nanobiotechnology, applied microbiology, material science and engineering. A Catholic diversity of methods has been reported in the literature for the synthesis of Fe304 MNPs such as hydrothermal process, sonochemical method, micro-emulsion techniques, co-precipitation method and electrochemical method [2]. But the synthesis of nanoparticles can be done by following two ways:

Bottom-up Approach: - Refers to methods where molecules and atoms create themselves by self-assembly using nanoparticle size.

Top-Down Approach refers to slicing or cutting of bulk material to get nano-sized particle [3].

Teeming plantain peels are available which are brought into use for the green synthesis of iron magnetic nanoparticles (MNPs); their significance has been stated in the literature. Plantain peels are rich in lignin, pectin, cellulose, polyphenols and carbohydrates that act as reducing agent as well as capping agents in the bioprocess of FeNPs[4]. Green synthesis of nanoparticles makes use of eco-friendly reagents. In the year 2012 (S.Phumying *et al.)* paper was published in which Aloe Vera leaf extract was synthesized using hydrothermal method thus produced magnetic iron nanoparticles[5].Many persistent reports stated that it is rich in glycoprotein and also describes antidiabetic, antibiotic activities[6] and anticancer[7]. Even in the same year (S. Venkateswarlu *et al.*,), plantain peels were used for the synthesis of (MNPs) [4]. Bhavika Turakhia et al., worked on Coriandrum Sativum leaves for the synthesis of zero-valent Iron nanoparticles[8]. Later than in the year 2013 (M. Mahadevi *et al*) work proceeded and they used seaweed named Sargassum muticum. Historically, the plant is readily available food source food in coastal region which is consumed habitually in many countries in South –East Asia [9].There is a diverse variety of seaweed available[10]. All types are rich in their medicinal values which can work against cancer , such as kinase inhibitor-based combinational therapy, instead of conventional therapy[11] obesity, thrombosis [12], diabetes [13],allergy [14], hypertensive and other degenerative diseases. Their phytochemicals comprise of hydroxyl, carboxyl and amino functional groups and a tremendous work were undertaken for the synthesis and characterization of magnetic iron oxide nanoparticles [15].Process continued and in the year 2015 a comparative evaluation of five plants (C. Mystrioti *et al*,) which included Camellia sinensis (green tea), Syzygium aromaticum (clove), Mentha spicata (spearmint), Punica gragatum juice (pomegranate) and red wine extracts and juices were used for the synthesis of FeNPs. Among all of the pomegranate juice and red wine extract were high in the production of Nano iron and when used together they formed a suspension which was further used for the chromium reduction [16]. A

recent research article of J. Jeyasundari et al., worked with the leaf extract of Psidium Guajava plant which is commonly known as guava plant, is very rich in antibacterial and antioxidant properties and its Nanoscale iron particles work tremendously for the remediation and removal of all types of pollutants from aqueous solutions. For this process to occur suitable precursor such as ferric chloride was used for reduction of plant extract [17].

Magnetic nanoparticles of iron have set up a base for research all because of their properties which are significantly different from those of their bulk counterparts [18]. Thus depending upon their unique physical, chemical, thermal and mechanical properties their surface characteristics & their power of super Para magnetism offer a great potential in many biomedical applications, such as magnetic resonance imaging (MRI) [19],drug delivery [20], tissue repair, cellular therapy [21], hyperthermia & magnetofection [22]. These particles can be used as markers in many biological screening test. After cellular uptake, they can kill cancerous cells [23]. Over the past few decades, there has been an enlargement in the development of targeted nanoparticle-based probes for tumour diagnostics, and therapeutics [24], which can reveal a cellular & molecular level two major operation related with this systems are MRI & controlled drug release (CDR) [25]. Different studies have shown that SPIONS (Superparamagnetic Iron Nanoparticles) are used as vehicles for drug delivery in- vitro and in-vivo [26]. Their specific dimensions make them ideal by all means of usefulness.They serve as a contrast agent for MRI and as targeted drug delivery in tumour therapy. That's the reason why targeted NPs are in a craze nowadays [27]. Fringe benefit has been seen in conventional contrast agents, containing high magnetic signal strength, [28] low toxicity, long-lasting contrast enhancement, [29] better depiction of tumour margins [30]and less sensitive towards the water molecules around them. Little attention has been devoted to the impact that the body metabolizes SPIONs in a body by reducing the potential for long-term cytotoxicity, and degradation of it produces iron [31]. SPIONs have cores which are made of iron oxides that can be used as targeting agents by applying an external magnetic field, and also a porous biocompatible polymer present in the cores in precipitated form. Due to their interesting properties, they do not show any kind of resemblance even after removing the external magnetic field which can be considered as a plus point. The man behind the concept of using external magnetic field coupled with magnetic carriers was Freman et al. [32]. In the years of late 1970s, various magnetic NPs and their carriers were developed to deliver drug at specific sites in vivo. The drug is complex materials of Ferrofluids which are injected into the circulatory system [33]. After reaching the particular site works enzymatically or by physiologically changes such as pH, temperature and osmosis [34].For SPIONs a significant characteristic which is must is its stability in water at neutral pH and salinity. And another important factor is charge and surface chemistry. As Cobalt and Nickel are high magnetic materials which tend to get oxidized. As a result, Iron oxide particles are used for

biomedical applications [35], Such as drug delivery and hyperthermia [36].Foliate linked therapeutics in which macromolecules involved where tumour cell membrane is accomplished with a barrier for macromolecules that enter target cells to cause cell death. Successful development of such strategies requires interdisciplinary collaborations involving researchers with expertise on e.g., polymer chemistry, cell biology, nanotechnology, systems biology, advanced imaging method and clinical medicine. Increased intratumor pressure enhances the macromolecular drugs to go deep inside the malignant mass if tumours lymphatic system is very poor [37]. For this, an alternative has been introduced by researchers that are cancer cell specific ligands as targeted moieties for drug delivery and retention of macromolecular drugs into the tumour tissue [38]. Folic acid is a vitamin acts by helping the body helping the body to produce and maintain new cells [39], the formation of RBC depends on this, which is prevalent targeting molecule for attached drugs to cancer cells [40]. Methotrexate [MTX] Modified Superparamagnetic NPs have also played a significant role in controlled drug release and MRI. The strategy of using them both together is to diagnose a disease and treat it at the same time [41]. For an MTX to get attached to a nanoparticle it has to be immobilised via poly (ethylene glycol) self – assembled monolayer (PEG SAM) [42].

EXPERIMENT SECTION

Synthesis of Iron nanoparticles using different plants and their Characterization

Materials and Method

Till now, iron nanoparticles has been synthesized using different plant extract was listed in Table: 1. The first step of synthesising of Iron nanoparticles is selecting plant material it can be a leaf, fruit, plantain peel or even root which is adequately washed. In some processes, the material used for extraction is dried and powdered, or it can be used fresh. Then it is ground using double distilled water and boiled by continuously stirring for 15 minutes. Followed by cooling of extract at room temperature and it was filtered and stored at -20°C for further process. 0.1 M $FeCl_3$ solution is made in double distilled water, and the extract was added to it in ratios, i.e., either 1: 1, 1: 5, 2: 3, and so on until there is the colour change. In case of Plantain peel along with $Fecl_3$ solution, Sodium acetate is also added [43.]Accordingly, the instant reduction can be seen. This is because the compound present in the extract acts as a reducing and capping agent which reduces the valency of iron to zero and this is detected by the initial colour change from pale yellow to dark brown or black colour. The mixture is stirred and allowed to stand for 30 minutes at 37°C. After that solution is centrifuged and washed using ethanol. After treatment, the Nanocomposites were magnetically separated, and the

supernatant was filtered through Whitman Grade GF/C filter paper [44] and dried in hot air oven at 40°C - 80°C for about 24 hours. Thus iron nanoparticles are synthesized as shown in Table-1.

Table-1 showing the plant used for green synthesis of Iron nanoparticles

Sr. no.	Author's Name	Year	Plant used	Methods Used	Characterization	
1.	Mahnaz Mahadevi, Farideh Namvar, Mansor B Ahmad, Rosfarizan Mohamad	2013	*Sargassum muticum*	Green biosynthetic method.	FTIR – XRD- TEM- and SEM UV– visibles pectra VSM-	Spectra over the range of 400-4000cm^{-1} were recorded. The crystalline structure and phase purity of Fe_3O_4 were obtained. These two were used for the detection of morphology. Peak formation was seen in 300-700nm range. Magnetic properties were checked under magnetic field up to 10kOe.
2.	SadaVenkateswarlu, Y.SubbaRao, T. Balaji , B. Prathima , N .V .V .Jyothi	2012-2013	Plantain peel	Biogenic method.	TEM- EDX– XRD– FTIR-	Size of Fe_3O_4MNPs were in between 30-50nm in diameter. The signals of Fe and O elements are present in the compound. The average particle size was obtained from peak 220,which was ~34nm which was quite close to TEM results. Different band stretching indicates the presence of polyphenols and other biomolecules.
3.	C.Mystrioti et al.	2015	Comparative study on : *Camellia sinensis,Syzygium aromaticum, Menta spicata, Punica grenatum juice,* Red wine	Green biosynthetic method	TEM-	Morphological analysis of the Nano iron suspension and operated TEM at 200kV.
4.	Gottimukkala KSV	2017	*Camellia sinensis* Green tea (Lipton green tea)	Bottom –up approach And Top – down approach	FTIR– SEM-	Absorption bands were observed at 2926 and 1383cm^{-1} Average diameter of FeNPs wasfound to be about 1116nm
5.	Santi Phumying , Srawuth Labuayai, Chunpen Thomas,	2012	Aloe Vera	Hydrothermal method	XRD-	When given an optimum temperature,No diffraction peaks of other impurities were observed, that denotes the purity of the product.

	Vittaya Amornkitbamrung, Ekaphan Swatsitang, Santi Maensiri				SEM - TEM-	Size of FeNPs were about ~25-50nm. Size of FeNPs were about ~6-10nm at initial stage and once temperature was increased particle size also increased to ~20-30nm.
6	J. Jeyasundari, P. Shanmuga Prabha,	2017	*Psidium Guajava*	Green synthesis Bottom-to- up method was used.	UV–Visibe specto FTIR– XRD – SEM - EDX	Peak of 245.12nm states the presence of Iron nanoparticles The carbonyl band at 1637cm^{-1} was shifted to 1690cm$^{-1,}$ and this shift in band is direct indication of FeNPs. All the four peaks in XRD pattern readily indexed to a hexagonal structure and the size was estimated to be about 27nm Morphology was determined using the SEM results and optical absorption at 7Kev denotes the presence of FeNPs.

STRUCTURE AND PHYSIOCHEMICAL CHARACTERIZATION

Table: 2 Different types of iron nanoparticles used in cancer therapy

Nanoparticles	Cancer cell line /tumour model used	Observed effects	Reference
1. SPIOs (Superparamagnetic Iron oxides)	Kupffer cells	Acts as drug reservoir in fight against their nearby neoplastic cells.	[45]
2. USPIO (Ultra smallSuperparamagneticIron oxides)	Blood pool and tumour imaging	Detection and characterization of lesions by vascular appearance.	[46]
3. LCDIO (Long-circulating dextran-coated Iron oxide)	In rat malignant brain neoplasms	Accumulate in the intra cerebrally implanted tumours	[47]
4. SPIONs	MCF-7 cells	Greater heating and cell killing capacityof ferromagnetic NPs compared to Superparamagnetic NPs with same uptake.	[48]
5. Uncoated IONPs	Female C3H mice bearing MTGB tumours	MNP induced hyperthermia (MNPH) caused slower tumour regrowth in mice with reduced normal tissue damage compared to 915 MHz microwave hyperthermia at the same thermal dose	[49]

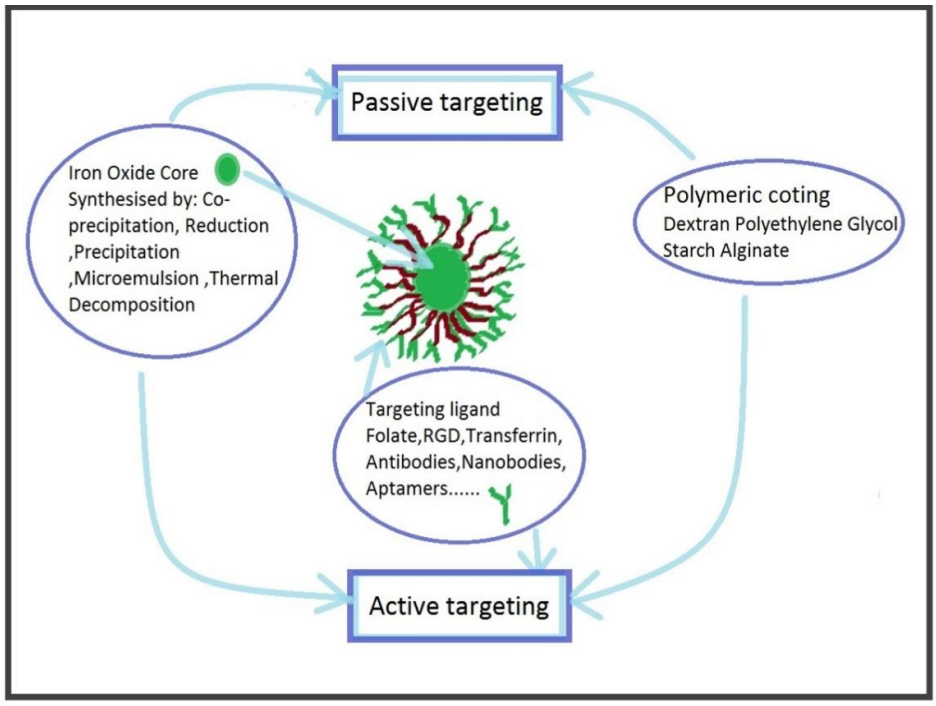

Figure 1: Iron oxide magnetic behaviour

Fig. 1 shows the different components that make up active targeting based and passive targeting based IONs (Iron oxide NPs) which project out themselves as an individual molecule because of its low cost, superparamagnetic behaviour, biocompatibility and biodegradability [50]. IONPs are the only metal oxide nanoparticles which are accepted for application in MRI. They can be used for removal of pollutants from water bodies such as nitrides. They can also be used as the nutritional supplement for Iron-deficient patients (anaemia).

Another best way of using magnetic nanoparticles is Folate- mediated conjugate uptake via receptor-mediated endocytosis[51]. Fig. 2 shows folate conjugate is uptaken by the mammalian cells via receptor-mediated endocytosis. Studies show that Folate receptor is organized into the submicron domain at cell surface due to GPI (Glycosyl Phosphatidyl Inositol) anchor. Later it was concluded that multimerization of GPI- anchored FR does not play any role, and the receptors may spread all over the plasma membrane till there is ligation of folate [52].Present result shows that FR is organised by GPI anchor into the receptor-rich complex. Thus it enters the cancer cell surface, and receptor-mediated endocytosis occurs. Only 15% -25% receptor bound to conjugates are released inside the cell.

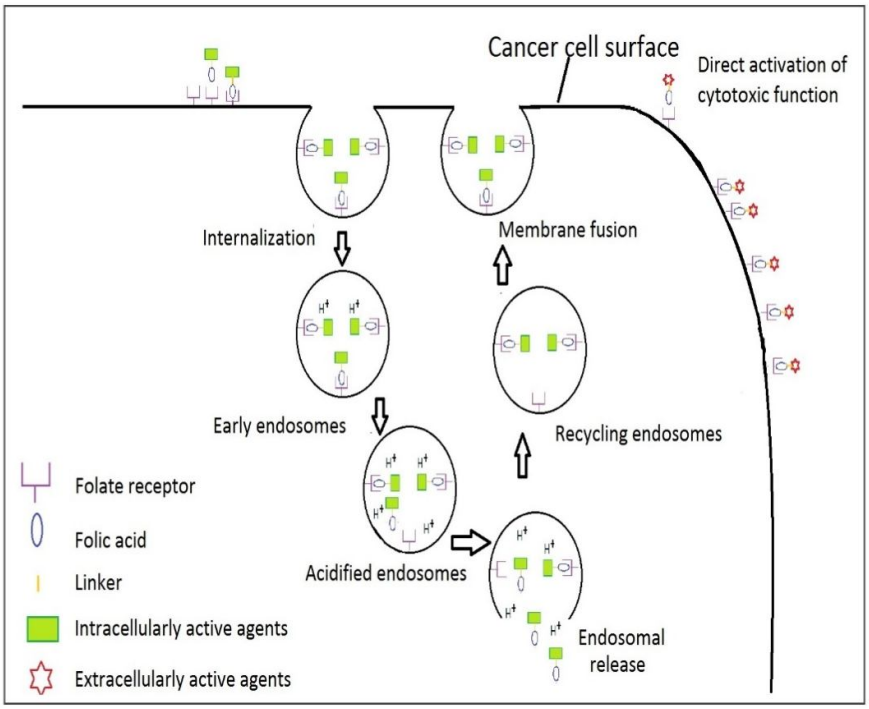

Figure 2: Folate mediate conjugate uptake via receptor – mediated endocytosis

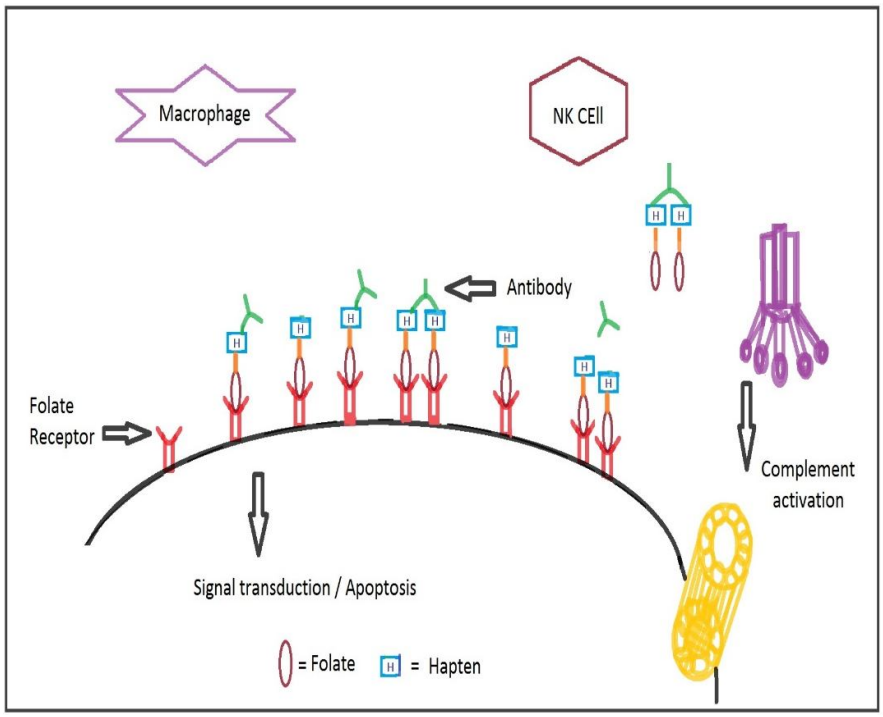

Figure 3: Folate –hapten targeted immunotherapy

Fig. 3 represents the cancer cell eradication using two-step strategy of Folate – Hapten targeted immunotherapy to create immunity against potent hapten. The cancer patient is injected with folate conjugate of hapten, which promotes opsonisation of the cancer cell with the anti-hapten antibody. Thus cancer cell is eliminated, the participation of NK cells and macrophages can also be seen in this process.Recent studies state that there is the maximum role of intronic polymorphism in cancer [53].

CONCLUSION

The low cost and environmental friendly features made iron nanoparticles into use.The possibility of achieving control on the shape of the nanoparticle can be purely done by green chemistry in future. Discovery of increased levels of folate receptor expression on many cancerous cells has improved. Folate receptor can be used as a vehicle for non- destructive trafficking as well as for extracellular therapeutic agents into the targeted tumour cell. It also acts as a marker for ligand-mediated drug delivery. While using a SPION, the main points such as surface charge, shape, size stability in the environment and saturation magnetisation are necessary. The issue of behind using Spoon for drug delivery is its route for elimination in the body and for this they are coated with biodegradable polymers, cross-linkable polymers are of great interest. Specificity is the must while selecting of a SPION for drug delivery to minimise the risk of toxicity. The most prominent share of SPIONs in cancer is that they work with the help of bodies own metabolism and also get degraded and do not get aggregated in the body. Magnetically directed microspheres containing radio nucleotides have been used for internal radiotherapy. And also magnetic particles could be used for repair of the human body with prosthetic or artificial replacement parts. Potential of using iron nanoparticles in future of diagnosis and therapeutics will significantly improve the survival rate and quality of life for many cancer patients.

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