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Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

Vasco Azevedo, Editor-in-Chief
Integrative Omics and Applied Biotechnology
(IIOAB) Journal

Prof. Vasco Azevedo
Federal University of Minas Gerais
Brazil

Editor-in-Chief

Integrative Omics and Applied Biotechnology (IIOAB) Journal Editorial Board:

ARTICLE

EFFECT OF POLYMER BASED SURFACE COATING ON DROPLET SIZE AND POTENTIAL PHARMACOLOGICAL PROPERTIES OF NEEM OIL NANOEMULSION

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ABSTRACT

Nano emulsion preparations of various phytochemicals are gained increased attention in drug delivery and agriculture than the micro emulsion due to their improved activity, high stability and biocompatibility. In the present study, neem oil based nano emeulsion was prepared under optimum condition by changing oil and surfactant ratio which yield decreased droplet sized particles was further coated with biocompatible polymer chitosan. Distinct effect on droplet size was recorded in chitosan coated neem oil Nano emulsion at the increased concentration of oil; surfactant ratio and increased time of sonication. Anti-bacterial activity was studied against human pathogenic bacterial strains Staphylococcus aureus and Pseudomonas aeruginosa adopting agar cup method and turbidometric method. Chitosan coated neem oil Nano emulsion showed improved spectrum of anti-bacterial activity against tested bacterial strains by showing increase in zone of inhibition and remarkable decrease in optical density under agar cup method and turibometric growth inhibition assay. Minimum Inhibitory concentration (MIC) and Minimum bacteriacidal concentration (MBC) also supported effective anti-bacterial activity by showing least concentration for the growth inhibition than the free Nano emulsion. Biocompatibility was done by determination of cell viability of Vero cells by MTT assay and haemo compatibility study against human peripheral blood cells which reveals less cytotoxicity against Vero cells and blood cells. Distinct effect on the viability was not affected. Further study will helpful to develop polymer based Nano emulsion as an effective pharmacological agent to prevent life threatening diseases.

KEY WORDS
Neem oil, nanoemulsion, Namasivayam, chitosan, droplet size, Professor phytochemical, antibacterial

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INTRODUCTION

Nano emulsion is defined as oil-in-water (o/w) emulsions with mean droplet diameters ranging from 50 to 1000 nm. Usually, the average droplet size is between 100 and 500 nm. Nano emulsions can be prepared using the spontaneous emulsification mechanism which occurs when an organic phase and an aqueous phase are mixed. The organic phase is a homogeneous solution of oil, lipophilic surfactant and water–miscible solvent, the aqueous phase consists on hydrophilic surfactant and water [1,2] Unlike micro emulsions (which are also transparent or translucent and thermodynamically stable) Nano-emulsions are only kinetically stable.

Nano emulsions hold great promise as useful dispersions of deformable Nano scale droplets that can have flow properties ranging from liquid to highly solid and optical properties ranging from opaque to nearly transparent. Moreover, it is very likely that Nano emulsions will play an increasingly important role commercially, since they can typically be formulated using significantly less surfactant than is required for nanostructured lyotropic micro emulsion phases [3]. Unlike micro emulsions (which require a high surfactant concentration, usually about 20% and higher), Nano-emulsions can be prepared using lower surfactant concentration, a surfactant concentration comprised between 3–10% may be enough. The small size of the droplets for cutaneous use allows them to deposit uniformly on skin [4].

Nano-emulsions are suitable for efficient delivery of active ingredients through the skin. The large surface area of the emulsion system, the low surface tension of the whole system and the low interfacial tension of the O/W droplets allow enhancing penetration of actives agents. Due to their small size, Nano-emulsions can penetrate through the “rough” skin surface and this enhances penetration of actives [5,6]. The fluidity nature of the system (at low oil concentrations) as well as the absence of any thickeners may give them a pleasant aesthetic character and skin feel. Nano-emulsions can be applied for delivery of fragrance, which may be incorporated in many personal care products. This could also be applied in perfumes, which are desirable to be formulated alcohol free [7].Nano-emulsions may be applied as a substitute for liposomes and vesicles (which are much less stable) and it is possible in some cases to build lamellar liquid crystalline phases around the Nano-emulsion droplets. Nano-emulsions constitute the primary step in Nano capsules and Nano spheres synthesis using Nano precipitation and the interfacial polycondensation combined with spontaneous emulsification [8].These two techniques require the spontaneous emulsification step in the same optimize conditions. The droplets size and size distribution are depending on the spontaneity of emulsification. The spontaneity of the emulsification is poorly defined, since it should account not only for the rate of the emulsification process, but also for the volume and the particle size distribution of the produced emulsion [9].In the present study, neem oil Nano emulsion stabilized with chitosan was prepared and the prepared Nano emulsion was evaluated against droplet size and anti-bacterial activity has been carried out.

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MATERIALS AND METHODS

Preparation of free neem oil nano emulsion

Initially, free neem oil Nano emulsion was prepared by emulsification process by changing the concentration of oil, water and surfactant ratio. Tween 80 (Hi media, India)was used as the surfactant The reaction mixture contains oil, surfactant and distilled water with the ratios of 1:1.5, 1:2.5, 1:5, 1:7.5,Reaction mixture thus obtained was kept under ultra-sonication for 15, 30, 45, 60 minutes. Droplet size was studied after every treatment using DLSI device.

Preparation of polymer coated nano emulsion

Chitosan was used in the study as the stabilizer. Chitosan (extra pure) was obtained from SRL, India Chitosan (0.5%) dissolved in distilled water containing 1% acetic acid. Dissolved chitosan suspension was kept under magnetic stirrer for 2 hours. After stirring, one ml of homogenized chitosan solution was added to the respective reaction mixture (prepared in the respective ratio), kept for sonication at different time periods as described earlier.

Evaluation of biological activities

Anti-bacterial activity

Anti-bacterial activity of Nano emulsion was studied against human pathogenic bacterial strains *Pseudomonas aeuroginosa* and *Staphylococcus aureus* adopting well diffusion assay. Both the strains were obtained from Microbial type culture collection (ATCC) and maintained on Tryptic soy agar (TSA) slants. A loopful of slant culture was inoculated into tryptic soy broth and incubated at 37°C for 12-16 hrs.to reach mild log phase. The respective broth culture was uniformly spread with sterile cotton swabs on sterile Mueller Hinton (MH) Agar Media (Hi-media, India). The wells were made using cork borer and aliquots (50 and 100 µl) was loaded into the wells. The plates were incubated at 37°C for 24 hrs.

Determination of Minimum Inhibition Concentration (MIC)

Anti-bacterial activity was also studied by determination of minimum inhibitory concentration micro dilution calorimetric assay using the chromogenic reagent 3-(4, 5-dimethyl thiazol-2-yl)-2-5-dephenyl tetrazolium bromide (MTT) concentration [10]. Minimum inhibitory concentration (MIC) value was defined as the lowest sample concentration that inhibited visible growth of the test bacterium, as indicated by MTT straining. Only living microorganisms can convert MTT to formaldehyde and a blue color appear.

Biocompatibility studies

Biocompatibility of chitosan coated neem Nano emulsion was studied by determination of cytotoxic effect on Vero cell line by MTT assay. Heamo compatibility against human peripheral blood was also used in this present investigation to evaluate biocompatibility.

Cytotoxicity assay

Chemicals

RPMI1640, fetal bovine serum (FBS), Trypsin, methylthiazolyldiphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from Hi media & Sigma Aldrich Mumbai.

Cytotoxicity assay

Inhibition of cell growth of Vero cell line using a tetrazolium dye (MTT) assay and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the untreated ones. Cell line was obtained from National center for cell sciences (NCCS), Pune, India. RPMI1640 was used as the source of cell growth medium and a humidified atmosphere (d 5% CO 2) was maintained for cell culture. Cells harvested in a logarithmic growth phase were seeded on 96 wells at a cellular density of 5x103 cells / ml followed by the addition of different concentrations, incubated for 24hrs at 5 % CO2 incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl–tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the Nano emulsion on the proliferation of cells was expressed as the % cell viability.

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In vitro blood cell compatibility

Further confirmation of biocompatibility was carried out by haemo compatibility under laboratory condition. Peripheral EDTA blood sample was used in the study. Collected blood samples were diluted with saline suspension. 0.9 ml of diluted blood sample and 0.1 ml of free Nano emulsion and chitosan coated Nano emulsion was taken in a centrifuge tube, incubated for 3 h at 37° C under shaking. After the incubation, plasma was collected by centrifuging the samples at 4500 rpm for 10 minutes. The concentration of hemoglobin in the plasma was quantified by spectrophotometry; plasma hemoglobin concentration directly correlates the percentage of lysed blood cells. Plasma hemoglobin concentration was quantified spectro photometrically.

RESULTS

Free and chitosan stabilized neem oil nano emulsion

Droplet size of neem oil Nano emulsion was greatly influenced by the amount of oil -surfactant ratio used and time of sonication Among the different condition, decreased droplet size was recorded in increase in surfactant concentration and time of sonication (Figure 1).The smallest droplet size of Nano emulsion was reported in 1:7.5 for oil and surfactant ratio and 60 minutes of sonication and the average droplet size was found to be 60.10 nm. .In the present study, emulsion with the mean droplet size as low as 169 nm was obtained in the presence of high oil-water concentration and maximum sonication time (60 minutes).

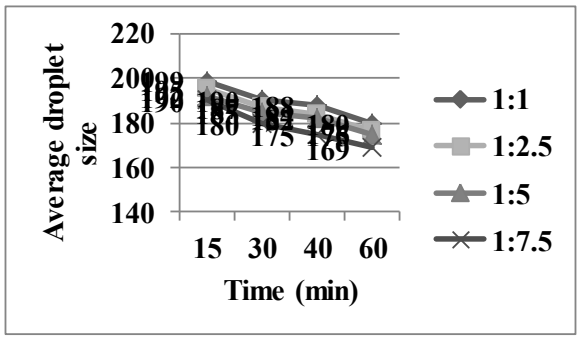

Fig. 1: Effect of sonication on droplet size of 1:7.5 ratio of oil-water; surfactant ratio.

Chitosan stabilization on Nano emulsion showed distinct effect on the droplet size. Droplet size was found to be decreased in all the tested parameters [Fig. 2]. As in free Nano emulsion, surfactant concentration and ultra-sonication influenced droplet size. Less droplet size was recorded in 1:7.5 oil-water; surfactant concentration at maximum ultra-sonication time. But in all the tested conditions (oil-water; surfactant ratio, ultra sonication time), lesser droplet size has been interfered from chitosan stabilized Nano emulsion than free neem oil Nano emulsion.

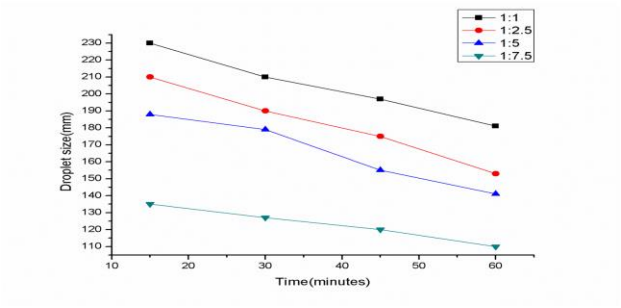

Fig. 2: Effect of chitosan coating on droplet size of neem oil Nano emulsion at different time of sonication.

Anti-bacterial activity

Anti-bacterial activity of chitosan stabilized neem oil Nano emulsion was studied against *P. aeruginosa* and *S. aureus* by well diffusion assay and micro dilution colorimetric MTT growth inhibition assay [Table 1].

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Table 1: Effect of chitosan stabilized neem oil Nano emulsion on antibacterial activity of pathogenic strains by agar diffusion assay

Treatment	Zone of inhibition (mm)	
	P.aeruginosa	*S. aureus*
Free neem oil Nano emulsion (50 µl)	12.0	11.0
Free neem oil Nano emulsion (100 µl)	14.0	12.5
Chitosan stabilized neem oil Nano emulsion (50 µl)	21.0[a]	19.0[a]
Chitosan stabilized neem oil Nano emulsion (100 µl)	23.0[a]	20.0[a]
Negative Control	0.0	0.0

[a] -Column carries alphabet is statistically significant at 5 % level by DMRT

Results showed that both the tested bacterial strains were susceptible to the Nano emulsion. But chitosan stabilized Nano emulsion brought about enhanced activity against the both tested strains. An increase in zone of inhibition was recorded in chitosan coated Nano emulsion than the free Nano emulsion. Improved anti-bacterial activity was also supported by colorimetric liquid MTT assay which reveals effective growth inhibition of chitosan coated Nano emulsion against both the tested bacterial strains as dose dependent manner. In *P.aeruginosa*, free Nano emulsion brought about 37 % of inhibition at high dosage and the inhibition was increased in chitosan coated Nano emulsion as 85 % [Fig. 3]. Similar finding was also recorded in *S. aureus*. High rate of growth inhibition was found in chitosan coated Nano emulsion treatment [Fig. 3, 4].

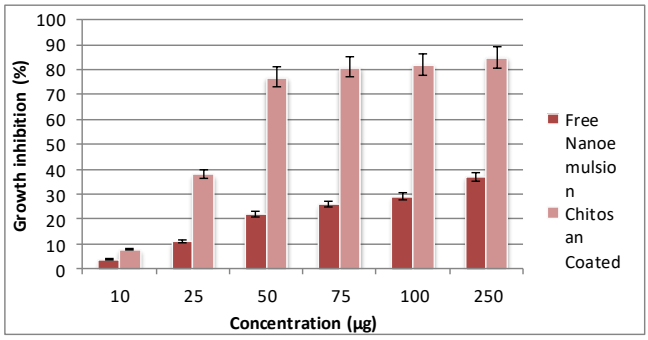

Fig. 3: Effect on free and chitosan coated Nano emulsion on growth inhibition (%) of *P.aeruginosa*.

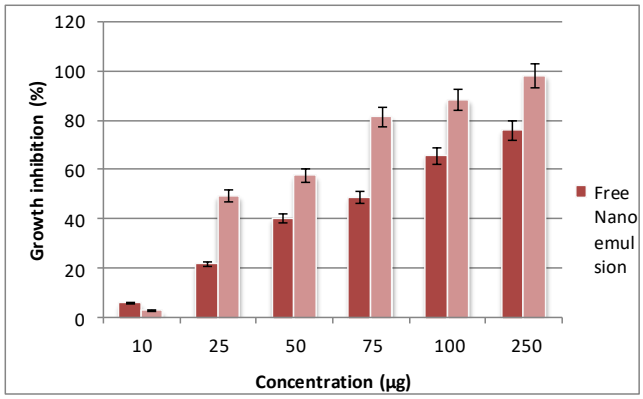

Fig. 4: Effect on free and chitosan coated Nano emulsion on growth inhibition (%) of *S. aureus*.

Biocompatibility studies

Nontoxic effect of tested Nano emulsion was also studied in the present study. Cytotoxicity tests against Vero cell line and peripheral cells (haemo compatibility) were carried out to determine biocompatibility. Colorimetric micro titre plate MTT assay was used to determine cytotoxicity which shows less toxic effect of both the free and chitosan coated Nano emulsion [Table 2].Cell viability was retained in maximum concentration. Above 99 % of cell viability was noticed in least dosage of Nano emulsion. Similar results have been observed in peripheral blood cells (hemo compatibility test. [Table 3] depicts hemolysis of tested emulsion against human peripheral blood which indicates both free and chitosan coated Nano

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emulsion exhibited very less toxic effect in all the dosages. Interestingly, hemolysis was low as chitosan coated Nano emulsion showed as The present findings would suggests the possible utilization of chitosan based Nano emulsion as an effective anti-microbial agent against life threatening disease causing organisms.

Table 2: Effect of free Nano emulsion (FN) and chitosan coated Nano emulsion (CS-NE) on hemolysis of peripheral blood

	Free Nano emulsion	Chitosan coated Nano emulsion
Concentration (µg)	Cell viability (%)	Cell viability (%)
1000	15.2	7.2
750	10.2	4.2
500	7.2	2.2
250	3.4	1.1
125	1.3	0.6
62.5	0.6	0.2
31.2	0.2	0.07
15.6	0.09	0.03

Table 3: Effect of free Nano emulsion (FN) and chitosan coated Nano emulsion (CS-NE) on the cell viability (%) of Vero cell line

	Free Nano emulsion	Chitosan coated Nano emulsion
Concentration (µg)	**Cell viability (%)**	**Cell viability (%)**
1000	87.4	91.2
750	89.2	92.1
500	92.2	94.2
250	94.1	96.2
125	96	97.2
62.5	97.1	99.2
31.2	98	99.4
15.6	98.5	99.5

DISCUSSION

Nano emulsions are emulsions with droplet size on the order of 100 nm. A typical Nano emulsion contains oil, water and an emulsifier. Nano emulsions have lot applications in the field of medicine as drug delivery agent, food sector and agriculture [2]. Success of Nano emulsion is primarily based on its stability and the various factors. There are various factors which control the stability. Nano emulsions are kinetically stable and given sufficient time, will separate into different phases. Different destabilization mechanisms of Nano emulsions namely flocculation, coalescence, Ostwald ripening and creaming/ sedimentation lead to reduce the uses of Nano emulsion [12].Destabilization can be controlled by various factors which have been improving the stability. In the present study, chitosan stabilized neem oil Nano emulsion was prepared by changing oil-water; emulsifier ratio under sonication at different time periods which exhibits distinct changes on the droplet size and biological activities. The present study, neem oil nano emulsion prepared under specific condition by changing oil-water surfactant ratio at different time of sonication which reveals decrease droplet size of emulsion was observed at increasing time of sonication, oil-water surfactant ratio. Similar finding has been reported by Ghotbi et al [13] Rather than surfactant concentration, ultra sonication play a vital role on the droplet size determination of Nano emulsion. Kentish et al [16] observed droplet size of flaxseed oil Nano emulsion was found be decreased in maximum ultra-sonication speed and time. Emulsion stability is dependent on role of surfactants, its composition and the drop size distribution. Nano emulsions exhibit stability against sedimentation or creaming due to the small size of droplets. Diffusion rate and Brownian motion exhibited by these droplets. Predominates over sedimentation/creaming rate is found due to gravity. Flocculation does not occur in Nano emulsions prepared by using nonionic surfactants as no attractive forces are created. Nano emulsions may remain stable for a short span to years depending on how they are formulated and other process parameters involved in formation. They are sometimes referred to as “approaching thermodynamics Coating of nano materials by polymer is an effective strategy for functionalization. In this study, chitosan was selected. Chitosan is an important plant based polymer Because of the biocompatibility, biodegradability, nontoxicity and adsorption properties of chitosan, it is recommended for stabilization [17].In this study, distinct reduction of particle size was recorded in chitosan coated neem oil nano emulsion which was observed in all the tested concentration of oil-surfactant water ratio and the sonication time. Anti-bacterial activity of both free and chitosan coated neem oil emulsion was studied

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against human pathogenic bacterial strains by well diffusion assay and MTT micro dilution tube assay. Maximum growth inhibition was recorded in chitosan coated Nano emulsion treatment. In general the droplet size reduction cause increased surface area, the number of active groups and determine the increase in antibacterial activity. Chitosan coating reduced the droplet size of the tested Nano emulsion known to improve the anti-bacterial activity as reported in the earlier studies [13]. Biocompatibility was studied by determination of cell viability and hemolysis against vero cell line and human peripheral blood cells which shows both the free and polymer coated nano emulsion did not induce any toxicity. Further study using animal model will be helpful to utilize nano emulsion based on chitosan coated neem oil as an effective anti-bacterial agent.

CONCLUSION

Functionalization of nano materials by polymers is an attractive field of nano science and nanotechnology which have a lot of application in the biomedicine field. The present study, preparation of chitosan coated neem oil nano emulsion prepared by changing oil-water-surfactant concentration under different time of sonication reveals reduced droplet sized particles which brought about enhanced antibacterial activity against human pathogenic bacterial strain and less cytotoxic effect against vero cell line and human peripheral blood cells .This study findings would suggest the possible utilization of polymer coated nano emulsion as an effective and biocompatible antibacterial agent.

CONFLICT OF INTEREST
There is no conflict of interest.
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FINANCIAL DISCLOSURE
None

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ARTICLE

COST ANALYSIS OF CATARACT SURGERY IN TWO EXTRACTION EXTRACAPSULAR CATARACT AND PHACOEMULSIFICATION

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ABSTRACT

A retrospective observational study to compare the cost of cataract surgery between extracapsular cataract extraction (ECCE) and Phacoemulsification (PEA) was conducted at Hospital Raja Perempuan Zainab II, Kota Bharu on May 2013. A total of 30 patients were included in this study. The cost of cataract surgery incurred by hospital up to two months after operation was included. The costs of training, loss of patient's income after discharge and intangible cost were excluded. Results showed that cataract surgery using (PEA) are most costly than using (ECCE) the cost include the calculation of salary of staff, pre & post opt as well as all the equipments involve in both surgery methods.

KEY WORDS
Cataract surgery, Cost analysis, Extracapsular cataract extraction, Phacoemulsification

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INTRODUCTION

Cataract is one of the important cause of blindness in Malaysia and worldwide. According to the World Health Organization (WHO), 50 million persons in the world are blind due to cataracts (WHO)[1]. Cataract usually affects people with age of over 65 years. The prevalence of blindness in the world is 0.7% with 0.3% in developed countries and up to 1.4% in less developed countries such as Africa (Dorland's medical dictionary, 2004. [1]. Based on Malaysian National Eye Survey (2007), cataract was found to be the commonest cause of blindness which was 39% [2]. According to World Health Organization (WHO), cataract can be defined as the presence of lens opacity that giving a grey or white appearance to the pupil during eye examination with an oblique light in a darkened area [3].

Based on Dorland's medical dictionary 27th edition (2004), cataract can be defined as opacity of the lens or lens capsule of the eye. Cataract must be treated early because it will eventually lead to blindness if left untreated. There are two types of cataract of surgery that can be performed in order to treat cataract which are phacoemulsification and extracapsular cataract extraction (ECCE) [4]. Phacoemulsification is relatively new method in treating cataract. Phacoemulsification is the extracapsular surgery in which the lens is softened with sound waves and removed through a needle. The posterior capsule remains in the eye. The older method to treat cataract is extracapsular cataract extraction (ECCE). Extracapsular surgery is the surgery in which the lens is removed and the back half of the capsule behind the lens (the posterior capsule) remains in the eyes. Different methods of surgery have their own advantages and disadvantages. Compared to ECCE, PEA requires only a smaller corneal incision and can be performed without needles. Besides that, the time for doing surgery is relatively short which only take a few minutes. Other than that, there is less inflammation occurring after operation. Furthermore, PEA also give a faster visual recovery, lower incidence of postoperative astigmatism, early stabilization of refraction and sustained intraocular pressure control during operation [1]. Compared to PEA, ECCE have more disadvantages and limitations. There are including prolonged surgery time, prone to get inflammation after operation, suture distortion of cornea, prolonged convalescence and restriction to activities. In Malaysia, there are several cataract surgery practice done including phacoemulsification which is about 65.7% and followed by extracapsular cataract extraction which is about 30.1% and intraocular lens implantation which is 98.2% [1]. the large number of cataract surgery performed in Malaysia, there is little knowledge or research regarding the cost or variation in costs between ECCE and PEA [2]. Therefore, we performed this study to analyze and compare the cost of cataract surgery by ECCE and PEA in Ophthalmology Department, Hospital Raja Perempuan Zainab II Kota Bharu. The objectives of this study includes assisting decision making process, carrying out the cost analysis for ECCE and PEA and performing cost effectiveness analysis and comparing between two types of operation.

MATERIALS AND METHODS

This study was a retrospective observational study done on May 2013 at the Ophthalmology Department, Hospital Raja Perempuan Zainab II Kota Bharu. A total of 30 patients were enrolled retrospectively from 11/05/2012 to 25/05/2013 in this study. The total of 20 patients underwent phacoemulsification while the rest of 10 patients underwent extracapsular cataract extraction. The cost perspective for this study was taken from that of Ministry of Health. The inclusion criteria were patients aged 40 years old or older and absence of preexisting ocular co morbidity such as glaucoma, maculopathy0, and difficult papillary dilatation. Besides that, the patients who involved in this study were limited to 2 months' post- operative period. The exclusion criteria were including costs borne by patients (e.g. spectacles), direct non treatment costs (e.g. transport to clinic), indirect costs (e.g. loss of work time), and intangible costs (e.g. pain and anxiety)[3]. Besides that, long term costs and outcomes beyond 2 months were not excluded in this study [5].

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The doctor in charge of this study will used some study tools such as medical record to review the patient' and progression note, interview with the ophthalmologists and nurses and also by using secondary data analysis. The costs of the preoperative and postoperative clinic visits, their admission to the ward, surgical procedures and medicines were calculated and documented. The sampling method is by simple random sampling to the patients who fulfilled the above criteria) [3].

ASSESSMENT OF COSTS

A cost incurred by the hospital (provider) was imputed in the study. The cost incurred by patients such as household costs was excluded in the study. Provider costs were further classified into capital and recurrent costs. These costs were based on the financial year 2013. Besides that, there was also micro costing (every input consumed by the patient during surgery and then its unit cost will be calculated) included in this study. Capital costs for provider included building, furniture and equipment costs. All capital costs were discounted at the rate of 5% per year. The useful life of building was assumed to be 20 years while the useful life of furniture was five years. Life span of equipment was based on article by Asimakis et al) [1]. The total capital costs then further divided by the total number of patients using the facilities to obtain the unit cost. Recurrent cost that included in this study were patient care medications and consumable costs (including pharmaceuticals, hospitalization and surgical consumables such as scalpel, gauze and others) and also staff and overhead costs. The staff and overhead costs which included were administration and supportive services such as laundry, cleaning services, building and equipment maintenance, utilities and telephone. Indirect cost due to loss of income for a working patient after discharge was not included in this study. This because the indirect cost is difficult to assess because patients may continue to work despite medical certification of being unfit for duty especially those who are self- employed. Assessment of intangible cost such as pain, anxiety and ability to interact with and support others were not included in this cost analysis study [1].

DATA ANALYSIS

The data for this study should include the socio demographic data (gender, age, ethnicity) and also costing (provider cost, micro costing, capital cost and recurrent cost). Then, the data collected were analyzed by using Microsoft Excel 2007 in order to calculate the cost of two surgical methods which were phacoemulsification and extracapsular cataract extraction.

FINDINGS

A total of 30 patients were enrolled for analysis. Out of this 10 underwent ECCE and 20underwent phacoemulsification. The mean age in both groups was similar, which was 67.59 years for ECCE and 63.17 years for phacoemulsification. There were more males than females in both groups. [Table 1]. shows the baseline characteristics of patients in the ECCE and phacoemulsification groups. Table 2 shows the comparison of treatment outcomes of ECCE and phacoemulsification. There was no difference in the mean length of stay in the hospital in the two groups; 2.4 days in ECCE group and 2.5 days in the phacoemulsification group. Characteristics of patientSocio-demographic characteristics such as gender, age and ethnicity were shown in the [Table 1]. [3].

Table 1: Patients characteristics

		Number of patients (n)	
		PEA (n)	ECCE (n)
Gender	Male	12	7
	Female	8	3
Age	0-30 years old	0	0
	31-60 years old	4	2
	61 -90 years old	16	8
Ethnic	Malay	19	7
	Chinese	1	2
	Others	0	1

Table 2: Length of stay table

	PEA (days)	ECCE (days)
Average length of stay (ALOS)	2	1

Table 3: Cataract Surgery Cost

	ECCE (RM)		PEA (RM)	
	Cost	%	Cost	%
Capital Cost				
Building	373	23.8	373	18.3
Equipment	284	18.1	442	21.7

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Subtotal capital	657	41.9	815	39.9
Recurrent Cost				
A) Staff Cost				
Pre-opt	15	0.9	15	0.7
Opt- day	3	0.2	3	0.1
Intra-opt	201	12.8	168	8.2
Subtotal staff	219	14	186	9.1
B) Non-staff cost				
Items For Pre Op. Assessment	17	1.1	17	0.8
Items for Cataract Operation	383	24.4	731	35.8
Consumables Day Activity	34	2.2	34	1.7
Consumables During Intra Opt	182	11.6	182	8.9
Medication Sub total	76 4.8 692 44.1		76 1040	3.7 51
TOTAL	1568 100		2041	100

The comparison of costs per cataract surgery is shown in [Table 3]. The cost of phacoemulsification, which was RM2041, was slightly more than that for ECCE, which was RM1568 The higher cost of phacoemulsification was attributed to higher equipment and consumable costs. The capital costs for both, ECCE at 657 and phacoemulsification at RM815 respectively. However, as expected the consumable cost for phacoemulsification (RM 1040) 51% was markedly more than that for ECCE 44.1% (RM692). The increased consumable cost contributes to 51 % of the total extra expenditure incurred by phacoemulsification. Based on the table above, there were more male patient compared to women in both ECCE and PEA groups. The mean age for both groups was 67.59 years for ECCE and 63.17 years for PEA [3]. In this study it was found that the average operation time for PEA is 21.8 minutes compared to 43.2 minutes for ECCE. The PEA technique is also less invasive where a smaller incision is required compared to ECCE. Through this small incision, the lens nucleus is phacoemulsified using a low flow/high vacuum machine. In ECCE, the lens nucleus was expressed using bimanual technique [1].

DISCUSSION

The results of this evaluation should be interpreted cautiously. The main weakness was the small sample size for the costing estimate. Though the cost per operation was calculated based on only30 patients (20 patients for PEA and 10 patients for ECCE), it was done through micro- costing where cost for every item or consumable used for the patient was quantified and was not based on budget assumption. Increasing the number of cataract surgeries performed may reduce the overhead cost, though restraints of paramedical staff and shortage of operating time may pose a problem. Besides, more MOH hospitals should perform day care cataract surgery so as to increase the volume of cataract surgery [3]. From the calculations of comparing these two techniques, PEA is costlier. The cost is lightly higher at capital cost because PEA is using more equipment such as Phacomachine and Phaco hand piece. Recurrent cost was calculated based on staff and non-staff cost. The recurrent cost of PEA is higher because the higher expenditure for the cataract operation equipment. The staff cost also different because the during intra operation surgery the time consume by PEA is shorter than ECCE techniques. The result of this study is different from a study by Loo et al in 2004 where the Conventional extra capsular cataract surgery with intraocular lens implant costs RM3442 (USD905.79) and phacoemulsification with intraocular lens implant costs RM4288 (USD 1128.42) [1]. This might be because cost of intraocular lens implant is not included in this study. This result however is comparable to the study done by Rizal et al in 2003 which showed average cost for one ECCE is RM1,664.46 (RM1,233.04 – RM2,377.64) and for PEA is RM1,978.00 (RM1,557.87 – RM RM3,334.50). The result of this study also concurs with study done by Asimakis et al. in 1996 where they found that the hospital costs for ECCE without any complication was ADD 1,000.85 and for PEA was ADD 1,231.00 (ADD 1.00 = RM 2.00). Another study which was conducted in Sweden has shown that the average cost for a cataract surgery performed at the eye clinic was 5,052 SEK (I SEK = RM0.37). The majority of their cases (90%) were performed using the PEA technique. There is a large variation in the cost of cataract surgery in different parts of the world. India shows the lowest cost per cataract in comparison to the USA where the cost per cataract surgery is nearly 20 times that of cataract surgery in the government sector in India. This huge difference in cost comes as no surprise and in fact reflects the economic stability and status of the country concerned. This study has shown that we are almost comparable to that of Australia for PEA cost, whose currency value is almost twice that of Malaysian Ringgit. There was no significant difference between the cost effectiveness of ECCE and

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phacoemulsification [3]. The results should be interpreted taking into account the limitations of the study. The main limitation is the time-frame where cases were followed-up for just two months after operation. PEA will not require any further visits after two months. Patients who had ECCE had to undergo two more visits to remove the sutures. They are also required to be followed-up till six months for refractive error correction. So it is possible then that if the study was extended to about six months, the cost of ECCE might increase and may be higher than the PEA technique. Another limitation is some costs that were not included in this study, for example, the cost of training the ophthalmologist in handling the PEA machine and the cost of patient's productivity loss, after being discharged from the ward. These costs were considered direct cost for the hospital as well as for the patient in the total cost of cataract surgery [1].Furthermore, the samples size in the two groups was imbalanced in its baseline distribution, having more subjects in the ECCE group and there was no randomization of surgical technique in the study subjects [3].

CONCLUSION

In conclusion, ECCE technique is less costly compared to PEA. Cost of equipment are the main reasons for the high unit cost of PEA compared to ECCE. However, in long term, it is likely that PEA cost will be less compared to ECCE [1]. The effectiveness of cataract surgery is also one of the aspects that should be considered in order to determine which technique is more cost effective. The study has also indicated that there is much room for improvement in the cataract surgery services provided by the MOH, with the aim to provide large volume, low cost and yet high quality cataract surgery. Such economic evaluation is a useful tool in the planning and operation of health care programmers, particularly in the public sector.

CONFLICT OF INTEREST
There is no conflict of interest.

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FINANCIAL DISCLOSURE
None

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ARTICLE

FABRICATION OF SILICA NANOPARTICLE-PEG-IONIC LIQUID SPME FIBER FOR DETERMINATION OF PESTICIDE RESIDUES IN TOMATO

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ABSTRACT

A novel SPME fiber was prepared for determination of pesticides in tomato samples. Different materials were synthesized as absorbent layer with Sol-Gel technology. These different adsorbing layers essentially were different combinations of polyethylene glycol (PEG), (Trimethoxysilyl) propyl methacrylate (TMSPA), polydimethylsiloxane (PDMS) with Silica Nanoparticles (Silica-NPs) and 1-Butyl-4-methylpyridinium tetrafluoroborate (BMPT) as the ionic liquid. These final synthesized absorbing layers were attached to the modified surface of porous copper self-assembled monolayer (SAM) with simple immersion. Then the fibers were used to extract 6 types of pesticides in tomato samples and the extraction parameters such as extraction temperature and time, Ionic strength, pH, desorption temperature and time were optimized. The fabricated fibers were compared with each other in terms of extraction efficiency and the selected fiber with best performance was PEG-Ionic Liquid-Silica NPs. The selected fiber was examined by SEM and figures of merit like LOD, LOQ, LDR, r^2 for each individual pesticide were calculated for this proposed technique under optimum conditions.

KEY WORDS
solid-phase micro extraction, sol-gel. ionic liquids, self-assembled monolayer (SAM), pesticide, silica nanoparticle

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INTRODUCTION

Pesticide residue analysis in environmental and biological samples has received increasing attention in the last few decades as can be deduced by the great number of papers published dealing with this subject [1]. Pesticides are substances intended for preventing, destroying, repelling or mitigating any pest and are widely used on a variety of crops in the current agricultural practices. In this regard, the pesticide residues are found in foods [2]. It has been shown that the majority of pesticides cause acute high toxicity in human beings [3-4].The residues are harmful not only for human being but also for all living organisms. The ongoing research in this area indicates the presence of pesticide residues in most agricultural products. When the contaminated products are taken by humans, they cause different diseases and may even be lethal.

Various methods such as supercritical fluid extraction, accelerated solvent extraction microwave-assisted extraction, ultrasonic extraction, and matrix solid phase dispersion have been employed to separate and measure these compounds in various matrices [5-9]. Many of these methods are time-consuming and require large amounts of organic solvents. In the literature, the need for rapid sample preparation methods for extraction and separation of analytes with high selectivity for the composition in matrix is emphasized [10-15]. To evaluate food safety and potential risks to human health, development of analytical methods to identify pesticide residues in aqueous and biological samples, fruits, and vegetables is necessary. A common sample preparation technique is solid-phase micro extraction (SPME). The technique was first described in 1990 by Pawliszynet.al [16] as a solvent-free pre-concentration and sample preparation method. It was introduced as a new way to insert the samples into the chromatography system. It is based upon development of a balance between the analyte in the sample solution and the fiber. After fulfillment of the extraction the analyte is desorbed into an analytical instrument such as GC or HPLC [17-20].

The fiber coated with typically an immobilized polymer, a solid adsorbent or a combination of them [17, 21]for sample introduction to GC and GC-MS, has been successfully and routinely applied to a wide variety of compounds from environmental, biological, and food samples[14, 21-24]. Among disadvantages of the commercial fibers used in SPME we can consider the large thickness of the stationary phase, fragility, high price, and lack of chemical bonding between the stationary phase and the substrate surface that causes low thermal stability of the aforementioned fibers. To tackle these disadvantages, a large number of home-made fibers have been prepared using different ways. Some of these methods include electrochemical deposition of conductive polymers, sol-gel technology, and using ionic liquids and nanoparticles [25]. Another approach to improve extraction efficiency in SPME is to use metals like Pt, Ni-Ti [26], Al [27], and Cu [28]as substrates to prevent the fragility of silica substrates. Increased porosity in the metallic substrate leads to higher thermal stability of the fiber and stronger bonding with the polymer network [28]. Metal substrate porosity has been achieved by electro deposition of a Cu thin layer on clean surface of Cu wire by applying a negative potential [29]. Other methods to enhance the extraction efficiency is promoting the formation of a covalent bond between the porous copper substrate and the polymer network (3-mercaptopropyl) trime thoxysilane (3MPTS) as binder using self-assembled monolayer (SAM) [30] as well as using carbon nanotubes[31-32]. Employment of silica nanoparticles is another way to improve the performance of SPME fibers [33].

Using SPME with ionic liquid coated fibers are highly proposed because ILs have some unique properties including negligible vapor pressure, good thermal stability, tunable viscosity and miscibility with water and

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organic solvents, as well as good extractability for various organic compounds and metal ions [34].Recently, solid-phase micro extraction (SPME) with ionic liquid (IL) coating was developed for headspace extraction of BTEX [35]. Liu and co-workers introduced the use of ILs in preparation of SPME fiber for the first time [36].There are several reports of the ionic liquids application in SPME such as extraction of polycyclic aromatic hydrocarbons (PAHs) in water samples by Nafion membrane based on ILs [37], extraction of various organic compounds and metal ions [38-39], headspace extraction of BTEX [40], and determination of amphetamine metabolite [41].

Conventional SPME including suffer from some drawbacks like operating temperature problems, instability, and swelling in organic solvents [34, 42]. Sol-gel technology which was suggested by Malik and co-workers [34] provides efficient approach by incorporation of the organic component into the inorganic polymeric structure in solution under very mild thermal condition [43]. Among many inherent advantages of sol-gel technology are the followings: i) high thermal stability achieved by strong adhesion of the coating to the substrate due to chemical bonding; ii) porous structure that provides high surface area and possibility of the use of thinner coatings to achieve acceptable stationary phase loading, sample capacities, and fast mass transfer characteristics; and iii) high degree of flexibility in coating composition and selectivity by varying the proportion of the sol solution ingredients or using a deactivation reagent[44].

In this study Cu porous wire was prepared by electro deposition of Cu thin layer on Cu substrate and metal surface was modified using SAM of (3-mercaptopropyl) trime thoxysilane (3MPTS) covalently bonded to the Cu substrate. Different combination of organic polymers, ionic liquids and silica NPs were prepared by sol-gel chemistry and the final synthesized mixtures were coated on the surface of the modified Cu-porous by SAM. Incorporation of silica NPs and ionic liquids enhances the fiber performance. Analysis of the surface morphology characteristics of the coated fibers was performed using SEM. Extraction efficiency of these fibers were investigated by direct immersion of the fibers in the aqueous sample for extraction of some pesticides in tomato samples. The most suitable fiber was PEG-Ionic Liquid-Silica NPs that caused highest extraction efficiency.

MATERIALS AND METHODS

Reagents

(3-Mercaptopropyl) trime thoxysilane (3MPTS) (95%) was purchased from Fluka (Buchs, Switzerland). Hydroxyl-terminated poly dimethyl siloxane (OHTSO), tetra ethylortho silicate (TEOS), tri fluoroacetic acid (TFA, 98%), poly methyl hydro siloxane (PMHS), silica NPs (10–20 nm), and 1-butyl-4-methylpyridinium tetra fluoro borate were obtained from Aldrich (Stein heim, Germany).Polyethylene glycol (PEG) (mono methyl ether, MW; 5000), and Superox-4 (PEG)were purchased from Sigma-Aldrich (Chemie, Stein heim, Germany), 3-(Trime thoxysilyl)propyl methacrylate (TMSPA),ionic liquid (1-butyl-4-methylpyridinium tetra fluoro borate) was purchased from Fluka (Buchs, Switzerland). All these materials were used as received, except TFA that was diluted to 95%and 99% with water. Before use, all plastic and glassware were decontaminated overnight in20% nitric acid and thoroughly washed with Milli-Q quality (Millipore, Billerica, MA) deionized water. Target pesticides include Phosmet, parathion, Phorate, desethylatrazine and terbuthylazine [Table1] were supplied from Riedel-deHaen (Seelze-Hannover, Germany) with purity higher than 95.5%. Sodium chloride (extra pure), ethanol, methanol and acetonitrile (all of analytical reagent grades) were purchased from Merck (Darmstadt, Germany). Characterize of pesticides used in this work are summarized in [Table 1].

Table 1: Specifications of the pesticides used

	S (mg/L)	Log Kow	VP (mPa)	MW	VP (mPa)
Terbuthylazine	6.6	3.4	0.12	229.71	0.12
Desethylatrazine	3200	1.51	12.44	187.63	12.44
Phorate	44	3.66	85.5	260.36	85.5
Parathion	11.75	3.73	2.3	291	2.3
Phosmet	15.2	2.96	0.065	317.323	0.065

Water solubility (S), vapor pressure (VP), Henry's law constant (H); data from Footprint Pesticide Properties Database.

Apparatus

A Hewlett-Packard (HP, Palo Alto, USA) HP 6890 plus series GC equipped with a split/split less injector and a Flame ionization detector system were used. Helium (99.999%) was employed as carrier gas and its flow velocity was constantly adjusted to 70 cm S^ (-1). The separation of pesticides was performed on a 30m× 0.25 mm HP-5 (0.25 µm film thickness). The column was held at 40 °C for 2 min, increased to 290 °C at a rate of 25 °C min−1 and kept constant for 4 min and then raised to 290°C at 40°C min−1 and kept at this temperature for 4 min. The injector temperature was set at 260 °C and fiber desorption was carried out in the split less mode.

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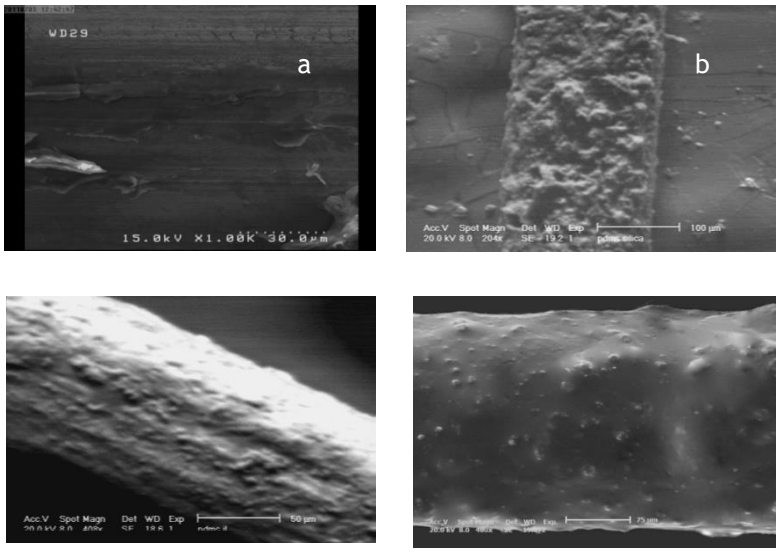
Fiber Fabrication

Preparation of SPME fibers

A Cu wire with a length of 2cm and diameter of 200μm was prepared and its physical contamination was removed by washing with acetone, ethanol and distilled water respectively. For achieving a porous Cu surface a system of two electrodes (anode and cathode; both copper) with an electrolyte solution of) 10% v/v) H2SO4 and) 5% W/W (CuSO4was applied by a voltage to -500 mv – DC for 5 min. All porous wires were kept in ethanol for later use. Then the wires were placed in a solution of 3-MPTS (10-3 M) in ethanol for two hours. In the following, the wire was immersed in a solution of 0.1 M NaOH for 90 min and then in a solution of HCl for 60 min and finally rinsed with distilled water. Cu surface porosity highly increases the level of adhesion of polymer network which was attached through 3-MPTSwith SAM procedure. [Fig. 1A] shows SEM images of Cu wire without electro deposition of the porous layer, 1B shows Cu-porous layer, 1C shows Cu-porous layer 3-MPTS modified and 1D shows Cu-porous- 3MPTS-PEG-IL-silica NPs fiber.



(C) (D)

Fig. 1: ACu wire without electro deposition of porous layer and - B shows Cu- porous layer –C shows Cu-porous layer 3-MPTS modified and D shows Cu-porous- 3MPTS – PEG-IL-silica NPs fiber.

Polyethylene glycol (PEG), (Trime thoxysilyl) propyl methacrylate (TMSPA), poly dimethylsiloxane (PDMS) with Silica Nanoparticles (Silica-NPs) and 1-Butyl-4-methylpyridinium tetra fluoroborate (BMPT) as the ionic liquid were the raw materials which was prepared with Sol-Gel procedure as PDMS, PDMS-Ils, PDMS-Ils-silica NPs, PA, PA-Ils, PA-Ils-silica NPs, PEG, PEG-Ils, and PEG-Ils-silica NPs. After preparing the Sol-Gel solution, the previous treated copper wire was simply immersed in it and after about 60 minutes the covalent bonding of absorbing layer with metallic substrate will be accomplished.

SPME procedure

Before extraction, all the fibers were conditioned in the inlet of GC at the temperature of 280 °C with helium carrier gas for one hour. For real sample analysis 300 g tomato (purchased from the local market) was crushed, homogenized and diluted with 500 mL of distilled water.

The stock solution of the pesticides contains terbuthylazine, desethylatrazine, Phorate, Parathion, and Phosmet were prepared in methanol at the concentration of 1000 ppm and was kept in the refrigerator and the standard solutions were prepared from that on daily basis. To spike a certain amount of pesticides in tomato samples 50 ml of chopped and homogenized tomato samples were mixed with appropriate values of standard solutions in a 100-ml flask and brought to the volume using double distilled water and became uniform by placing in an ultrasonic bath for 30 min. Then, 10 ml of the top solution was transferred to a Falcon tube, blended smoothly, and was centrifuged at 8000 rpm for 10 min. After that few milliliters of the supernatant in the test tube was transferred to a glass vial and the fabricated fibers were placed in it for 45 min at 30 °C. Then the fibers were removed and injected into the GC device. [Fig. 2] shows chromatogram obtained after SPME of pesticides at 500 ppb. As can be seen main peaks started from around 6 min and ended at 9 min. These five peaks represent desethylatrazine, Phosmet, terbuthylazine, Phorate and parathion respectively.

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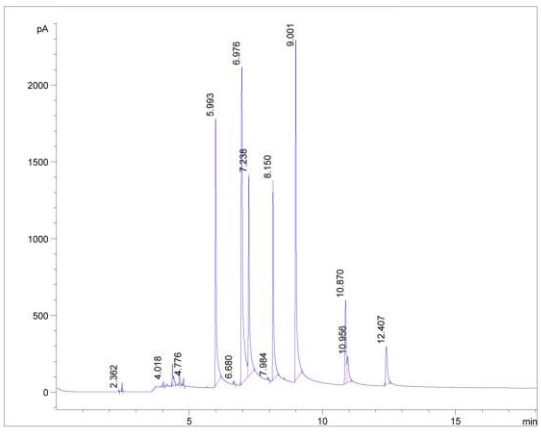

Fig. 2: Final chromatogram after SPME procedure for extracted pesticide from tomato samples. Five main peaks from 6 min to 9 min are desethylatrazine, Phosmet, terbuthylazine, Phorate and parathion respectively

RESULTS

For optimization of effective parameters of extraction, the concentration of 500 ppb of the pesticides was spiked in to the tomato samples. According to the results demonstrated in [Fig. 3], absorbent fiber with PEG-IL-silica NPs was selected as the most suitable fiber for extraction. The extraction parameters including extraction time and temperature, pH, ionic strength, stirring speed for the solution, and time and temperature of desorption were evaluated and the extraction efficiency values were compared under various condition.

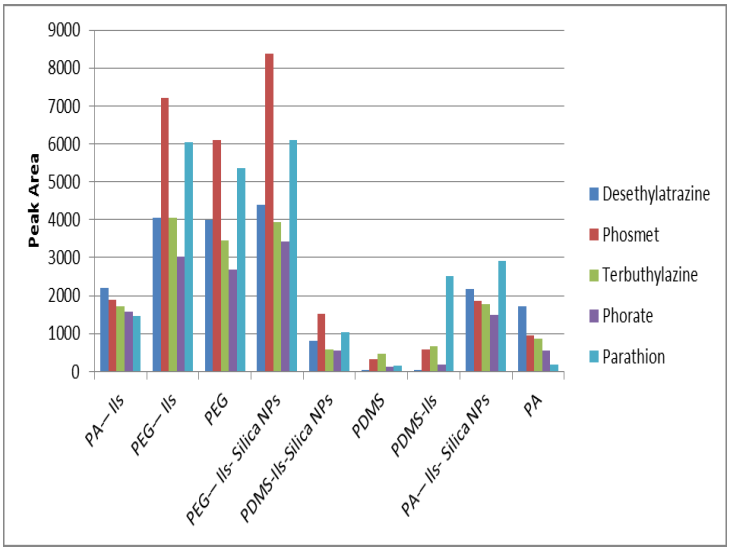

Fig. 3: Comparison of the extraction efficiency of five pesticides spiked in tomato solution by SPME with different fibres. The sample concentration was 500 ppb.

DISCUSSION

Camel Optimization of SPME procedure

To determine optimal conditions for separation and measurement of the amount of pesticides, we compared the area of the peak of the pesticide as studied in the chromatogram obtained by direct fiber injection with a SPME syringe in to the GC inlet. To achieve accurate and reliable results, each analysis was repeated three times. This optimization was performed by keeping other parameters constant at values obtained from the literature.

The best extraction temperature for the pesticides studied was 30 °C due to increasing diffusion coefficient of analytes in higher temperatures and 45 min was chosen as the duration of extraction as the best equilibration time.

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Adding salt may lead to increased or decreased extraction efficiency where-as ionic strength of the solution may reduces water solubility of organic compounds and several folds increases partition coefficient of the analytes present in water. After optimization the salt concentration in the solution was selected as 15%.The finding is probably due to different physicochemical properties and polarity of the pesticides.

Stirring the solution can speed up the process of mass transfer between the fiber and the solution but much higher speeds can remove the analytes or absorbent layer. Optimization resulted in tostirring rate of 400 rpm as the optimal stirring rate.

When the analytes are in ionic, acidic or basic formor the analytes are compounds that their decomposition or formation is affected by pH, it is necessary to adjust the pH value. Therefore effect of pH value on extraction of pesticides was studied in the range of 2-13 by adding hydrochloric acid or sodium hydroxide. Some pesticides such as terbuthylazine showed considerable signal loss at high pH values. This is while Phosmet and Phorate showed lower signal loss as pH value changes from the neutral state. So pH = 7 was chosen as the optimal pH value which caused the best extraction efficiency for majority of investigated pesticides.

By finishing the extraction process the fiber was directly injected into the GC inlet by SPME syringe. The inlet temperature and the time which SPME fiber remained in it were studied because of their remarkable effect on the final achieved signal. The results showed that the peak area of each individual pesticide was at its highest level at 250 °C and 4 min placing SPME fiber in the GC inlet.

Method validation

To assess method validation of this proposed method, specific values of each pesticide spiked into the tomato samples so that final concentration of each of them was between 50 to 1000ppb and the whole process was conducted on them in the optimum conditions. The coefficient of correlation was higher than 0.98 for all the pesticides measured. The values obtained for R2, RSD, LDR, LOD and LOQ are summarized in [Table 2].

Table 2: Specific values of each pesticide spiked into the tomato

Analyte	% RSD	R^2	LDR	LOD	LOQ
Phosmet	1.2	0.989	60-1000	20	60
Parathion	2.5	0.998	150-1000	50	150
Phorate	4.6	0.987	270-1000	90	270
Desethylatrazine	2.3	0.985	120-1000	40	120
Terbuthylazine	3.8	0.996	240-1000	80	240

CONCLUSION

Regarding the importance of pesticide residues in agricultural products, a new SPME fiber coated by sol-gel technology based on ionic liquids, on a copper porous substrate using silica NPs was fabricated. Different parameters in extraction of pesticides from real sample solution containing the pesticides such as extraction time and temperature, stirring rate, pH, ionic strength, desorption temperature and time were optimized.

Extraction of pesticides from tomato samples using fibers showed that the PSG-Ils-silica NPs fiber has the highest efficiency in the extraction and determination of pesticides in real samples. This new method was successfully able to extract different pesticides from tomato samples with high reliability and reproducibility.

CONFLICT OF INTEREST
There is no conflict of interest.

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None

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ARTICLE

AN ASSESSMENT MODEL TO EVALUATING THE QUALITY OF SOCIAL MEDIA DATA USING QUALITY ATTRIBUTES FOR IMPROVING BUSINESS DECISION MAKING

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ABSTRACT

Background: *Big data describe volume amount of structured and unstructured data. Big data sources like social media, Facebook collected large number of unstructured data. When unstructured data collected from different sources, maintain the quality of data is also important. Big data sources provide insights to businesses for improve business decision making. The proposed system improves the quality of business data which are collected for business decision making. Data quality Assessment is a way for practitioners to understand the scope of how poor data quality effects on business process and develop a business case for data quality management.* ***Methods:****This paper contributes to providing a solution by introducing new assessment model to evaluate and manage the quality of social media data. Sentiment analysis is used for monitoring real-time data. Generate the new rules and attributes to assess the quality of data. Apply quality attributes on input data and assess only those data which are fit into the quality attribute dimensions. Evaluate data quality using large data set.* ***Result:*** The system provides the visualized data and generates a report based on sentiment analysis. ***Conclusion:*** *The proposed system improve solution by provide real time and validate data for the user.*

KEY WORDS

Data quality, social media data, Sentiment Analysis, Quality attributes, evaluating quality attributes.

INTRODUCTION

Big data is a term that describes large volume of data. Data are both unstructured and structured format. Now day's large number of data are generated in business and this data important in business point of view. Analyzing large data set in big data architecture is most important. Analytics process in big data has the capability to disclose patterns, movement and associations. Especially process of big data affecting to businesses to achieve the goal of decision making. Extracting information from large data set is very important. For extracting large data set carefully do the planning and provide relevant input to decision layers. When adopting solution for big data, businesses are needed to use new technology and framework for large data set.

Social media data is one of big data's most influential origins. The big data dimensions: Volume, variety, velocity, and veracity, produce some challenges not only to data analytics but also to the data system that manage the data. In big data system, input data is eventual source of knowledge. In big data, lifecycle data travel in four phases as shown in [Fig. 1]: data generation, data acquisition, data storage and data analytics. In data generation phase data are created, data are created from larger number of sources, for example, social media sites, Facebook. Data acquisition phase consist of collection of data, transmission of data and pre-processing of data. Generated data are in structured, semi-structured and unstructured format. After collecting data cleansing, data deduplication, filtering is done in preprocessing phase. When data is collected form data sources maintain the data quality is also important. Improve the quality of data by applying quality attributes on input data. Quality attributes are accessed, controlled and improve the data that impact the result of the analysis phase. After data preprocessing data are stored for analysis the results.

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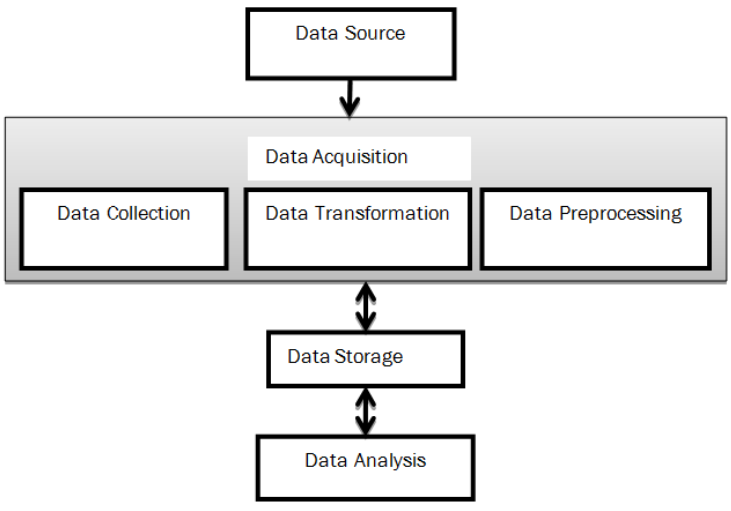

Fig. 1: Lifecycle of big data.

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Social media platform offers marketers significant amount of data which can be used to make a marketing decision in the future [1]. Goals of social media data are an improvement of customer service, instant feedback on products and services through sentiment analysis. Use different metrics to reach different goals. When Social media data collected from various sources (Facebook, twitter), common challenges are arises in data like missing or incomplete data, unavailable of data streams, old data. The user wants to ensure the reliability of the data while collecting. When data is proceeding for analyzing some data, the user intends to make sure that the relevancy and quality of data are appropriate the particular solution. Reliable and valuable data enhance decision making of business. The evaluation of data quality happens in data processing phase in big data architecture, data extraction, data processing, and decision making. Quality evaluation of big data considers while data goes through the pipeline of big data system [2].

When unstructured data collected from different sources, maintain the quality of data is also important. Unstructured data (Input data) are important for marketer for taking a right decision and gain valuable customer insights, reduce marketing expenses and improve sales. The proposed system ensures the quality and trustworthiness of social media data for business and marketers decision-making [3]. Data quality management is one where evaluate data quality and improve business decision making. Data quality management is continuous analysis, observation and improvement overall quality of the organization. The purpose of this paper is to how to evaluate the quality of data and provide trustworthiness data to business for decision making. Introduce solution for data evaluation, in which data customer can select relevant quality attributes and metrics and evaluate quality attributes with evaluation metrics.

LITERATURE SURVEY

Big data and big data quality
Big data

Big data is a term that describes large volume of data. Data are both unstructured and structured format. Unstructured data like document files, social media data, and website content. Structured data like SQL database stores. Big data is large data set and use this data to analyze computationally association, trends and human interaction and behavior.

Data quality

Data quality is a process of assessment of data. It defines set of values to gather qualitative and quantitative data. Data is consider high quality if data use in decision making, planning and operation. Quality of data is measure using attributes of data quality.

Quality attributes

Data quality dimension is term used to in business to evaluated data quality. Associations select the information quality measurements and related measurement edges in light of their business setting, prerequisites, and levels of hazard and so on.

Data quality attributes might be used to:

1. Recognize which information things should be evaluated for information quality, normally this will be information things considered as basic to business operations and related administration detailing.
2. Survey which information quality measurements to utilize and their related weighting.
3. For every information quality measurement, characterize values or ranges to great and quality information.
4. Apply the appraisal criteria to the information things.
5. Audit the outcomes and decide whether information quality is worthy or not.

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Fig. 2: Data quality attributes.

Quality attributes definition [Fig 2]

1. *Accuracy*: Ensure that the input data is error free
2. *Completeness*: Check the extracted data is not missing
3. *Consistency*: Implies that not two or more values conflict with each other
4. *Relevancy:* The extracted information is helpful for the task. Non relevant data should not be considered.
5. *Validity*: Input data is valid in its purposed used.
6. *Timeliness:* The extracted input data is not old data. The timestamp is necessary when retrieving the data.
7. *Believability:* the extracted data is valid and credible

Quality metric

Quality metrics are components of an effective quality management plan and measure properties of quality attributes. To assess and analyze the quality in any system, first need to characterize any quality attributes which are relevant to that system. In proposed system quality attributes are consider:

- Relevancy: The extracted information is helpful for the task. Non relevant data should not be considered.
- Timeliness: The extracted input data is not old data. The timestamp is necessary when retrieving the data.
- Believability: the extracted data is valid and credible.
- Accuracy: Ensure that the input data is error free

RELATED WORK

Big data is relevant to many components like government, healthcare, business management, social media, education, life science. Using big data these components improve their decision making, transparency, quality by providing continuous monitoring. The challenges are arises when the volume of structured and unstructured data coming from different sources. The reason for generating a large amount of data, big data-based application introduced new challenges and issues for quality assurance engineers [4]. These challenges are not only limited to data analysis but also to a big data system that manages all the information [1].
Recently social media data such as Twitter, Facebook increase business by providing insights into customer opinions, thoughts, and preferences. Design the platform that supports to monitoring and analyzing customer feedback in social media network and identifies issues which are faced by customers. Internet users communicate and express their thoughts with thousands of other people. People use a social media platform to share their ideas and experiences with different customer products and services [2].

When data are coming from different sources maintaining or evaluating the quality of data is also important. Quality metrics are components of an effective quality management plan and measure properties of quality attributes. To assess and analyze the quality in any system, first need to characterize any quality attributes which are relevant to that system. Quality attributes such as Accuracy, performance, consistency, timeliness, completeness, relevancy [3] [4] are used to evaluate quality in social media data. Research aim [4] is providing

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trustworthiness metrics for information provenance and quality evaluation.Quality metrics measure the performance of product and processes. Each metrics has following properties [2].

- Description: Metric description.
- Purpose: The purpose of metric.
- Target: Where metric are used.
- Formula: How the value of metric is achieved.
- Range value: Value of range for the metric evaluation.
- Acceptable Value: Minimum value for accepted quality attributes.
- Rules: The set of measurement value range and a set of constraints which define a set of target measurement.

The above observations and literature studies [1-6] indicate that quality evaluation is limited to only a few qualities attributes, the purpose is to increase the quality assessment to introduce more quality attributes. Data quality management is one where evaluate data quality and improve business decision making. Data quality management is continuous analysis, observation and improvement overall quality of the organization. Unstructured sources like social media need data quality management for improving their data quality. Data quality management is one where evaluate data quality and improve business decision making. Data quality management is continuous analysis, observation and improvement overall quality of the organization. Ensuring data quality involves following steps [Fig 3]:

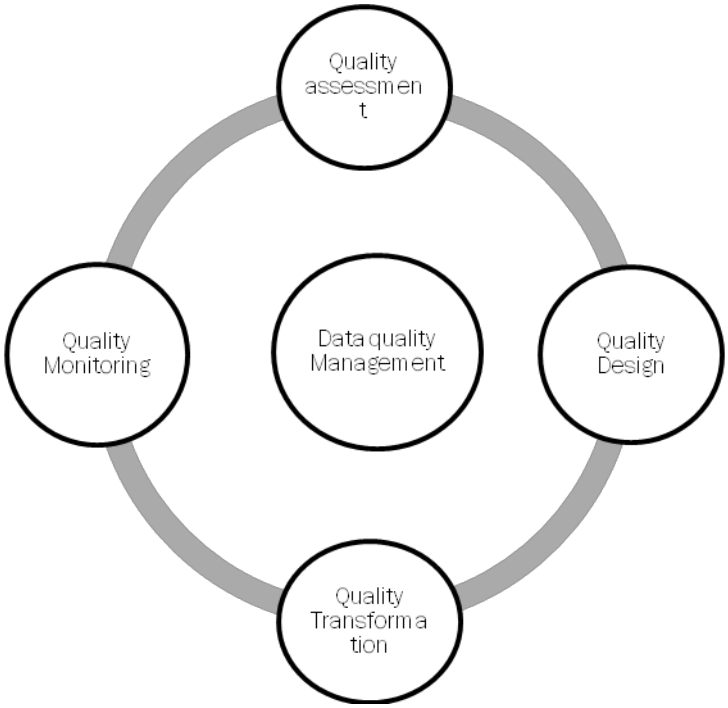

Fig.3: Data quality management.

Quality assessment- In quality evaluation phase, decide data source type to extract the data. It is a way for practitioners to understand the scope of how poor quality data affects on business process and develop a business case for data quality management.

Quality design

In the quality design phase, design and analysis the quality process and concentrate on the data elements that are consider based on the selected business user needs.

Quality transformation

In quality transformation phase, define business related quality rules and perform measurement using metrics

Quality monitoring

In quality monitoring phase, review expectations and refine rules and monitor data quality versus target.

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MATERIAL AND METHODS

Architecture of proposed system is given in Fig-4.

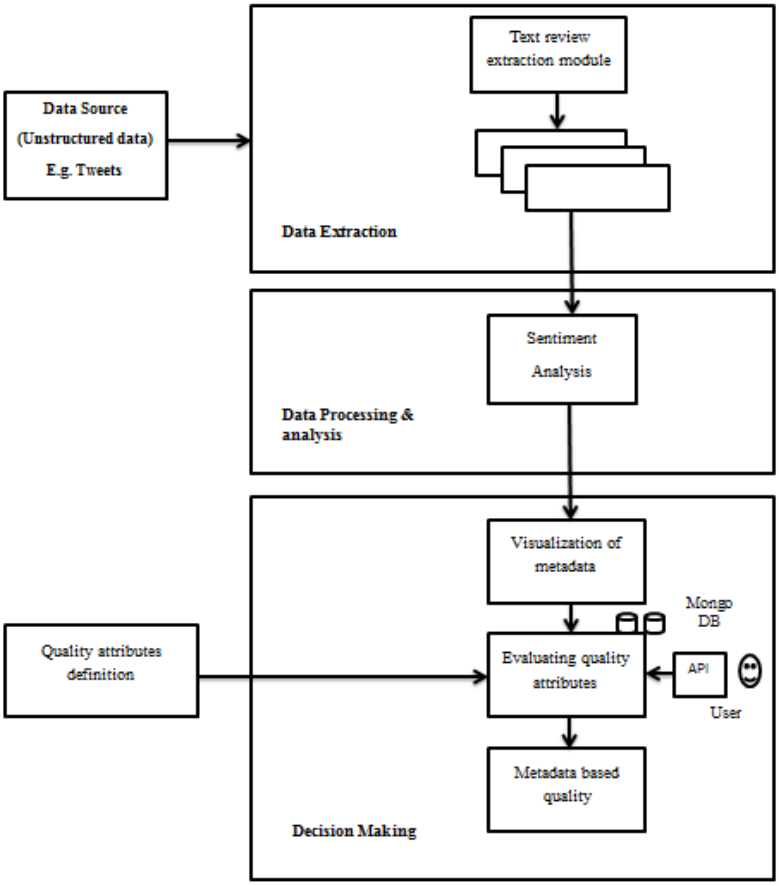

Fig.4: Architecture of proposed system.

Data Source

The main purpose of the system is to collect social media data (e.g. tweets) to achieve customer insight that can be used in business decision making. The decision-making policies have a great importance in quality evaluation. Social media source like tweeter where peoples are connecting to share their opinions and find out what happening in the world right now (Input data). Input data (unstructured data) are useful to business for improve data quality and decision making.

Data Extractor

Data extractor extracts tweets or document. Input data comes in preprocessing phase where data gets filtered. The links and punctuations are removed in that data and data become relevant to the system. Extracted data are in .json format. The design has been presentedfrom utilizing the developed tool for quality management of Twitter or .json based data sets. First, parse .json file by following attributes: Battery, Charger, display, phone, price, processor, ram, rare camera, screen, SD card, and weight.

Quality attributes

Apply quality attributes on input data and assess only those data which are fit into the quality attribute dimensions. Sentiment analysis is applied on assess data. Sentiment analysis is the process of recognition whether extracted data is positive, negative or neutral. Input data is scanned for positive or negative words like sad, happy, great, and terrible. Algorithms are used to score the document to decide whether they indicate positive or negative sentiment. Evaluate The Sentiment Analysis by Review Text Polarity and Polarity Confidence. Store the Polarity Confidence in Unstructured Database MongoDB which store data as documents. Then applying Decision Making Quality Attributes is:Accuracy, Timeliness, Confidences, relevance, popularity Tweets are created based on metadata, and related quality attributes. Metadata are a help to users to validate the quality and value of data for business usage. Manage quality metadata and

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attributes; rules are needed to define in quality, i.e. which quality attributes can be used and where. The system applies below Quality attributes [5].

- *Accuracy*-Ensure that the input data is error free
- Timeliness- The extracted input data is not old data. The timestamp is necessary when retrieving the data.
- *Confidence* – How quality based data is important for business decision making is decide by confidence attribute.
- *Relevance*- The extracted information is helpful for the task. Non-relevant data should not be considered.
- *Popularity*- Source of the system provides correct information and this information having numbers of followers.

Visualization of metadata

The relevant data is visualized to the end user, and decision-making policy defines the valuable data for decision making by selecting only those data sets with the correct quality attribute value.

Sentiment analysis algorithm

Input is text string. Input is always in JSON format. JSON syntax is a subset of JavaScript syntax. The JavaScript function JSON.parse(text) can be used to convert a JSON text into a JavaScript object. It involves the breaking down text into component parts with explanation of function and syntactic relationship of each component part. . First load positive words and negative words from text. If in text find positive word then count of positive word is incremented by 1 or if find negative word then count of negative word is incremented by 1. After finding positive and negative words calculate positive ratio and negative ratio. That is positive ratio is count by positive count divide by count of all words and negative Ratio is count by negative count divided by count of all words. Positive ratio set text is applicable and negative ratio set text is not applicable.If polarity is Applicable Then find positive Count in text and Apply range in between 0 -5.

Algorithm 1: Sentiment analysis

Input- text string
Output- Confidence

1. positiveWords = load positive words
negativeWords = load negative words
2. for each tweet:
parse the tweet
3. tweetWords = all the words in the tweet text
positiveCount = 0
negativeCount = 0
4. if candidate is in the text:
if a positive word is in the text:
positiveCount = positiveCount + 1
if a negative word is in the text:
negativeCount = negativeCount + 1
5. positiveRatio = positiveCount / count of all words
Polarity = Applicable
negativeRatio = negativeCount / count of all words
Polarity = Not applicable
6. Confidence: If polarity is Applicable Then find positiveCount in text and Apply range in between 0 - 5
ElsePolarity is not applicable

A. System Algorithm

Algorithm 2: System Algorithm

Input= data set
Output= complete data set

1. Set of input R= {r1, r2, r3,....., rn}
Where,
rn = total number of reviews

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2. Parse document Pd= Pd(r1,r2,r3,.....rn)= json(r1,r2,r3,.....rn)
Where,
Pd = total numbers of reviews parsed

3. Text extraction
Fp= {f1, f2, f3,.....fn}
Where,
Fp= product features
Therefore,
Text extraction= $\sum_{f=1}^{n}(Pd\{r1,r2,r3 \dots \dots rn\})$
Tr= $\frac{|(Fp)\cap(Pd|}{|(Pd)|}$

Where,
Tr= Total number of reviews extracted from features of product
Fp= Product Features
Pd= total numbers of reviews parsed

4. Let, W= Wordcount
WTq= bw (Tq1, Tq2, Tq3.....Tqn) and gw (Tq1, Tq2,Tq3.....Tqn)
Where,
bw= badword
gw= goodword

WTq are the words count of bw and gw for finding support.

5. Quality attribute
Set of quality attribute Q= {q1, q2, q3, q4}
Tq= $\sum_{q=1}^{n}(Tr\{tr1,tr2,tr3 \dots \dots trn\})$
Where,Tq= Total number of reviews extracted from quality attributes

6. Supp (Tq)= $\frac{|tegw;Tq\subseteq t|}{|gw|}$
t= data set
gw= good words
Tq= support

$$\text{Confidence}(Tq \rightarrow gw) = \frac{|supp(Tq \cup gw|}{|supp(gw)|}$$

7. Accuracy (Tr) = $\frac{\sum_{Fp=0}^{n} Tr1}{\sum_{Fp=0}^{n} Fp1}$

Label= Complete data or Incomplete data

B. Mathematical model

Mathematical model

1. Tr = $\frac{|(Fp)\cap(Pd|}{|(Pd)|}$

Where,
Tr= Total number of reviews extracted from features of product
Fp= Product features
Pd= total numbers of reviews parsed

2. Supp (Tq)= $\frac{|tegw;Tq\subseteq t|}{|gw|}$
t= data set
gw= good words
Tq= support

3. $\text{Confidence}(Tq \rightarrow gw) = \frac{|supp(Tq \cup gw|}{|supp(gw)|}$

4. Accuracy (Tr) = $\frac{\sum_{Fp=0}^{n} Tr1}{\sum_{Fp=0}^{n} Fp1}$

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RESULTS

The proposed system provides real time and quality data to business for decision making. Identify data items or input data need to be assessed for business data quality. Existing system taking decisionfor evaluate quality of data based on rating. If reviewer give the four rating to particular product and write comment product is not good that time businesses consider only rating not comment. From this analysis system businesses taking wrong decision. In proposed system taking decision based on comments. Apply quality attributes on input data and evaluate only those data which are fit into the quality attribute dimensions. For each quality, attribute defines the range to reprinting good or bad quality data. Using relevancy attribute businesses check for which product user said product is good or product is bad. For analysis, the result consider five reviews [Table 1]. First, apply timeliness attribute, the extracted input data is not old data. In proposed system consider only after 2014 year reviews. Then apply relevancy attribute, forextracted information is helpful for the task and in this situation,non-relevant data should not be considered. From relevancy find accuracy of data. Accuracy defineinput data is error free. Then find popularity of data. Form popularity attribute find which data is useful and which data is not useful.

Table 1: Results of five reviews

No. of Reviews	Confidence	Analysis
1	0.65497869	Incomplete data
2	0.90458458	Complete data
3	0.59680178	Incomplete data
4	0.98198456	Complete data
5	0.98235877	Complete data

Sentiment analysis is applying on usedfull data and finds confidence of the data. Sentiment analysis determines positive and negative sentiment from text. Sentiment analysis API (or document) provides very accurate analysis of the emotion of the text from sources. The analysis of text presented in range (e.g. range between 0 to 5). The result of scores closer to 5 considered to be positive sentiment and scores closer to 0 will be of negative sentiment. Based on above principle figure show the result is, 63% data is incomplete and 96% data are complete, which is shown in [Fig 5].

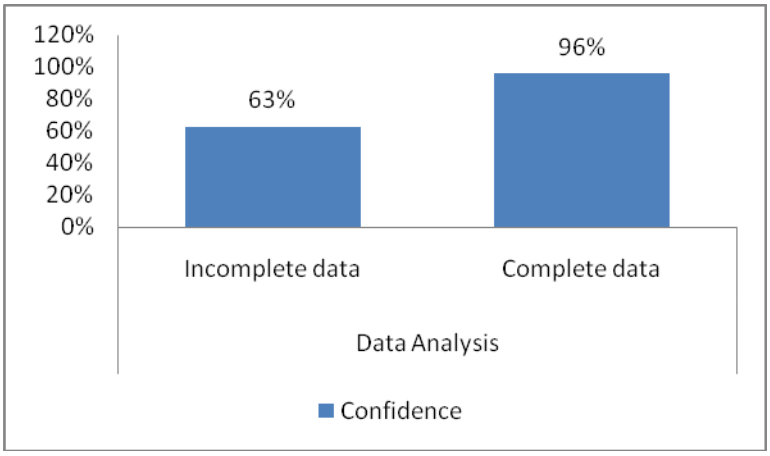

Fig. 5: Result of data analysis.

CONCLUSION

Most of existing research addresses different solutions to evaluate data quality in big data such as data cleansing using quality rules based on different functional dependency, data provenance. The quality of data is assessed in each of data processing phase. The proposed system provide preprocessing quality framework for big data quality. Generate quality rules which are applied on preprocessing activities to data analysis. Apply quality attributes on input data and evaluate data quality. The system analysis various data across the social web and helps to convert data into actionable insights. Sentiment analysis is used to review the social media data. The system provides the visualized data and generates a report based on sentiment analysis. Generation of data quality process on sample data set provides very faster result of data quality evaluation and instance updates are done when quality rules are inserted or deleted.

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CONFLICT OF INTEREST

There is no conflict of interest.

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FINANCIAL DISCLOSURE

None

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ARTICLE

AN EFFICIENT DUAL FLUIDIZED BED BIOMASS GASIFICATION SYSTEM BASED ON FLUID PARTICLES VERTICITY

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ABSTRACT

A combined modelling of system is established for a dual fluidized bed (DFB) biomass gasifier and a rotary biomass dryer utilizing a grouping of built in unit and user defined operations. In the proposed method the idea of verticity is considered for compensating the excessive fuel and air supply in the fast fluidized bed (FFB) reactor in which with the rotating movement of air a single source of fire can flared up with no extra fuel supply. In order to achieve this the proposed methodology initially models the DFB gasification system based on the quasi equilibrium model. Then with the help of rotary drier model the moisture content with the biomass feedstock are eliminated and is fed to the gasification reactor. Next to introduce the verticity of the fluid particles, different air feeding angular fixtures are used in the FFB reactor. With this proposed model we can reduce the excessive air and fuel required in the FFB reactor considerably and also reduce the impact of energy efficiency of the system. The proposed method will be implemented on the MATLAB platform and the experimental results are validated based on the operation parameters such as feed air to the FFB reactor, gasification temperature, steam to biomass (S/B) ratio and initial moisture content of the feed biomass.

KEY WORDS
Dual fluidized bed gasifier; biomass gasification; biomass drying; verticity; fast fluidized bed (FFB).

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INTRODUCTION

Gasification could be a method that converts organic or fossil fuel based carbonic materials into monoxide, hydrogen and dioxide [1]. This is often achieved by reacting the fabric at high temperatures (>700 °C), while not combustion, with a controlled quantity of gas and/or steam. The ensuing gas mixture is termed syngas (from synthesis gas or artificial gas) or producer gas and is itself a fuel. The energy derived from chemical action and combustion of the resultant gas is taken into account to be a supply of renewable energy if the vaporized compounds were obtained from biomass. It's a thermochemical method, which means that the feedstock is heated to high temperatures, manufacturing gases which may bear chemical reactions to create a synthesis gas. This examination of technology for the chemical action of biomass and wastes 'syngas' in the main contains hydrogen and monoxide, and may then be accustomed turn out energy or a spread of chemicals, together with liquid and aerosolized transport fuels. Gasification could be a key technology for the utilization of biomass [2]. It delivers a high flexibility in utilizing different quite feedstock materials in addition as within the generation of various merchandise. In main, all differing types of biomass is regenerate by chemical action into syngas in the main comprising hydrogen, monoxide, dioxide and alkane series [3].

Biomass refers to all or any organic materials that are created from plants [4]. It's wide thought-about to be a significant potential fuel and natural resources for the longer term [5] [6]. Based on the resource size, there's the potential to supply a minimum of five hundredth of Europe's total energy demand, from purpose full-grown biomass mistreatment agricultural land now not needed for food, and from wastes and residues from agriculture, commerce and shoppers [7]. There are many completely different generic styles of gasification technology that are incontestable or developed for conversion of biomass feed stocks. Most of them are developed and commercialized for the assembly of heat and power from the syngas, instead of liquid fuel production. They're draft fastened bed, draught fastened bed, Entrained flow gasifiers (EF), Bubbling fluidized bed gasifiers (BFB), circulating fluidized bed gasifiers (CFB), dual Fluidized Bed and alternative steam blown, indirectly heated gasifiers and Plasma gasifiers [8].

Here, we tend to see concerning the DFB gasifier and its technologies. The DFB gasification process, offers varied benefits for biomass chemical change moreover because the utilization of different solid feed stocks [9]. This technology is eventual as a result of it yields high caloric product gas freed from N2 dilution even once air is employed to get the desired endoergic heat via in place combustion [10]. Indirect biomass gasification in a DFB system will be accustomed convert solid biomass into raw gas, which might be more upgraded to be used as substitute fossil fuel, city gas, liquid transport fuels or fuel in gas turbines [11]. DFB gasification advantages from the expertise gained with BFB and CFB, though square measure at associate in earlier stage of development than EF, BFB and CFB. DFB systems solely presently in operation in little scale heat [12] [13] and applications of power, and that they still got to be incontestable at pressure – but, if developed, these pressurized systems have the potential to supply low price, N free syngas.

The players concerned have a shorter log of expertise, however have with success operated plants at high availabilities, and a few have plans for liquid fuels production within the future. Twin systems have intermediate feedstock necessities, having the ability to just accept larger particle sizes and a wider vary of wetness contents [14]. The cluster of twin technologies even have many different attainable comes

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mentioned (such as Silva Gas for Process Energy, Taylor Biomass for Abengoa), and consequently the ECN MILENA 3.8odt/day pilot plant, operational since 2008, has fairly formidable scale-up goals (480odt/day by 2015). DFB gasifiers have had a scattered development within the past, however recent victorious demonstrations and interest in BTL applications are hopeful [15].

The organization of the paper is summarized as follows. Section 2 gives some of the recent research done in fluidized gasification system. In section 3, we explained our proposed methodology and the experimental results are shown in section 4 followed by conclusion in section 5.

RELATED WORKS

The very recent works related to the Fluidized Bed Biomass Gasification System are listed below:

A. Gomez-Barea and B. Leckner [16] have reviewed a Modeling of biomass chemical change in fluidized-bed (FB) reactors. Most of the modeling components from FB combustor models utilized in models of FB biomass chemical change (FBBG). There have been variations, although like within the mode of conversion of the char particles and within the quantity of heat transferred to surfaces. Char conversion was, in distinction, acknowledge however, revealed FBBG models haven't forbidden the offered info. The assorted approaches applied for reactor modeling, from black box models to machine fluid-dynamic models, were delineated, demonstrating their state of development and also the quality of every approach looking on the aim of the model. The fluidization model, wherever the fluid-dynamics of the FB was shortened by semi-empirical correlations, is that the commonest approach up thus far, utilized with major success. Most of the FB biomass chemical change models match moderately well experiments elite for validation, despite the assorted formulations and input file, additionally the validation of models with information from complete FB biomass chemical change units remains to be done.

Ion Iliuta et al. [17] have projected to investigate the new thought of all thermal cyclic multi-compartment BFB steam biomass gasifier. The active, one-dimensional, multi-component, non-isothermal model established for this idea accounts for elaborate solid and gas flow dynamics wherever upon chemical process or combustion reaction mechanics, thermal effects and freeboard-zone reactions were secured. Within the Multi-compartment effervescent fluidized bed (BFB) hybrid steam gasifier, all compartments were of rectangular cross-sections, contiguous and were divided by extremely thermally semiconducting sheets whereby an economical heat transfer happens. They showed that char combustion produces ample heat to sustain chemical process at warmth by tolerating up to twenty percentage heat losses. A non-diluted high hydrogen output and comparatively large hydrogen content may well be obtained from biomass chemical process in two-compartment effervescent fluidized-bed reactors. All thermal operation needed burning extra fuel so as to keep up a warmth within the combustor, and afterward within the gasifier itself, conjointly this operation may well be achieved with a switch periods of a moment supporting practicability of this new thought.

K. Goransson et al. [18] have conferred a preliminary check all thermal biomass gasifier at middle Kingdom of Sweden University (MIUN). The MIUN gasifier joined a fluidized bed gasifier and a CFB riser as a combustor with a style appropriate for in-built tar/CH4 chemical process restructuring. The check was dispensed by 2 steps, fluid-dynamic study and measurements of gas composition and tar. These tests give basic data for temperature management within the combustor and also the gasifier by the bed material circulation rate. For the gas composition measurements, the syngas was drawn by an air pump through a gas acquisition stage and tested manually in a very gas sampling bag (Cali-5-bond) and examined off-line in a very parallel FID and TCD detection GC-system. The biomass chemical action technology at MIUN was straightforward, inexpensive, and dependable.

Thanh D.B. Nguyen et al. [19] have developed a three-stage steady state model (TSM) for biomass steam gasification during a DFB to compute the producer gas composition, carbon conversion, heat recovery, price potency, and heat demand required for the energy-absorbing gasification reactions. These models divided into 3 stages, the biomass shift to char and volatiles, the solid–gas reactions between biomass char and gasifying reagents (carbon oxide and steam) in fluidized- bed, and also the gas part reactions among the vaporized species within the free board of the gasifier. At every stage, associate degree empirical equation was calculable from experimental information to calculate carbon conversion and vaporized parts. It had been assumed that each unpersuaded char and extra fuel were fully combusted at 950°C within the combustor and also the heat needed for chemical change reactions was provided by the bed material. These have assessed the method performance of DFB that specialize in the electrical power generation, victimization the TSM. A completely unique procedure was initially mentioned there to search out effective in operation conditions of DFB on the idea of seven method performance criteria.

F. Miccio et al. [20] have planned the combined gasification of biomass and brown coal in an interior circulating fluidized bed (ICFB) for generating a valuable gas. The ICFB additionally also known as dual bed, had been practical to biomass gasification. The main advantage of this technology was the likelihood to hold out the method in two interconnected vessels, the primary operative beneath gasification conditions, whereas the other permits for partial combustion of the fuel and char burn-off. The heat and mass transfer between the vessels was delivered by the high bed circulation rate. The most advantage of associate degree ICFB gasifier was the assembly of gas with doubtless high heating price and made in flammable species, decreased dilution with element. The reliableness of the devices that enable the

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convective mass and warmth transfer between the combustor and also the gasifier ought to be improved to utterly mitigate leaks. The analysis of bed samples once utilization within the gasifier for over forty hours confirmed that a decrease of each the typical size and also the expanse occurred for the catalyst. Finding these ascribed to the mechanical stresses imparted by the sand at the high fluidization speed within the riser. These features were the main focus of their in progress investigations.

AfsinGungor Associate and UgurYildirim [21] have planned a 2-D model for an atmospheric circulating fluidized bed (CFB) biomass gasifier that utilized the particle based approach conjointly integrated and at the same time expected the hydraulics and gasification aspects. They separated the biomass gasification modeling into 3 classes, physical science equilibrium models, kinetic rate models and neural network models. These dimensional models self-addressed each hydraulics parameters and reaction kinetic modeling. The gasifier operation needed understanding of the result of assorted operational parameters on the performance of the system. The consequences of the operational parameters like gasifier temperature of an atmospheric biomass CFB gasifier valid with experimental knowledge within the literature for sensitivity analysis.

PROPOSED METHODOLOGY OF THE GASIFICATION SYSTEM

The energy demand of BFB reactor depends on the excessive fuel and air supply in FFB reactor to produce sufficient heat for gasification. Thus within the proposed methodology the thought of verticity is taken into account for compensating the excessive fuel and air supply within the FFB reactor during which with the rotating movement of air one supply of fireside will increasing up with no further fuel supply. So as to attain this the proposed methodology primarily models the DFB gasification system supported the quasi equilibrium model. Then rotary drier model is employed to get rid of the moisture content with the biomass feedstock and is fed to the gasification reactor. Next to introduce the verticity of the fluid particles, completely different air feeding angular fixtures area unit employed in the FFB reactor. The schematic diagram of the proposed methodology is shown in [Fig. 1].

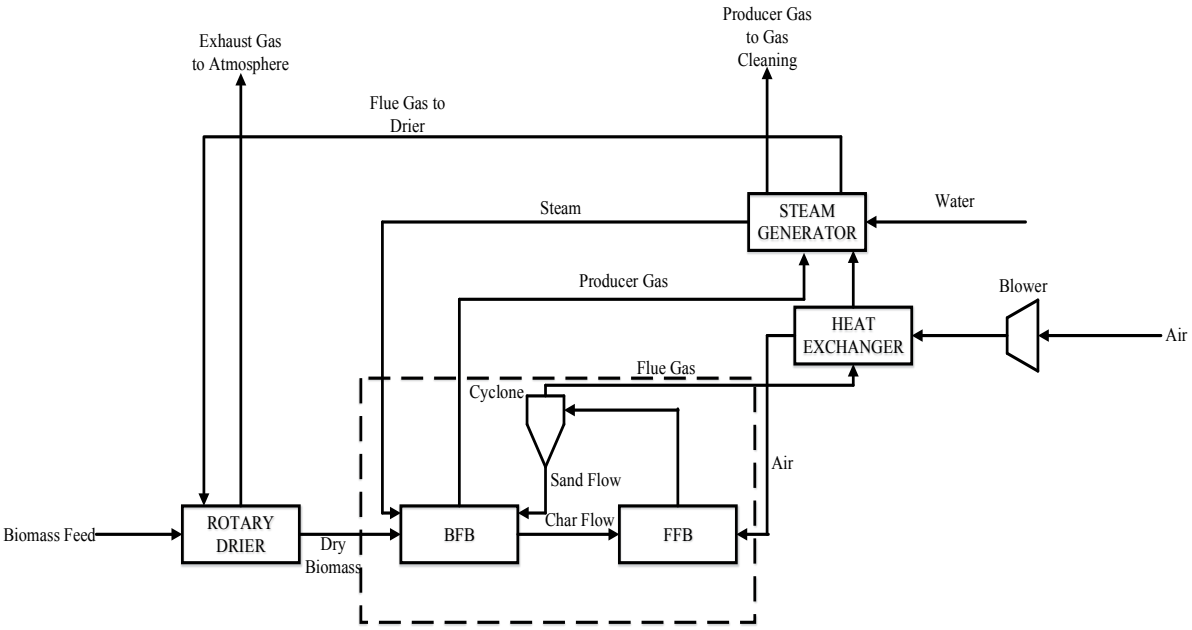

Fig. 1: Schematic diagram of proposed method

From [Fig. 1], the biomass first enter the rotary drier to diminish the moisture content. At that point, the dried biomass is given to the BFB reactor for steam gasification. The gasifier which provides the producer gas is chilled off to recoup for gas cleaning. The heat of the producer gas is recuperated for steam generation which is utilized in BFB reactor. The hot flue gas from FFB is utilized for preheating of air took after by generation of steam and toward the end for drying of biomass. If there should be an occurrence of typical operation, the FFB reactor temperature is higher than BFB reactor gasification temperature to convey the required heat with the end goal of gasification. The FFB reactor flue gas subsequent to preheating of air, generation of steam and drying of biomass is discharged to environment. Steam generation and air preheating are indirect procedure while biomass drying is direct procedure where biomass and flue gas are in direct contact. Thus, the exhaust gas water is from humidity of air, vaporization of food biomass moisture amid drying and excessive fuel ignition. The water imported to framework is utilized for source of steam produced in framework. The water present in the producer gas is acquired from the steam mixed to the BFB reactor, the water vaporized from gasification reactions and biomass.

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Characteristics of Biomass

In this proposed method, chips from Pinus radiata wood are utilized as feedstock in which the chemical formula is considered to be $C_{31}H_{45}O_{24.5}$ with proximate analysis and ultimate analysis shown in [Table 1] [22]. The LHV (Lower Heating Value) of biomass, ash free basis and water is computed by using the below correlation [23].

$$LHV_{BM} = 34835z_C + 93870z_H + 6280z_N + 10465z_S - 10800z_O \quad (1)$$

where is mass fraction of carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen (O).

Table 1: Proximate analysis and ultimate analysis results of Pinus radiate

Proximate Analysis wt % (od)		Ultimate Analysis wt % (od)	
H_2O	0	C	51.2
Volatile	84	H	6.1
Fixed Carbon	15.6	O	42.3
Ash	0.4	N	0.2
-	-	S	0.02

The physical and thermal properties of Pinus radiata are calculated as a hypothetical compound. The feedstock heat formation is computed from reaction of the combustion, equation (2), and the capacity of heat of moisture free feedstock is computed using equation (3) [24].

$$C_{31}H_{45}O_{24.5} + 30O_2 \rightarrow 22.5H_2O + 31CO_2 \quad (2)$$

$$C_{P_{BM}} = 0.003867T + 0.1031 \quad (3)$$

where T is the temperature in Kelvin (K).

Rotary Drier Modelling

The modelling of the rotary drier's model is demonstrated in [Fig. 2]. The feedstock is thought to be from green log preparingor from forestresides, thusly, the moisturecontent is evaluated to be somewhere around 50-60%. A rotary drier was chosen for biomass drying in light of the fact that it is moderately straightforward and adaptable for utilizing distinctive types and sizes of biomass feedstock. Co-current design is received for the rotary drying, which avoids direct contact between dry biomass and thehot drying medium in this way lesser potential fire danger [30].

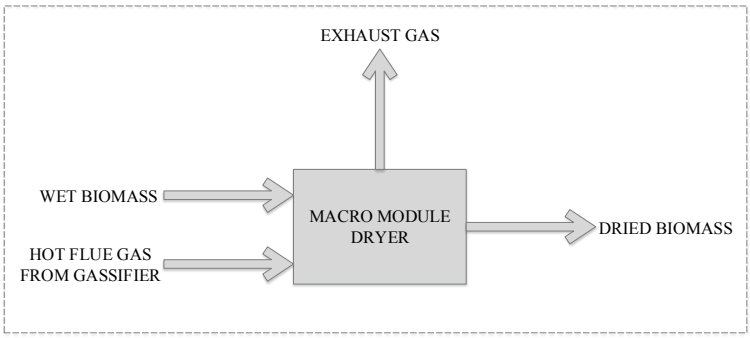

Fig. 2: Rotary dryer model

The computation of drying rate of actual biomass can be complex, which includes heat and mass transfer inside the solid biomass and among the surface of biomass and the drying medium. The absolute moisture content is relatively high, the rate of drying is still sensibly fast at drying end, and hence the process of drying is mainly controlled by the rate of heat transfer.

Mass Equilibrium of Water

The general mass equalization for the water over dryer is moderately basic on the grounds that there is no chemical reaction included in the process and the water is the main segment exchanging between stages. The water lost by the feed biomass is picked up by the gas stage, as is depicted in below equation

$$\dot{M}_{FG}(X_2 - X_1) = \dot{M}_{BM}(Y_1 - Y_2) \quad (4)$$

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Where $\dot{M}_{FG}$ - rate of mass flow of flue gas (kg/s)

$\dot{M}_{FG}$ - Biomass on dry basis (kg/s)

$X_1 \& X_2$ - Flue gas humidity at inlet and outlet (kg/kg)

$Y_1 \& Y_2$ - Moisture content of biomass at inlet and outlet (kg/kg), which can be calculated by using the below equation

$$Y = MC/(100 - MC) \quad (5)$$

where MC indicates the moisture content of feed biomass. The humidity and rate of mass flow of inlet flue gas is calculated from operation of gasifier unit. Thus, if we know the target moisture content of the biomassandinlet, the outlet humidity of flue gas can be calculated from equation (4).

Energy Balance

The balance of energy for the drying system depends on the assumption that the provided heat by flue gas is equal to the gained heat by biomass for heat-up and vaporization of water plus the heat loss.

$$H = H_1 + H_2 + H_3 + H_4 + H_5 + H_L \quad (6)$$

In which

$$H = \left(\dot{M}_{FG} C_{p_{FG}} + \dot{M}_{FG} X_1 C_{p_{VW}}\right)(T_{FG} - T_{OUT}) \quad (7)$$

H_1 gives the heat for moist biomass to be heated to temperature of wet bulb which is given by

$$H_1 = \left(\dot{M}_{BM} C_{P_{BM}} + \dot{M}_{BM} Y_1 C_{P_{VW}}\right)(T_W - T_B) \quad (8)$$

H_2 gives the heat for vaporization of water at the temperature of wet bulb which is given by

$$H_2 = \dot{M}_{BM}(Y_1 - Y_2)\Delta Q_{VW} \quad (9)$$

H_3 gives the heat for biomass to be heated to the temperature of outlet temperature which is given by

$$H_3 = \left(\dot{M}_{BM} C_{P_{BM}}\right)(T_{OUT} - T_W) \quad (10)$$

H_4 is the heat utilized to heat moisture remaining in the biomass to the temperature at the outlet which is given by

$$H_4 = \left(\dot{M}_{BM} Y_2 C_{P_{LW}}\right)(T_{OUT} - T_W) \quad (11)$$

H_5 is the heat utilized to heat the water vapor to the temperature of outlet which is given by

$$H_5 = \dot{M}_{BM}(Y_1 - Y_2) C_{P_{VW}}(T_{OUT} - T_W) \quad (12)$$

H_L is the estimation of heat loss which is given by

$$H_L = 0.15H \quad (13)$$

In the above equations, is the latent heat of vaporization (kJ/kg), and are the inlet temperatures of biomass and flue gas, is the flue gas wet bulb temperature (0C), is the drier outlet temperature (0C), and and are the specific heat of biomass, flue gas, water, and liquid water (kJ/kg 0C) which are assigned as constants while drying.

Modeling Of DFB Gasification System

In this proposed method, DFB gasification system is modelled based on quasithree phase equilibrium model[19].Biomass steam gasification procedure is modeled in three phases including pyrolysis, char-gas reactions and reactions among gases. For modeling FFB reactor, a conversion reactor is described for combustion of unreacted char and unnecessary fuel. The DFB gasifier model developed is shown in [Fig. 3].

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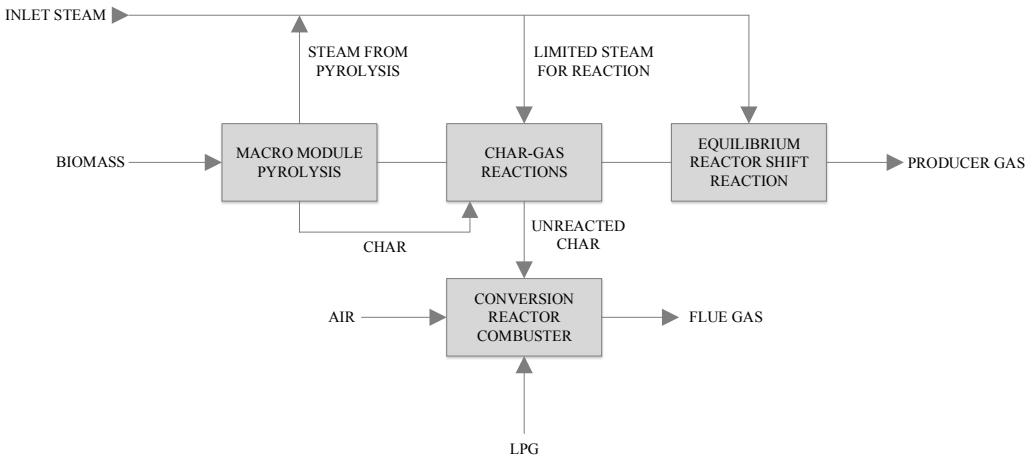

Fig. 3: DFB Gasification system model.

Pyrolysis Step Modelling

In Pyrolysis, biomass is transformed to char mixture and combustible gases. The exact prediction of the pyrolysis is considered as the most significant phase of the gasification [25]. Pyrolysis gas generally contains $H_2, CO_2, CO, H_2O, N_2, CH_4, H_2S, NH_3$ and tar vapors. Then by introduction of two empirical factors of molar ratio of CO/CO_2 (Φ_{CO}) and molar ratio of CH_4/H_2 (Φ_{CH_4}), five equations produced depends on elemental equilibrium of C, H and O components of biomass and gas to compute the concentration of five major elements of gas comprising of H_2, CO_2, CO, CH_4 and H_2O. In modelling, methane characterizes traces of other light hydro carbons and tar.

$$m_{CH_4} + m_{CO_2} + m_{CO} = m_C \quad (14)$$

$$4m_{CH_4} + 2m_{H_2} + 2m_{H_2O} = m_H \quad (15)$$

$$2m_{CO_2} + m_{CO} = m_O \quad (16)$$

$$m_{CO} - \Phi_{CO} m_{CO_2} = 0 \quad (17)$$

$$m_{CH_4} - \Phi_{CH_4} m_{H_2} = 0 \quad (18)$$

where, m_i is molar flow rate of each element (kmol/s). Φ_{CH_4} and Φ_{CO} are computed by the subsequent correlations as a function of temperature.

$$\Phi_{CH_4} = 1.4 \times A_2 \times \exp\left(-B_2/T_G\right) \quad (19)$$

$$\Phi_{CO} = A_1 \times \exp\left(-B_1/T_G\right) \quad (20)$$

where T_G is gasification temperature (K), $A_1 = 4.7 \times 10^3, A_2 = 2.28 \times 10^{-3}, B_1 = 7163.6$ and $B_2 = 5404.85$ which were attained from curve fitting of experimental data [26]. The composition and amount of tar will change considerably from pyrolysis to final gasification. However, in this transition is ignored and the absolute tar content of producer gas were considered as function of gasification temperature, which has been taken from data in [27]. Then, composition of methane was altered by subtracting the hydrogen and carbon content of tar and its composition is given in [28].

$$Tar(wt\%) = -5.61 \times 10^{-3} \times T_G(K) + 6.95 \quad (21)$$

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The other species concentration ($N_2, H_2S,$ and NH_3) are computed from fundamental balances for S and N and their reaction formation. The hydrogen composition is changed by subtracting the hydrogen content of hydrogen sulfide and ammonia.

Char Gas Reactions and Reactions among Gases Modelling

In this proposed method, boudouard, primary and secondary gas reactions were considered as char gas reactions whereas steam gas shift reaction was considered as steam gas reaction [29].

- Boudouard: $CO_2 + C \rightarrow 2CO$
- Primary Reaction: $H_2O + C \rightarrow H_2 + CO$
- Secondary Reaction: $2H_2O + C \rightarrow 2H_2 + CO_2$
- Shift Reaction: $H_2O + CO \rightarrow H_2 + CO_2$

It has been demonstrated that steam commitment to the primary and secondary reactions is restricted, along these lines, the steam commitment to reactions at balance can be computed utilizing a straightforward connection as follows [19].

$$\beta = \frac{m_{H_2O,CON}}{m_{H_2O}} = 51.4 \exp\left(-7542.8/T_G\right) \quad (22)$$

Where $m_{H_2O,CON}$ is the moles of steam that contributes to the reactions, m_{H_2O} represents the total moles of steam and T_G represents gasification temperature.

The requirement of heat for BFB gasification reactor is given by the sum of requirement of heat of pyrolysis, char reactions and steam gas reactions. The requirement of heat at each part is computed from enthalpy balance. The produced char from biomass gasification and the FFB reactor excessive fuel are combusted with supplied air to deliver the required energy for BFB reactor. The excessive fuel to FFB is denoted by ratio of excessive supply of fuel energy to feed biomass energy.

$$\omega = \frac{\dot{M}_{FUEL} \times LHV_{FUEL}}{\dot{M}_{BM} \times LHV_{BM}} \quad (23)$$

Where $\dot{M}_{BM}$ and $\dot{M}_{FUEL}$ are the mass flow of biomass and excessive fuel, and LHV_{BM} and LHV_{FUEL} are the equivalent lower heating values. The quantity of supplied air to FFB reactor is vital in design of DFB system. The need for supplying air to FFB reactor is for char oxidizing and excessive fuel as well as performing as fluidizing agent. The excessive factor for supplied air is defined as follows.

$$\lambda = \dot{M}_{AIR} / \dot{M}_{AIR,STOICH} \quad (24)$$

where $\dot{M}_{AIR}$ is the mass flow rate of supplied air to FFB (kg/s), $\dot{M}_{AIR,STIOCH}$ is the mass flow rate of air at stoichiometric condition.

Biomass and air Feeding Angular Fixtures

In the proposed method, we have considered verticity for compensating the excessive fuel and air supply in the FFB reactor in which with the rotating movement of air from a single source of fire can flared up with no extra fuel supply. In order to achieve this we have designed the FFB reactor with different angular fixtures of biomass and secondary air inlet to study the effect of fluid particles in different characterized nature of verticity and their effects on axial temperature profiles for different angular fixtures of biomass and secondary air inlet in the fluidized bed zone. Four different angular (450,600,750and 900) biomass-feeding attachments are made and the required amount of biomass is filled in the hopper, which is connected to one end of the biomass feeding angular attachment. Similarly four angular (450,600,750and 900) air-feeding attachments and capacity blowers are connected to one end of the each air feeding angular attachment. Both attachments are fastened on the reactor chamber at maximum expandable bed height.

RESULTS & DISCUSSIONS

The proposed method is implemented on MATLAB working platform and the results obtained are given in this section. The results for the effects of temperature of gasification from 750 °C to 850 °C are shown in

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[Fig. 4] and for the S/B ratio effect from 0.6 to 1.2in [Fig. 5]. It can be seen in [Fig. 4 and [Fig. 5], with increase in temperature of gasification, the composition of H2 increases considerably while composition of CO decreases.

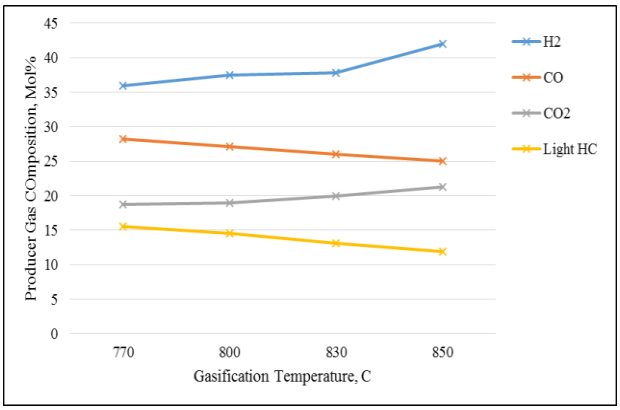

Fig. 4: Producer gas composition,*S/B* = 0.84

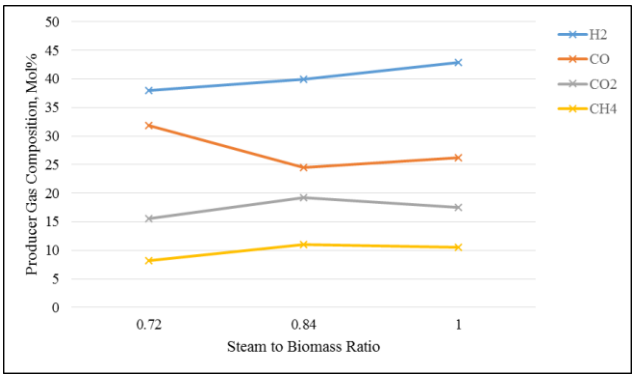

Fig. 5: Comparison of the producer gas composition, *T* = 850 °C

The effect of temperature of gasification and ratio of S/B on the performance of gasification has been observed utilizing the model established and the experimental results are presented in [Fig. 6] for the effect of gasification temperature and in [Fig. 7], for the effect of S/B ratio. From the Fig. 6 it can be observed that both the temperature of gasification and the S/B ratio have positive effects on gas output and ratio of H2/CO in the producer gas. The experimental results further more shows that the char output decreases with temperature of gasification and the ratio of S/B when more carbon is transformed to gas thereby more gas output. Though, as can be understood in [Fig. 6] and Fig. 7], the temperature of gasification has more important effect than the ratio of S/B on the gas output. For that reason, increasing the temperature is more efficient on reactions of char-gas than adding more steam to the system.

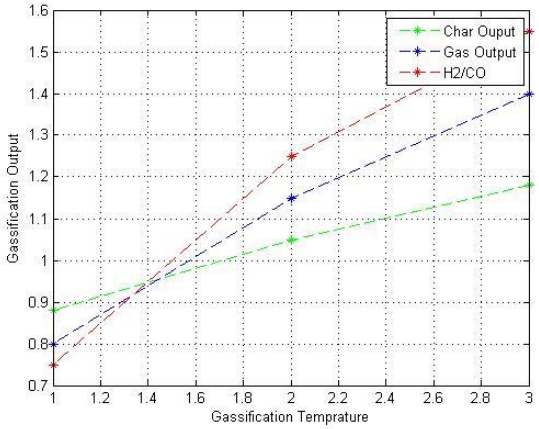

Fig. 6: The effect of gasification temperature on the system outputs, S/B = 0.80

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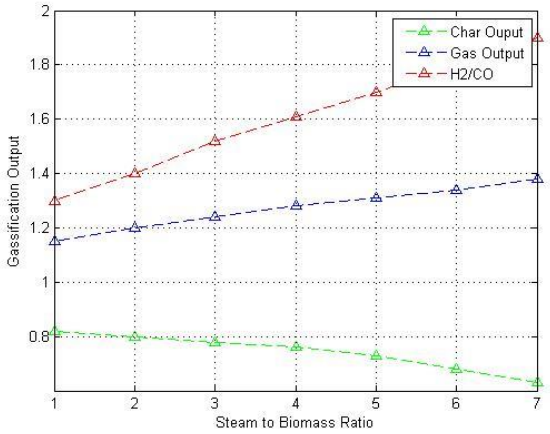

Fig. 7: The effect of steam to biomass ratio on the system outputs, T = 850 °C.

From [Fig. 7], it can also be understood that with rise in the ratio of S/B the gas output rises slightly but theratio of H2/CO of the produced gas rises more significantly. Both the temperature of gasification and ratio of S/B favor the steam-gas shift reaction near hydrogen production found according to the principle of Le Chatelier's, in which higher H_2/CO ratio results. The results obtained by different angular axis of biomass and Air feeding angular fixtures is shown in [Table 2] which gives the value of temperature, pressure drop and air velocity.

Table 2: Results of Biomass and Air feeding Attachment angle combination

Biomass and Air feeding Attachment angle combination	Temperature	Pressure Drop (N/m^2)	Air Velocity (m/s)	Mass Flow Air (Kg/s)
90^0-60^0	812	2901	0.023	0.0301
90^0-75^0	828	2845	0.020	0.0284
90^0-90^0	705	3470	0.047	0.0589
75^0-60^0	725	3345	0.042	0.0541
75^0-75^0	702	3478	0.048	0.0589
75^0-90^0	687	3712	0.051	0.0625
60^0-60^0	742	3648	0.044	0.0569
60^0-75^0	764	3601	0.035	0.0521
60^0-90^0	801	2941	0.020	0.0280

The effect of bed characteristics is another impact in the gasification system. The variation in the residence time with bed height is shown in [Fig. 8] in which the increase in bed height from 0.5 to 2.0D increased the residence time from 0.89 sec to 4.20 sec.

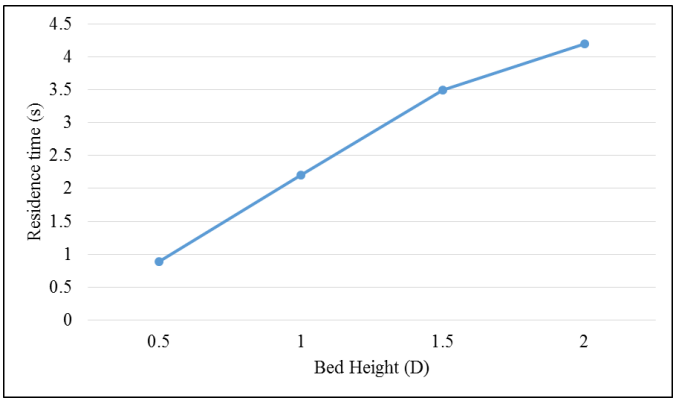

Fig. 8: Bed Height Characteristics with residence time

The impact of feed biomass moisture content by the exhaust gas on energy and exergy losses is shown in [Fig. 9]. As seen from the [Fig. 9], even though the exhaust gas energy loss rises intensely with rise in the content of feed biomass moisture, the exergy loss is not as much of exaggerated. The energy loss over exhaust gas rises with content of feed biomass moisture as its flow rate rises with more evaporation of

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water. Though, its exergy somewhat variate as its temperature is kept nearly constant with increase in content of feed biomass moisture. In the drying model, the inlet condition varies while temperature of exhaust gas and target biomass moisture content of are constant.

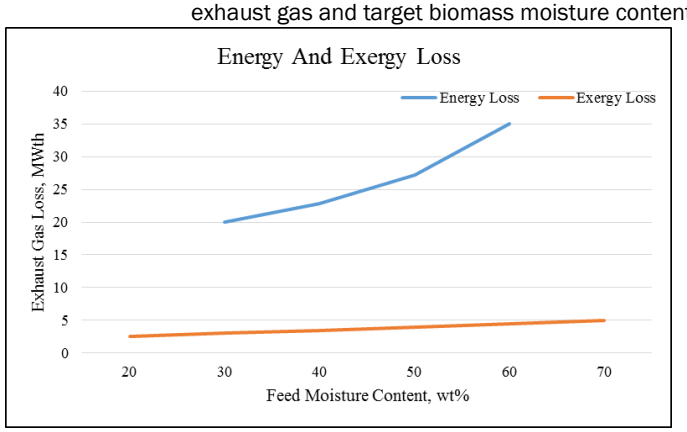

Fig. 9: The effect of feed moisture content on energy and exergy losses

The irreversibility of unit operations and the exhaust steam of flue gas are two sources of exergy loss though the irreversibility of dissimilar unit operations is the foremost contributor to the exergy loss of the system. The distribution of dissimilar unit operations in interior exergy loss of the system at different feed biomass moisture content is shown in [Fig. 10].

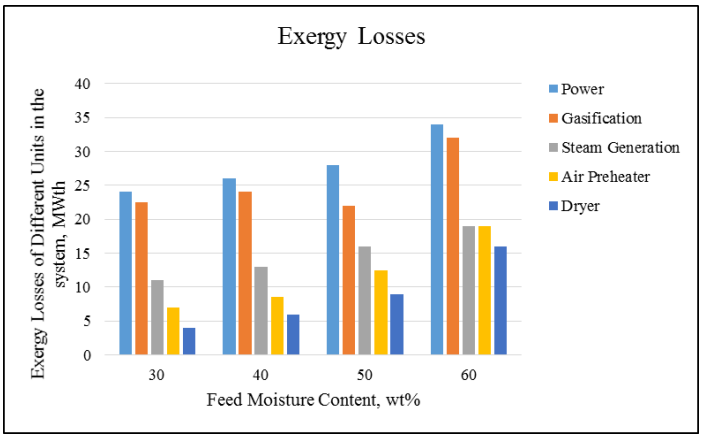

Fig. 10: The exergy losses of different unit operations in the system

The total exergy loss by the model rises considerably by increasing the moisture content of feed biomass. The exergy loss due to the drying is unimportant at low feed biomass moisture contents of below 30%. On the other hand, the exergy loss raises with feed biomass moisture contents intensely and the exergy loss from drying was higher than the gasification with feed biomass moisture content is at 50% or higher. Exergy loss due to steam generation falls significantly by the increase of the feed biomass moisture content.

CONCLUSION

The energy requirement of BFB reactor depends on the excessive fuel and air supply in FFB reactor to deliver sufficient heat for gasification. An integrated model system for DFB gasification and rotary dryer is established in mathematical modelling. Flue gas from the FFB reactor was employed for biomass drying. In the proposed model, the idea of verticity is considered for compensating the excessive fuel and air supply in the FFB reactor in which with the rotating movement of air from a particular source of fire can flared up with no extra fuel supply. The proposed method is implemented on the MATLAB platform and the experimental results are validated based on the operation parameters such as gasification temperature, feed air to the FFB reactor, S/B ratio and initial moisture content of the feed biomass.

CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this paper.

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OPINION

IMPRESSION MAKING IN FIXED PARTIAL DENTURE: TRADITIONAL OR GO DIGITAL?

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ABSTRACT

Digital technology, today controls every aspect of our life and dentistry is no exception to it. Impressions made using elastomeric impression material is an everyday procedure in almost every general dental practice, for the production of bridges, crowns and dentures. The great success of the indirect fabrication of intracoronal/extracoronal restorations such as inlays and onlays, advancing to full coverage gold, metal-ceramic,on all ceramic crowns, have been facilitated by the development of accurate elastomeric impression materials and die stones.[1] The concept of digital impression is emerging rapidly on the horizon and it is believed that digital impression will solve the challenges and difficulties of the conventional impressions

KEY WORDS
digital impressions, CEREC, CAD/CAM

History

The history of today's traditional impression materials began in the mid-1930's with the introduction of reversible hydrocolloids. By the year 1955, the polysulfides were introduced and for the first time an elastomeric impression material was used. There was a great improvement in reproducing the characteristics of prepared teeth, but still there were inherent problems like shrinkage and dimensional instability of material.

In 1966, further improvements in impression materials occurred with the introduction of polyether. This material proved to be far superior to the hydrocolloids followed by silicones in 1976. Although they are hydrophobic by nature, they are highly dimensional stable even in the presence of a moist environment, resulting a superior elastic recovery. With the advancement of time and technology improvements are made to these materials to reduce tearing, chairtime and enhance the patient comfort.

Evolution of digital impression

Dr. Duret first introduced the CAD/CAM concept to dentistry in 1973 in Lyon, France in his thesis entitled 'EMPREINTE OPTIQUE', which translates to optical impression. The concept of CAD/CAM systems was further developed by Dr. Mormann, a swiss dentist and Mr. Brandestini, who was an electrical engineer. The first commercially available digital impression system for use in the field of dentistry was introduced in 1980 pioneered by PROCERA and CEREC. Over the last 10 years, systems like 3M LAVA COS, Cadent iTero,E4D dentist and 3Shape Trios have been introduced. Each employs a specific, distinct technique for making impression.

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Disadvantages of conventional impression materials

In comparison to digital impressions, conventional elastomeric impressions have various limitations which could be a direct or indirect result of factors such as choice of tray, choice of material, manipulation of the materials or certain inherent properties of the material used. The errors or misjudgment on the dentist part to select a tray is the foremost error that can take place. While placing the tray or during its removal, movement by patient can cause errors in the impression. Dimensional stability of the set materials is also another major limitation. Flow of the material, hydrophilicity, voids, inadequate wetting, tearing, and deformation are some of the disadvantages of elastomeric impression. The errors that could occur during the fabrication and steps in making the prosthesis can be eliminated by the digital impression.

Types of CAD/CAM production concepts

The three different production concepts are available, depending upon the location of the components are

- Chairside production
- Laboratory production
- Centralized fabrication in a production center

(a) Chairside Production

All of the CAD/CAM systems are located in the dental clinic. The chairside production, fabricates final dental restoration at chairside without laboratory procedures. The intra-oral camera (the digitization

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component) replaces a conventional impression in most clinical situations. This saves the patient multiple appointments and also the cost for indirect fabricated restorations. Although this production concept is very convenient, it's not very economical.

(b) Laboratory production

In this production system, the traditional working sequence between the dentist and the laboratory is carried out. The dentist makes an elastomeric impression and sends the cast/model to the laboratory. The CAD/CAM steps are carried out completely in the laboratory. By the assistance of the scanner, 3D data is produced on the basis of the master die. This data is produced by the means of a dental design software, and then sent out to the milling device that produces real geometry in the dental laboratory. After this, the exact fit of the framework is evaluated and corrected on the basis of the master cast, if necessary. The ceramist then carries out the veneering of the framework in the powder layering or overpressing technique.

(c) Centralized Production

In this computer-assisted production of dental prosthesis, centralized production is done in a milling center. The 'satellite scanners' in the dental laboratory are connected with a production center via the internet. The data is sent to the production center for the production of the final restoration with a CAD/CAM device. Finally, the production center sends the prosthesis to the responsible laboratory [2].

Components of CAD/CAM

CAD/CAM machinery, irrespective of the system, consists of three basic components:

Scanner

The data collection tools are called as scanners. It scans and collects three dimensional data of jaw and tooth structures and transforms them into digital data sets.

Design Software

The software is provided by the manufacturers for the design of various kinds of dental restorations. With such software, various restorations like, crowns and fixed partial dentures (FPD's) frameworks, full anatomical crown, partial coverage crowns, inlays, inlay retained FPD's as well as adhesive FPD's etc are fabricated. The software of CAD/CAM systems available are continuously upgraded in the market. Various data formats are available for storing the data of the three dimensional construction. The basis for data storage is often standard transformation language (STL) data.
Many manufacturers use their own data formats, specific to that particular manufacturer.

Processing Devices

The data, which is produced by the CAD software is converted into milling strips for the processing of CAM and then finally loaded into the milling device.
Processing devices are distinguished by means of the number of milling axes:-

- 3-milling axes devices
- 4-milling axes devices
- 5-milling axes devices[2]

Milling variants

Dry processing

Dry processing is applied with respect to zirconium dioxide blanks with a low degree of pre-sintering.
This offers several benefits:-

- Minimal investment costs for the milling device.
- No moisture absorption by the die (zirconium dioxide mould), as a result of which there are no initial drying times for the zirconium dioxide frame prior to sintering.

Disadvantages: The low degree of pre-sintering results in higher shrinkage values for the frameworks.

Wet milling

In this process the milling diamond or carbide cutter is protected by a spray of cool liquid to protect against overheating of the milled material. This kind of processing is necessary for all metals and glass

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ceramic, materials in order to avoid damage through heat development. Wet processing is recommended, if zirconium oxide ceramic with a higher degree of pre-sintering is employed for the milling process. A higher degree of pre-sintering results in a reduction of shrinkage factor and enables less sinter distortion.

Open and closed architecture

There are two important categories of digital impressions systems in terms of data files created during scanning:- open and closed architecture. Open architecture files, typically termed as STL files, are not dependent on the manufacturer and can be used in virtually any design software to fabricate a final restoration. Open architecture systems allow the individual dentists to work with several different laboratories and maximize the potential of their investment with options such as implant restorations etc. Closed system software architecture collects and manipulates data modules by the same manufacturer, offering laboratory owners security and a one stop shop for resolving problems. For laboratories which do not want to immerse themselves in all the new technologies and software from each different manufacturer, closed architecture systems generally do a great job of taking the user by the hand from the start to finish [3].

Procedure

The dentist captures the image of the tooth/teeth involved using the digital impression system. Some digital impression systems use a reflective powder which is a specially formulated titanium dioxide powder in order to scan both arches and bite and this is done once the area to be treated is anaesthetized and free of saliva and blood. Some other systems for example, Cadent iTero allow dentist to create a three dimensional image of the patient's teeth without the use of reflective powder. The intraoral wand/scanner is inserted into the patient's mouth and moved over the surface area of the tooth or teeth. Chairside monitors are usually used to display the captured picture of the impression image. Approximately 90 seconds are required to capture the digital impression of prepared teeth and 45 seconds for the opposing arch.

Enlargement and adjustments can be done for enhanced detail to ensure any possible mistakes to be identified and corrected onscreen before sending it to be milled. The delivery workflow depends upon the digital impression system used by the dentist [4].

Tissue management

Management of soft tissue during the preparation and impression taking stages is critical for the success of the final impression, for both traditional and digital methods. Although supragingival margins are the most effective way to achieve visualization of the margin, in many situations, an equi-gingival or a sub-gingival margin may be required. A dual-cord retraction technique is recommended in such situations.

After the preparation, a thin cord is placed and an initial scan is made. The areas on the preparation that need modification are identified and the preparation is refined. A hemostatic agent maybe used, and a thicker cord is placed for 5minutes. The final scan is then accomplished. A great deal of attention needs to be placed on the soft tissue management to ensure the camera has an unobstructed view of the margin, as the scanner cannot distinguish debris and soft tissue from the sound tooth structure[5].

Scanning systems

CEREC (chairside economical restoration of esthetic ceramic [SIRONA]) uses optical scanning and requires the entire area to be captured in the impression to be coated with a reflective powder. It is based on the principle of laser triangulation.

Table 1: Various CEREC Systems

YEAR	HARDWARE	SOFTWARE CAPABILITY	RESTORATION TYPE	DEVELOPER
1980	Basic concept	Two dimensional	Inlays	Mormann(university of Zurich) and Brandestini(brandestini instruments, Zurich)
1985	CEREC 1	Two dimensional	First chairside inlay	Mormann and Brandestini
1988	CEREC 1	Two dimensional	Inlays(1), Onlays(2), and veneers(3)	Mormann and Brandestini

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1994	CEREC 2	Two dimensional	1-3, partial(4) and full crowns(5) and copings(6)	Sirona (Munich, germany)
2000	CEREC 3 & inLab	Two dimensional	1-6 and three unit bridge frames	Sirona (Bensheim, germany)
2003	CEREC 3 & inLab	Three dimensional	1-6, and three and four unit bridge frames	Sirona
2005	CEREC 3 & inLab	Three dimensional	1-5, and automatic virtual occlusal adjustment	Sirona

Courtesy- The evolution of the CEREC system. JADA 2006:137:7S-13S.[6]

The E4D dentist system (D4D Technologies) uses laser scanning (high speed swept laser scan) and requires no reflective powder. Like the CEREC system, the E4D system can be connected directly to a milling machine to create the restoration.

There are two digital impression systems, introduced in 2008 that are not connected directly to a milling machine. It uses a red light laser to reflect off the tooth structure. The iTero system (cadent) uses a camera that takes several views (stills) and uses a strobe effect and the use of reflective powder is not required with this system. This system does not require the aid of the reflective powder to facilitate the impression .The LAVA COS (Chairisde oral scanner) (3M ESPE) uses light powder to facilitate scanning by an optical video system. It takes a completely different approach by using the continuous video stream of the teeth. Adequate tissue retraction and fluid control is very important for all of these systems.[7]

Other Dental CAD/CAM systems:-[8]

- ZENOTec (Weiland Dental & Technik GmbH & Co KG)
- Hint-ELs DentaCAD system (Hint-ELs, Griesheim, Germany)
- Cerasys (Cerasystems, Buena Park, CA)
- Wol-Ceram (XPdent corporation, Miami FL)
- BEkGO Medifacturing (BEGO Medical GmbH, Bremem, Germany)
- Tturbodent System (u-Best technology Inc. Anahiem, Germany)
- Etkon system (etkon USA, Arlington, TX)
- iTero (Cadent, Carlstadt NJ, US

Table 2: Comparison of Common Dental CAD/CAM Systems

SYSTEM	MARKET LAUNCH	PROCESS CENTRE	SCANNING MECHANISM	CAD PROGRAM	CAM PROCESS
Cerec 3	2000	Chairside	Optical	Yes, custom design and database	Fully Automatic
Cerec InLab	2001	Dental Lab	Laser	Yes, custom design and database	Fully Automatic
DCS Precident	1989	Dental Lab	Optical	Yes, custom design and database	Fully Automatic
Procera	1993	New Jersey or Sweden	Manual	Yes, custom design and database	Fully Automatic
Lava	2002	Dental Lab	Optical	Yes, custom design and database	Fully Automatic
Everest	2002	Dental Lab	Optical	Yes, custom design and database	Fully Automatic
Cercon	2001	Dental Lab	Laser	No	Fully Automatic

Courtesy: Panorama of Dental CAD/CAM restorative systems. Compedium, July 2005:26(7):507-512.[9]

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Images retention and transmission

Following image acquisition, the final image is either stored in the system and used for chairside fabrication or digitally transmitted to a laboratory for use. The form that digital transmission takes for the indirect CAD/CAM methods depends on the system used. The lab can create a physical model and fabricate restorations traditionally from any material, or design and fabricate restorations using CAD/CAM, depending upon the system.

Materials used

As materials evolve, there is a continual push towards strong-yet esthetic restoration. Depending on the milling unit, there are many material choices now available in the form of CAD/CAM blocks. Restorations can be milled from a variety of materials such as composites, feldspathic porcelain, leucite-reinforced ceramic, lithium disilicate ceramic and zirconia.

Wax patterns and acrylic provisional restorations can also be milled. Metals, resins, composites and ceramics can also be milled by the processing devices. Commercially pure titanium, titanium alloys and cobalt chrome alloys are metals commonly used in the devices. Resins can be milled to create lost wax frames for casting and also for long-term provisional prosthesis. Composite blanks that are prefabricated to mimic enamel and dentin in their translucency and color can be milled to create final anterior restorations. Zirconia is a high performance ceramic with excellent mechanical characteristics. It is used in milling devices for crowns, fixed partial prosthesis and implant abutments [10].

DISCUSSION

The conventional impression procedure involves the necessary steps of tray preparation, impression making and disinfection of the impression. All the dental lab steps are followed and the prosthesis is fabricated and then delivered to the patient. Multiple steps and visits are required for the prosthesis to be finally delivered to the patient. In comparison to the conventional methods, the occlusion, the fit, the quality of contacts and the long-term survival rates of the CAD/CAM crowns have been found to be better[10].It provides improved precision and consistency and allows the clinician to visualize the preparation on a computer display from many perspectives. The occlusion, fit, accuracy can be checked on the computer by the software which cannot be done with the traditional methods. It allows the clinician to design the restoration on a computer while visualizing the opposing dentition. It provides a clean and streamlined impression. It also helps in reduction of the environmental impact of disposing the materials required for conventional impressions. The clinical outcomes have shown that performance of restorations produced by the CAD/CAM systems have improved drastically in the last decade.

However, due consideration should be given to the fact that the equipment required for the digital impressions and milling is expensive. It neither economical for the patient or the dentist. Digital equipments are complex and require trained personnel for its operation and to maintain the device. Up-to-date lab support is also essential. Those with limited mouth opening may have difficulty with the scanner.

Inspite of all the benefits of these new methods, the dentists working procedures will have to be adapted to the methods of CAD/CAM and milling technology. These include appropriate tooth preparations with creation of a continuous preparation margin, which is clearly recognizable to the scanner. Shoulder-less preparations and parallel walls should be avoided. On the basis of present knowledge, a tapered angle of between 4 degree and 10degree is recommended. Subsections and irregularities on the surface of the prepared tooth as well as the creation of troughs with a reverse bevel preparation margin can be inadequately recognized by many scanners. In addition, sharp incisal and occlusal line angles are to be rounded. Sharp and thinly extending edges as well as 90degree shoulder margin in a ceramic restoration can result in a concentration of tension and at the same time, sharp edges cannot be milled exactly using rounded grinders in the milling device [2]. A radial (120°) shoulder or a chamfer would probably be the most preferred finish line.

CONCLUSION

Over the past 10 years, CAD/CAM has developed at a rapid pace, and it is likely that integration of different systems will become the industry norm. Smaller intraoral scanners that require no cart are already appearing in the market, as well as those that do not require contrast medium. More and more dentists are purchasing dental impression systems with numerous advantages of digital impression over traditional impression and the ability to benefit from the digital impression taking and/or CAD/CAM. It will likely be a routine procedure in most dental clinics in the near future, as dentists, lab technicians and patients all reap benefits. CAD/CAM technology has already changed dentistry and will replace more and more of the traditional techniques in fabricating dental restorations. One thing is for sure that, from material selection to technique, CAD/CAM is changing the way clinicians look at dentistry.

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CONFLICT OF INTEREST
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ARTICLE

PREVALENCE OF CUTANEOUS LESION OF CLOPIDOGREL AND MANAGEMENT WITH ANTIHISTAMINE

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ABSTRACT

Background: *Hypersensitivity reactions to clopidogrel are complicated reaction and difficult to management.* ***Objectives:*** *The aim of this study was to describe Prevalence of cutaneous lesion of clopidogrel and manage of it.* ***Methods:*** *Patient's received clopidogrel evaluated for cutaneous manifestation. If diagnose established they received oral antihistamine.* ***Results:*** *Twenty five patients representing 1.4% of the patients receiving clopidogrel developed cutaneous lesion during the study period. The mean age was 57 ± 6 years, 58% of patients were male, and 20% reported prior adverse drug reaction. Cutaneous lesions are manifested as generalized exanthema in 84%, localized skin reaction in 12%, and urticarial in 4% of patients. Complete resolution of cutaneous lesion was observed in 61 patients [96%] with a short course of oral antihistamine.* ***Conclusions:*** *Cutaneous lesion of clopidogrel is manifested commonly as generalized exanthema. This can be managed with oral antihistamine.*

KEY WORDS

Clopidogrel, cutaneous lesion, antihistamine

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INTRODUCTION

Background: Clopidogrel is an oral thienopyridine widely used in the management of patients with cardiovascular disease [1–3]. In addition, long-term administration of clopidogrel with aspirin is recommended after discharge from cardiac care unit due to acute coronary syndrome [4,5]. Development of hypersensitivity reactions after clopidogrel administration is a recognized complication and is difficult to manage. The characteristics and prevalence of clopidogrel hypersensitivity are poorly understood, and treatment options remain limited. In this report, we characterize prevalence and morphological features of clopidogrel hypersensitivity and describe successful resolution with oral antihistamine. The aim of this study is Prevalence of cutaneous lesion of Clopidogrel and Management with Antihistamine

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MATERIALS AND METHODS

Patients

Twenty five patients with suspected clopidogrel hypersensitivity after discharge from cardiac care unit due to acute coronary syndrome at Emam Reza Hospital from March 2016 to March 2017 were included and evaluated. Detailed history and physical examination were performed. All patients diagnosed with clopidogrel hypersensitivity were prescribed a 1-week course of oral antihistamine [cetirizine] with10 mg twice per day. Long-term follow-up was completed in all patients by office visit. Written consent was taken from each subject and the study was approved by the hospital authority.

Hematologic analysis

Complete blood counts with automated differential for leukocyte, lymphocyte, eosinophil, and platelet count were obtained before initiation of clopidogrel therapy and repeated after complete resolution.

Statistical analysis

Continuous data are expressed as mean ± SD, and dichotomous data are expressed as absolute values and percentages. All analyses were performed using SPSS version 17. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 25 patients were referred and evaluated for clopidogrel hypersensitivity during the study period. This represented 1.4% [25 of 3,500] of the patient population undergoing discharge from cardiac care unit due to acute coronary syndrome during the study period. Baseline and procedural characteristics of the patients are shown in [Table 1]. The information for presence of asthma, family history of allergy, and prior drug allergy in individual patients was not systemically collected except for patients presenting with clopidogrel hypersensitivity. There were no significant differences in cardiac risk factors, concurrent medications, and procedural variables between patients with clopidogrel hypersensitivity and the entire patient admitted in cardiac care unit.

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Clinical outcomes

Clinical follow-up was completed in all patients for a median of 100 days. During follow-up, there was 1 target vessel revascularization procedures in patients who underwent percutaneous coronary intervention. No patient died. All patients had completed the minimum recommended duration of clopidogrel therapy.

Table 1: Demographic characteristic

	Patient with cutaneous lesion
Baseline characteristics	
Age, yrs	57 ± 6
Female	11 [44%]
Diabetes	8 [32]
Hypertension	9 [36]
Dyslipidemia	10 [40]
Prior drug allergy	5 [20]

Clinical manifestations of clopidogrel hypersensitivity

Patients with clopidogrel hypersensitivity presented with 3 distinct clinical patterns. Category 1 contained 21 patients [84%] who developed a generalized, pruritic, exanthemata's rash predominantly affecting the trunk with or without involvement of the upper and lower extremities. The rash was limited to the chest, abdomen, and back in 18[86%] and extended to the proximal part of the upper and lower extremities in 4 patients [14%]. Category 2 consisted of 3 patients [12%], in whom the rash was limited to a localized area. These reactions involved the neck, face, back, axilla, palm of the hand, and/or sole of the feet. Category 3 had 1 patients [4%]; with generalized urticarial. The median time to development of clopidogrel hypersensitivity after clopidogrel use was 6 ± 1 days for category 1, 4 ± 3 days for category 2, and 2 ± 1 days for category 3 [p = 0.01].All patients were able to eat antihistamine as prescribed, and all patients report improvement at 5 ± 2 days. The patient with urticarial was prescribed oral steroids. All patients were able to continue clopidogrel for the recommended duration without recurrence of clopidogrel hypersensitivity.

DISCUSSION

The main findings of the present study were that clopidogrel hypersensitivity in most patients was characterized by a generalized exanthemata's rash. Patients with allergic reactions after PCI present difficulty in diagnosis and management. Many patients first have exposure to contrast media and then more recent initiation to standard therapy for coronary artery disease including statins, angiotensin-converting enzyme inhibitors, aspirin, and clopidogrel. No specific assays are available for confirming most drug-induced allergic reactions. In view of these limitations, detailed history of exposure and timing of allergic manifestations is critical for appropriate diagnosis and management.

Clopidogrel is a platelet adenosine diphosphate receptor antagonist that inhibits platelet aggregation by irreversible binding of its active metabolite to the P2Y12 receptor, and it has been shown to reduce adverse cardiac events in patients with both ST and non–ST-segment elevation myocardial infarction [1, 2]. Clopidogrel hypersensitivity is a recognized complication of clopidogrel therapy and presents difficulty in management. The potential ways to manage patients with clopidogrel hypersensitivity include clopidogrel desensitization or treatment with ticlopidine or possibly prasugrel. However, alternative therapy with ticlopidine and prasugrel may not be suitable for all patients because of allergenic cross-reactivity [6, 7] and potential for serious side effects [8–11]. In addition, clopidogrel desensitization as reported previously may not be suitable after PCI because of the need for drug discontinuation and lack of well-defined criteria for selection of patients [8-10].

Adverse drug reactions are classified as type A for predictable reactions related to pharmacological activity of a drug and type B for unpredictable and rare hypersensitivity reactions. Although diverse manifestations for clopidogrel-related type B hypersensitivity, including urticaria [11], angioedema [12], arthritis [13, 14], serum sickness–like reaction [15], and fixed drug reactions [16], have been reported, cutaneous reactions remain the most common presentation.

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Diagnostic confirmation of drug hypersensitivity reactions in clinical practice is difficult because challenge and drug-specific testing are not routinely employed. Drug allergy testing with skin prick and intradermal challenge has been standardized for several drugs responsible for immediate hypersensitivity reactions, but the use of patch testing as a means of controlled challenge in patients with delayed-onset drug hypersensitivity is not widely used. The administration of a single course of oral steroids resulted in complete resolution of clopidogrel hypersensitivity in all but one patient and offers an important treatment option for patients requiring prolonged clopidogrel therapy without drug discontinuation, switching, or desensitization. Furthermore, the steroids were effective in alleviating clopidogrel hypersensitivity in patients presenting with cutaneous manifestations, urticarial, or angioedema. The mechanism of action for successful treatment of clopidogrel hypersensitivity by oral steroids is unclear but is likely related to suppression of the immune response followed by development of immunologic tolerance in sensitive individuals. In our study we treat patients with antihistamines successfully.

CONCLUSIONS

Clopidogrel hypersensitivity was commonly manifested by a generalized exanthemata's eruption. The manifestations of clopidogrel hypersensitivity were successfully treated with an antihistamine.

CONFLICT OF INTEREST

There is no conflict of interest.

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ARTICLE

CHARACTERISTICS OF BEADS FROM LACTOBACILLUS ACIDOPHILUS PROBIOTIC MICROENCAPSULATION WITH CALCIUM ALGINATE AND RESISTANT STARCH

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ABSTRACT

Background: *Lactobacillus acidophilus has large beneficial impacts as a probiotic and the low viability of the bacteria and other probiotics in the extreme acidic- biliary conditions of gastrointestinal tract and food products have always encouraged researchers to seek approaches for improvement of these indicators.* ***Material and method:*** *Microencapsulation as one of the newest methods has revealed sub stantial out comes in this respect. The aim of this study was to evaluate the morphological and protective features of beads obtained from the microencapsulation of L. acidophilus probiotic. Microencapsulation by calcium alginate and resistant starch was carried out through extrusion.* ***Result and discussion:*** *The strength of beads was determined within 12 h and the survival rate of encapsulated bacteria was as certained in the hydrochloric acid solution, 0.1 M phosphate buffer, and a solution containing digestive powder during 120 min.* ***conclusion:*** *The results showed that the stability levels of beads were different in various media, whit physical tension playing an important role. Under adverse environmental conditions, microencapsulation with calcium alginate and resistant starch plays a critical role in the protection of L. acidophilus probiotic. The survival rate of microencapsulated bacteria in all conditions was significantly higher than free bacteria [p < 0.05].*

KEY WORDS
Microencapsulation, probiotic, Lactobacillus acidophilus, calciumalginate, resistant starch

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INTRODUCTION

Probiotics are live microorganisms that are found in the gastrointestinal tract with a specific number and exert one or more beneficial effects on the health of the host. Some notable beneficial effects are increased food digestion, boosted immune system, and increased resistance to infection, reduced blood cholesterol, as well as anti-mutagenic and anticancer properties [1]. In addition, probiotics are today introduced as viable alternatives to antibiotics to deal with pathogens in humans and animals leading to considerable acceptance and consumption of probiotic foodstuffs and drugs [2]. The foods containing such bacteria are classified as functional foods. According to the recommendation by the International Dairy Federation [IDF], such foods should contain 107 cfu/g probiotic bacteria and the consumer should use at least 100 g/day of the food to acquire the beneficial effects of the foods in this category [1 &3].

The low viability of the bacteria and other probiotics in the extreme acidic- biliary conditions of gastrointestinal tract and food products have always encouraged researchers to seek approaches for improvement of these indicators. Microencapsulation as one of the newest methods has revealed substantial outcomes in this respect [3,4]. From a microbiology perspective, microencapsulation includes covering a layer of hydrocolloid around living cells that protects them from adverse conditions of surrounding environment and raises the survival of these cells [5]. Different materials such as gelatin, chitosan, etc. have been used for the encapsulation of probiotic bacteria. However, microencapsulation with calcium alginate has largely been applied for this purpose, particularly on lactic acid bacteria, due to numerous advantages such as non-toxicity, safety to the human body, reasonable prices, and convenient handling [6, 4, 3].Alginate combined with starch and in particular resistant starch has shown better results because it leads to the formation of an additional capsule around the beads raising the wall stability and subsequently the viability of bacteria therein[7].

L. acidophilus is the predominant natural intestinal flora of humans and animals. It is also found in the usual fermentation products and possess many beneficial effects listed above as the most important and most widely used probiotic [8]. It is, therefore, of particular importance to examine and enhance the survival of this bacterial species within food products through a fitting and strong microencapsulation and probiotic drugs and also to enable the transfer and safe release of the bacteria in appropriate locations of the gastrointestinal tract. Moreover, the translocation of a variety of other probiotic bacteria such *as L. casei* was studied in this respect [9].

On the one hand, it is of paramount importance to study the morphology of beads obtained from microencapsulation such as shape, size, numbers of wall layers and beads capsules lying as a buffer between the bacteria and the exterior space, bacterial distribution within the beads, matrix density surrounding bacteria, and finally bacterial release from the beads [7, 10, 11].Furthermore, several studies have been conducted on the stability and protective capacity of the beads [maintaining a high viability of probiotic bacteria] in the unfavorable conditions of gastrointestinal tract and internal milieu of some food products including cheese, yogurt, and honey [12, 13, 14]. The strengthening effect of using UV radiation has recently been examined for the proper inherent stability of beads [15]. The aim of this study was to evaluate the morphological and protective characteristics of beads from encapsulated *L. acidophilus* probiotic as the prevailing normal flora of human gut by calcium alginate and resistant starch.

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MATERIALS AND METHODS

This is an in vitro experimental study conducted on the beads obtained from microencapsulation *of L. acidophilus* and assessing the survival rates of both free and encapsulated bacteria.

Activation of L. acid ophilus Freeze-dried starter culture is usually released in the market. The starter culture package for *L. acidophilus* La5 [CHR-Hansen, Horsholm, Denmark] was opened with care in sterile conditions. One gram of starter was uniformly mixed in 100 ml of MRS broth [de Man-Rog as a-Sharpe] and incubated at 37 °C for 24 h in order for the starter to completed or mancy with the absorption of water and enter the logarithmic growth phase. The next day, one ml of the culture obtained was diluted again with 99 ml of fresh medium [1%] and incubated at 37 °C. During the week, the culture was transferred to the fresh medium three times and after incubation for 24 h, stored at 4 °C in order to permanently access to probiotic bacteria in the logarithmic growth phase [7].

Purification of bacteria

In this study, almost 2 ml of medium prepared in the starter culture activation and stored at 4 °C was transferred to ca. 200 ml of fresh medium and incubated at 37 °C for 18 h, which was used for the purification of the target bacteria. For this purpose, the medium was thoroughly stirred to a homogeneous state and centrifuged [Centurion centrifuge, Model 2010, West Sussex, BNI8OHY, UK] in 10000 rpm for 10 min. After centrifugation, the supernatant was discarded and the bacterial pellets in the micro tubes were centrifuged twice by normal saline [0.09%] to be thoroughly washed [16]. The bacterial emulsion was used for microencapsulation process.

Microencapsulation

In this study, the probiotic was microencapsulated by calcium alginate and resistant starch by extrusion method using a multi-nozzle extruder micro encapsulated device [17]. To do this, a mixture of sodium alginate [Sigma, USA] and corn resistant starch, Hi-maize [Merk, Darmstadt, Germany] with a purity of 99.9% and a significant amount of the bacteria was prepared by purification from the medium in sterile distilled water.

Sodium alginate [20 g] was added to 200 ml of distilled water followed by sterilization. Alginate solution was then refrigerated overnight in order for alginate particles to adequately absorb water. Afterwards, alginate solution was transferred to the laboratory at the same temperature. The resistant starch [20 g] was gently added to the alginate solution and stirred by a magnet at regular rounds on a hot plate unit [I KA Labortechnik, Model 79219 staufen, KG, Germany]. Afterwards, 10 micro tubes containing the bacterial emulsion [totally 10 ml] prepared in the previous step were evacuated into the alginate/starch mixture followed by the addition of circa 0.5 mltween 80 [Merk, Hohenbrunn, Germany]. The resulting mixture was slowly placed in the micro encapsulated tank to complete the microencapsulation process. After injecting the mixture into 1.0 M calcium chloride solution, the capsule walls were perfectly formed as a result of alginate contact with calcium ions. The beads were deposited as droplets in calcium chloride solution [7, 16, 18]. The beads obtained were finally collected from the tank outlet.

Release of bacteria from beads

To release *L. acidophilus* bacteria from the beads, one g of beads was stirred in 1.0 M phosphate buffer [9 ml, pH=7] on a clipped shaker [IKA- Model Janke & Kunkel GMBH. Type VX5-Germany] for 30 min to dissolve the beads as a homogenate [7 and 16].

Assessing the stability of beads

The stability of microencapsulated beads was evaluated through the production of beads [1 g] with a diameter of approx. 50- 200 µ affected by the following processes at each stage:

1. Hydrochloric acid [9 ml, pH = 2] with and without mechanical stress [conducted by magnets at 400rpm for each experiment];
2. Phosphate buffer solution [1.0 M, 9 ml, pH = 7] with and without physical tension;
3.Distilled water [9 ml] containing digestive powder [including 4500units of amylase, 6000 units of lipase, 50 ml of hemicellulose, 25 ml of bovine bile extract, and pH = 8.3] with and without physical tension incubated at 37^{0}C.

The stability of beads and viability of the containing bacteria were evaluated under the influence of the above conditions at different times [from 30 min up to 12 h]. In case the beads were destructed, one ml of the solution in which the beads were dissolved was harvested, transferred to MRS broth medium, and incubated for 24 h to detect the survival of bacteria released to the medium. If the beads were preserved within the maximum time [12 h], the bacteria were initially released, transferred to MRS broth medium, and incubated for 24 h to realize bacterial growth/lack of growth within the beads.

Evaluation of encapsulated bacterial survival in hydrochloric acid solution, phosphate buffer solution, and a solution containing digestive powder, with and without physical tension were carried out.

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For this purpose, the beaded microencapsulated bacteria [1 g] and 1 ml of the free bacteria [one of the tubes containing bacterial pellet emulsified by saline] were added under similar conditions to hydrochloric acid solution [10 ml, pH= 2], 1.0 M phosphate buffer solution [10 ml, pH = 7], and the solution containing digestive powder [10 ml, including 4500 units of amylase, 6000 units of lipase, 50 ml of hemicellulose, 25 ml of bovine bile extract, and pH = 8.30], all of which were already autoclaved [121°C, 15 min] and incubated at 37° C in two conditions of with and without mechanical stress. In order to evaluate the survival rate of microencapsulated cells at zero, 30, 60, 90, and 120 min, each medium was diluted by peptone water [0.1 percent] and incubated in MRS- Salicin-agar medium as a mixed culture at 37°C for 72 h [19]. Independent t-test [α = 0.05] was used to compare the number of live bacterial cells at each of the time periods stated above.

RESULTS

Assessing the stability of beads in hydrochloric acid solution [Fig. 1] revealed that the structure of beads was maintained within 12 h without physical tension and the containing bacteria were capable of growing after release and transfer to the medium. The structure of beads was completely dissolved with physical tension after3 h, after which the bacteria were immediately able to grow in the medium.

Evaluating the stability of beads in phosphate buffer solution [Fig. 1] showed that the structure of beads was maintained within 12 h without physical tension and the containing bacteria were capable of growing after release and transfer to the medium. The structure of beads was completely dissolved with physical tension after 30 min, after which the bacteria were able to grow in the medium.

Examining the stability of beads in digestive powder solution [Fig. 1] clarified that the structure of beads was entirely dissolved within 10 h without mechanical tension and the containing bacteria were capable of growing after release and transfer to the medium. The structure of beads was completely dissolved with physical tension after 1 h, after which the bacteria were able to grow in the medium.

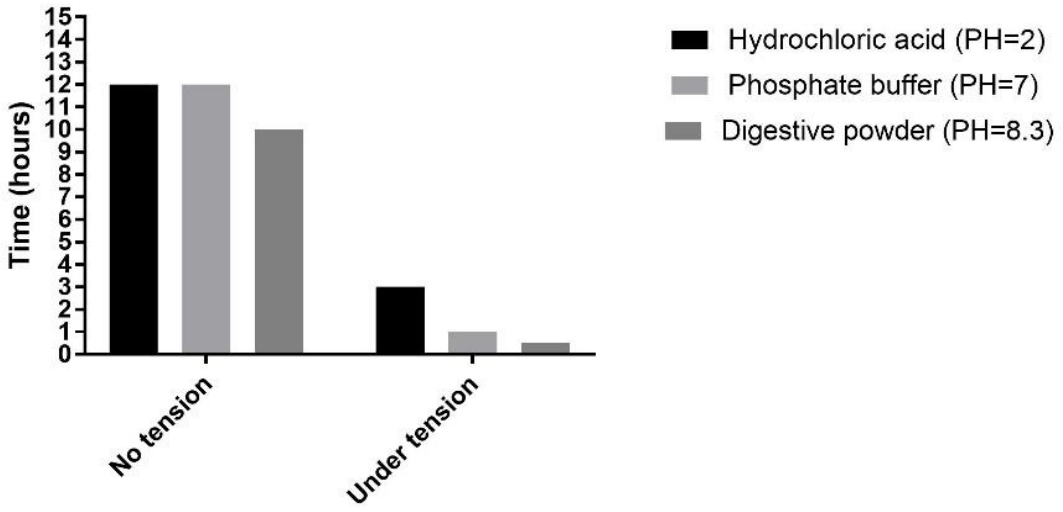

Fig. 1: Stability of beads in hydrochloric acid [pH = 2], phosphate buffer [pH = 7] and digestive powder [pH = 8.3] in two physical states [no physical tension and under physical tension].

[Fig. 2] shows the survival rates of free and encapsulated cells of *L. acidophilus* La5 after incubation in hydrochloric acid, phosphate buffer, and digestive powder solutions with and without physical tension, respectively, at 37 °C within 2 h. Independent t-test [α = 0.05] compared the number of live bacterial cells at each time period stated. In all circumstances, the number of microencapsulated live bacterial cells was significantly greater than free forms [$p < 0.05$]. Additionally, the number of free and microencapsulated live cells under no physical tension was significantly higher than those under physical tension [$p < 0.05$].

DISCUSSION

Several studies conducted on the survival of *L. acidophilus* indicate the particular importance of the bacteria as a natural and important human gut flora as well as an important bacterial species in most fermentation products [20, 21, 22, 23]. Micro encapsulation is considered one of the newest methods to increase the viability of probiotic bacteria. In the present study, the morphological features of beads obtained from micro encapsulated *L. Acidophilus* probiotic was shown by calcium alginate and resistant starch using extrusion method. In numerous studies, morphological characteristics of beads from encapsulated probiotic bacteria have been studied via different methods and using light and electron microscopy [24, 11, 10, 6]. Our results showed that the stability of beads was different under the influence

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of diverse media, with physical tension playing an important role. In a study by Simpson et al. [2004], it was concluded that calcium chloride increases alginate gel stability, and that greater levels of Ca+2 resulted in elevated capsule thickness, hence, the stability [25]. Anselmi et al. [2002] used a new technology to improve the microencapsulation characteristics. They applied the strengthening effect of UV radiation and discussed the proper inherent stability of beads, low toxicity, a better resistibility, and a simple cheap formulation as the main goals of the technology [15].A number of recent and ongoing investigations conducted in this regard suggests the importance of increasing the stability of beads containing probiotics both in the product until use and during passage through the digestive tract until it reaches the target area of probiotics' function, i.e. the colon [26, 30].

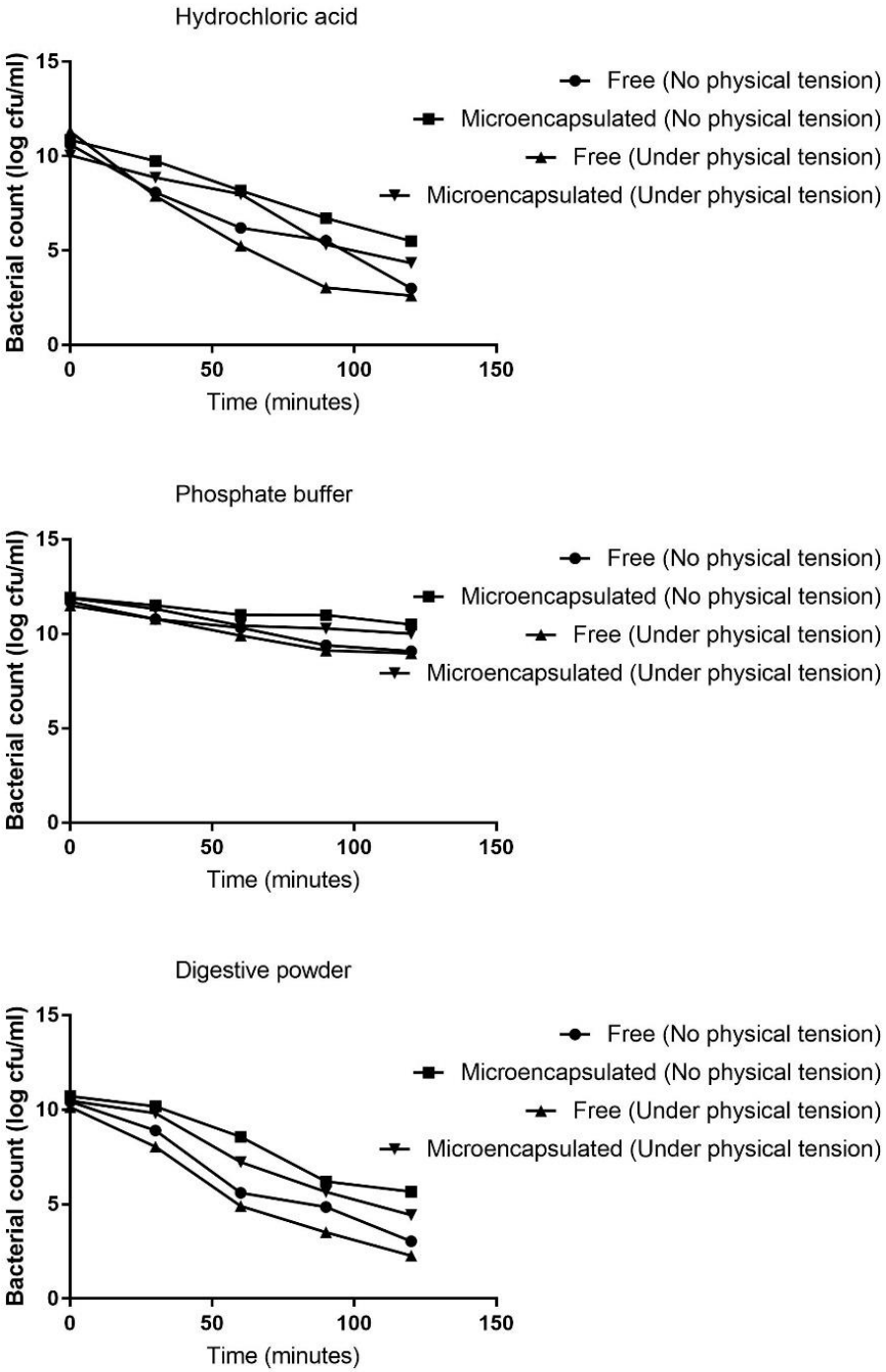

Fig. 2: Free and microencapsulated *L. Acidophilus* viability during 2 h incubation at 37°C in three chemical treatments [hydrochloric acid, buffer phosphate, and digestive powder] and two physical sates [no physical tension and under physical tension].

Note: In each graph, the viability of microencapsulated bacteria is significantly higher than free forms and viability significantly decreased under physical tension in both free and microencapsulated bacteria [P <0.005].

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The results of bacterial counts at zero, 30, 60, 90, and 120 min of incubation at 37 °C in hydrochloric acid, phosphate buffer, and digestive powder solutions with and without physical tension showed that microencapsulation with calcium alginate and resistant starch plays a crucial role in protecting *L. acidophilus* probiotic. The survival rate of microencapsulated bacteria was significantly higher than free forms in all conditions. This means that microencapsulation as a barrier reduces the adverse effects of unfavorable conditions on the bacteria leading to their extended survival. This is consistent with the findings of Krasaekoopt et al. [2004] [10]. Likewise, Sultana et al. [2000] encapsulated probiotic bacteria with alginate-starch and studied the survival of bacteria in conditions similar to gastrointestinal fluid and in yogurt. Their results presented evidence on the improvement oflive bacterial encapsulation using high-amylose starch or Hi-maize [as a prebiotic] in comparison with no starch application. They further observed that the survivability of *L. acidophilus* and encapsulated Bifi do bacteria strains decreased as much as 0.5 log and 1 log in free cells within 8 weeks of incubation in yogurt. The combined effect of alginate-starch in increased survival of probiotic bacteria atadverse conditions corresponds our observations [24].

Kailasapathy et al. [2002] [30] studied encapsulated probiotic bacteria, probiotic products, and increased survival of these organisms in products, especially in the gastrointestinal tract of human. They noted that encapsulation is carried out with the aim to provide a physical barrier to protect probiotics against adverse environmental conditions as well as immobilization of probiotic bacteria in biotechnology. Different micro encapsulation methods were also discussed in their study stating that calcium alginate is the major part of this technology indicating the importance of this substance. Other materials other than alginate with the highest usage were introduced as Kappa-carrageenan, gellan gum, gelatin, and starch. Research needs in this area include the design of small-sized beads at micro and nano scales with high stabilities and many commercial applications. Food carriers reported include yogurt, cheese, ice cream, and mayonnaise [6].

CONCLUSION

The overall results of this study show that the microencapsulation of *L. acidophilus* probiotic, known as important flora of the human gut and fermented products, is of particular importance resulting in significant increased survival of the bacteria at difficult environmental conditions. It is recommended to investigate other substances such as gelatin, chitosan and poly-L-lysine for microencapsulation of *L. acidophilus* and other beneficial intestinal bacteria.

CONFLICT OF INTEREST
There is no conflict of interest.

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LETTER TO EDITOR

SMART GREEN TECHNOLOGY FOR MICROBIAL GHOSTS PREPARATION

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Dear Editor

Recently a simple but smart tool has been introduced to control pathogens [1, 2]. This tool is concerned with evacuating the microbial cells including the viruses from their internal content and leave the cell wall or the virus envelop intact with correct 3D surface structure and surface antigens [3]. The tool has been given the name Sponge Like protocol where pore or pores were introduced in the microbial cell walls or the DNA/RNA genomic materials of the viruses are degraded [2, 4-9]. The tool is so simple but efficient. It is based on determining the minimum inhibition concentration (MIC) and the minimum growth concentration of the used chemical compounds. The chemical compounds themselves are inexpensive. The most used ones till now are H_2O_2, NaOH, $CaCO_3$, $NaHCO_3$, SDS, NaCl, ethanol and water. The serial dilution method for such compounds (each used alone) is utilized to determine their effect on a particular microbe. The correct 3D structure could be judged using the light microscope and the crystal violet staining. In advanced labs, electron microscopes could be also used. To evaluate the surface antigens, classic immunological studies could be used as well as advanced ones.

I suggest reading the original protocol and also the reduced protocol as well as the papers published in such a topic carefully. One should observe that each microbe is different from the other. Even two closed *E. coli*, the JM109 and BL21 strains show different MIC and MGC which encourage some, not to use such a method in preparing the Bacterial ghosts or the Microbial ghosts but to be used as a tool for strain differentiation.

Such a tool will help a lot in controlling pathogens, strain identification and differentiation, study the cell wall, vaccine production, immunological applications etc.

For introducing a simpler method that could be used in emergence cases, egg lysozyme was used to do the same target following the same steps. The native lysozyme was used after determining their MIC and MGC against the microbe [6; 7]. Lysozyme which existed everywhere even in our saliva could prepare vaccine!

By introducing this topic, I have the hope that such tool and such concept could be useful in tackling pathogenic bacteria. The differences between the MIC and the MGC could be smarter and can be used in killing unwanted cells by compounds that affect differently on normal and cancer cells. Or at least could cause the minimum side effect.

The scientist are invited to use such a tool or more better ones, but I have a hope that this protocol and such tools will be introduced for free to the better of the humanity.

CONFLICT OF INTEREST
There is no conflict of interest.

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