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SYNTHESIS OF 2-ARYL THIO ARYL CYANAMIDES FROM 2-IODO ARYL ISOTHIOCYANATES IN ONE POT THREE COMPONENT REACTION BY USING IRON AS A CATALYST

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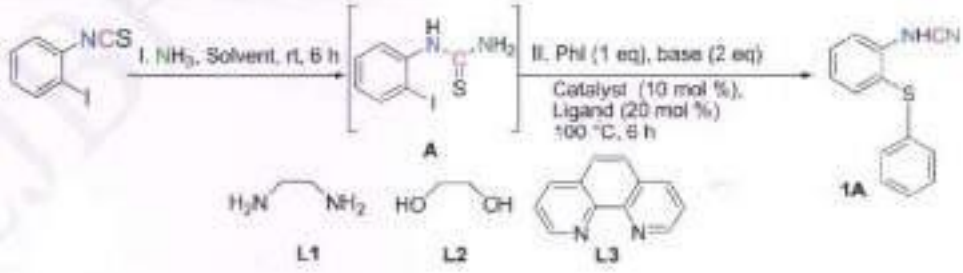
INTRODUCTION

Due to its unique reactivity, cyno group is recognized as important building block and is found in various bioactive molecules and functionalized materials.[1] Cyanamides are useful precursors and important synthetic intermediates for the synthesis of biological, medicinal and pharmaceutically important hetero-cycles.[2] Since the cyano group is easy removal from cyanamide and N-alkyl or N-aryl imides,[3] they often represent as a useful protecting groups in the synthesis of secondary and tertiary amines containing heterocycles.[4] Aromatic cyanamides have also been prepared by both classical and ancient methods.[5]

In recent years, the formation of carbon-heteroatom bonds[6] towards the synthesis of heterocyclic compounds has been developed through cross-coupling reactions using transition-metal-catalysis. Among these, carbon-sulfur bond formation has received much attention due to the presence of this moiety in many molecules that are of biological, pharmaceutical and material interest.[7] Recently the above said moieties containing compounds like 2-(arylthio) aryl cyan-amides from 2-halophenyl thiourea via domino C-S cross-coupling reaction using copper as catalyst.[8] But to the best of our knowledge no one has reported in the presence of iron.

Therefore, herein, we wish to demonstrate the one-pot synthesis of 2-(arylthio) aryl cyanamides from 2-iodoaryl isothiocyanate and aryl iodides using cheap, readily available and air stable iron source as catalyst under milder conditions.

Table 1: Standardization for the reaction conditions.

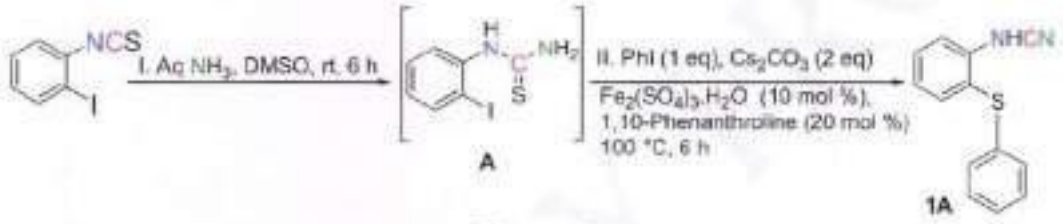


Entry	Solvent	Catalyst	Base	Ligand	Conversion[b]	
					A	1A
1	EtOH	$Fe_2(SO_4)_3.H_2O$	$K_3PO_4.3H_2O$	L3	100	n.d.
2	EtOAc	$Fe_2(SO_4)_3.H_2O$	$K_3PO_4.3H_2O$	L3	100	n.d.
3	n-Hexane	$Fe_2(SO_4)_3.H_2O$	$K_3PO_4.3H_2O$	L3	n.d.	n.d.
4	n-Heptane	$Fe_2(SO_4)_3.H_2O$	$K_3PO_4.3H_2O$	L3	n.d.	n.d.
5	H_2O	$Fe_2(SO_4)_3.H_2O$	$K_3PO_4.3H_2O$	L3	70	n.d.
6	DMF	$Fe_2(SO_4)_3.H_2O$	$K_3PO_4.3H_2O$	L3	45	55
7	DMSO	$Fe_2(SO_4)_3.H_2O$	$K_3PO_4.3H_2O$	L3	45	55

8	DMSO	$Fe_2(SO_4)_3.H_2O$	KOH	L3	25	75
9	DMSO	$Fe_2(SO_4)_3.H_2O$	K_2CO_3	L3	40	60
10	DMSO	$Fe_2(SO_4)_3.H_2O$	Cs_2CO_3	L3	n.d.	100
11	DMSO	$Fe_2(SO_4)_3.H_2O$	Cs_2CO_3	L1	75	25
12	DMSO	$Fe_2(SO_4)_3.H_2O$	Cs_2CO_3	L2	55	45
13	DMSO	$Fe(NO_3)_3.9H_2O$	Cs_2CO_3	L3	n.d.	100
14	DMSO	$FeCl_2$	Cs_2CO_3	L3	n.d.	100
15[c]	DMSO	$Fe_2(SO_4)_3.H_2O$	Cs_2CO_3	L3	50	50
16[d]	DMSO	$Fe_2(SO_4)_3.H_2O$	Cs_2CO_3	L3	35	65
17	DMSO	$Fe_2(SO_4)_3.H_2O$	Cs_2CO_3	-	79	21
18	DMSO	-	Cs_2CO_3	-	100	n.d.

[a]Reactionconditions: 2-Iodophenyl isothiocyanate (1 mmol), solvent (2 mL), Aq NH_3 (2 ml), 6h, room temperature, then, iodo benzene (1 mmol), catalyst (10 mol %), ligand (20 mol %), base (2 mmol), 6 h, 100 °C. [b] Isolated yield. [c] Catalyst (5 mol %) was used. [d] Cs_2CO_3 (1.5 equiv) was used. n.d. = not detected.

2-Iodo phenyl isothiocyanate reacts with aq ammonia in the presence of DMSO to afford 2-iodo phenyl thiourea as an intermediate, which reacts with iodo benzene using iron source as catalyst under below shown reaction conditions to obtain the target product as 2-phenylthio phenyl cyanamide via domino intra and inter molecular C-S cross-coupling reaction (**Scheme 1**).

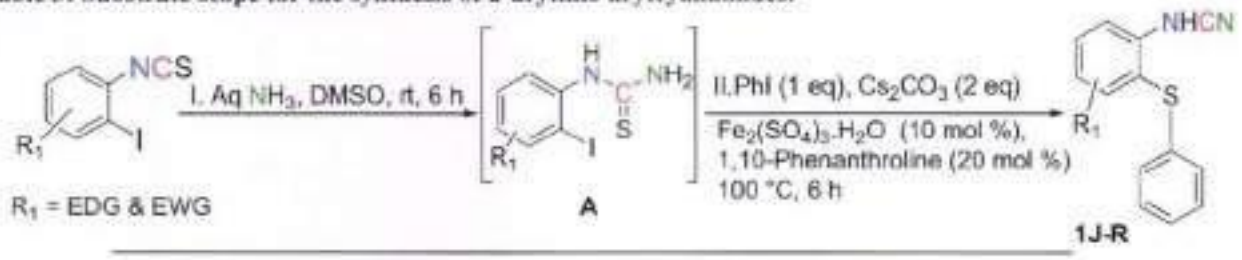


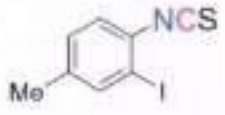
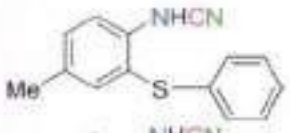
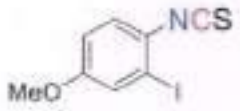
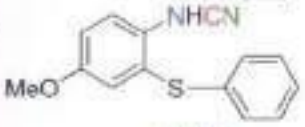
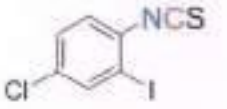
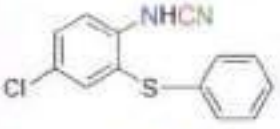
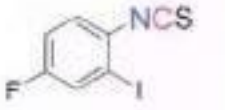
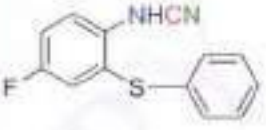
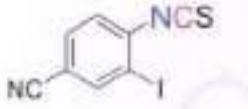
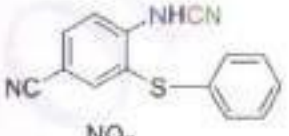
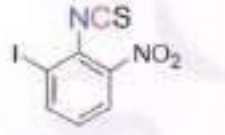
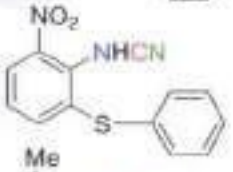
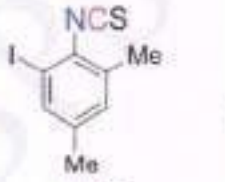
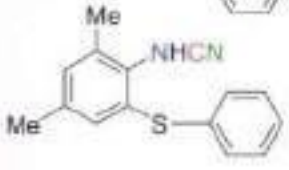
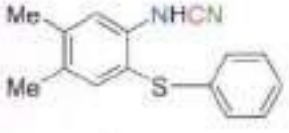
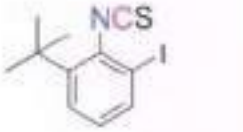
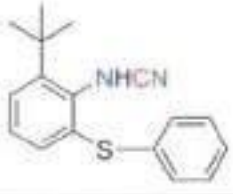
Scheme 1

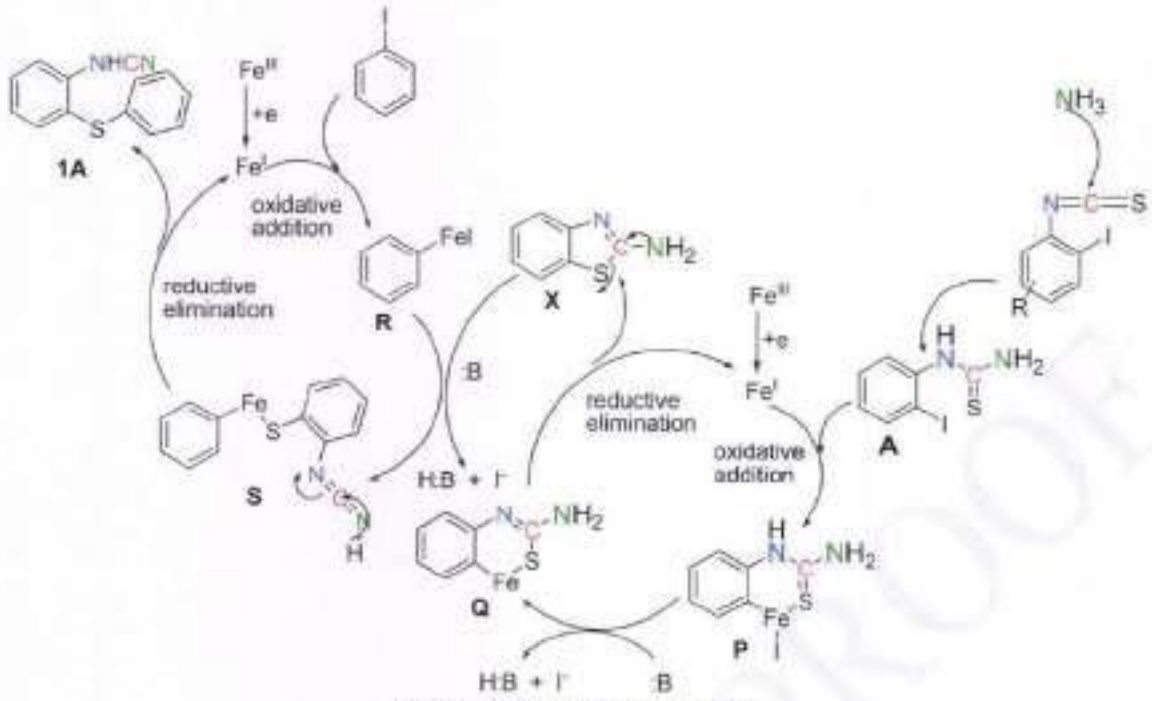
Initially, the optimization of the reaction conditions was performed using 2-iodophenyl isothiocyanate as model substrates with different solvents at room temperature (Step 1). We could observe that that the substrate proceeded reactions with aq NH_3 and in the presence of EtOH, EtOAc, DMF and DMSO at room temperature to get the corresponding thiourea A in complete conversion, that, gratifyingly, proceeded an domino intra and inter molecular C-S cross-coupling reaction with iodobenzene using 10 mol % $Fe_2(SO_4)_3.H_2O$, 20 mol % Ligand (1,10-Phenthraiene) and 2 equiv Cs_2CO_3 at 100 °C temperature to give target product 1A in complete conversion. In case of solvent optimization, non-polar solvents like n-hexane and n-heptane couldn't proceed the reaction. Green solvent H_2O could give thiourea (1st step) in 70% conversion, that couldn't proceed the domino C-S cross-coupling reaction (2nd step). Various bases were examined and among them Cs_2CO_3 could give target product in complete conversion (Table 1, entry 10). Other bases ($K_3PO_4.3H_2O$, K_2CO_3 and KOH) could give less effect for the formation of target product. In a set of ligands L1-L3 screened, L3 was found to be the most effective in comparison to L1-L2 (Table 1, entries 11-12). Both iron (II) and iron (III) sources ($Fe_2(SO_4)_3.H_2O$, $Fe(NO_3)_3.9H_2O$ and FeCl2) exhibited a similar catalytic activity (entries 10 and 13-14). Lowering the amount of base (1.5 equivalent) or the iron source (5 mol %) led to the domino C-S cross-coupling reaction to afford target product in less conversion (Table 1, entries 15-16). The reaction was also checked in the absence of ligand, unfortunately, the reaction could give target product in 21% conversion only (Table 1, entry 17). Control experiments confirmed that the reaction didn't proceed (step 2) in the absence of the catalyst and ligand and the thiourea was recovered intact (Table 1, entry 18).

Having optimization reaction conditions in our hand, we further pursued the scope of the process with respect to the other substrates. Aryl iodides having electron donating groups 4-Me and 4-OMe proceeded reactions to afford their domino coupled products 1B and 1C in 95% and 97% yields, respectively. Aryl iodides bearing weak electron withdrawing substituent's 4-Cl and 4-F could give their respective target products 1D and 1E in 91% and 88%. Aryl iodides possess strong electron withdrawing groups 2-NO_2 and 4-CN gave their final products in less yield. It might be occurred as these have strong electron withdrawing capacity. Finally, we have also checked reaction with 2,4-diMe iodobenzene and it could give target product in decent yield. Aryl iodide contain bulkier group 2-^{t}Bu carried out the reaction under optimized reaction conditions to give respective 2-(arylthio) aryl cyanamide 1I in 82% yield. Similarly, phenyl isothiocyanate bearing 4-Me, 4-OMe, 4-Cl, 4-F, 4-CN, 2-NO_2, 2,4-di-Me, 3,4-di-Me and 2-^{t}Bu substituents readily carried out reaction with iodobenzene to give their final products 1J-R in 70-95% yields. The above-mentioned results clearly confirm that the substrates having electron donating

Table 3: Substrate scope for the synthesis of 2-arylthio arylcyanamides.



Entry	Substrate	Product		Isolated yield (%)[b]
1			**1J**	95
2			**1K**	95
3			**1L**	90
4			**1M**	83
5			**1N**	75
6			**1O**	70
7			**1P**	82
8			**1Q**	85
9			**1R**	77



Scheme 2: Proposed mechanism.

We have developed neat, clean and efficient methodology for the synthesis of 2-halo aromatic cyanamides. During the reaction process we couldn't observe any other byproducts (no other products could observe except cyanamide only). The reactions are rapid and facile and accomplished under mild reaction conditions. All the substrates could obtain their target products in good to excellent yields.

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REFERENCES AND NOTES

1. (a) Ricci, A.; Amino Group Chemistry from Synthesis to the Life Sciences; Wiley-VCH: Weinheim, **2008**; (b) Fatiadi, A. J.; Patai, S.; Rappaport, Z. Ed. In Preparation and Synthetic Applications of Cyano Compounds; Wiley: New York, 1983; (c) Kleemann, A.; Engel, A.; Kutscher, B.; Reichert, D. Pharmaceutical sustance: Synthesis Patents, Applications, 4th ed.; Georg Thieme: Stuttgart, **2001**.
2. (a) Sandler, S. R.; Karo W. In Organic Functional Group Preparations; Academic: New York, **1972**; Vol. 3; (b) Sandler, S. R.; Karo, W. In Organic Functional Group Preparations; Academic: New York, **1972;** Vol. 2; (c) Nickon, A.; Fieser, L. F. J. Am. Chem. Soc. **1952**, 74, 55-66; (d) Jia, Q.; Cai, T.; Haung, L. M. H.; Xian, M.; Poulos, T. L.; Wang, P. G. J. Med. Chem. **2003,** 46, 2271; (e) Cai, T.; Xian, M.; Wang, P. G. Bio Org. Med. Chem. Lett., **2002;** 12: 1507.
3. (a) Kumar, V.; Kaushik, M. P.; Mazumdar, A. Eur. J. Org. Chem. **2008,** 1910; (b) Saneyoshi, M.; Tokuzen, R.; Maeda, M.; Fukuoka, F. Chem. Pharm. Bull., **1968;** 16: 505; (c) Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th ed.; Pergmon Press: New York, **1990**; (d) La Mattina, J. L. J. Heterocycl. Chem., **1983;** 20: 533.
4. Stephens, R. W.; Domeier, L. A.; Todd, M. G.; Nelson, V. A. *Tetrahedron Lett.*, **1992;** *33:* 733.
5. (a) Donetti, A.; Omodei-Sale, A.; Mantegani, A.; Zugna, E. *Tetrahedron Lett.*, **1969;** *39:* 3327; (b) Pala, G.; Mantegani, A.; Zugna, E. *Tetrahedron* **1970**, *26*, 1275; (c) Currie, A. C.; Newbold, G. T.; Spring, F. S. *J. Chem. Soc.* **1961;** 4693.
6. (a) Kaupp, G.; Schmeyers, J.; Boy, J.; *Chem. Eur. J.* **1998**, *4*, 2467-2474. (b) Sato, R.; Itoh, K.; Itoh, K.; Nishina, H.; Goto, T.; Saito, M. *Chem. Lett.* **1984,** 1913-1916. (c) Bakunov, S. A.; Rukavishnikov, A. V.; Tkachev, A. V. *Synthesis,* **2000;** 1148-1159.
7. Chen, C.-Y.; Wong, F. F.; Huang, J. J.; Lin, S.-K.; Yeh, M.-Y. *Tetrahedron Lett.*, **2008;** *49:* 6505-6507.
8. Chaudhuri, K. H.; Mahajan, U. S.; Bhalerao, D. S.; Akamanchi, K. G. *Synlett,* **2007;** 2815-2818.
9. Beletskaya, I. P.; Cheprakov, A. V. *Coord. Chem. Rev.* **2004**, *248*, 2337-2364. (b) Hartwig, J. F. *Synlett,* **2006;** 1283-1294. (c) Ley, S. V.; Thomas, A. W. *Angew. Chem. Int. Ed.*, **2003;** *42:* 5400-5449. (d) Corbet, J.-P.; Mignani, G. *Chem. Rev.*, **2006;** *106:* 2651-2710.
10. (a) Evano, G.; Blanchard, N.; Toyni, M. *Chem. Rev.*,**2008;** *108:* 3054-3131. (f) Kondo, T.; mitsudo, T.-A. *Chem. Rev.*, **2000;** *100:* 3205-3220. (b) Choi, T. A.; Czerwonka, R.; Forke, R.; Jaeger, A.; Knoell, J.; Krahl, M. P.; Krause, T.; Reddy, K. R.; Franzblau, S.G.; Knoelker, H.-J. *Med. Chem. Res.*,

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DEVELOPMENT AND VALIDATION OF A SENSITIVE AND STABLE HSGC-MS/MS METHOD FOR SIMULTANEOUS DETERMINATION OF TWO N-NITROSAMINE IMPURITIES IN OMEPRAZOLE SODIUM DRUG SUBSTANCES AND DRUG PRODUCTS BY USING QUALITY BY DESIGN APPROACH

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Abstract:

Nitrosamine impurities in angiotensin II receptor antagonists containing a tetrazole group represent an urgent concern for active pharmaceutical ingredient (API) manufacturers and global regulators. Regarding safety, API manufacturers must develop methods to monitor the levels of each nitrosamine impurity before individual batch release. In this study, we developed and validated a sensitive, selective, and high throughput method based on headspace gas chromatography-mass spectrometry with Multiple reactions monitoring mode (MRM) (HSGC−MSMS) for the simultaneous determination of two nitrosamines, namely, N-Nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in Omeprazole sodium drug substances and products by using quality by design approach.

The quantification of two nitrosamines in Omeprazole sodium drug substances and products ranged from 0.07 ppm with respect to the sample concentration of 1000 mg/mL with good sensitivity in LOQ level. The limit of quantification is 0.021 ppm and limit of detection is 0.007 ppm for NDMA and NDEA with respect to the sample concentration of 1000 mg/mL with good sensitivity in the proposed method. The calibration curves of the assay ranged from 0.035 to 0.105 ppm with limits of quantitation of 0.021 ppm for NDMA and NDEA. The recoveries of two N-nitrosamines in selected Omeprazole sodium drug ranged from 85% to 115%. The precision was in the acceptance criteria of bellow 10% for NDMA and NDEA. Other validation parameters, including specificity, robustness, ruggedness, solution stability met the validation criteria.

Therefore, this proposed HSGC-MSMS method exhibited good sensitivity and precision, high accuracy, and fast analysis speed, which provide a reliable method for quality control of two N-nitrosamines in Omeprazole sodium drug substances and products. This method is applied for pharmaceutical dosage forms, results met the with the specifications. In conclusion, it will be useful to quantify the low-level nitrosamines in Omeprazole sodium drug substances and products.

Index Terms: **Omeprazole sodium, N-Nitroso dimethyl amine, N-nitroso diethyl amine, HSGC-MS/MS, method development and validation.**

I. INTRUDUCTION

Nitrosamines are organic chemical compounds that may be present in low levels in a variety of products that people are exposed to every day. Air, water, and consumer products such as processed meats, alcoholic beverages, cosmetics, and cigarette smoke all contain nitrosamines [1]. N-nitrosamines have been classified as 'probable human carcinogens' by the International Agency for Research on Cancer [2] and were listed as a representative "cohort of concern" of mutagenic carcinogens in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidance for industry M7 (R1) [3]. In 2018, the nitrosamines N-nitrosodiethylamine (NDEA) and N-nitrosodimethylamine (NDMA) were detected in several API used in the treatment of hypertension and related medicines. After thorough investigations, regulatory agencies have outlined that the formation of nitrosamines is possible in the presence of secondary, tertiary, or quaternary amines and nitrite salts under acidic reaction conditions. The starting materials, intermediates, reagents, solvents, catalysts, active pharmaceutical ingredient (API), or API degradants may participate in reactions and form nitrosamines in certain processing conditions. Contaminated reaction materials in the manufacturing process such as recycled solvents are a potential root cause of nitrosamines in drug products. Excipients and packing materials may contain nitrosamine precursors to generate nitrosamines in drug products. Regulatory agencies have guided manufacturers to follow risk assessment, acceptable intake (AI) limits, and multiple analytical test procedures to control nitrosamines [4, 5].

The U.S. FDA has reported that low concentrations of NDMA have been detected in some metformin products, and no sample of metformin exceeds the acceptable daily intake for NDMA [6]. When nitrosamine impurities have been detected in several pharmaceutical products, many countries announced interim limits for NDMA and NDEA recall based on the acceptable daily intake of the sartan drug substances (valsartan, irbesartan, fimasartan, rosartan potassium, olmesartan, candesartan), metformin and ranitidine [7,8,9].

Since the Omeprazole sodium products produced by several pharmaceutical companies were proven to contain potential contamination with carcinogenic nitrosamine impurities, namely, N-nitrosodimethylamine (NDMA) and N-nitroso diethylamine(NDEA) (Fig. 1). 5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)sulfinyl)-1H-benzo[d]Imidazole is a substituted benzimidazole compound and a prototype anti-secretory agent, being the first "proton pump inhibitor" widely used for the prophylaxis and treatment of gastro-duodenal ulcers and for the treatment of symptomatic gastro-doesophageal reflux [10]. There is a potential for the presence or formation of NDMA and NDEA in the Omeprazole drug substance and drug product.

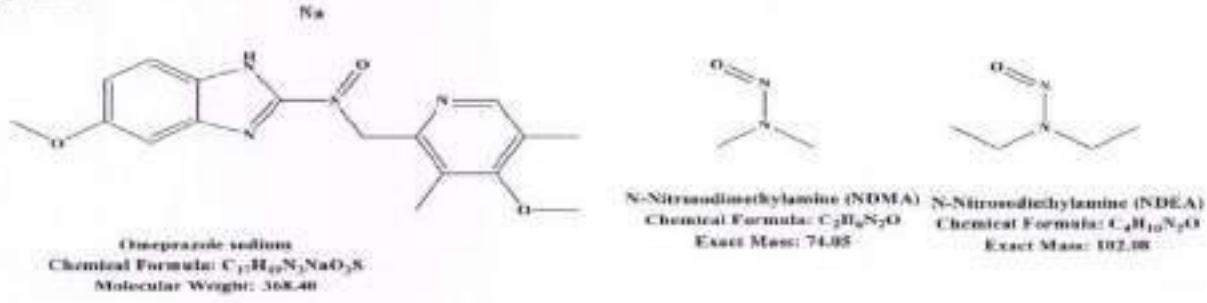

Figure 1: Molecular structures of Omeprazole sodium and two N-nitrosamines

Literature review has been proved that the multiple analytical methods are also published by regulatory agencies for the determination of Genotoxic impurities, nitrosamines in various drug substances and drug products by using [11-17]. And determination Omeprazole assay in its bulk and pharmaceutical dosage forms [13],[18-21]. However, no approach is reported for the trace-level quantification of NDMA and NDEA in the Omeprazole sodium drug substance and drug products by using HSGC-MS/MS so far. So, our proposed work is very necessary for the quantification of two N-nitrosamine impurities at low level in Omeprazole sodium drug substances and products. However, these were not suitable for rapid and high-throughput analysis in the pharmaceutical industry. Here in, we have developed a simple, sensitive, accurate, and reproducible HSGC-MS/MS method for the detection of two N-nitrosamines in Omeprazole sodium drug substances and products. The obtained LODs and LOQs met the sensitivity requirements set by FDA. Then, this developed method was validated according to the International Council for Harmonization (ICH) guidelines [22,23] in terms of sensitivity, linearity, accuracy, precision, specificity, and stability.

Based on Control of Nitrosamine Impurities in Human Drugs requirements, FDA has established the interim acceptable daily intake limits for N-nitrosamines. For two N-nitrosamine impurities specifications are calculated by the maximum daily dosage (360mg/day) of Omeprazole sodium. The specifications of NDMA and NDEA impurities were calculated by using 'Accepted Intake limit' is divided by 'maximum daily dosage' of Omeprazole sodium (360mg/day). European Medicines agency has published the maximum daily intake as 96 ng for NDMA and 26.5 ng for NDEA and no guidance on suitable acceptable daily intake for rest of the possible nitrosamines. By considering the worst case, 26.5 ng will be considered for the NDMA & NDEA nitrosamine which was considered in the evaluation study. The calculated Interim limits for NDMA and NDEA are shown in Table-1.

Table 1: Interim limits for NDMA and NDEA in Omeprazole set by FDA

Name of Nitrosamine Impurity	Accepted Intake limit (ng/day)	maximum daily dosage (mg/day)	Accepted Intake limit (ppm=AI/MDD)	Taken limits (ppm)
NDMA	96	360	0.27	0.07
NDEA	26.5	360	0.07	0.07

METHODS

Chemicals and reagents

The N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA) standards and Omeprazole sodium drug substance were purchased from local well known Laboratories, Hyderabad, India. Millipore Milli Q purification system was used from Bangalore, India. HPLC grade DMSO was purchased from Rankem (Mumbai, India). Millipore Milli Q purification system purchased from Bangalore, India.

Instrumentation and optimized HSGC-MS/MS conditions

Analyses of two N-nitrosamines were performed on a SHIMADZU GC-MS-TQ8040NX system. VF-Wax ms capillary column (30m × 0.25mm i.d., 1.0 µm) was used as the analytical column in this work. MS/MS detection was carried out on a SHIMADZU TQ-GC triple quadrupole mass spectrometer with electron ionization (EI) ion source. The GC oven program utilized an initial oven temperature of 40 °C, held for 5 min, raised firstly at 20 °C·min−1 to 240 °C finally held for 5 min. The total run time was 20 min. Helium as the carrier gas was set at a flow of 1.0 mL/min. The injection volume was 1 µL in the split ratio is 10:1, HS Oven temperature, Sample line temperature, Transfer line temperature were set to be 150°C, 160°C & 170°C. HS vial Equilibrating time, GC cycle time and Injection time were set to be 15 min, 30 min & 1.0 min.

Both the interface temperature and ion source temperature were set to be 250°C & 230°C. The MS was operated in EI mode at 70 eV with a quadrupole temperature of 150 °C. The solvent cut time was 5 min and end time was 15 min and multiple reactions monitoring (MRM) mode was selected as the data acquisition for the quantitative determination of two kinds of Nitrosamine GTIs. The precursor ions and product ions of two N-nitrosamine GTIs, as well as the optimized collision energy (CE) were summarized in Table 2.

Table 2: Optimized MRM fragments and CE

Impurity Name	CH1 m/z	CH1 CE(V)	Q3 Resolution
NDMA	74.00 > 42.00	20	High
NDEA	102.00 > 85.10	5	High

Preparation of Sample and Impurity Standard solutions

Preparation of Diluent

Prepared the homogeneous mixture of DMSO and water in the ratio of 80:20 (v/v) respectively and mix well.

Preparation of Nitrosamine Impurities standard solution (0.07 ppm)

Accurately weight and transfer about 70 mg of each nitrosamine impurity in 100 mL of volumetric flask and diluted to the volume with diluent and sonicated about 1 min (stock solution-1). Pipette 0.5 mL of the stock solution-1 into a 50 mL volumetric flask and dilute to the desired concentration with diluent (stock solution-2). Further pipette 0.5 mL of the above stock solution-2 into a 50 mL volumetric flask and dilute up to the mark with diluent (stock solution-3). Further pipette 0.5 mL of the above stock solution-3 into a 50 mL volumetric flask and dilute up to the mark with diluent (Standard solution was prepared with respect to 1000 mg/mL of Omeprazole sodium sample). The standard Heads pace vials were prepared with 1 mL of the Standard solution and sealed the vial with aluminum closure.

Preparation of Omeprazole sodium Drug substance (1000 mg/mL)

Weigh and transfer 1000 mg of Omeprazole sodium sample into a 20 mL Head Space vial and add 1.0 mL of diluent and immediately sealed with aluminum closure.

Preparation of Omeprazole Drug product (1000 mg/mL)

Crush the appropriate number of Omeprazole tablets equivalent to 1000 mg of Omeprazole sodium sample and accurately weigh and transfer into 20 mL of headspace vial, adds 1 mL of diluent and immediately sealed with aluminum closure.

RESULTS

Method Optimization

The stationary phase, column length, film thickness, and column diameter have a large impact on the retention and peak shape of the analytes. Based on the trial-and-error methodology with the literature review, the optimal separation of nitrosamines was achieved when the capillary surface was modified by polyethylene glycol residues at the length of 30 m. The

optimal chromatographic separation and retention were achieved using a column dimension of 30 × 0.25 mm i.d. and a film thickness of 1 µm coated with polyethylene glycol stationary phase. The temperature programming is also a very important parameter to influence peak separation. An initial constant temperature at 40°C for 5 min followed by an increase at a rate of 20°C can provide the best separation with a resolution greater than 1.5 between NDMA and NDEA at the retention times of 11.85 and 12.47 min, respectively. We optimized, HS oven temperature (140-160°C), Sample line temperature (150-170°C), and Transfer line temperature (160-180°C). After optimized, choose the HS oven temperature 150°C, Sample line temperature 160°C, and Transfer line temperature 170°C. Different diluents were checked, NMP, DMSO and DMF etc. for sample and impurities solubility. For this study, DMSO: Water (80:20v/v) was used, and advantages in terms of volatility and polarity were achieved. More importantly, Omeprazole sodium and impurities are soluble and can be extracted entirely and dissolved in pre mixed solution of DMSO: water (80:20v/v).

The GC-MS/MS conditions for the simultaneous determination of two nitrosamines in Omeprazole sodium was optimized to ensure reliability, selectivity, and sensitivity. Initially, the mass parameters were optimized to achieve the highest sensitivity with a consistent response. NDMA (m/z 74) and NDEA (m/z 102 were tuned on a single quadrupole mass spectrometer in the electron ionization (EI) mode under the selected ion monitoring (SIM) acquisition method. These particular ions were used for quantification. In addition to the parent ions, the major daughter ions of NDMA (m/z 42) and NDEA (m/z 85.10) were qualitatively monitored for confirmation. Alternatively, the ion ratio calculation between the parent and daughter ions was also quantitatively used in the confirmatory objective.

METHOD VALIDATION

The proposed determination method for two N-nitrosamine GTIs has been validated according to the (ICH) guidelines [19] through the following parameters, such as specificity, system precision, method precision, linearity, LOD, LOQ, accuracy, Robustness, Ruggedness and solution stability.

Specificity:

To demonstrate the specificity of the proposed method, Omeprazole sodium and the mixture solution of two N-nitrosamine standards were subjected to the HSGC-MS/MS analysis. In Fig. 2, no interference peaks in the N-nitrosamine standard and the Omeprazole sodium were observed at the retention times of two N-nitrosamines and resolution was greater than 1.5 between NDMA and NDEA at the retention times of 11.85 and 12.47 min, respectively. This indicates that HSGC-MSMS method for the determination of two N-nitrosamines in Omeprazole sodium showed good specificity.

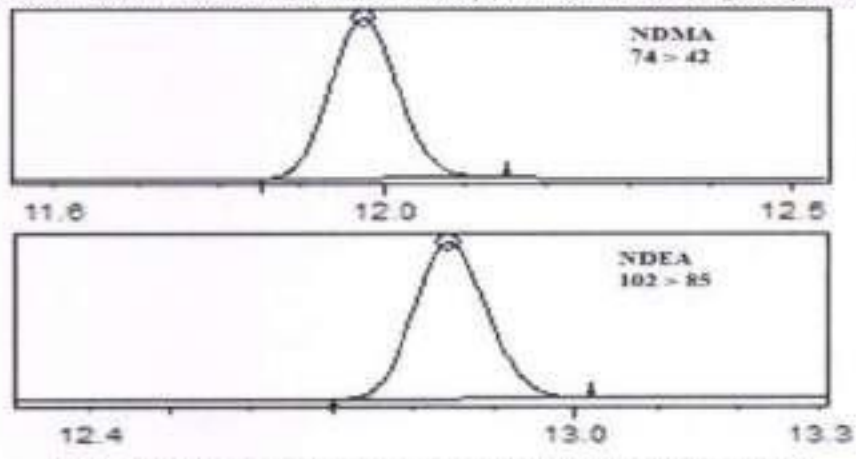

Figure 2: Standard two N-nitrosamine impurities (NDMA & NDEA)

System precision

System precision was established by six measurements of the standard solution at the 100% concentration levels on the same day. Six injections of standard two N-nitrosamines solution were injected into the HSGC-MS/MS system to evaluate the system precision of developed method. The obtained %RSD two N-nitrosamines impurities were not more than 10%. The corresponding data is shown in Table 3.

Method precision

The gas chromatographic method precision was verified by analyzing the Omeprazole sodium drug sample spiked with two N-nitrosamines impurities at specification limits. The method precision was vented as mean concentration quantified and relative standard deviation of six quantified values of two N-nitrosamines impurities. The relative standard deviation was noticed as ≤ 10%, which proved that HSGC-MSMS method was precise for quantification of two N-nitrosamines impurities in Omeprazole sodium drug. The corresponding data was shown in Table 4.

Table 3: System precision data for two N-nitrosamines impurities

No. of Injections	NDMA area	NDEA area
1	53425	9355
2	55632	9456
3	58025	8465
4	52506	8726
5	57456	8399
6	55693	8589
ACVG	55456	8832
STDV	2169	459
% RSD	3.91	5.20

Table 4: Method precision data for two N-nitrosamines impurities

No. of Preparations	NDMA area	NDEA area
1	59862	9632
2	58269	9745
3	59023	9526
4	55789	9756
5	56128	9285
6	58692	9968
ACVG	57961	9652
STDV	1640	233
% RSD	2.83	2.41

Limit of Detection (LOD) and Quantization (LOQ)

The LODs were defined as 3 times the signal-to-noise (S/N) ratio, and the corresponding LOQs were S/N = 10. The matrix effect (ME) value was calculated according to the following equation. LODs and LOQs results were summarized in Table 5.

$$ME(\%) = \frac{Slope\ of\ matrix\ matched\ curve - Slope\ of\ solvent\ curve}{Slope\ of\ solvent\ curve} * 100$$

Table 5: LOD and LOQ data for two N-nitrosamines impurities

Nitrosamine Impurity	LOQ Concentration (in ppm) w.r.t sample	LOQ Avg. Area(n=6)	LOD Concentration (in ppm) w.r.t sample	LOQ Avg. Area (n=3)
NDMA	0.021	16833	0.007	5481
NDEA	0.021	2714	0.007	887

Linearity

The approach was verified for linearity in accordance with ICH recommendations. Prepare five different concentrations and LOQ concentration from stock solution. The peak area of each level is assessed after injection into the chromatographic apparatus. A graph with concentration on the x-axis and peak area on the y-axis issued to compute the correlation coefficient. The linear regression coefficients of determination (r) for two N-nitrosamines were over 0.995 in the corresponding concentration range, which meant a good linearity and suitable for quantitative analysis. The results and correlation graphs were summarized in Table 6 & Fig.3.

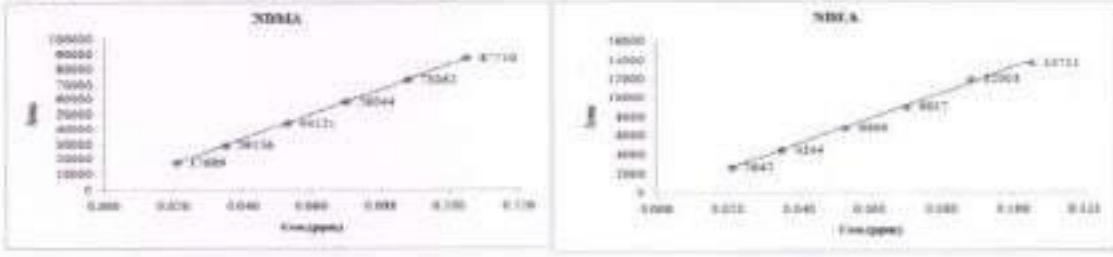

Fig.3: Correlation coefficient graph for two N-nitrosamines impurities

Table 6: Linearity data for two N-nitrosamines impurities

Con.(ppm)	NDMA Avg. area (n=2)	NDEA Avg. area (n=2)
0.021	17689	2642
0.035	29156	4544
0.053	44121	6800
0.07	58044	9037
0.088	73062	12001
0.105	87710	13711
Correlation coefficient (r)	1.000	0.999
SLOPE	831873	134187
Intercept	54	-197

Precision at LOQ

The quantification limit values for two N-nitrosamines impurities were confirmed by precision examination *i.e.*, the determined percent relative standard deviation of the responses of six injections. The relative standard deviation was noticed as ≤ 10%. This confirmed that, HSGC-MS/MS quantification method is very sensitively precise at LOQ level. The corresponding data is summarized in Table 7.

Table 7: LOQ precision data for two N-nitrosamines impurities

No. of Injections	NDMA area	NDEA area
1	16856	2933
2	16236	2599
3	17865	2611
4	15999	2778
5	16456	2498
6	17586	2866
ACVG	16833	2714
STDV	752	171
% RSD	4.47	6.29

Accuracy

The accuracy of the procedure was evaluated by spiking two N-nitrosamines with Omeprazole sodium samples individually at 0.021 ppm (LOQ), 0.035 ppm, 0.07 ppm and 0.105 ppm with respect to sample concentration of 1000 mg/mL. At each level three preparations were done. The results exhibited that the recoveries for NDMA and NDEA in Omeprazole sodium drug substances ranged from 91.69 to 111.31%. The accuracy of the method was demonstrated by the fact that the recovery for each impurity at each level was within 100 ± 15%. The obtained results indicated that the proposed method is accurate for nitrosamine analysis in the proposed Omeprazole sodium drug substances and drug products.

Robustness

It assists to find out the effect of slight variations in the different chromatographic conditions. Robustness of the method was checked by varying the HS oven Temperature 145°C & 155°C and Ion source Temperature 225°C & 235°C. The standard two N-nitrosamine impurities were injected for six times under each changed method parameter. The relative standard deviation was calculated at each varying condition and obtained results (% RSD) were not more than 10%.

Ruggedness

Ruggedness of the method was evaluated by performing the sample analysis in six replicates using different analysts on different days. The % RSD was calculated for day wise and analysts wise as well as individual and cumulative for two N-nitrosamine impurities and the results were obtained within the acceptance criteria (RSD is not more than 10.0%) indicating the method is rugged within the specified range.

Solution stability

Solution stabilities of two N-nitrosamines impurities in diluent solutions were evaluated by preparing specification level (0.07ppm) standard solutions and analyzing them every 6 h, 12 h, 24 h & 48 h against a freshly prepared standard. All the solutions were kept in the dark place at 25°C. Clearly, the percentage variation of these stock solutions was in the range of 100±10% which indicated that these stock solutions were stable for at least 48 h.

Applications in Omeprazole dosage forms

This HSGC-MS/MS analytical method was used to determine two N-nitrosamine GTIs in commercial Omeprazole products, and no N-nitrosamines were found in commercial Omeprazole products. Therefore, the proposed method can be confidently employed for the Omeprazole products containing the pharmaceutical two N-nitrosamines impurities. So, it can be used in the routine quality control of dosage form in Pharma industries.

DISCUSSION

Analytical quality by design (QbD) [23] with HSGC-MS/MS method was developed for quantification of two N-nitrosamine GTIs in Omeprazole sodium drug substances and combined dosage forms. Target profile was retention time, plate count and resolution. The developed method was validated according to ICH guidelines [19]. Different method validation parameters like linearity Co-relations should not less than 0.995. The percentage recoveries at each level are between 85.0% and 115.0%. LOD for NDMA and NDEA is 0.007ppm and LOQ for NDMA and NDEA is 0.0021ppm with respect to Omeprazole sodium sample concentration. The %RSD is obtained less than 10% for system precision, method precision and ruggedness and robustness. As per our proposed HSGC-MSMS method, sample and standard solutions were stable up to 48 hours. And this is method is applied for pharmaceutical dosage forms. So our proposed work is when compared with other literature works, method was found to be novel, simple, sensitive, accurate, precise, economical, and rapid for the estimation of two N-nitrosamine impurities in Omeprazole sodium drug substances and combined dosage forms.

CONCLUSION:

In this work, the HS-GC−MS method was developed and validated for the quantitation of NDMA and NDEA in Omeprazole sodium drug substances and drug products. The headspace injection technique was applied to serve as an online sample preparation in residual impurity analysis, thereby offering benefits concerning the efficient removal of potential interference and High-throughput routine analysis. The complete method validation was performed using the Q2(R1) ICH guidelines [19]. The method should be further fully investigated for its accuracy, precision, and other validation parameters using the acceptable limits of nitrosamines in each substance. The LOQ and LOD of the developed method were far below the US FDA and EMA interim limits of the corresponding nitrosamines in Omeprazole sodium drug substances. The overall results indicated that the developed method is reliable and useful for determining the levels of two nitrosamines in Omeprazole sodium drug substances and drug products.

LIST OF ABBREVIATIONS:

APCI: Atmospheric pressure chemical ionization; HSGC-MS: Head space gas chromatography mass spectroscopy; MRM: Multi reaction mentoring; APIs: Active pharmaceutical ingredients; FDA: Food and Drug Administration; GTI: Genotoxic impurity; EI: Electron ionization; SIM: Selected ion monitoring; ICH: International Council for Harmonization; LOD: Limit of detection; LOQ: Limit of quantification; MRM: Multiple reactions monitoring; NDMA: N-nitrosodimethylamine; NDEA: N-nitrosodiethylamine; DMSO: Dimethyl sulfoxide; RSD: Relative standard deviation; S/N: Signal to noise.

REFERENCES:

[1] Nitrosamines are formed by a reaction between nitrates or nitrites and certain amines (2014).
[2] Encyclopedia of Toxicology (third Ed.).
[3] IARC Monograph (1987). 17(7).
[4] ICH Guidance for Industry M7 (R1) (2018) Assessment and Control of DNA Reactive
[5] (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. March.
[6] The EDQM's response to nitrosamine contamination.
[7] FDA Guidance for industry: Control of Nitrosamine impurities in Human drugs.
[8] US FDA updates and press announcements on NDMA in metformin(2020), announcements.
[9] NDMA- metformin.
[10] Korea Food and Drug Administration(2020). Sartent's Hypertension Drug Impurity Management Standards Disclosure, Establishment of Sartan NDMA, NDEA temporary management standard and test method education.
[11] US FDA Statement (2019-2020) announcements/FDA-requests-removal-all ranitidine-products- Zantac-market.
[12] USFDA (2020), FDA updates and press announcements on angiotensin II receptor blocker (ARB) recalls (valsartan, losartan, and irbesartan) availability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan.
[13] Cristina Iuga, Marius Bojiţa, Sorin c. Ieucuţa (2009). Development of a validated RP-HPLC Method for separation and Determination of process-related Impurities of Omeprazole in bulk drugs. Farmacia, 57(5):534-541.
[14] Gudibanda Chandrasekhar reddy, Pulipaka Shyamala, Rallabhandi Murali Krishna, Kapavarapu maruthi Venkata Narayanarao and Mannem Durga babu(2022). Characterization of Loratadine API and simultaneous quantification of Seven potential genotoxic nitrosamine impurities in single method by LC-MS/MS in Loratadine API and its dosage forms. Asian journal of chemistry, 34(6):1505-1512.
[15] Mannem Durga babu, Surendra babu K, Uma maheswar K (2016). Simultaneous determination of 2-chloro methyl propionate, 1,4-di-bromo butane and para anisic aldehyde in Mebeverine hydrochloride API by gas chromatography-

mass spectrometric with selected-ion-monitoring mode. Asian Journal of pharmaceutical and Clinical Research, 9(2):168-175.

[16] Mannem durga babu, Surendra babu K, Kishore M(2016). Development and validation of a gas chromatography-mass spectrometry with selected ion monitoring method for the determination of trace levels of methane sulfonyl chloride as an impurity in Itraconazole active pharmaceutical ingredient. Asian Journal of pharmaceutical and Clinical Research, 9(5):403-407.

[17] Mannem Durga babu, Medikondu Kishore, K. Surendra babu(2015). Development and validation of a headspace gas Chromatographic method for the determination of Methyl bromide content in Itraconazole API. International Letters of Chemistry, Physics and Astronomy, 14(59):160-169.

[18] H. Rebiereа, Y. Grangeа,b, E. Deconinckb, P. Courselle, J. Acevska, K. Brezovska, J. Maurin, T. Rundlöf, M.J. Portela, L.S. Olsen, C. Offerle, M. Bertran(2021). European fingerprint study on omeprazole drug substances using a multi analytical approach and chemometrics as a tool for the discrimination of manufacturing sources. Journal of Pharmaceutical and Biomedical Analysis, 208:114444.

[19] Aishwarya Dande, Gummadi Rishitha Reddy, Gaddam Saithya (2021). Stability Indicating Method Development and Validation for Assay of Omeprazole Sodium for Injection by a Rapid RP-HPLC. Asian Journal of Pharmaceutical Research and Development, 9(2):18-32.

[20] Samar S. Elbaramawi1 Mohamed E. El-Sadek1 Mohamed M. Baraka Lobna M. Abdel-Aziz Mahmoud M. Sebaiy (2020). Instrumental analysis of some anti-ulcer drugs in different matrices. Chem Rep, 2(1):156-172.

[21] Asma Rahman, Mohammad Rashedul Haque, Md. Zakir Sultan, M. Muhibur Rahman and Mohammad A. Rashid (2017). Enantiomeric Determination of Omeprazole and Esomeprazole by a Developed and Validated Chiral HPLC Method and Stability Studies by Microthermal Analysis. Dhaka Univ. J. Pharm. Sci., 16(2):221-233.

[22] Rockville, USA (1996). Validation of Analytical Procedures Methodology in Proceedings of International Conference on Harmonization. ICH Guidelines, Q2B:1-10.

[23] Thakor NS (2017). Implementing quality by design [QbD] in chromatography. AJAPC 4:1078.

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Study the Impact of Endosulfan Pesticide on behavioral responses in the Fresh Water Fish Labeo Rohita

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ABSTRACT ***Static renewal test was conducted to determine the toxicity of technical grade (91.06% purity) insecticide Endosulfan on the freshwater fish Labeo Rohita. Fishes were exposed to various concentrations of insecticide Endosulfan for 96 hours and the percent mortality was recorded. Behavioral responses and morphological deformities were studies in the experimental periods. Fish in toxic media exhibited irregular erratic and darting swimming movements. The behavioral and morphological changes may be due to the formation of amino acids by degradation of proteins with concentration of pesticide.***

Keywords: Endosulfan, Labeo Rohita, Behavioral changes, Amino acids and Proteins.

INTRODUCTION

Endosulfan is an off patent organo chlorine insecticide and acaricide that is being phased out globally. Endosulfan becomes a highly controversial agrichemicall due to its acute toxicity. Potential for bioaccumulation, and role as an endocrine disruptor, because of its threats to human health and the environment, a global ban on the manufacture and use of Endosulfan was negotiated under the Stockholm convention in April 2011. More than 80 countries including the European Union, Australia, New Zealand several west African nations, the United States, Brazil, and Canada had already banned it or announced phase outs by the item the Stockholm convention ban was agreed upon2. It is still used extensively in India, China, and few other countries. It is produced by Makhteshim Agan and several manufacturers in India and China despite laws against its use. It is also used in few countries. Endosulfan is a derivative of hexachlorocyclopentadiene, and is chemically like aldrin, chlordane and heptachlor and it is obtained by Diels – Alder reaction3. Endosulfan is one of the most toxic pesticides in the market today. It is used in agriculture sector to control insect pests including white fly, aphids, leafhoppers, Colorado potato beetles and cabbage worms. Due to its unique mode of action, it is useful in resistance management; however, as it is not specific, it can negatively impact populations of beneficial insects. It is, however, considered to be moderately toxic to honey bees, and it is less toxic to bees than organophosphate insecticides. Endosulfan is acutely neurotoxic to both insects and mammals, including humans. The US EPA classifies it as Category I: "Highly Acutely Toxic" based on a LD50 value of 30 mg/kg for female rats, while the World Health Organization classifies it as Class II "Moderately Hazardous" based on a rat LD50 of 80 mg/kg. It is a channel antagonist, and a Ca2+, Mg2+ATPaseinhibitor13

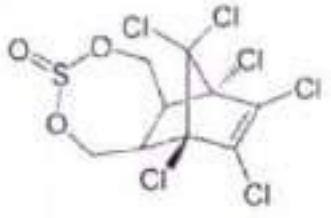

Figure 1. Structure of Endosulfan

A major part of the world's commercial food is being supplied from fish source and it is essential to secure the health of fishes. In India, as much as 70% of the chemical formulations employed in agricultural practices are believed to affect non-

target organisms and to find their way to freshwater bodies ultimately polluting them. Labeo Rohita (rohu) is an herbivorous cyprinid fish that inhabits the tropical lowland river systems of Pakistan, northern India, Nepal, Bangladesh, and Myanmar. Rohu is the most important of the Indian 'major carps' and is the world's 10th highest cultured finfish by production volume. The major producing countries are India, Bangladesh, and Myanmar. Rohu is also a substantial component of stock enhancement programs in floodplains and open waters in these countries, and it has been widely trans located both within and external to its natural range. Its high growth potential, coupled with high consumer preference, have established rohu as the most important freshwater species cultured in India, Bangladesh, and other adjacent countries in the region.

Figure-1a: Fresh water fish Labeo Rohita

MATERIALS AND METHODS

Labeo Rohita finger lings weighing 5+0.5 grams and average length of 7 cm were collected from the Government fish seed form at Ponnuru, Guntur District, Andhra Pradesh, India and acclimatized to laboratory conditions for 7 days in large plastic tubs previously washed with potassium permanganate to free walls from any microbial growth physic chemical characters of water was followed APHA Method4. Acclimatized conditions are temperature 27+ 10c, pH: 6.8+0.05 at 270c and dissolved Oxygen (D.O) 6.9 to 7.4 mg L-1. In actualization time, we have supplied food regularly by commercially available fish feed. For this investigation technical grade Endosulfan (91.06%) was used and brought from Agrochemical Industries Limited Guntur, A.P. India. Pesticide column 250 mm length and 4.8 mm diameter samples filtered through PALL life sciences filter paper 0.45 mic. Membrane 13 mm diameter size. Preparation of sample solution: We have taken the effected organs form pesticide induced fish into boiling test tube and added the pure Hexane (AR) then boiled. After heated fish absorbed pesticide settled at inside of the test tube walls, and then removed the fish organ from the test tube bottom and again added hexane for dissolving the pesticide in the test tube walls. This sample solution is used for the behavioral study of pesticide induced fish by using HPLC analysis. Analysis of Endosulfan by HPLC: Concentrations of Endosulfan in the test medium was confirmed by High-performance liquid chromatography (HPLC) and this method described by Mr. Johnson5. The HPLC analysis was operculated by using a UV detector with a mobile phase. Mobile phase consisting of acetonitrile (20%), water (20%), and methanol (60%) then run though a C18 column with a flow rate of 1.0 ml/ minute and then analyzed the peaks of Endosulfan in sample solutions.

Table 1. Concentration of Endosulfan pesticide observed in fish organs.

Conc. of Pesticide(ml)	Gill(mg/ml)	Brain(mg/ml)	Digestive system(mg/ml)	Liver(mg/ml)	Muscle(mg/ml)	Total observed pesticide(mg/ml)
1.0 ml	0.0433	0.0460	0.0525	0.0280	0.0180	0.1780
2.0 ml	0.0580	0.0470	0.0570	0.0310	0.0154	0.1831
3.0 ml	0.0517	0.0540	0.0588	0.0328	0.0271	0.2068
4.0 ml	0.0580	0.0601	0.0610	0.0329	0.0281	0.2119
5.0 ml	0.0625	0.0642	0.0703	0.0360	0.302	0.2688
6.0 ml	0.0646	0.0690	0.0681	0.0410	0.0309	0.2744
7.0 ml	0.0710	0.0782	0.0685	0.0482	0.0317	0.2716

The protein changes are observed in various tissues after the pesticide induced along with control was graphically represented in Figure 2.

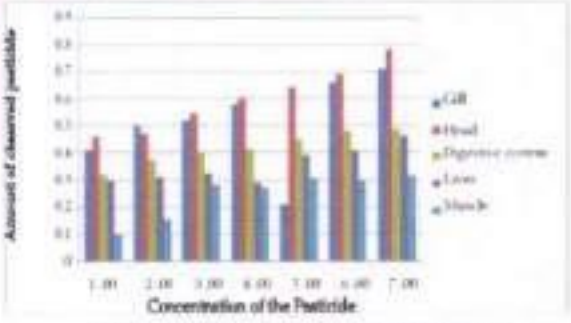

Figure 2. Pesticide absorbance by fish organs in different volumes

RESULTS AND DISCUSSION

Proteins are primary importance of the living world and only because of their peculiar biological specificity among various types and these are responsible for many metabolic changes in total fish organs. These proteins are main energy sources to

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play an important role in the maintenance of blood glucose6 (Fig 2). It is the most fundamental biochemical constituent present abundantly in the body of fish and this result presented in Table 1. The insignificant alteration of proteins at the end of 45 hours and it was observed in the tissues suggesting that the fish tend to resist the sudden stress for shorter duration7. Later with increases of time the decreases of protein content. The significant and maximum depletion was observed in the head and minimum in muscle. However, increases of consumer concentration of the pesticide decreases the protein in tissues of the fish Labeo Rohita the present analysis coincides with the reported data that the protein content in muscle and liver8-12. Formation of Amino Acids: After consuming of the pesticide, proteins were degraded and to form amino acids, then change the sequence of amino acids from original sequence in the isopropyl alcohol medium. In this chapter, we can report the measuring of amino acid quantity after degradation of proteins in fish body by applying the chlorpyrifos pesticide. In this research work, after the consumed the pesticides, proteins are degraded and to form amino acids, then change the sequence of amino acids from original sequence in the isopropyl alcohol medium. In this paper we have report the measuring of amino acids quantity after degradation of proteins in fish body by applying the Endosulfan pesticide in fish. These results are shown in Table 2.

Table 2. Endosulfan pesticide effect on fish body

Conc. of pesticide(mg/lit)	Leucine (mg/lit)	Tyrosine (mg/lit)	Tryptophan (mg/lit)	Valine (mg/lit)	Phenyl alanine(mg/lit)
1.00	24	72	18	62	33
2.00	37	83	17	58	52
3.00	49	94	19	89	67
4.00	92	104	20	83	61
5.00	74	98	19	98	73
6.00	88	118	22	113	94
7.00	113	262	18	123	122

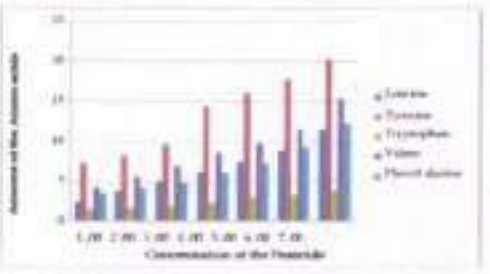

Figure 3. Change in Amino Acids by increasing concentration of Pesticide

Tryptophan amino acid is not affected in protein degradation at low concentrations. But Tyrosine valine, Leucine is degraded at all concentration of Endosulfan pesticide. These results are shown in figure4.

APPLICATIONS

Due to the formation of amino acids by degradation of proteins with concentration of pesticide the behavioral and morphological changes occur in the freshwater fish LabeoRohita.

CONCLUSIONS

The present study proved that the Endosulfan is highly toxic and detrimental impact on the behavioral responses of Labeo Rohita at sub level concentration and any alterations caused by the pesticide may lead to variations of total proteins in fish body. Therefore, the amount of Endosulfan pesticide in the aquatic systems should be monitored then control the usage pesticide because the decreasing nutria valent value and mortality of fish. The histopathological changes in certain vital tissues like head, gill, liver, muscle and digestive system in the fish Labeo Rohita exposed to sub lethal and lethal concentrations of Chlorpyrifos, Cypermethrin and Endosulfan were studied. Endosulfan caused highly marked pathological changes in the gill than liver and digestive system. Endosulfan also caused profound pathological changes under chronic exposures in liver tissues of the fish Labeo Rohita. All these changes indicate the hepatoma toxic nature of Endosulfan. Since Endosulfan is an Chlorinated Hydrocarbon compound, it is neuro poisons.

REFERENCES

[1] "Bayer to stop selling Endosalfan" Australian Broadcasting corporation, July 17, 2009.

[2] The Hindu.com / News / National / article 198299 ece.

ISSN:-2349-3585 |www.ijrdt.org

[3] "Endosulfan Supreme court to hear seeking ban on Monday" The Hindu (Chennai, India) 1 May 2011.

[4] Agency of Toxic substances and Disease Registry. Toxicological profile for Endosulfan, 2000.

[5] Cone, Morla. EPA Bans pesticide found on cucumbers, Zucchini, Green Beans and other vegetables the Daily Green. June 10, 2010.

[6] P.B Bhushan. K.M Singh. M. Rani. Nature Environ. Poll Technol2002.1 (2). 147-150

[7] H.M Jruger. J.B.Saddler. G.A Chapman. I.J. Timely. R.R.Lowry. American Zool.1968 .8. 19

[8] F.E.J. Frey 1971 in: Fish physiology. Eds. W.S. Hoar and D.J. Randall. Academic press. New York. 6.1Journal of Ecotoxicology and Environmental monitoring. 2009.19.

[9] S. Durairaj. V.R.J.Selvarajan. Environ. Biol.1992. 13(3). 181-185.

[10] A.Anusha Amali. L.Ciryl Arun Kumar. F.X. Elizabeth Jayanthi. M.J.Selvanayagam Environ. Biol1996. 17(2. 121 – 124)

[11] S.G. Yeragi. S. Koli Vand Yeragi. J. Ecotoxical. Monit2000. 10(1). 59-62)

[12] K.S. Tilak. K.Veeraiah. D.K.Koteswara Rao. J. Environ. Biol2005. 26 (2Suppl). 341 – 347.

[13] P. Srinivasa Rao. N. Bujji babu. R Ramesh Raju. IJRPB. 2010. Vol.1. Issue 1. 1-5.

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FORM 2

THE PATENT ACT, 1970

(39 OF 1970)

&

THE PATENT RULES, 2003

COMPLETE SPECIFICATION

[SEE SECTION 10 AND RULE 13]

TITLE: COMPOSITION AND METHOD FOR THE PREPARATION OF NANOPARTICLES WITH CONTROLLED RELEASE PROPERTIES FOR DRUG DELIVERY APPLICATIONS	
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COMPOSITION AND METHOD FOR THE PREPARATION OF NANOPARTICLES WITH CONTROLLED RELEASE PROPERTIES FOR DRUG DELIVERY APPLICATIONS

FIELD OF THE INVENTION

The present invention generally relates to drug delivery applications. More specifically, the invention relates to composition and method for the preparation of nanoparticles with controlled release properties for drug delivery applications.

BACKGROUND OF THE INVENTION

The field of drug delivery has witnessed significant advancements aimed at enhancing therapeutic efficacy and patient compliance. One promising approach involves the use of nanoparticles as carriers for controlled release of therapeutic agents. These nanoparticles provide numerous benefits, such as improved drug stability, targeted delivery, and prolonged release kinetics.

Traditional drug delivery systems often suffer from limitations such as rapid drug degradation, non-specific distribution, and insufficient drug concentrations at the desired site of action. To overcome these challenges, researchers have explored the use of biocompatible polymers to encapsulate therapeutic agents within nanoparticles. This approach offers several advantages, including protection of the drug from enzymatic degradation, controlled release kinetics, and enhanced bioavailability.

Poly(lactic-co-glycolic acid) (PLGA) is a commonly employed biocompatible polymer in nanoparticle-based drug delivery systems. PLGA possesses desirable properties, such as biodegradability, biocompatibility, and tunable release kinetics. By adjusting the ratio of lactic acid and glycolic acid units in the polymer, the degradation rate and release profile of encapsulated drugs can be precisely controlled.

would offer precise control over the release kinetics, enable targeted delivery to specific cells or tissues, and improve therapeutic outcomes.

In light of these considerations, the present invention provides a novel composition and method for the preparation of nanoparticles with controlled release properties. The invention addresses the limitations of existing drug delivery systems, offering an innovative approach to optimize drug delivery for various applications, including oral, intravenous, transdermal, and inhalation routes.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: Schematic diagram of the pulse-heat aerosol reactor (PHAR) system used for synthesis of nanoparticle matrices with controlled properties comprised a pulse-heating zone followed by a perforated diluter and an isoaxial sampler. The key variables in aerosol reactor design were maintaining laminar flow of the aerosol and minimizing particle losses by diffusion and sedimentation and provision for imposing pulse-heat with alternate heating and quenching by dilution air.

FIG. 2: Number particle size distributions of nanoparticle aerosol lipid matrices synthesized using stearic acid in cyclohexane solutions at gas temperature of 298 K and 383 K (pulse), of varying concentrations, (a) 0.01 mg·cm−3, (b) 0.1 mg·cm−3, (c) 1 mg·cm−3 and (d) 10 mg·cm−3, measured using scanning mobility particle sizer. The mobility diameters ranged from 47-183 nm with a unimodal distribution and geometric standard deviation of 1.5-1.8.

DETAILED DESCRIPTION OF THE INVENTION

The present invention discloses a composition and method for the preparation of nanoparticles with controlled release properties for drug delivery applications. The composition comprises biocompatible polymer matrix nanoparticles encapsulating therapeutic agents, while the method involves a series of steps to achieve efficient and targeted drug delivery.

centrifugation, filtration, or dialysis, ensuring the production of high-quality nanoparticles suitable for drug delivery applications.

In addition to the core steps, the method can also involve optional modifications to the surface of the nanoparticles. This can be achieved by attaching targeting ligands to enhance the specificity of drug delivery. Targeting ligands can include antibodies, peptides, aptamers, or other molecules that recognize specific receptors or markers expressed on target cells or tissues.

The resulting nanoparticles exhibit controlled release properties, allowing for sustained drug delivery over a predetermined period of time. The release kinetics can be precisely controlled by adjusting the properties of the polymer matrix, including its molecular weight, composition, and degradation rate. The composition and method provide versatility in tailoring the release profile to match the therapeutic needs of different drugs and diseases.

The nanoparticles prepared using this composition and method can be formulated into a drug delivery system by incorporating them into a pharmaceutically acceptable carrier. Such carriers can include gels, creams, or injectable formulations, depending on the intended route of administration and desired application.

The following specific example is not intended to be limitive but only illustrative.

FIG. 1, a prototype pulse-heat aerosol reactor (PHAR) was designed and fabricated for control of particle properties through aerosol dynamics. The pulse-heating zone has an internal diameter and heated length of 38 mm and 80 mm, respectively. The aerosol flow rate was fixed at 3 L·min-1, with a flow Reynolds number of ~109 and a pulse time of one second. Stokes number for atomized droplets (mean diameter 300 nm) flowing in the reactor is in the order of 10−5, implying that droplets follow gas streamlines and do not undergo impaction. Evaporating droplets flowing along with the gas streamlines are expected to undergo negligible drop breakup, internal solute circulation/motion and asymmetric solute concentration distribution. Thus the droplet evaporation process is expected to be uniform leading to isotropic particle properties. In the prototype PHAR, heating was provided to the pulse-heat zone to attain a gas temperature of (low and high 383±1 K) using heating tape of 250 W. Gas temperature in the pulse-heat zone was measured using a platinum resistance temperature detector (RTD, PT100) interfaced with a digital controller. The magnitude of temperature was fixed based on the required evaporation rate to produce particles of

proteins, peptides, nucleic acids, vaccines, antibiotics) for treatment of various diseases and disorders.

Example Illustrating the Disclosure

Experiments were done to produce stearic acid nanoparticles, in PHAR, with controlled size and morphology at varying evaporation rates. The pulse-heat aerosol reactor (PHAR) system used to study effect of pulse-heat on synthesis of nanoparticle matrices (FIG. 4) comprises of a collision-type air jet atomizer. The atomization device could also comprise of any device based on ultrasonic, electro spray, evaporation-condensation or FEAG principle of aerosol generation. The PHAR is designed with a pulse-heat zone, wherein a heat pulse of controlled temperature (heating element) and duration (flowrate of gas) is applied to the droplet aerosol to control the rate of evaporation. A perforated-wall aerosol diluter is provided to quench the temperature and aerosol dynamics mechanisms immediately after the pulse-heating. A scanning mobility particle sizer was placed downstream for measurement of mobility diameter. Any other nanoparticle size distribution measurement device including, ELPI, hypersonic impactor can be used in-lieu of or in addition to the SMPS. The standard upstream pressure of the atomizer was 35 psig. The solution, of lipid in a selected organic solvent (stearic acid in cyclohexane of varying concentrations, 0.01 mg·cm−3, 0.1 mg·cm−3, 1 mg·cm−3 and 10 mg·cm−3), was fed with a syringe pump at a flow rate of 0.6 mL/min. The resulting atomized droplets were suspended in a nitrogen flow through the PHAR, where droplet evaporation at a controlled rate, followed by quenching of aerosol dynamics was used to produce nanoparticles with controlled size, morphology and crystallinity.

Stearic acid nanoparticle matrices of mobility diameters of 47-183 nm, with a unimodal size distribution of geometric standard deviations (GSD) (1.5-1.8), were obtained in PHAR by fixing the gas temperatures, at 298 K and 383 K (pulse), to obtain the varying evaporation rates. For a given concentration, stearic acid nanoparticles of smaller mobility diameters were synthesized at lower evaporation rates, while nanoparticles with larger mobility diameters were synthesized at higher evaporation rates. The differences in the mean mobility diameters of stearic acid nanoparticles synthesized at higher evaporation rates, using larger concentrations (1 mg·cm −3 and 10 mg·cm−3), were statistically significant (at the 95% confidence level; P=0.002, by t-test) than those synthesized at lower evaporation rates (Table 1). TEM images of nanoparticle matrices (FIG. 3 a), synthesized at 298 K using stearic acid in cyclohexane solution of 10 mg·cm−3, showed

I/WE CLAIM:

1. A composition for drug delivery comprising:
    - Nanoparticles comprising a biocompatible polymer matrix; and
    - A therapeutic agent encapsulated within said nanoparticles.

2. The composition of claim 1, wherein said biocompatible polymer matrix comprises poly (lactic-co-glycolic acid) (PLGA).

3. The composition of claim 1, wherein said therapeutic agent is selected from the group consisting of small molecules, proteins, peptides, nucleic acids, and combinations thereof.

4. A method for preparing nanoparticles with controlled release properties, comprising the steps of: a. Dissolving a biocompatible polymer in an organic solvent to form a polymer solution; b. Adding a therapeutic agent to said polymer solution to form a drug-polymer solution; c. Emulsifying said drug-polymer solution in an aqueous phase to form an emulsion; d. Removing the organic solvent from said emulsion to obtain nanoparticles with controlled release properties; and e. Collecting and purifying said nanoparticles.

5. The method of claim 4, wherein said organic solvent is selected from the group consisting of dichloromethane, chloroform, ethyl acetate, and combinations thereof.

6. The method of claim 4, wherein said aqueous phase comprises a surfactant to stabilize the emulsion.

7. The method of claim 4, further comprising the step of modifying the surface of said nanoparticles with a targeting ligand.

COMPOSITION AND METHOD FOR THE PREPARATION OF NANOPARTICLES WITH CONTROLLED RELEASE PROPERTIES FOR DRUG DELIVERY APPLICATIONS

ABSTRACT

The present invention relates to a composition and method for the preparation of nanoparticles with controlled release properties for drug delivery applications. The composition comprises biocompatible polymer matrix nanoparticles encapsulating therapeutic agents, providing a platform for efficient and targeted drug delivery. The biocompatible polymer matrix, such as poly (lactic-co-glycolic acid) (PLGA), ensures biocompatibility and controlled release of the therapeutic agents. The therapeutic agents can include small molecules, proteins, peptides, nucleic acids, or combinations thereof. The method involves dissolving the biocompatible polymer in an organic solvent to form a polymer solution, followed by adding the therapeutic agent to obtain a drug-polymer solution. This solution is then emulsified in an aqueous phase, forming an emulsion. Subsequently, the organic solvent is removed from the emulsion, resulting in the formation of nanoparticles with controlled release properties. The nanoparticles are collected, purified, and optionally modified with targeting ligands for enhanced specificity.

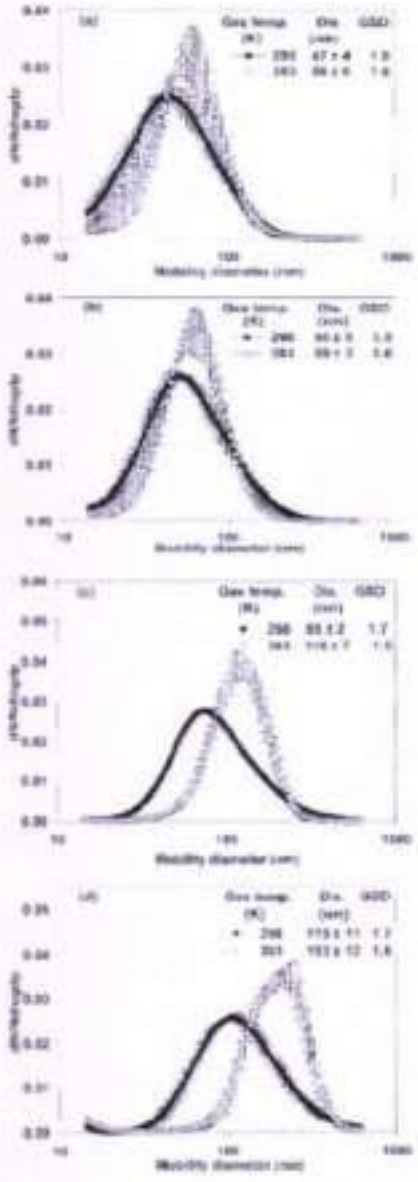

FIG. 2

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(51) International classification :B82Y 100000, B82Y 200000, H01G 092000, H01L 310320, H01L 310352
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(57) Abstract :
METHOD FOR THE SYNTHESIS OF QUANTUM DOTS WITH TUNABLE OPTICAL PROPERTIES FOR USE IN PHOTOVOLTAIC DEVICES ABSTRACT The present invention discloses a method for the synthesis of quantum dots with tunable optical properties for use in photovoltaic devices. The method involves the controlled growth of quantum dots by manipulating reaction conditions and adjusting parameters to achieve desired sizes, compositions, and optical characteristics. The synthesized quantum dots offer tunability in terms of bandgap, absorption, and emission wavelengths, making them suitable for efficient light absorption and power conversion in photovoltaic devices. The method begins by providing a precursor solution containing semiconductor materials and capping ligands. The reaction conditions, including temperature, pressure, and reaction time, are carefully controlled to promote the growth of quantum dots with specific properties. The size of the quantum dots is controlled by adjusting the concentration of the precursor solution and the reaction time, while the composition is manipulated by varying the stoichiometry of the semiconductor materials.

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(57) Abstract :
The present invention relates to the synthesis and application of copper manganese doped nickel oxide nanoparticles as a versatile agent for antimicrobial and anticancer treatment. The nanoparticles are carefully synthesized with controlled sizes and compositions. They exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and viruses, while selectively inducing cytotoxicity in cancer cells. The nanoparticles can be functionalized with targeting ligands for specific delivery to tumor sites, enhancing their efficacy in inhibiting tumor growth. Coating medical devices with these nanoparticles reduces the risk of device-associated infections. Additionally, the nanoparticles can be used in pharmaceutical compositions for combined antimicrobial and anticancer therapy.

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(57) Abstract :
7. ABSTRACT This research delves into the evaluation and enhancement of a Fe3O4 magnetic nanocomposite tailored for the effective removal of heavy metals from diverse aqueous solutions. The significance of this work lies in its direct application to environmental and industrial contexts, where heavy metal contamination poses a severe threat. The study scrutinizes the nanocomposite's resilience under various environmental conditions, encompassing shifts in pH levels, ionic strengths, and temperature ranges, all of which are pivotal in real-world applications. Moreover, it delves into the fine-tuning of its characteristics, focusing on optimizing its heavy metal adsorption capacity and magnetic behavior for efficient post-capture separation. The culmination of this research holds great promise for addressing critical challenges in heavy metal remediation, offering a sustainable, efficient, and customizable solution with substantial benefits for environmental and human well-being.

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(57) Abstract :
7. ABSTRACT The invention pertains to the synthesis and characterization of a chitosan stabilized Fe3O4 magnetic nanocomposite. This nanocomposite combines the magnetic properties of Fe3O4 nanoparticles with the biocompatibility and stability of chitosan. The synthesis involves coating Fe3O4 nanoparticles with chitosan, crosslinking with glutaraldehyde, and thorough characterization using techniques such as TEM, XRD, FTIR, and TGA. The resulting nanocomposite exhibits a core-shell structure with a chitosan shell, making it suitable for a broad spectrum of applications. It finds utility in drug delivery, serving as a magnetic resonance imaging (MRI) contrast agent, and as an efficient tool for environmental remediation by separating pollutants from fluid media. The nanocomposite's advantages include biocompatibility, stability, and versatility. Experimental results confirm its potential for diverse applications, such as controlled drug delivery and environmental cleanup.

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SECURITY THREATS AND MEASURES AND HOW TO OVERCOME FROM IN SUPERIOR CLOUD

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Abstract—**Cloud computing is a highly popular technology known for its cost-effectiveness, reliability, rapid access, flexibility, and scalability for computer operations. The IT industry has witnessed significant growth in cloud computing adoption. However, IT organizations have raised concerns regarding security in cloud computing due to the outsourcing of essential services to third parties, making it challenging to maintain data security and confidentiality. This paper offers insights into security threats associated with cloud computing and suggests potential countermeasures.**

Keywords—Cloud Computing, Security, Threats, Confidentiality.

Introduction

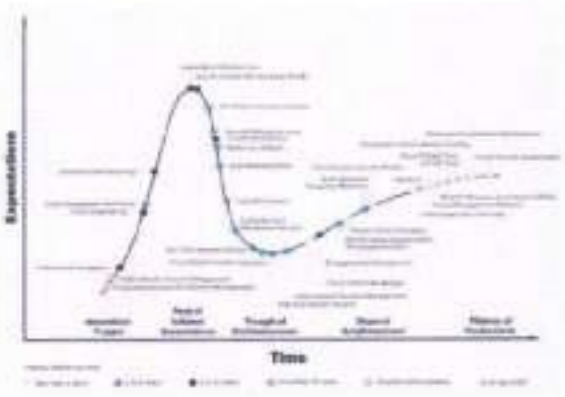
Cloud computing offers a range of computing services, including servers, storage, databases, networking, software, and analytics, delivered over the internet. It functions as a network for storing and sharing resources. In the cloud, you pay only for the services you use, such as storage on a pay-as-you-go basis, reducing operating costs and improving organizational efficiency. In cloud environments, multiple virtual machines can coexist on the same physical server infrastructure.

he main concerns of many IT organizations revolve around areas like external data storage, reliance on the public internet, limited control, multi-tenancy, and integration with internal security systems. Cloud computing differs from outdated technologies due to its vast scale and complete distribution and visualization of resources. To ensure security in the cloud, it is crucial to implement mechanisms like robust authentication, confidentiality for personal information, and measures to prevent data loss.

1.1 Virtualization:

Cloud computing relies on virtualization, where various virtual machines can operate with different operating systems and run multiple applications while sharing a single underlying physical computer. This approach prevents cloud vendors from the burden of providing individual physical resources to each customer. Instead, it enables efficient resource utilization in the cloud, making virtualization a smart choice.

Fig. 1. Hyper Cycle for Cloud Security, 2020.



1.2 Types of Cloud Environments:

Public, Private, Hybrid and Community clouds.
Public Cloud: A public cloud is standard model which providers make several resources, such as applications and storage, available to the public. Public cloud services may be free or not.

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Private Cloud: A private cloud refers to cloud computing resources used exclusively by a single business or organization. A private cloud can be physically located on the company's on-site data center. Some companies pay to third-party service providers to host their private cloud. A private cloud is one in which the services and infrastructure are maintained on a private network [5].

Hybrid Cloud: Hybrid cloud is a combination of both public and private cloud that provides and controls some resources internally and has some others for public use.

Community Cloud: The cloud infrastructure is shared among organizations that share the same concerns such as the mission, security requirement and policy. It may own by more organization and it exists on premises or even off-premises.

II.related work

2.1 Types of Cloud Services:

Most cloud computing services fall into three broad categories: Infrastructure as a service (IaaS), Platform as a service (PaaS), and Software as a service (SaaS).

1. Infrastructure as a Service (IaaS): This is the fundamental category of cloud computing services. IaaS allows you to lease IT infrastructure, including servers, virtual machines (VMs), storage, networks, and operating systems, from a cloud provider. You pay for these resources on a usage basis.

2. Platform as a Service (PaaS): PaaS is a cloud computing service that offers an on-demand environment for developing, testing, deploying, and managing software applications. It simplifies the development process by handling the underlying infrastructure, including servers, storage, networks, and databases, allowing developers to focus on creating websites and mobile apps.

3. Software as a Service (SaaS): SaaS is a method of delivering software applications via the internet, where users access and pay for the software, they use on-demand. In the SaaS model, the cloud provider hosts and manages both the software application and its underlying infrastructure. This includes tasks like software updates and security patches. Users can connect to the application through a web browser on various devices [5].

In the SaaS model, the responsibility for security primarily falls on the cloud provider because of the high level of integration and limited customer control. In contrast, the PaaS model offers more extensibility and greater control to the customer [1].

2.2 Issues in Service Models:

Software as a Service Security Issues (SaaS): Clients relying on service providers must trust them to implement adequate security measures. The provider's responsibility includes ensuring that multiple users cannot access each other's data, which is crucial for user data privacy. Therefore, it's essential for users to verify that appropriate security measures are in place. However, guaranteeing the availability of the application when needed can be challenging.

SaaS software vendors have two common deployment options. They may host the application on their private servers or utilize a third-party cloud computing infrastructure service (e.g., Amazon, Google, etc.) for deployment [3].

Platform-as-a-Service Security Issues (PaaS): In the PaaS environment, data must be accessed, modified and stored. This means data will require decryption and re-encryption, thus introducing key management issues. Encryption challenges are far from the only security issue with PaaS.

Infrastructure-as-a-Service (IaaS) Security Issues: IaaS provides a pool of resources such as servers, storage, networks, and other computing resources in the form of virtualized systems, which are accessed through the Internet [4]. Users are entitled to run any software with full control and management on the resources allocated to them. With IaaS, cloud users have better control over the security

compared to the other models as long there is no security hole in the virtual machine monitor. They control the software running in their virtual machines, and they are responsible to configure security policies correctly.

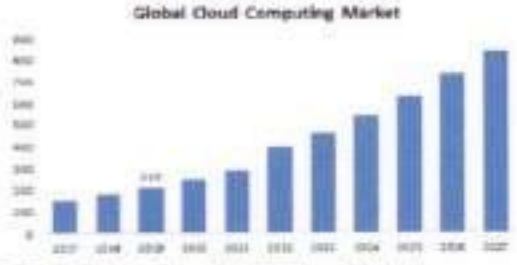

Fig. 2. Global Cloud Computing Market.

2.3 Common Security Threats in Cloud Computing:

Here are descriptions of various cybersecurity threats in cloud computing:

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Threats	Description
Third-party Relationships	If a third party fails to maintain the proper coverage and an uncovered event or situation occurs, your organization may face additional risk
Malicious Code Injection	Any code in any part of a software system or script that is intended to cause undesired effects, security breaches or damage to a system. Malicious code is an application security threat that cannot be efficiently controlled by conventional antivirus software alone.
Insecure permissions on Cloud Data	Giving permissions to the unauthenticated & unauthorized users may compromise the data security

Table 2. Types of possible attacks in PaaS

Threats	Description
Data Leaks	Data in the cloud is exposed to the same threats while it is being transferred, stored, audited or processed.
Account Hijacking	Account of User or an Organization is hijacked by Hacker/Attacker gains access to a user's credential. Then the hacker has full authority to perform Unauthorized Activities.
Vulnerabilities in Virtual Machine	Possible covert channels in the colocation of VMs, Unrestricted allocation and deallocation of resources with VMs
Denial of Service Attack	It is a type of attack that tries to make a website or network resource unavailable.

Table 3. Types of possible IaaS

1. Account Hijacking: Account or service hijacking is a type of identity theft that's on the rise. Attackers aim to deceive end users by taking control of their accounts or services.

Threats	Description
Data Access Risk	Data access issue is mainly related to security policies provided to the users while accessing the data. Some of the employees are not given access to certain amount of data.
Identity Theft	Identity theft is the act of a person obtaining information illegally about someone else. They try to find such information as full name, maiden name, address, date of birth, social security number, passwords, phone number, e-mail, and credit card numbers.
Network Security	All data flow over the network needs to be secured in order to prevent leakage of sensitive information. This involves the use of strong network traffic encryption techniques such as SSL and TLS for security
SQL Injection	An unauthorized user tries to access the confidential data in the database by injecting malicious code in to a standard SQL code. This can be reduced by using automatic generated SQL code. And does not allow unauthorized users to enter the database to access the private data.
Phishing Attacks	Phishing" refers to an attempt to steal sensitive information, typically in the form of usernames, passwords, credit card numbers, bank account information or other important data in order to utilize or sell the stolen information.

Table 1. Types of possible attacks in SaaS

2. Malware Injection: In this attack, malicious actors attempt to inject malware or malicious services into the cloud. They create their own malicious service modules or virtual machine instances, trying to make them appear as valid services. If successful, legitimate users are redirected to the malicious service, allowing the attacker's code to execute.

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3.Denial of Service (DoS) Attacks: Dos attacks are aimed at making a website or network resource unavailable. Attackers flood the target with a large number of packets in a short time, overwhelming the host and causing it to ignore legitimate requests.

4. Data Breach: Data breaches involve unauthorized access, theft, or use of data. Such breaches have become increasingly common and can have severe consequences.

5. Man-in-the-Middle (MITM) Attack: In MITM attacks, intruders intercept or modify messages between two entities that believe they are communicating directly. This exploit often relies on network packet sniffing and can compromise the security of transactions and data transfers.

6. Side Channel Attacks: Attackers try to place a malicious virtual machine on the same host as the target, using side channel attacks to decode ciphertext and calculate encryption keys. Strong firewall systems can help prevent this.

7. Malicious Insider: Malicious insiders are individuals within an organization who intentionally misuse confidential data for personal gain, potentially compromising the organization's integrity. Privilege planning and security auditing can mitigate this risk.

8. Insecure API: Poorly designed user interfaces (UIs) and APIs can provide hackers with easy access to sensitive data, leading to significant financial, reputational, and business losses.

These threats underscore the importance of robust security measures and practices to protect cloud computing environments and the data they contain.

Proposed work

3.1 Measures for Major Threats in Cloud Computing

Here are some key security practices for maintaining security in a cloud environment:

1.Up-to-Date Intrusion Detection System: Employ an intrusion detection system that can identify unusual network traffic and provide early warnings based on credentials and behavioral patterns. This system acts as an alarm for potential security breaches. Blocking IP addresses that are the source of an attack can help mitigate threats.

2.Select a Reliable Cloud Provider: Choose a reputable and trustworthy cloud service provider that prioritizes security and compliance.

3.Patching and Updating: Keep software and systems up to date by promptly applying security patches and updates as they become available. This helps protect against known vulnerabilities.

4.Data Encryption: Implement robust encryption mechanisms to safeguard data both at rest and during transit. Cloud encryption involves transforming data using mathematical algorithms to conceal it from unauthorized access.

5.User Behavior Monitoring: Continuously monitor the behavior of users with access to the cloud environment. Maintain control over encryption processes and keys, segregate duties, and restrict access to minimize potential risks.

6.Proper Password Management: Enforce strong password management practices, including regular password changes and the use of complex, unique passwords.

7.Access Restrictions: Restrict access to cloud services to authorized users only. Implement multi-factor authentication (MFA) systems, such as smart cards, one-time passwords (OTP), and phone authentication, to enhance security.

8.Use of First-Class APIs: Pay attention to abnormal activities and conduct regular audits. Implement strong protection measures to secure API endpoints, as these are often targeted by attackers.

By following these security best practices, organizations can enhance the security of their cloud environments and reduce the risk of data breaches and unauthorized access.

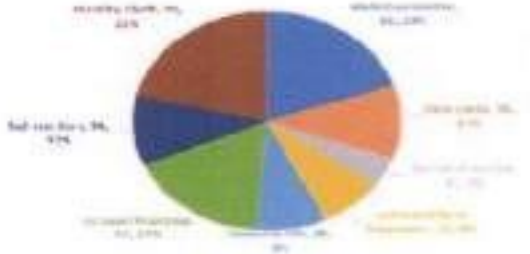

Fig. 3. Possibility of Threat after implementing Security Measures.

IV. Conclusion & future work

The use of cloud computing has become the norm for businesses as well as people in society. Its growth has been seen mainly in the past decade and will continue

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to evolve. As rapid growth in cloud computing era the security for private data is also essential, should be given proper authentication.

This paper surveyed the key security issues of Cloud Computing being faced today and the challenges and opportunities that it brings for business community . This research paper analyzed what exactly cloud computing security-related issues are, and discussed data security and privacy protection issues associated with cloud computing across all stages of data life cycle.

By this paper we know various threats in the cloud computing and some measures are proposed to reduce the risks and threats in cloud computing .The cloud still has much to improve on with security and ease of integration, but cloud computing will continue to grow and advance the ability to share and store data in the technological world.

Threats	Impact (High -2, Low -1)	Risk Percentage before Measures	Risk Percentage after Measures
Malicious Insider	2	65	20
Data Leaks	2	36	11
Denial of Service	1	15	5
vulnerability in Hypervisor	2	25	8
Insecure	2	28	8
Account Hijacking	2	57	17
SQL Injection	2	34	10
Identity	2	70	21

Table 4. Reduction in vulnerability after implementing Securing Methods.

VIII. References

[1] Keiko Hashizume et.al, An analysis of security issues for cloud computing ,Journal of Internet Services and Applications volume 4,

[2] Priyanka SP et.al, A Review Paper on Security in Cloud Computing, IJERT,ISSN:2278-0181

[3] C. Lakshmi Devi, Security issues in SaaS of cloud computing , International Journal of Scientific & Engineering Research Volume 8, Issue 5, May-2017 35 ISSN 2229-5518.

[4] Morsy MA, Grundy J, Müller I: An analysis of the Cloud Computing Security problem. In Proceedings of APSEC 2010 Cloud Workshop. Sydney, Australia: APSEC; 2010.

[5] Bader Alouffi et.al, A Systematic Literature Review on Cloud Computing Security: Threats and Mitigation Strategies , IEEE , Volume: 9.