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# TOE WAVING IN THE BROWN MARSH FROG RANA BARAMICA: PEDAL LURING TO ATTRACT PREY?

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**Abstract:** Rapid vertical flicking movements of the long middle toe of the hind foot of four *Rana baramica* males are interpreted as luring behaviour used to catch prey. Males were observed to toe wave in the field between bouts of calling. Two males responded to the beam of a laser pointer simulating prey approach towards the waving toe by lunging towards it. This response suggests that toe waving is used to attract prey.

# Introduction

Anurans use visual signals for a variety of functions, including signalling to conspecifics, to warn predators, and to lure potential prey (Myers and Daly, 1983; Hödl and Amézquita, 2001; Grafe and Wanger, 2007; Hagman and Shine, 2008). Of these behaviours, use of visual lures has been the least investigated. Recent observations by Hagman and Shine (2008) provide strong evidence that toe waving in cane toads (*Chaunus marinus*) is used by medium-sized toads to attract prey and thus functions as a lure. In this brief communication, toe waving with very similar patterns of movement using the same toe is reported in the very distantly related brown marsh frog *Rana baramica*, a common denizen of freshwater swamp forests in Peninsular Malaysia, Singapore and Borneo (Das, 2007).

#### Methods

Adult male brown marsh frogs (*Rana baramica*) were located in the field and observed between 6-10 minutes. The localities of observations were the Sime Forest, Singapore (103°48'E, 1°21'N), and a peatswamp forest along Labi Road in Brunei Darussalam (114°30'E, 4°35'N). To determine if leg waving is used to attract prey, a beam of a red laser pointer was slowly moved into the direction of the waving toe in two males. Responses of the male to the laser beam were recorded.

#### **Results and Discussion**

Four observations of toe waving were conducted (Table 1). The initial observation of toe waving was made in Sime Forest, Singapore on March 6, 2008. A calling male *R. baramica* perched on a branch approximately 60 cm above ground was observed for ten minutes. Between bouts of advertisement calling, the long middle toe of either hind foot was vibrated vertically for about a second. Approximately 50 toe waves were given within the ten minutes in which the male was observed. Toes from both hind feet were waved in all four males, but

**Figure 1:** Male *Rana baramica* observed in the Sime Forest, Singapore. White arrow points to the lifted long hind toe used in waving. Length of black bar equals one cm.



In response to the presentation of the laser light, both males tested responded by repeatedly lunging forward into the direction of the light beam. Lunging responses were elicited only when the light beam was moved to within 2 cm from the tip of the waving toe.

**Table 1:** Date and location of toe waving behaviour and response to laser beam in four *Rana baramica* males.

Individual	Date	Location	Distance at which laser beam
			elicited response
Male 1	6.3.2008	Sime Forest, Singapore	not tested
Male 2	19.3.2008	Peatswamp forest, Brunei	not tested
Male 3	24.3.2008	Peatswamp forest, Brunei	<2 cm
Male 4	24.3.2008	Peatswamp forest, Brunei	<2 cm

This behavioural response suggests that *R. baramica* probably uses toe waving to attract prey to approach within its reach. The toe lure is likely to mimic a small food item consumed by prey species in both size and movement pattern. Although no suitable prey items were seen at the time of observations, it appears likely that small arthropods, especially predatory ants, would be attracted by the moving toe.

The possibility of toe waving being used by males to attract females seems very unlikely

Nothing is known about the foraging mode of the brown marsh frog. However, given the putative function of toe waving as a lure, *R. baramica* males are ambush foraging predators, at least during nights of calling. Advertisement calls are produced to attract females and in would be an advantage for males to remain stationary to allow females to locate them. Locatability can be impaired in dense foliage and exacerbated if males move around. Catching prey while waiting for females to arrive would be a useful tactic. Several species of anurans are known to feed opportunistically in this way (Wells, 2007), however, none have been reported to use lures during advertisement calling.

Toe waving has been reported in several anurans (Murphy, 1976; Bertoluci, 2002; Hagman and Shine, 2008). Cane toads wave the long middle toes of their hind feet only when being fed crickets or in the presence of smaller conspecifics. Experimental evidence strongly suggests that toe waving in cane toads is a pedal lure (Hagman and Shine, 2008). In contrast to cane toads, toe waving was seen in *R. baramica* in the absence of potential prey. Use of the same toe and similar movement patterns of toe waving in cane toads (Bufonidae) and brown marsh frogs (Ranidae) indicates convergence and suggests that similar selective pressures have shaped this intriguing behaviour in these two distantly related frog species. It will be fascinating to investigate the prevalence of this behaviour and which prey is attracted to toe waving in *R. baramica*.

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# NO ORANG -UTANS (PONGO PYGMAEUS) IN BRUNEI DARUSSALAM

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**Abstract:** A reported sighting of an orang-utan in Brunei Darussalam in 1992 was investigated further but no evidence of its presence was gathered. Only maroon langurs were seen in the area. The probability of a nomadic proboscis monkey moving into the area from the nearby swamp is high. It is inferred that this report was one of misidentification. There have been no records of orang-utans in Brunei and their patchy distribution in Borneo is discussed.

Keywords: Orang-utan, Pongo pygmaeus, Brunei Darussalam, Bornean distribution.

Figure 1: An orang-utan, *Pongo pygmaeus* found in Sabah, Sarawak & Kalimantan (J.K. Charles).



I refer to the report of a sighting of an orang-utan, *Pongo pygmaeus*, published in the Brunei Museum Journal (Leuy, 1992) which has been erroneously cited a faw times as evidence that

The author of that paper, who is not a field mammalogist, discussed her sighting with me but there were insufficient details to provide positive confirmation. No binoculars were used and the distance of the observer from the animal was too great for reliable naked-eye identification. From my extensive field experience with mammals in the tropical rainforest, binoculars are essential to confirm field observations and in many cases repeated rigorous observations are necessary before reliable conclusions can be drawn. If there is any doubt as to the validity of a record it should be rejected. For the purpose of confirming Levy's observation on 23 February 1992, I visited the area of the original sighting at Bukit Teraja on four separate occasions, 27 February, 3, 10 and 18 March 1992.

I found a group of 15 maroon langurs *Presbytis rubicunda* during the 4 field trips, feeding on the vegetation in the valley that Levy spoke about, and I used a pair of binoculars to confirm the sightings. The red coat colour of a male maroon langur can be easily confused with that of an orang-utan and the question of size of the animal becomes subjective and irrelevant, especially when binoculars are not used. Another animal that resembles an orangutan when seen at a distance is the male proboscis monkey *Nasalis larvatus* which has a reddish coat of fur on the dorsal side and a white tail which is not easily visible when the animal moves about in the foliage. Again binoculars must be employed to confirm its presence.

Nomadic proboscis monkeys have been seen straying many kilometres away from their riverine habitats, into dipterocarp forests of Ulu Temburong and also into kerangas forests (personal observation, Bennett and Gombek, 1993). It is possible that a male proboscis monkey could have moved into the Bukit Teraja area from a nearby swamp forest during one of its feeding forays. I therefore reject Levy's inference that orang-utans occur in the Bukit Teraja area.

There are no records of *Pongo pygmaeus* in Brunei Darussalam in recent historical times, but remains, dating to 35,000 years ago have been found in the Niah cave deposits (Medway, 1977; Medway, 1979, Bennett 1998; Rijksen and Meijaard, 1999; Cranbrook 2000,). Orangutans which were once widespread in Borneo are now patchily distributed. This has been attributed to a lack of essential minerals in the forest soils, high sea levels in the Holocene period, in combination with high rainfall (Payne, 1992) and, more recently, hunting coupled with extensive loss of forest habitat (Payne et al., 1985; Payne and Andau, 1989; Bennett 1998,). Habitat loss during the 1997-98 fires amounting to well over 50,000 km<sup>2</sup> in Borneo has further shrunk the distribution of these apes and only 7% of the Bornean population estimated at the beginning of the twentieth century, survives today (Rijksen and Meijaard, 1999).

There are three peculiar gaps in orang-utan distribution in Borneo – between Sg Rajang (Sarawak) and Sg. Padas (Sabah), the lowlands between Sg Barito (South Eastern Borneo) and Sg Mahakam (East Kalimantan) and the Tawau region of Sabah (Bennett, 1998; Payne et al., 1985). Hence their natural occurrence in Brunei at the present time is highly improbable and any seen in the wild are likely to be escaped captive animals.

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#### JOB SCHEDULING IN ECONOMY-BASED GRID

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**Abstract:** The existing Grid is uncoordinated in nature as their scheduling and resource management strategies do not take into account the needs of the users. This has led current Grid developers to use economic models as an approach to support efficient resource management and scheduling. To achieve the supply and demand equilibrium, economic models are used to take into account of the users' behaviour in their scheduling mechanism. This paper aims to study the approaches in economic models and scheduling mechanisms used by other systems. To achieve economic stability, it is necessary that the economic model enforces fairness in its job scheduling mechanism, taking into account of the users' behaviour, usage and income distribution.

*Keywords:* Grid, economic models, scheduling, resource management, discrete-event simulation.

#### 1. Introduction

The Grid is a way to aggregate resources that are located at different places around the world to obtain massive computing power in order to solve large and complex computational problems. It is a computing infrastructure that allows the sharing of unused computational resources, storage systems, data sources, databases, servers and scientific instruments (Sun Microsystems, 2003; Foster, 2002; Baker et al., 2002).

A current problem with the Grid is that resources either get overloaded with work or under-utilised. Many users may be submitting large jobs to be processed and completed with conflicting deadlines or when few jobs may be submitted at less popular times. During the peak time, other users may be denied the services they require while at off-peak times when there are less users and the resources become wasted as they are left idle. This creates an imbalance in the supply and demand of resources.

The uncoordinated nature of existing Grid systems makes their resource management and job scheduling operations inefficient. They do not take into account the priorities and work load of the submitted jobs or the behaviour of the users. This problem becomes crucial when time critical jobs experience a long waiting time in a heavily congested queue until resources become available. Low priority jobs may get processed before higher priority ones creating unfairness in resource access for users.

Existing Grid systems such as Nimrod/G (Buyya et al., 2000a), TeraGrid (TeraGrid, 2006) and Legion (Natrajan et al., 2002) make use of high speed communication technologies and global process scheduling to organise computing resources into clusters to create general-

# 2. The Grid Economy

There have been many proposals made for the architecture of an economy-based Grid. One such proposal is the Grid Architecture for Computational Economy. Other examples of economy-based Grids are those proposed by (Venugopal and Buyya, 2004), (Ferguson et al., 1996) and (Amir et al., 2000).

# 2.1 The Grid Architecture for Computational Economy (GRACE) (Buyya et al., 2005)

Figure 1 shows a typical architecture for an economy-based Grid.

The architecture is made up of four main components:

- The **Grid Consumer** is a Grid user with applications that require the computational power provided by Grid resources.
- The **Grid Resource Broker** is a mediator between the Grid consumer and the Grid service providers using the Grid middleware services. One important component in the resource broker is the scheduler or the schedule advisor. The scheduler is responsible for the discovery of resources and the selection of suitable resources that meet the user requirements before the assignment of jobs to these resources.
- The **Grid Middleware** provides services to help couple a user and remote resources through a resource broker or an application which supports Grid. These services include communication, resource reservation, status and health monitoring components, resource allocation and process management, authentication and security and so on.
- The **Grid Service Providers** are the parties that provide resources to be traded in the Grid market to users who require them.

Figure 1: Grid Architecture for Computation Economy (Buyya et al., 2005).



### 2.2 Economic Models

In the Grid, economic models (Buyya et al., 2001) are used as service-price negotiation protocols to provide algorithms or policies and tools for resource sharing or allocation. These help both the service providers and the users to realise each other's requirements.

The business of Grid resources is conducted using different types of economic models. Some of these are (Buyya et al., 2001):

- The **commodity market model** lets the resource owners specify their service price and the users are charged according to the amount of resource they use. The pricing can be at a particular price based on the resource supply and demand.
- The **posted price model** allows the resource owners to advertise their service price including special offers that will attract users. Unlike the commodity market model, the resource brokers do not have to negotiate the price directly with the service providers but use the posted price.
- The **bargaining model** allows the resource brokers to bargain with the resource providers for lower price or higher usage duration. Negotiation stops when they both agreed on a price or when one is reluctant to continue further negotiation.
- The **tendering model** allows the user or resource broker to advertise its requirements and for resource providers to bid.
- The **auction model** allows negotiation between one resource provider and many users. Resource providers initiate negotiation by announcing their services and users are invited to bid. There are many types of auction such as the English auction, First-price sealed-bid auction, Vickrey auction and Dutch auction.
- The **bid-based proportional resource sharing model** allows the allocation of a certain percentage of resource shares to the users' application that is proportional to the bid value of the user.
- The **community model** allows resource providers who contribute their resources to a common pool to get access to resources in that pool.
- **Monopoly** is a model such that a single resource provider is the provider of a particular service.

# 2.3 Mechanisms for Pricing, Accounting and Payments

A mechanism for pricing (Ranjan et al., 2004) is required to attract users to use the resources, ensure resources are utilised efficiently and to maximise the profits of resource providers and users. The mechanisms for accounting and payment (Ranjan et al., 2004) are required to keep records of the resources used by the user and to charge them based on these records.

The pricing schemes (Ranjan et al., 2004) are determined by various parameters and they include a flat price model, time of usage, usage period and duration, demand and supply, foresight-based, loyalty of customers, historical data, contract, calendar-based or bulk purchase.

One of the ways to keep an account of how much is used by the user is to measure the amount of the type of resources they use. Some of these resources are the CPU, memory, storage, software and libraries and network activities. A combination of pricing schemes may be used to form a costing matrix to take into account multiple resources in pricing.

Payment mechanisms supported by economy-based Grid can be in the form of prepaid, use and pay later, pay as you go or credit-based.

#### 3. Scheduling

Resource management is extremely important in the Grid to optimise the performance of the entire Grid system (Chun and Culler, 1999). Resource allocation is performed by schedulers in the resource management structure to ensure fairness and efficiency (Buyya et al., 2000b). Schedulers vary from one another depending on the parameters found in their algorithm (Buyya et al., 2000a). In economy-based Grids, these parameters include both system and user requirements such as resource architecture and configuration, resource capability, resource state (busy or free), resource requirement of an application, access speed, priority of the user, queue type and length, network bandwidth, load and latency, reliability of resource and connection, user preference, application deadline, resource cost, historical information and users' willingness to pay for usage.

#### 4. Methodology

#### 4.1 Discrete-Event Simulation

Discrete-event simulation (DES) (Ball, 1996) is used in this project to study the stability between the supply and demand of resources in Grid systems. Using this, models can be built and simulated to study their time-based behaviours. DES changes value or state at discrete instances in time, rather than in continuous flow (Fishman, 2001). This efficiently speeds up the simulation as time jumps from the time of an event happening to the time of the next event.

Figure 2: The three major components of a discrete event simulation.



In a typical DES, there are three major components (Figure 2); the steering component, the simulation core and the model. The steering component handles the overall running of the simulation system. It starts the simulation core and runs it in a loop after initialising the simulation core and the model. It also stops the simulation at a preset time.

The simulation core is made up of the event queue, the clock and the simulator. The simulator processes the events in the queue and updates the clock to the time of the current event.

The model assigns timestamps to each event it generates and put these events into the event queue in chronicle order. The simplest model is the M/M/1 model which is a queuing model. This model has a source, a queue and a server with the arrival time and service time of jobs following the exponential distribution.

Figure 3 shows that when the simulator takes an event from the event queue to process, it would trigger the model to generate new events to be put in the event queue. There are two

with this arrival time as its due time is enqueued in the event queue. When a server event is processed, the server is triggered to process the next piece of work in the (model) queue if it is free. A server event with the service time of work as its due time is added into the event queue. When an event in the event queue is processed, the clock is updated.

Thus, the simulation core uses information generated by the model to simulate the queuing scenario of the model. When simulated, the model will in turn update the event queue with new information as new events are inserted.



Figure 3: The simulator, event queue and clock in the simulation core.

# 4.2 Correctness of the Simulator

Figure 4: Mean delay time against loading graph using M/M/1.



The M/M/1 model is first used to validate the discrete-event simulator. When this has been achieved, a simple closed network model is used for further validation as the experiments to be carried out are closed networks.

In the M/M/1 model,  $\lambda$  and  $\mu$  are the average arrival and service times respectively. Random arrival and service times can be generated as follows:

arrival time, at = -  $\lambda \log (r)$  and service time, st = -  $\mu \log (r)$  where r is any random number between 0 and 1.

Figure 4 shows a graph of the average time taken for a job to be completed against loading. Loading is equal to  $\mu/\lambda$  during each simulation run where  $\mu$  varies from 1 to 9 on each run with  $\lambda$  staying at a constant value 10. The graph shows that the mean delay time increases with increasing service time. The graph is increasing according to the theoretical formula. This means that the model is working correctly on the simulation core.





A closed network is one that allows a fixed number of customers (or jobs) in its system (Gelenbe et al., 1987).). The queue length in an open system is bounded. The arrival of the next job depends on the server (Sauer and Mani Chandy, 1981). If the server is still serving a customer, the arrival of the next job cannot be determined. A closed network reflects closely to economy-based Grids as users have to register before using the service. Thus, there is a finite number of users.

Figure 5 shows that the total number of jobs completing a job at a particular time. This follows a normal distribution.

#### 4.3 Economic Model Used

Every time the user uses a resource, his usage is updated as usage + = priority\*size. Everyday, at intervals of 24\*60 minutes, income is deposited into his account.

#### 4.4 Scheduling Algorithm Used

The scheduling algorithm in the scheduler involves the computation of a merit value which will determine the position of the job in the job queue. A good merit value will secure a front position for the job in the queue so that it can reach the server sooner.

The merit value is formulated as  $merit = (priority^*C)/(usage+(priority^*C))$  where C is a constant,  $10^4$ . C is needed to ensure the merit value is in a known range. Otherwise, the merit values are very small, falling within the range of  $10^{-3}$  if the usage is 200 or -1000. This makes it difficult for us to tackle these numbers. If a user has a high usage, that is he is using more resources than he can pay, he will be given a lower merit.

Figure 6: An economic model using a scheduler to allocate jobs in the queue.



Figure 6 illustrates the structure of an economy-based scheduling system. The users have a registered account with the resource providers. Users submit jobs to the scheduler who then computes merit values to the jobs by doing various checks on the costs of the jobs, the priorities specified by the users and the health of the users' accounts. A healthy account is one that is free from debts. The scheduler takes charge of where to insert the job in the queue. An idling server is prompted by a new job to process the job. After the job has been processed, the results are sent back to the user before he decides on the submission of the next job.

#### 4.5 Aging Process

Figure 7: A possible user behaviour without the use of the aging process.



An important reason for implementing the aging process is to ensure consistency and fairness in job submission among users. A user may realise that he will not get a good merit value with a high usage in his account at a particular time. Due to this, his job will be placed at the back of the queue (see 1 in Figure 7). He may consequently cancel a submitted job. He then waits for time to pass so that his usage will eventually decrease and resubmit the same job (see 2 Figure 7). With a lower usage at this time, the job he submiss will obtain a better merit value and thus, be nearer to the front of the queue for the same price.

In the experiments, the merit value is incremented by 3 at regular interval of 30 minutes. This number is chosen because the aging merit increment should be in the range with the merit value.

#### 5. Experiments, Results and Discussion

To conduct the following experiments, 20 users are set in the system. Each user belongs to a group and there are equal number of users in the two groups; a group with a fixed priority and a group with varying priority. 10 simulation runs are conducted with the fixed priority group having a fixed priority of 5 throughout the simulation. The varying priority group has priority varying from 1 to 10 in each run. We run the simulation for 100 days which is 100\*24\*60 minutes, the end time of each simulation run.

#### 5.1 Experiment 1 with Additive Income

**Figure 8**: Graph of average usage for the group against time in the experiment with additive income, where f1 means fixed group at priority=5 when varied group is at priority=1 or v10 means varied group at priority=10 when fixed group is at priority 5.



The additive income economy model works by increasing the income with a fixed increment and with time at regular intervals like getting your wage on a pay day. The usage is updated with income as usage=income at every regular intervals of 24\*60 minutes. The economic model with the additive income behaves like the finances today where a person has a fixed regular income going into his bank account.

 $\mathbf{F}$  and  $\mathbf{0}$  illustrates that the economic model using addition income connet calibrate

#### 5.2 Experiment 2 with Multiplicative Income

The multiplicative income used in this experiments means that the usage of a user is updated as  $usage^{*}=0.8$ . This means that the updated income will always be 0.8 times of his usage. The value 0.8 is just an arbitrary value. This economic model works like the bank interest in a typical savings account where the bank debits a certain percentage of a customer's bank balance at the end of the month or year depending on the type of savings account.

**Figure 9**: Graph of average usage for the group against time in the experiment with multiplicative income when priority for varied group is 1. f1 means a graph of the fixed group during the simulation run where varied group's priority=1. Fixed group's priority remains at 5 in all simulation runs while varied group's priority is different for different runs.



Interestingly, Figure 9 shows that initially, f1 has a large increase in usage before it tumbles down and reaches equilibrium. During the same time, v1 makes a big plunge before attaining equilibrium. From here, the fixed group takes the advantage of the varied group's plunge to submit more jobs to be processed.

# 5.3 Experiment 3 with Different Aging Merit Value for the Multiplicative Income Economic Model

Three simulation runs were conducted, with the aging merit at 0, 0.001 and 0.01. From Figure 10, at priority 1, the varied group does not get any job done as mean delay is at infinity and the fixed group gets more done. Due to this, the fixed group has a high usage as shown in Figure 11. The fixed group is taking advantage of the varied group's poor priority by having more jobs done and hence, the higher usage. Without an aging merit value, jobs with low priority may be forever stuck in the queue and do not get processed.



Figure 10: Mean delay against priority graph with aging merit=0.

Figure 11: Usage against priority graph for aging merit=0.





Figure 12: Mean delay against priority graph with aging merit=0.001.

Figure 12 shows that fairness is guaranteed with the use of an aging merit value. Despite its low priority, jobs submitted by the varied group get processed at a finite time. Every job submitted into the queue eventually gets processed.

#### 5.4 Experiment 4 with Varied Users Submitting Jobs with Different Priorities

In this experiment, the effect of having varied users submitting jobs with different priorities on the scheduling mechanism is investigated. The multiplicative income model is used to study this effect. The formula,  $5\pm 0.5*priority$  difference is used to generate the priority of the job. The varied group would generate 50 percent of the jobs with priority, 5+0.5\*priority difference, and 50 percent with priority, 5-0.5\*priority difference. At simulation run 1, the varied group is submitting jobs of either priority 5.5 or priority 4.5, thus having a priority difference of 1. At simulation run 2, the varied group is submitting jobs that have priority difference of 2, that is of either priority 6 or 4 and so forth. The fixed group will continue to generate jobs with fixed priority. Each simulation run corresponds to the priority difference.

From Figure 13, the mean delay of the varied group is increasing as the difference in priorities of the job the users submit becomes larger. Thus, the fixed group is benefiting from this behaviour of the varied group as their mean delay decreases. The reason is shown in Figure 14 as the usage of the fixed group is increasing as they are having more jobs done while the opposite is happening for the varied group.

Despite the high priority difference of say, 8, in the varied group that is submitting jobs of priorities 9 and 1, a low usage is observed. This usage has been brought down by the jobs of priority 1 while it brings up the mean delay. While the merit value may be higher for jobs with priority 9 for the varied group than for jobs with priority 5 for the fixed group the jobs



Figure 13: Graph of mean delay against priority difference.

Further tests is required to be conducted to find out in this case if the varied users can make use of the low usage caused by the priority 1 jobs to obtain higher merit values for jobs with priority 9.

Figure 14: Graph of usage against priority difference.



### 6. Conclusion

scheduling algorithms that support efficient resource management with the economic models are available.

In this paper, steps were taken to build a correct simulation using the M/M/1 model as a validation test. A simple closed network model is then used for further validation before moving onto developing economic models. The fairness issue is considered by using the aging process. Merit value calculations are used in the scheduling algorithm. Experiments using two economic models; one with an additive income and the other with a multiplicative income are conducted.

The economic model with the multiplicative income established stability. But, it does not go along with the laws of finance today as users do not go bankrupt and this raises questions in social ethics. The results from the additive income experiment showed that the usage of the users does not reach equilibrium but either decreases or increases with time depending on the amount of the users' income.

From the experiment that with aging merit value 0, jobs with priority 1 never gets done as they wait forever in the queue. In the other experiment, varied users may possibly use large priority difference to obtain very high merit values for their high priority jobs while their low priority jobs will have very small merit values.

Based on the economic models and scheduling aspects presented in this project, future studies include the development of other economic models with different user behaviours and more importantly, models with income distribution of users that will abide with good finance rules.

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# APPLICATION OF ELECTROSPRAY MASS SPECTROMETRY OF SOME METAL COMPLEXES TO COORDINATION COMPOUNDS

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**Abstract:** Electrospray ionisation mass spectrometry (ESI-MS) is a soft vaporisation and ionisation technique applicable to both neutral and charged species. Naturally, neutral species need to become charged to be detectable by MS. This can occur via:

loss of an acidic hydrogen to give an anion

protonation or attachment of an alkali metal ion to form a cation (either deliberately or serendipitously)

by reduction.

In this paper we describe the ESI-MS of some neutral and cationic metal complexes with some O and N-donor ligands.

The interpretation of the spectra shows that ESI-MS is in some cases a useful technique for coordination complexes. The method should be applicable to similar complexes of other metals.

*Keywords*: metal complexes, coordination compounds, electrospray mass spectrometry

### 1. Introduction

Mass spectrometry (MS) is a gas-phase technique in which molecules are ionised and the ions are separated and detected according to their m/e values; this gives both the mass of the ions and their abundance. Separation is commonly done via a quadrupole. The standard technique for the ionisation of organic molecules is bombardment by a stream of electrons (EI).

Obviously, the limitation is that the compound under study needs to be volatile and this criterion applies to many organic compounds. What if the compound has a high molar mass, is thermally sensitive or is charged and non-volatile? A variety of techniques have been developed to overcome these limitations (field desorption, chemical ionisation, fast-atom bombardment, laser desorption).

The most recent technique is electrospray ionisation mass spectrometry (ESI-MS), which is a soft vaporisation and ionisation technique applicable to both neutral and charged species. The technique was developed by John Bennett Fenn who awarded the Nobel Prize in Chemistry in 2002 for his work in the field of mass spectrometry, specifically for the electrospray ionisation technique. It is often used to identify and analyze biological macromolecules. His Nobel Lecture (December 8, 2002) was entitled (Fenn, 2002):

ELECTROSPRAY WINGS FOR MOLECULAR ELEPHANTS

The electrospray technique is believed to work as follows (Cole, 1997; Stewart and Horlick, 1996; Henderson et al., 1998a). In the atmospheric pressure region of the spectrometer, a solution of the compound in question is sprayed from a needle, the tip of which is held at a high voltage (Figure 1). The resulting droplets acquire a positive charge.





The droplets shrink in size due to evaporation. When the Rayleigh limit is reached the electrostatic repulsion equals the surface tension. Below this, the droplets explode yielding ultimately desolvated ions (Fig. 2) which are sent to the mass analyser.

Figure 2: The desolvation process.



electrostatic repulsion = surface tension

Electrospray Mass Spectrometry (ESI-MS) can be summarised as:

- Mild ionisation technique
- Solution volatilised in an electrostatic field
- Solvent evaporates and the substrate is in gas phase
- Applicable to ions and neutral molecules

Naturally, neutral molecules need to be ionised. There are a variety of processes that generate ions:

Loss of an acidic proton  $\rightarrow$  [M - H]<sup>-</sup>

Adduct formation:

Attachment of a proton  $\rightarrow [M + H]^+$ 

Attachment of an incidental ion, e.g. sodium 
$$\rightarrow [M + Na]^{\dagger}$$

chloride 
$$\rightarrow [M + Cl]^{-1}$$

Oxidation and reduction

Typical ions produced by electrospray ionisation are:

- Positive mode:
  - $[\mathbf{M} + \mathbf{U}]^+$  proto

protonated molecula

•	Negative mode:	
	$[M - H]^{-}$	deprotonated molecule
	$[M + HCOO]^{-}, \ldots$	adducts

Typical positive-ion ESI mass spectra of a cation and a neutral molecule are shown in Figs. 3 to 5. Note the proton and sodium adducts in Fig. 4 and the lack of fragmentation.

**Figure 3:** Positive ion ESI mass spectrum of  $[Ru(bpy)_2(en)](ClO_4)_2$  (bpy = 2,2'-bipyridine, en = ethylenediamine) in acetonitrile (0.1 mM) (Note the ion pair and the M/2 peak) (Arakawa, 1996).



The inset shows the expansion of the ion cluster due to the molecular dication.

**Figure 4:** Positive mode mass spectrum of 1,5-diacetylbenzimidazole (note the proton and sodium adducts) (University of Oxford).





Figure 5: Positive mode mass spectrum of reserpine (proton adduct only) (Asahikawa).

A typical negative-ion ESI mass spectrum, that of 6-hydroxyflavanone (this work) is shown in Fig. 6. The base peak is the loss of the proton. Note the dimer,  $[L + LH]^-$  and the sodium complex,  $[2L + Na]^-$ .

Figure 6: Negative ion mass spectrum of 6-hydroxyflavanone (this work).



The objective of this project was to see whether the ESI technique gives useable spectra for coordination compounds and whether fragmentation is reduced relative to the EI method.

#### 2. Materials and Methods

#### 2.1 Reagents and syntheses

All reagents were from Sigma-Aldrich and were used as supplied.

 $[Cr(acac)_3]$  (Woolins, 1994a),  $[Cr(3-Bracac)_3]$  (Cardwell and Lorman, 1984, 1986),  $[Cu(NH_3)_4]SO_4$  (Clareen et al., 2000), SnI<sub>4</sub> (Schaeffer et al., 1997; Woolins, 1994b) and SnI<sub>4</sub>.2OPPh<sub>3</sub> (Woolins, 1994b) were prepared according to the previously described methods. Toluene was used as the solvent for the preparation of SnI<sub>4</sub>.

 $[Al(flav)_3] \ (flav = anion \ of \ 3-hydroxyflavone, \ chrysin) \ was \ prepared \ as \ pale \ yellow \ crystals \ from \ Al_2(SO_4)_3.18H_2O \ in \ water \ and \ the \ flavone \ in \ methanol.$ 

 $[Mo(CO)_4(L)]$  (L = 2,2'-bipyridine) was prepared by heating Mo(CO)<sub>6</sub> with the ligand (1:1) in toluene under nitrogen for 2 h (Stiddard, 1962).

The platinum complexes were prepared from  $K_2PtCl_4$ . [Pt(terpy)Cl]Cl was obtained by heating  $K_2PtCl_4$  and terpy in water (Morgan and Burstall, 1934; Howe-Grant and Lippard, 1980). [Pt(DCMB)Cl\_2] was prepared by heating  $K_2PtCl_4$  and DCMB in water/ethanol (Dholakia et al., 1985). *Trans*-[Pt(py)\_2I\_2] was prepared according to (Souchard et al., 1990).

[2,2'-bpyMe]I was prepared by heating 2,2'-bpy and methyl iodide in 1-butanol. The complexes were prepared according to (Dholakia et al., 1982).

#### 2.2 Mass Spectrometry

GC-MS were recorded on a Hewlett-Packard model 5890 series II instrument with a flame ionisation detector and an ultra five column. The complexes were dissolved in dichloromethane, the carrier gas was helium with a flow rate 20 cc min<sup>-1</sup>, the column temperature was 100° for 5 min followed by a ramp of 8°/min., The retention time for [Cr(acac)<sub>3</sub>] was around 17 min.

ESI mass spectra were recorded using a Finnigan TSQ7000 spectrometer with the following parameters:

•	Spray Voltage:	4.5 kV
•	Cone Voltage:	20 V
•	Capillary Temperature:	200°C
•	Scan Range:	100 - 2500 m/z

The solvents were mainly methanol and  $H_2O$ .

#### 3. Results and Discussion

#### **Positive ion mass spectra**

# 3.1 Chromium compounds

The chosen test compound was  $[Cr(acac)_3]$  (I) (acac = anion of 2,4-pentanedionate ion, II pentanedione). This compound is sufficiently thermally robust to pass through the column of a GC-MS without decomposition. The subsequent EI-MS shows extensive fragmentation of the molecular ion (16% RA) (Table 1) (only peaks due to <sup>52</sup>Cr (isotope shown, 83.8% mass abundance) isotope are shown )



<u>The reported EI spectra of Comparison with</u>  $[M(acac)_3]$  (M = Al, Cr) (Macdonald and Shannon, 1966; McLafferty and Stauffer, 1989) are similar with  $[M(acac)_2]^+$  being the base peak and-

The cationic ESI MS of  $[Cr(acac)_3]$  shows considerably less decomposition of the molecular ion <u>having a relative</u> abundance <u>of around 8%</u>. Pierce et al. (1982) in a SIMS (Ar ion beam) mass spectral study of  $[Cr(acac)_3]$  in a NaCl matrix observed peaks in roughly equal abundance for  $[Cr(acac)]^+$  (98%) and  $[Cr(acac)_2]^+$  (100%) and a minor peak (14%) for  $\{[Cr(acac)_3] + Na\}^+$ .

<u>The positive ion ESI-MS of  $[Cr(acac)_3]$  reveals practically no decomposition of the complex</u>, with the base peak now being the molecular ion as the  $\{[Cr(acac)_3] + Na\}^+$  adduct. A small amount of the bis-adduct,  $\{[Cr(acac)_3]_2 + Na\}^+$ , is also present.



ion ESI-MS <u>of product obtained from one such reaction</u> is summarised in Table 2. Bromine has two isotopes:  $^{79}$ Br (50.5 %) and  $^{81}$ Br (49.5 %); only the peaks due to the former are tabulated.

Table 2: Positive ionIon ESI-MS of the product from the bromination of [Cr(acac)<sub>3</sub>]

Species	m/e <sup>a</sup>	RA (%)
$[Cr(Bracac)_3] + Na^+$	608	100
$[Cr(Bracac)_2(acac)] + Na^+$	530	45
$[Cr(Bracac)(acac)_2] + Na^+$	450	22
$[{Cr(Bracac)_3} + {Cr(Bracac)_2(acac)} + Na^+]$	1115	10
$[{Cr(Bracac)_3}_2 + Na^+]$	1194	15
0 1 + 520 + 1790 + 1300		

<u>a.</u> Only the  ${}^{32}$ Cr and  ${}^{79}$ Br peak is given.

<u>Incomplete reaction would result in a mixture of the three potential bromination products</u> <u>together with unreacted starting material. These can been separated by either tlc, gas</u> <u>chromatography</u> or hplc\_(Cardwell, 1984, 1986) and characterized by MS.\_Cardwell also showed that the ease of bromination is mono > di > tri and an excess of NBS is required for complete reaction.

The mass spectrum shows only tris(ligand) species with no fragmentation. The dominant species is the tri-brominated complex, along with some di- and a lesser amount of the monobrominated compound. The relative intensities of the bis-adducts,  $[(complex)_2 + Na]^+$  are consistent with the relative abundances of the mono cations.

In the spectrum of the product of a different reaction, bromination was less successful and the three bromo complexes were minor components, along with unreacted  $[Cr(acac)_3]$  as the major species. Thus, ESI-MS is useful for monitoring reactions that modify the acac ligand and ought to be applicable to other metals and other reactions, e.g. nitration.

Cardwell and Lorman (1984) found that the EI MS of  $[Co(Bracac)_3]$  has  $[Co(Bracac)_2]^+$  as the base peak;  $[Co(Bracac)_2(acac)]$  has  $[Co(Bracac)(acac)]^+$  as the base peak, Bracac;  $[Co(Bracac)(acac)_2]$  has  $[Co(acac)_2]^+$  as the base peak; selectively loss of Bracac over acac is preferred. In the ESI spectra there is no loss of ligand.

3.2 Organometallic compounds

<u>The group 6 homoleptic carbonyls,  $[M(CO)_6]$  (M = Cr, Mo, W) give EI-MS with</u> extensive fragmentation (NIST). All the ions down to the metal are present. In our hands,  $[Cr(CO)_6]$  gave a similar satisfactory EI spectrum.

Table 3:	EI-MS	of [Cr(C	$(O)_6$
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Species	m/e <sup>a</sup>	RA (%)
Cr <sup>+</sup>	52	85
$[Cr(CO)]^+$	80	100
$[Cr(CO)_2]^+$	108	63
$[Cr(CO)_3]^+$	136	5
$[Cr(CO)_{4}]^{+}$	164	3

<u>The ESI-MS results for metal carbonyl compounds were rather disappointing. The</u> <u>spectrum for [Mo(CO)<sub>6</sub>] was all noise with no peaks. The spectrum for [Mo(bpy)(CO)<sub>4</sub>] (IV), although noisy, showed the following isotope patterns:</u>

254, 283-287, 317 which may be due to the following species: Mo(bpy)H<sup>+</sup> Mo(bpy)(CO)H<sup>+</sup>/Li<sup>+</sup> Mo(bpy)(CO)<sub>2</sub>/Li<sup>+</sup> Molybdenum has a range of isotopes:  $\frac{92}{9}$ Mo\_(15.1\_{\%}),  ${}^{94}$ Mo\_(9.3\_{\%}),  ${}^{95}$ Mo\_(15.8\_{\%}),  ${}^{96}$ Mo\_(16.5\_{\%}),  ${}^{97}$ Mo\_(9.6\_{\%}),  $\frac{98}{9}$ Mo\_(24.0\_{\%}),  ${}^{100}$ Mo\_(9.7\_{\%}),

The fragment assignment given above is rather tentative as it supposes extensive fragmentation of the complex and a combination of protonation and adventitious lithium. Extensive fragmentation is generally not the usual behaviour in ESI-MS.

Although anionic and cationic carbonyl complexes give good ESI spectra, neutral complexes give poor results, as there is no suitable site to fix a proton (Henderson et al., 1998). Successful ESI-MS have been reported for neutral homoleptic carbonyls in the presence of a reagent such as methoxide, or silver or sodium ions (Henderson et al., 1998, Hori et al., 1998).



Hori et al. reported that the addition of sodium ions to a solution  $[Re(bpy)(CO)_3Cl]$  results in a strong peak at  $\{[Re(bpy)(CO)_3Cl] + Na^+\}$ . In the absence of sodium ions, no peaks were detected. Moreover, the bpy ligand facilitates the attachment of a sodium ion since  $[Re(CO)_5Cl]$  gives no detectable species. They obtained no spectrum for  $[M(bpy)(CO)_4]$  (M = Cr, Mo) at low drift voltages and only  $\{[M(bpy)(CO)_3] + Na^+\}$  and  $\{[Cr(bpy)(CO)_2] + Na^+\}$  at higher drift voltages.

#### 3.3 Tin compounds

<u>The Sn(IV)</u> halides,  $SnX_4$  (X = Cl, Br) give good EI-MS (NIST), albeit with extensive fragmentation. These compounds are both reasonably volatile.

<u>We examined  $[SnI_4]$ </u>, which is a solid, and its derivative  $[SnI_4(OPPh_3)_2]$  with the objective of using the spectra for interpretation in an undergraduate experiment.

Tin has the following major isotopes:

 $^{116}$ Sn (14.3%),  $^{117}$ Sn (7.6%),  $^{118}$ Sn (24.0%),  $^{119}$ Sn (8.6%),  $^{120}$ Sn (32.8%),

 $^{122}$ Sn (4.7%),  $^{124}$ Sn (5.9%)

giving tin-containing fragments a distinctive isotope pattern (iodine has one isotope, <sup>127</sup>I).

Neutral tin complexes could potentially manifest themselves in an ESI-MS either by addition of a cation or via loss of iodide. Further, tin has two oxidation states (II, IV) of comparable stability, giving rise to the possibility of reduction. Cleavage of the Sn $\Box$ I bond may be either homolytic, giving rise to a neutral fragment and is tantamount to reduction or

 $Sn \square I$  bond, the nearness of Sn and I in terms of their electronegativity and the relative evergies of the two processes.

 $SnI_4 \longrightarrow SnI_3^+ + I^-$  (homolytic)  $SnI_4 \longrightarrow SnI_3^+ + I^-$  (heterolytic)

The ESI-MS of  $SnI_4$  is complex with extensive fragmentation, and only a weak peak for the molecular ion as the Li<sup>+</sup> adduct (Table 4). The minor peak at m/e 768 is consistent with Li<sub>2</sub>SnI<sub>5</sub>, which is reasonable considering the chemistry of tin. Addition to the coordination sphere of SnI<sub>4</sub> is facile resulting in the formation of  $[SnI_6]^{2-}$  (Dillon and Marshall, 1987, Greenwood and Earnshaw, 1984a). Likewise,  $[SnCl_5]^{-}$  is a known ion (Shamir et al., 1985).

Table 4: ESI-MS of tin compounds

$SnI_4$			$SnI_4(PPh_3O)_2$		
Species	m/e <sup>a</sup>	RA (%)	Species	m/e <sup>a</sup>	RA (%)
$[SnI_2] + Li$	381	100	Ph <sub>3</sub> POH <sup>+</sup>	279	100
$[SnI_2] + K$	413	24			
			Sn(Ph <sub>3</sub> PO)I <sup>+</sup>	525	35
[SnI <sub>3</sub> ] +Li	508	16			
$[SnI_3] + K$	540	6	$Sn(Ph_3PO)I_3^+$	779	28
$[SnI_4] + Li$	635	10			
$[SnI_5] + 2Li$	769	4			
	7	120			

a. <sup>1</sup>Li and <sup>120</sup>Sn isotope

The major decomposition fragment was a 14 line isotope pattern centred at m/e2 380 (base peak). This lies in the region of a  $SnI_2$  fragment; however the values are too high for  $SnI_2^+$ , they fit more reasonably with  $SnI_2Li^+$  (adventitious  $Li^+$ ?). This suggests homolytic fission of the  $Sn\Box I$  bond and thus reduction to Sn(II).  $SnCI_2$  is formed in the gas phase on heating the solid. It has a bent structure (Greenwood and Earnshaw, 1984b)and the lone pair of electrons on the tin is able to coordinate to any suitable Lewis acid. The  $SnI_2Li^+$  ion only accounts for 7 of the 14 lines; other species may include  $SnI_2H^+$  and/or a dicationic di-tin species (13 principal lines for a  $Sn_2$  ion).

The minor isotope pattern centred around m/e 413 is consistent with  $SnI_2K^+$ ; there is no sign of a sodium adduct.

The cluster at m/e 508 is due to  $SnI_3Li^+$ . Again, there are between 11 and 14 lines, suggesting another species, possibly a dimer based on  $Sn_2I_6$ . The minor potassium analogue is also present.

The ESI-MS of  $[SnI_4(Ph_3PO)_2]$  shows extensive fragmentation and no molecular ion. The base peak is due to Ph<sub>3</sub>POH<sup>+</sup>. The highest mass peak is Sn(Ph<sub>3</sub>PO)I<sub>3</sub><sup>+</sup>, followed by loss of I<sub>2</sub> to give Sn(Ph<sub>3</sub>PO)I<sup>+</sup>:

 $"SnI_4(Ph_3PO)_2" \xrightarrow{-PPh_3O} SnI_3(Ph_3PO)^+ \xrightarrow{-I_2} SnI(Ph_3PO)^+$ 

#### <u>3.4 Classical complexes</u>

 $[Cu(NH_3)_4]SO_4$  failed to give a spectrum. The m/2 ion was not observed (c.f. Fig. 3); possibly, the complex-sulphate ion pair is tightly bound and is not subject to protonation.

#### 3.5 Aluminium compounds

<u>The aluminium compound [Al(acac)<sub>3</sub>] gives a useful EI MS</u> (NIST). It would be of interest to see whether ESI-MS is applicable to Al(III) complexes.

<u>Al<sup>3+</sup>(aq) reacts with flavones to afford [Al(flav)<sub>3</sub>] (flav = anion of a flavone):</u>

 $AI^{3+}$  + flavH  $\longrightarrow$  [Al(flav)<sub>3</sub>] + 3H<sup>+</sup>

where flavH = 3-hydroxy-flavone (V) and chrysin (VI). The structure of the tris(chelate) complexes is similar to that of the acac complex (I).



The positive-ion ESI spectrum of 3-HF itself shows a peak at 239 (3-HF +  $H^+$ ) and another at 261 (3-HF +  $Na^+$ ); chrysin has the corresponding peaks at m/e 255 and 277 respectively.

<u>The ESI-MS of the aluminium complexes are summarised in Table 5. Note that Al has</u> only one isotope: <sup>27</sup>Al.

#### Table 5: Positive Ion ESI-MS of [Al(flav)<sub>3</sub>]

Flavone	3-HF		Chrysin	
Species	m/e	RA (%)	m/e	RA (%)
$flavH + H^+$	239	55	255	70
$[Al(flav)_2]^+$	-	-	533	85
$[Al (flav)_2]^+ + MeOH$	533	25	-	
$[Al (flav)_3] + H^+$	739	100	-	
$[Al (flav)_3] + Na^+$			809	100
$[{Al_2(flav)_4} + OMe]^+$	1033	45	1097	10
$[Al_2(flav)_5]^+$	1239	5		

The spectra are consistent with the formulation of a tris-complex. The chrysin complex shows more fragmentation than the 3-HF analogue, while both complexes have more fragmentation than the acac complex.

Negative ion spectrum for the <u>chrysin</u> complex shows only peaks for flav and  $[flay + flayH]^2$  (similar to that for 6-bydroxyflavanone Fig. 6)

A series of complexes of the cationic ligand N-methyl-2,2'-bipyridinium (L) (VII) were prepared by mixing hot ethanolic solutions of  $MX_2$  and [L]X.



The complexes were of the formula  $[M(L)X_3]$  (**VIII**) where X is a halide (Cl, Br, I) and M is a divalent 3*d* metal (Mn, Co, Ni, Cu, Zn). These complexes are only soluble in aprotic dipolar solvents, such as MeCN, MeNO<sub>2</sub> and DMF. The conductivity indicates a 1:1 electrolyte.

The cationic electrospray mass spectrum showed only one peak at m/e 171, due to the ligand and no peaks due to metal-containing species. Thus, in solution, complete solvolysis of the complex has occurred, as shown by the equation:

 $M(L)X_3 + S \rightarrow [M(S)X_3] + L^+ \rightarrow [M(S)_2X_2] + L^+ + X^- \qquad (S = solvent)$ 

3.5 Platinum(II) complexes

Platinum has several isotopes. The isotope distribution is as follows: <sup>192</sup>Pt (1%), <sup>194</sup>Pt (33%), <sup>195</sup>Pt (34%), <sup>196</sup>Pt (25%), <sup>198</sup>Pt (7%) Chlorine has two isotopes: <sup>35</sup>Cl (75%) and <sup>37</sup>Cl (25%). In this study, we looked at the neutral and monocationic complexes. as well as dimers and cyclometallated species (neutral and cationic).

#### 3.5.1 Neutral complexes

Figure 7: Positive ion mass spectrum of [Pt(DCMB)Cl<sub>2</sub>].



The neutral 3,3'-dicarbomethoxy-2,2'-bipyridine (DCMB) complex [Pt(DCMB)Cl<sub>2</sub>] shows a straightforward spectrum with a sodium adduct of the complex (m/e 558) and no fragmentation (Fig. 7). The loss of chloride is therefore unfavourable. There are another peak at higher m/e value, possibly due to a potassium adducts.

Figure 8: Positive ion mass spectrum of *trans*-[Pt(4-pic)<sub>2</sub>Cl<sub>2</sub>] (sodium adducts).



The dominant peak in the spectrum of trans-[Pt(4-pic)<sub>2</sub>Cl<sub>2</sub>] is the sodium adduct of the molecule (Fig. 8) at m/e 657. Some fragmentation is observed in the loss of one picoline ligand to give the three-coordinate species [Pt(4-pic)<sub>2</sub>I] (m/e 564). Loss of the neutral picoline is preferred to that of iodide (c.f. SnI<sub>4</sub>). There are a number of minor peaks at low m/e values - possibly noise.

#### 3.5.2. Monocationic complexes

The complex  $[Pt(terpy)Cl]^+$  is a natural cation. The spectrum is simple - one peak (Fig. 9). The lack of fragmentation is due to the robust nature of the terpy ligand and the strong Pt-terpy chelation.

Neo et al. (2004) recorded the ESI spectra of  $[M(Ph_2P(CH_2)_5PPh_2)Cl_2]$  (M = Pd, Pt) in MeOH-pyridine. At low cone voltage, the sole peak was due to  $[M(Ph_2P(CH_2)_5PPh_2)(py)Cl]^+$ . Increasing the cone voltage results in the loss of the pyridine. Likewise, the spectrum of the cyclometallated complex  $[Pd(Ph_2P(CH_2)_2CH(CH_2)_2PPh_2)Cl]$  in MeOH-CH<sub>2</sub>Cl<sub>2</sub> shows complete loss of chloride, while in MeOH-MeCN the dominant peak is due to  $[Pd(Ph_2P(CH_2)_2CH(CH_2)_2PPh_2)Cl]$ .


Figure 9: Positive ion mass spectrum of [Pt(terpy)Cl]Cl.2H<sub>2</sub>O.

# Negative ion mass spectra

The <u>negative ion ESI spectrum of 6-hydroxyflavanone</u> (LH) is shown in <u>Fig. 6.</u> Peaks are observed due to the ions: L<sup>-</sup>,  $[L + LH]^-$  and  $[2L + Na]^-$ . The latter can be viewed as a Na<sup>+</sup> complex. Flavones (e.g. chrysin) give an L<sup>-</sup> peak.

Apart from the anion of chrysin and iodide in the spectrum of  $[SnI_4(Ph_3PO)_2]$ , no other negatively-charged species were observed.

<u>4.</u>

## Conclusion

A wide variation in the quality and usefulness of the spectra was observed, ranging from the complete lack of a spectrum (all noise) for homoleptic metal carbonyls to useful low-noise spectra for platinum complexes.

The following tentative conclusions are drawn concerning fragmentation reactions in the gas phase <u>for metal complexes</u>:

Neutral monodentate ligands are rather easily lost Chloride ligands tend to remain bonded to the metal Loss of iodide is easier than loss of chloride Chelating ligands remain bonded to the metal Dicationic complexes appear

either at m/2 or form an ion pair Several gas phase reactions with the solvent are observed

## 5. Acknowledgements

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## 6. Abbreviations

GC	gas chromatography
MS	mass spectrometry
EI	electron ionization
ESI	electrospray ionization
SIMS	secondary ion mass spectrum
RA	relative abundance
tlc	thin layer chromatography

acac	acetylacetonate anion
Bracac	<u>3-bromoacetylacetonate anion</u>
flav	flavonate ion
3-HF	3-hydroxyflavone
bpy	2,2'-bipyridine
DCBM	3,3'-dicarbomethoxy-2,2'-bipyridine
4-pic	4-methylpyridine ( ]-picoline)
terpy	2,2',6',2''-terpyridine
NBS	N-bromosuccinimide

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# FABRICATION AND CHARACTERIZATION OF POLYGALACTURONIC ACID-MODIFIED CARBON PASTE ELECTRODE FOR ELECTROCHEMICAL DETECTION OF LEAD

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Abstract: Carbon paste electrodes (CPE) modified with polygalacturonic acid were prepared and used as working electrodes in the electrochemical determination of lead (II) in aqueous samples. The voltammetric responses of the modified CPE indicated that this electrode is a suitable substitute for mercury in anodic stripping voltammetry of some heavy metal species. The overall detection procedure involves pre-concentration of the targeted metal ions on the electrode surface at open circuit, then cathodic electrolysis of the pre-concentrated species in an acidic medium followed by a detection step using square wave anodic stripping voltammetry (SWASV). The electrode used contained 20% (w/w) of polygalacturonic acid and 80% (w/w) of carbon paste. The optimum operating conditions were 2-5 minutes for the pre-concentration period in metal ion solution, followed by a 30-second cathodic electrolysis of the pre-concentrated species and SWASV detection in 0.1 M HCl. Under these conditions, the voltammetric responses after a 4-minute pre-concentration period were linear with respect to lead ion concentration in the range of 0.2 - 20 ppm. Ultra-trace levels of down to 2 ppb were detected after a 2-hour pre-concentration period. The deposition potential range was varied and this had an effect on the voltammetric response. CPEs were regenerated using 0.4 % ethylenediaminetetraacetic acid (EDTA) for 4 minutes.

## 1. Introduction

Pollution or contamination of the environment, by heavy metals, is one of the most serious problems in the world today(Bott 1995). Some metals, which are toxic, may get incorporated into drinking water and food chains. Because of their toxicity, it has become increasingly important to detect and monitor the presence of heavy metals in the environment. Over the past decades, scientists have been able to employ several analytical methods including voltammetric techniques for this cause (Dias, Caetano et al., 2006; Dias and Carmo, 2006; Prior and Walker, 2006; Rivas, 2006; Yuan et al., 2006; Zejli et al., 2006).

Stripping analysis is one of the most sensitive electroanalytical technique available. Thus, it is highly suitable for the task of field monitoring of toxic metals (Wang, 1991; Murray et al., 1994). In previous years, anodic stripping voltammetry (ASV) at a mercury electrode has been used for this purpose (Bott, 1995; Gherghi, et al., 2005). However, mercury itself is a heavy metal and recently, research in this field have concentrated on finding suitable substitutes for mercury electrodes (Li et al., 2004; Li et al., 2004; Buckova, et al., 2005; Manivannan 2005; Widmann and van den Berg 2005). Over the past three

of these studies, the carbon paste electrodes are firstly chemically modified with nucleophilic or metal-binding functionalities, and only then are they used for trace metal analysis (Shamsipur et al., 2005; Svancara, et al., 2005; Tonle et al., 2005; Shams et al., 2006; Svancara, 2006).

The aim of this study is to fabricate and evaluate the electrochemical behavior of a CPE modified with a biopolymer polygalacturonic acid to determine its suitability as a reliable electrode for the anodic stripping voltammetric analysis of lead (II) in aqueous samples. The influences of various experimental parameters were examined to establish the optimum conditions that bring about reliable results. These parameters include (a) accumulation or preconcentration time which is defined as the amount of time the modified CPE is immersed in a sample containing Pb (II); (b) deposition potential which refers to the applied potential in which the modified CPE will be subjected after accumulation; (c) varying the concentration of the Pb (II) in the sample to establish the linear concentration range as well as the limit of detection; (d) examining the possibility of regenerating the CPE by immersing the electrode in aqueous solution of ethylenediamineteraacetic acid (EDTA).

### Carbon paste electrodes (CPE)

One of the most widely used chemically modified electrodes is carbon paste electrode. These electrodes have been used and successfully employed over the past three decades in electroanalysis (Cresphillo and Rezende, 2004; Collila et al., 2005; Cui et al., 2005; Dias and do Carmo, 2005; Dong et al., 2006). CPEs are composed of a matrix of graphite and an organic paste. Such electrodes offer the advantages of very low background current, a wider potential window, miniaturization, easy preparation, as well as renewed and modified surfaces. The organic binder, which is responsible for many of the attractive properties of CPEs accounts for the slow rates of electron transfer and hence to the substantial over voltages for certain important analytes (Rice et al., 1983).

## Polygalacturonic acid as an electrode modifier

Polygalacturonic acid as shown in Figure 1 is a polymer, which consists of chains of 300 to 1000 units of the galacturonic acid monomer, joined with  $\alpha 1 \rightarrow 4$  linkages. This acid forms a methylated ester called pectin, which is a polysaccharide that acts as a cementing material in the cell walls of all plant tissues. The white portion of the rind of lemons and oranges contains approximately 30% pectin. Pectin is an important ingredient of fruit preserves, jellies and jams. To date, modifiers have been mixed with carbon powder to modify a carbon paste electrode, enhacing its selectivity towards a certain analyte of interest. Recent findings show that many compounds are able to be reliable modifiers for carbon paste electrodes (Honeychurch et al., 2001; Honeychurch et al., 2001; Hocevar et al., 2002; Hu et al., 2003; Majid et al., 2003; Jureviciute and Malinauskas, 2004).

Figure 1: Chemical structure of polygalacturonic acid.



media, which, in this study, are both aqueous. This polymer is water insoluble and does not undergo electrochemical oxidation or reduction within the potential range where most heavy metals are electroactive. In addition, polygalacturonic acid possesses functional groups that are capable of attracting heavy metal ions and hence establish the possibility of accumulating these metals on the electrode surface upon prolonged contact. In aqueous solutions, polygalacturonic acid has -COO<sup>-</sup> groups that have strong affinity towards metal ions. One possibility for the accumulation of lead ions on polygalacturonic acid-modified electrode is given by the equation below where R represents the inert polymer network:

 $2(\text{R-COOH}) + \text{Pb}^{2+}(aq) \implies \text{Pb}(\text{R-COO})_2 + 2\text{H}^+(aq)$ 

# 2. Materials and Methods

Analytical-reagent grade chemicals which included 1000 ppm lead solution (Sharlau, Barcelona, Spain) and polygalacturonic acid isolated from orange peel (Fluka, Buchs, Switzerland) were used as received, while all aqueous solutions were prepared using double distilled water. Mineral oil, graphite powder and ethylenediaminetetraacetic acid were purchased from Sigma-Aldrich and were used as received.

All chemical experiments were carried out using a BAS 100B Electrochemical System (BioAnalytical System) utilizing carbon paste electrodes modified with polygalacturonic acid as working electrodes, an Ag/AgCl reference electrode and a platinum wire counter electrode. Stripping voltammetry was performed in the Osteryoung square wave stripping voltammetry mode (OSWSV) under the following conditions, unless stated otherwise: (a) sensitivity: 100  $\Box$ A, (b) deposition potential: -200 mV to -800 mV, (c) deposition time: 30 s and (d) accumulation time: 5 min. Other specific experimental conditions are given in the captions of relevant figures. The solvent used for the accumulation of Pb(II) was distilled water while 0.1 M HCl was used in the voltammetric stripping step.

## Preparation of the modified carbon paste





slightly flaky paste. The modified carbon paste electrodes were prepared using 200  $\Box$ L pipette tips (Poulten & Graf Ltd., Sussex, U.K.). These tips were fitted onto the flat end of 3-mm copper rods forcefully until they were tightly fastened and secured. The extra length was cut off using a cutter, leaving a small empty space at the tip. This was the crucial step in the process because the absence of a smooth clean cut would result in the paste getting trapped in small rough areas. Prior to use, the surface of the carbon paste electrode was physically packed by pressing it down into the carbon paste mixture. This process was done meticulously to ensure that the paste was securely compact at the tip. Figure 2 depicts a typical carbon paste electrode prepared in this manner.

## Accumulation (pre-concentration) technique

A beaker containing the lead (II) solution to be analysed is placed on the magnetic stirrer. The CPE is then held in place using an adjustable stand and a crocodile clip, then immersed in different concentrations of Pb (II) with continuous stirring. A stopwatch is used to keep track of the time of immersion.

The accumulation time was set to 5 minutes unless stated otherwise for certain experiments. After the pre-concentration period, the CPE was then removed and rinsed thoroughly with water and transferred into the voltammetric cell in the Faraday cage for the subsequent electrochemical stripping step of accumulated metal on the electrode surface. The effects of accumulation time, along with deposition potential and concentration were also studied. To determine the effect of accumulation time, a sample of 50 ppm of Pb (II) was tested while varying the accumulation time from 30 s, 1 min, 2 min, 3 min, 4 min, 5 min to 10 min.

## Anodic stripping voltammetry

Prior to recording the OSWSV response, the accumulated metal was first allowed to be converted into its metallic form by applying a fixed potential ranging from -200 to -800 mV on the CPE in a 10-mL cell containing 0.1 M HCl. The stripping step was performed by gradually increasing the applied potential using OSWSV technique to transform the deposited Pb (0) back to its +2 oxidation state.

### Electrode regeneration

Using several different CPEs did not give good or reliable results. Furthermore, it was time consuming to continually fit each CPE with the pipette tip. Therefore, several tests were carried out to try and re-use the same CPE for several experiments. The CPE would have to be treated with ethylenediaminetetraacetic acid (EDTA). It follows the concept that most heavy metal ions, including Pb (II), form stable complexes with EDTA. It was expected that there would be a noticeable difference between a new CPE and a treated one.

### 3. Results and Discussion

Voltammetric characterization of the polygalacturonic acid-modified carbon paste electrode by cyclic voltammetry (CV):

Cyclic Voltammetry (CV) was used to determine the electrochemical behaviour of the polygalacturonic acid-modified carbon paste electrode at different working conditions. Figure 3 compares the cyclic voltammograms obtained with a glassy carbon electrode and the modified carbon paste electrode.

CPE shows inactivity over a potential range of about -700 mV to 1300 mV. That is the only significant difference when comparing the two cyclic voltammograms.

**Figure 3:** Cyclic voltammogram of 0.1 M HCl using (a) glassy carbon and (b) polygalacturonic acid-modified carbon paste electrodes at a scan rate of 100 mV/s.



The cyclic voltammogram showed reliable results for its use in determining the trace amounts of lead (II) in the experiment. Firstly, one desirable characteristic of a carbon paste electrode is a low residual current and this is clearly shown in the CV of the modified CPE. Also, the potential range where the characteristic potential of lead (II) arises (around -500 mV) was even flat. Therefore, any signal observed during voltammetric measurements of Pb (II) could be solely attributed to the analyte and not to any component of the working electrode or cell solution.

## Effect of pre-concentration period (accumulation time)

As previously thought, when the accumulation time was increased, the peak current also increased. Figure 4(A) shows the OSWSV for a 50 ppm Pb (II) solution using various accumulation times ranging from 30 s to 10 min. On the other hand, Figure 4(B) shows the plot of the peak currents at these accumulation times. This is the same kind of trend observed in previous stripping techniques. The reason behind this is that the longer the electrode is exposed to the solution containing the analyte; more materials will be deposited on the surface, which consequently provides a higher current. From the experiment, only voltammetric responses up to a 4-minute accumulation time period were linear with respect to 50 ppm of lead.

**Figure 4:** (A) Effect of accumulation time on OSWSV response for 50 ppm Pb (II): (a) 30s, (b) 1 min, (c) 2 min, (d) 3 min, (e) 4 min, (f) 5 min and (g) 10 min.; (B) Plot of accumulation time versus peak current derived from (A).



### Effect of deposition potential

Figure 5 shows the dependence of voltammetric current on deposition potential. The results showed that as the deposition potential becomes more negative, the peak current also increased. The reason behind this is that the accumulated metal can be more effectively reduced when the applied potential becomes more negative. The figure also revealed that only voltammetric responses from -600 mV to -900 mV were linear with the sample.

**Figure 5:** (A) Effect of varying the deposition potential on OSWSV response for 50 ppm Pb (II): (a) -600 mV, (b) -700 mV, (c) -800 mV, (d) -900 mV; (B) Plot of applied potential versus peak current derived from (A).



## Effect of concentration

The effect of concentration was studied using different concentrations of Pb (II). Figures 6 and 7 show the OSWSV responses of high and low concentration ranges of Pb (II) with polygalacturonic acid-modified CPE. The corresponding plots of peak currents versus concentration are also shown in the figures.

**Figure 6:** (A) Effect of high Pb(II) concentration on OSWSV response: (a) 1 ppm, (b) 2 ppm, (c) 5 ppm, (d) 10 ppm, (e) 20 ppm, (f) 50 ppm, (g) 100 ppm on Pb (II); (B) Plot of concentration versus peak current derived from (A).



Consistent with what one might expect, when the concentration is increased, the corresponding peak currents also increased. This trend has been observed in previous studies and is due to the fact that as the concentration increases, there are more materials to be deposited at the surface of the CPE during the pre-concentration period. Linearity of relationship between peak current and lead concentration shown in Figure 7 (B) gave a correlation coefficient of 0.9989. The slope of the curve is 105  $\mu$ A/ppm corresponding to the sensitivity of the method at that particular set of experimental conditions.

**Figure 7:** (A) Effect of trace Pb(II) concentration on OSWSV response: (a) 0.2 ppm, (b) 0.3 ppm, (c) 0.4 ppm, (d) 0.5 ppm, (e) 0.6 ppm, (f) 0.7 ppm, (g) 0.8 ppm, (h) 0.9 ppm, (i) 1 ppm, (j) 2 ppm on Pb (II); (B) Calibration curve derived from (A).



### Analysis at the ultra-trace level

Figure 8 shows the OSW voltammograms and plot of peak current versus analyte concentration for the ultra-trace levels of lead (II) in solution. The results showed that concentrations as low as 2 ppb can be reliably detected using the polygalacturonic–acid modified CPE using a pre-concentration period of 2 hours.

**Figure 8:** (A) OSWSV response for (a) 2 ppb (b) 7 ppb and (c) 10 ppb of Pb (II) and (B) calibration curve for ultra-trace levels of lead(II) using 2-hour pre-concentration time.



### Electrode regeneration

The old electrodes after analysis were further studied for possible regeneration of the surface through the use of EDTA cleaning solution. In principle, the high formation constant of Pb-EDTA complex would prove effective in the removal of remaining Pb (II) ions bound to the electrode surface. A solution containing 750 ppb of Pb (II) was used as the sample. After stripping analysis, 0.4 % EDTA was used to regenerate the CPE with a pre-concentration period of 4 min. After treatment, the EDTA-treated electrodes gave no peaks after the subsequent stripping step. The graph shown in Figure 9 shows the effects of CPE regeneration on a sample of Pb (II). Curve (a) shows the original OSWSV response using freshly prepared electrode and curve (b) was obtained after treatment with EDTA. The superimposed curves labelled (c) are stripping peaks after reusing the electrode that has been regenerated using EDTA. These results indicated that EDTA cleaning solution has successfully restored the polygalacturonic acid to its protonated form, effectively displacing the adsorbed metal on the electrode surface.

**Figure 9:** Multi-graph showing effect of regeneration of CPE with 0.4% EDTA: (a) original OSWSV response for 50 ppm Pb (II); (b)  $1^{st}$  run after addition of 0.4% EDTA and (c) are the following runs after regeneration.



## 4. Conclusion

The present study investigated the voltammetric behavior of fabricated polygalacturonic acidmodified carbon paste electrode prior to and after accumulation with the analyte, lead (II). Electroinactivity of the prepared electrode and its ability to preconcentrate the analyte was experimentally established through the electroanalytical techniques cyclic and Osteryoung using prolonged accumulation time and by applying a more negative deposition potential, the current signal is enhanced, thus improving the sensitivity and detectivity of the analyte. Using a 2-hour preconcentration period, ultra-trace levels of down to 2 ppb showed excellent linear relationship with peak current. Regeneration of the electrode surface utilizing EDTA was demonstrated, and results showed that the electrode may be cleaned and reused again. Although the results of the study provided initial evidence on the possibility of using stripping voltammetric method using polygalacturonic acid-modified CPE for lead (II) analysis, several other variables must still be studied and optimized before such a method is definitively established. Among these are the various voltammetric waveforms, the nature of the supporting electrolyte, the presence of interfering ions, reproducibility and accuracy of analytical data as well as applications to real samples. In addition, a further study on the full implications of regeneration of the electrode is recommended.

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# ANTIBACTERIAL PEPTIDES FROM *EURYCOMA LONGIFOLIA* (TONGKAT ALI) AND *LABISIA PUMILA* (KACIP FATIMAH) LEAVES IN MALAYSIA

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Abstract: Extracts from Eurycoma longifolia and Labisia pumila leaves were evaluated and analyzed for their antibacterial activity against human pathogenic gram positive (Staphylococcus aureus) and gram negative (Pseudomonas aeruginosa) bacteria by agar well-diffusion method, and expressed as the average diameter of bacterial inhibition zones surrounding wells. The extracts were prepared in different solvents (acetone, methanol, ethanol, and phosphate buffer) and in various concentrations ranging from 5 mg/ml to 100 mg/ml. Most of the extracts showed relatively high antibacterial activity against the tested bacteria with diameter of inhibition zones ranging between 7 and 25 mm. From the tests conducted, the minimum concentration of E. longifolia and L. pumila extracts which inhibited the growth of S. aureus and P. aeruginosa was 75 mg/ml in ethanol and 25 mg/ml in phosphate buffer, respectively. The extracts were subjected to size exclusion chromatography for partial purification. The purified fractions slightly showed antibacterial activity against S. aureus and P. aeruginosa. Peptides purified from E. longifolia and L. pumila leaf extracts were analyzed by 15% SDS-PAGE and silver stained to give an estimated molecular weight of 7.5 kDa and 6.0 kDa, respectively, comparable to the molecular weights range of most of the available antibacterial peptides purified and characterized from plants.

*Keywords:* antibacterial, plant extract, medicinal plants, *Eurycoma longifolia* (tongkat ali) and *Labisia pumila* (kacip fatimah).

# 1. Introduction

The medicinal properties of plants have been known for many years and have become a valuable source of natural products especially for therapeutic treatment. For example, herbal medicines have been the basis of treatment and cure for various diseases in traditional methods (Subrahmanyam et al., 2001; Samy et al., 1998). Plants usually hold some active compounds synthesized in secondary metabolism, and these secondary metabolites possess medicinal properties such as antimalarial, anti-fertility agent, analgesic, antihypertensive and so forth. Thus, the properties, safety and efficiency of plant secondary metabolites need to be carefully studied to take the medicinal benefits inherent in these compounds. In addition, plants become more preferable as a source of natural products since they are not affected by environmental factors and more consistent in product yield and quality.

than doubled from the 115 articles published between 1966 and 1994. Most of the researches focused on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds, i.e., alkaloids and flavonoids, or focused on the study of natural flora of a specific region or country. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs (Nascimento et al., 2000).

There are a number of new antibiotics against various pathogens which have been produced since several decades ago. However, resistance to these drugs by the pathogens has also increased. In fact, some of them are multi-resistant bacteria, which mean that they have the genetic ability to transmit and acquire resistance to a range of drugs. This growing of drug-resistance microorganism would lead to new infections and diseases (Nascimento et al., 2000). Therefore, extensive research has been conducted to better understand the genetic mechanism of resistance, as well as to develop new synthetic or search for natural drugs against those microorganisms.

Plants generally do not have an immune system such as that found in higher vertebrates to protect themselves against pathogens. According to Bruno et al. (1994), plants' resistance is mainly based on a dynamic defense system, which composed of antimicrobial agents of different molecular weight classes. The first group covers low molecular weight compound such as phytoalexins, a secondary products of plant metabolism. It works by rapidly accumulate at the site of infection to act as antibiotics against invading pathogens. The second group of plants' defense mechanism is formed by higher molecular weight compound. These include structural proteins such as hydroxyproline-rich glycoproteins which is able to incorporate into the cell wall to participate in confinement of pathogens. However, it was reported that the best-known group of stress- or infection-induced plant proteins is formed by diverse classes of pathogenesis-related (PR) proteins. This group forms the third class of plant antibiotic compound. Well-studied examples of the PR proteins are chitinases and  $\beta$ -1, 3-glucanases which are believed to kill pathogens by hydrolyzing their cell walls (Bruno et al. 1994). The frequency of Staphylococcus aureus infections has risen dramatically in the past few decades. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Staphylococcus aureus* strains which are resistant to some or all of the standard antibiotics. This phenomenon has created a demand for new anti-microbial agents, vaccines, and diagnostic tests for this organism. Pseudomonas aeruginosa rarely causes disease in healthy individuals. It is a serious pathogen for patients with immunocompromised or chronically debilitated patients. Patients with cystic fibrosis, haematological malignancies or neutropenia are at risk of severe bacteraemic pneumonia. Most strains of *Pseudomonas Aeruginosa* are resistant to relatively high levels of most antibiotics in use. Acute life-threatening infections are treated by a combination of tobramycin and an anti-pseudomonal b-lactam. It is very demanded to discover alternative sources for those different groups of bacteria to overcome the antibiotics resistant.

*Eurycoma longifolia* and *Labisia pumila* are among the most well known traditional medicinal plants which are widely found, whether naturally or cultivated in South East Asia, particularly Malaysia, Indonesia, Thailand, Laos, Cambodia and Vietnam (Farouk and Benafri, 2007). They belong to the family of *Simaroubaceae* and *Myrsinaceae*, respectively. In Malaysia, *Eurycoma longifolia* is known as *Tongkat Ali*, while *Labisia pumila* as *Kacip Fatimah*, and both plants were traditionally used for various medicinal practices for a long time. For example, the puts of *Eurycoma longifolia* are locally used for treating malaria and

from *Eurycoma longifolia* have been known to possess antiamoebic, cytotoxic, antitumoral and plasmodial activities (Jiwajinda et al. 2002).

On the other hand, traditionally, the roots of *Labisia pumila* were utilized by the female population to increase their libido. Numerous other uses have also been known. Jamia et al. (2004) mentioned that the plant extracts helps women achieve a better abdominal muscle tone. It is also used as a post-partum medication to help women contract the birth channel and regain strength. In addition to that, the herb can also be utilized to treat edema, which a condition where there is a swelling of an organ or a tissue due to accumulation of excess lymph fluid without increase in the number of cells in the affected tissue. In the study, Jamia et al. (2004) reported that the herb's extract tested positive against some bacteria namely – *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. Both *Eurycoma longifolia* and *Labisia pumila* were known to be promising natural sources of biologically active compounds (Jamia et al., 2004). Hence, the present study aims to recover these compounds, which were expected to show antibacterial properties.

## 2. Materials and Methods

### 2.1 Plant materials and extract preparation

Fresh leaves of *Eurycoma longifolia* were taken from the trees which have been planted nearby the Faculty of Engineering, International Islamic University Malaysia, while *Labisia pumila* plants were supplied by Dynamic Venue Sdn Bhd in Kuala Lumpur. They were airdried under shade at room temperature before being used, and washed thoroughly with 70% ethanol and distilled water. The leaves were powdered by using an electric grinding machine. From each plant, 5 mg to 100 mg powder materials were loaded and dissolved in 1 ml ethanol, methanol, acetone, and phosphate buffer pH 7, to obtain extract concentrations ranging from 5 mg/ml to 100 mg/ml. Each concentration of extract was prepared in triplicates. These extracts were then tested against the selected strains of bacteria. The aim was to determine the minimum concentration of extract that would inhibit the bacterial growth.

### 2.2 Bacterial cultures

Bacterial strains used for testing the antibacterial activity of extracts were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These were obtained from the Institute of Medical Research, Kuala Lumpur. The stock culture was maintained on Luria Bertani (LB) agar medium at 37°C, and subcultures were only freshly prepared before use.

### 2.3 Antibacterial assay

Antibacterial activity for the four different solvents and various concentrations of plant extracts were assayed by using agar-well diffusion method (Tepe at al., 2004; Abolhassani, 2004). Top agar (35-40°C) containing fresh overnight bacteria culture was poured and evenly distributed on Petri plates containing LB agar medium. After 15 minutes, wells (5 mm diameter) were made on each plate by using sterile pipette tips. Then, 50  $\mu$ l of each solvent extract was loaded into each of the well. As a negative control, 50  $\mu$ l of the solvents (acetone, methanol, ethanol and phosphate buffer) alone was used. All the plates were then covered with lids and incubated overnight at 37°C. The presence of inhibition zones on LB plates containing bacterial culture around the wells was observed. Then, the diameters of each inhibition zone ware measured. Antibacterial activities of each solvent extract ware avpressed

## 2.4 Extraction in 1 liter solvent

After identifying the minimum concentration of *Eurycoma longifolia* and *Labisia pumila* extracts that were capable of inhibiting the growth of *S. aureus* and *P. aeruginosa*, the extraction process was scaled up in 1 liter volume of solvent. The solvent was then dried by evaporating the extracts by using rotary evaporator to obtain the crude extract. The extract was then reconstituted in 0.1 M phosphate buffer pH 7.

### 2.5 Purification

Extracts of both *Eurycoma longifolia* and *Labisia pumila* that gave optimum activity of antibacterial were fractionated and purified by using size exclusion chromatography. For purification of both *E. longifolia* and *L. pumila* extracts, a chromatography column of 70 cm x 5 cm, packed with Sephadex<sup>TM</sup>G-50 Fine was used. Fraction volumes of 7 ml and 10 ml were collected.

## 2.6 Antibacterial assay and SDS-PAGE analysis

Optical density at 280nm of each chromatographic fraction was then measured to plot the graph of absorbance versus elution volume. The fractions were also concentrated (10x) and tested against the selected bacteria by using agar-well diffusion method. Purified fractions that showed positive antibacterial activity against the bacteria were then analyzed by SDS-PAGE. This analysis was considered as the final step in this study that functioned to show the purity of the fractions obtained after chromatography process, as well as to estimate the molecular weight of the peptides, compared to the available protein/peptides. Protein SDS-PAGE Molecular Weight Standards (broad range, from 200 kDa to 6.5 kDa) and Polypeptides Standards (from 26 kDa to 1.4 kDa) were used as markers.

## 3. Results and Discussion

## 3.1 Antibacterial evaluation

Tables 1, 2, 3 and 4 show the effect of different solvents and extracts concentration of both *E. longifolia* and *L. pumila* against *S. aureus* and *P. aeruginosa*. They were expressed in terms of diameter of inhibition zones surrounding the wells. Among the four solvents with the concentrations ranging from 5 mg/ml to 100 mg/ml, the minimum concentration of *E. longifolia* extract that inhibited the growth of *S. aureus* and *P. aeruginosa* was found to be 75 mg/ml in ethanol (Table 1 and 2), meanwhile for *L. pumila* extract, 25 mg/ml extract in phosphate buffer was found to be the minimum concentration to inhibit the growth of *P. aureginosa* (Table 4). However, only *L. pumila* extract in acetone, ethanol and phosphate buffer showed antibacterial activity against *S. aureus*, while no activity was observed for the extract in methanol (Table 3).

**Table 1:** Antibacterial activity caused by *Eurycoma longifolia* extracts on *S. aureus* as evaluated based on the diameter of inhibition zones in agar well diffusion test.

Extract	Diameter of Inhibition Zone (mm)				
concentration (mg/ml)	Acetone	Methanol	Ethanol	Phosphate buffer pH 7	Negative Control
75	11	7	10	20	

**Table 2:** Antibacterial activity of *Eurycoma longifolia* extracts on *P. aeruginosa* as evaluated based on the diameter of inhibition zones in agar well diffusion test.

Extract	Diameter of Inhibition Zone (mm)				
concentration	Acatona	Mathanal	Ethanol	Phosphate Buffer	Negative
(mg/ml)	Acetone	Methanoi	Ethanoi	pH 7	Control
75	17	18	19	16	-
100	15	16	17	15	-
125	15	15	14	17	-

**Table 3:** Antibacterial activity of *Labisia pumila* extracts on *S. aureus* as evaluated based on the diameter of inhibition zone in agar well diffusion test.

Extract	Diameter of Inhibition Zone (mm)				
concentration (mg/ml)	Acetone	Methanol	Ethanol	Phosphate Buffer pH 7	Negative Control
25	0	0	9.7	10	-
50	10	0	9	0	-
75	11.3	0	11.3	10	-
100	11.3	0	11.3	0	-

**Table 4:** Antibacterial activity of *Labisia pumila* extracts on *Pseudomonas aeruginosa* as evaluated based on the diameter of inhibition zone in agar well diffusion test.

Extract		Diameter	r of Inhibitic	on Zone (mm)	
concentration (mg/ml)	Acetone	Methanol	Ethanol	Phosphate Buffer pH 7	Negative Control
25	12.7	14.7	10.7	18.7	-
50	14	16.3	11.7	19.7	-
75	15.3	16.3	11.7	22	-
100	16.3	16	13.3	23	-

3.2 Purification: size exclusion chromatography

Extracts of *Eurycoma longifolia* (Fig 1a) and *Labisia pumila* (Fig 1b) were fractionated using size exclusion chromatography. Two significant peaks were observed for *E. longifolia* extract and 18 active fractions against the selected bacteria were identified between 1092 ml (fraction 156) and 1330 ml (fraction 190) of elution volume ( $V_o$ : Void volume:  $V_r$ : Elution volume within the separation range of Sephadex<sup>TM</sup> G-50 Fine;  $V_t$ : Total volume). Sephadex<sup>TM</sup> G-50 Fine resin is capable of separating proteins/peptides within the size range of 1 to 30 kDa (Voet and Voet, 2004). The second peak of the graphs represented the proteins/ peptides in the size range of 1 to 30 kDa. For *Labisia pumila*, activity was observed between 910 ml (fraction 61) and 1330 (fraction 103). The clustered peaks in Figure 1b indicate that the extraction in phosphate buffer had yielded peptides of similar size.

After chromatography process of the *E. longifolia* and *L. pumila* extracts, it was found that slightly low antibacterial activities of the fractions were observed compared to the activities before purification. A hypothesis could be made that the antibacterial properties of

on identification of the active compounds by means of bio-guided assay must be continuously done. This is necessary to uncover as much as potentially data as possible, including toxicity against animals and human cells, mechanisms of action, effects in vivo, positive and negative interactions with common antibiotics and so forth, as mentioned by Rios and Recio, (2005).

**Figure 1a:** Sephadex<sup>TM</sup> G-50 Fine elution profile of antibacterial peptides sample extracted from *Eurycoma* longifolia. Protein/polypeptides sample were eluted between 470 ml (fraction 67) to 1750 ml (fraction 250); sample volume: 10 ml, buffer: 0.1 M phosphate buffer (pH 7).



**Figure 1b:** Sephadex<sup>TM</sup> G-50 Fine elution profile of antibacterial peptides sample extracted from *Labisia pumila*. Protein/polypeptides sample were eluted between 600ml (Fraction 30) and 1800ml (Fraction 150); sample volume: 10 ml, buffer: 0.1 M phosphate buffer (pH 7).



# 3.3 SDS-PAGE analysis

After being concentrated by using centrifugal concentrator and tested against *S. aureus*, 18 chromatographic fractions of *E. longifolia* have shown positive antibacterial activities but much lower compared to extract activities before purification. These active fractions were found between fraction 156 and 190, located in the second peak of the graph as shown in Figure 1a. On the other hand, the active fractions of *L. pumila* were found between fraction 61 and 103, located at the middle of the peak, as shown in Figure 1b. Those active fractions eluted from the chromatography column were further analyzed by using 15% SDS-PAGE and stained with silver nitrate. From the analysis, *E. longifolia* fractions between 158 and 164, and *L. pumila* fractions between 85 to 97 showed the results, where bands slightly have appeared on the gel after staining with silver nitrate. To estimate the molecular weight of the target peptides, a standard curve of log molecular weights versus distance traveled from the well by each band of the marker was plotted. As a result, antibacterial peptides having estimated size of 7.5 kDa and 6.0 kDa were in *E. longifolia* (Fig 2a) and *L. pumila* (Fig 2b), respectively.

**Figure 2a:** 15% SDS-PAGE loaded with  $25\mu$ l of antibacterial peptides extracted from *Eurycoma longifolia* for the chromatographic fractions of 158 to 174, stained with silver nitrate. (M = protein marker).



# 4. Conclusion

The results obtained showed that the extracts of *Eurycoma longifolia* and *Labisia pumila* leaves were capable of inhibiting the growth of *S. aureus* and *P. aeruginosa*. This seems to confirm the traditional therapeutic claims of these herbs. It would most probably present an alternative source of new antibacterial compounds to treat infectious diseases caused by resistant microbes in the future.

**Figure 2b:** 15% SDS-PAGE loaded with  $20\mu$ l of antibacterial peptides extracted from *Labisia pumila* for the chromatographic fractions of 85 to 97, stained with silver nitrate. (M = protein marker).



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# A RAPID EXTRACTION METHOD FOR DNA FROM MALAYSIAN FOOD SUITABLE TO DETECT EVEN TRACES OF GENETICALLY MODIFIED SOYA

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**Abstract:** A quick and cheap method for DNA extraction prior to PCR to detect even traces of genetically modified (GM) material in food has been established. PCR is used because it is a rapid and sensitive detection assay. Effective PCR-based detection relies on the extraction of high quality DNA without PCR inhibitors. A simple and modified method for DNA isolation has been designed prior to the application of a conventional Polymerase Chain Reaction (PCR) - based assay to detect the soya lectin gene and the 35S promoter in either highly processed or non-processed foods. Using the principle of microwave irradiation of lysed food material, the DNA extract after centrifugation was directly used in standard PCR protocol after their dilution. The 118 bp lectin gene fragment could be detected in 35 food samples of which two were positive for GMO through detection of 195 bp fragment of the 35S promoter. The detection technique for GMO is highly demanded in compliance to GMO regulation. The developed low-cost microwave-based DNA extraction method was shown to be able to replace the use of expensive commercial kits for DNA extraction. *Keywords:* GMO, Microwave, DNA extraction, PCR.

# Introduction

It is known that certain traits of genetically modified (GM) plants have contributed positively towards increased yields in the agricultural sector, however, the use of GM for public consumption has been very controversially discussed from the day it was initiated until now (Hart, 2004). There have been huge concerns among consumers as well as farmers related to the marketing and the development of GM crop derived foods. Farmers become more dependent on GM herbicides resistant plants especially soya bean (Robert F. Service, 2004). The increasing concerns about GM foods have resulted in the increased awareness of food labeling and its monitoring in the market. These modified foods have not gained worldwide acceptance due to the factor of environmental and public health and safety issues (Malarkey, 2003; Maynard, 2004; Mohamed and Abdur Rahim, 2004; Pence and Gregory, 2004) as well as intellectual property rights (Farid 2002).

order to meet\_the mandatory labeling of GM food. Therefore, GM screening is performed to determine the presence of GM soybean in current food products, and also to allow the separation of GM and non-modified food products.

Identification of RR soy by PCR was performed using primers specific for the 35S promoter (Screening approach) (Pietsch et al., 1997) and species-specific gene (soya lectin) (Jankiewicz et al., 1999). Originally, the 35S promoter was introduced in almost all genetically modified plants and was therefore used to get information about a possible contamination with material derived from genetically modified plants, whereas the lectin gene was used as a taxon-specific reference gene to check the suitability of the extracted DNA for PCR. The methods involved are expected to be a benefit to GM food testing and labeling efforts to be regulated and implemented in Malaysia.

## **Methods and Materials**

Two main food materials were used: non-processed raw soya beans and processed food containing traces of soya. From each food sample, 200 mg of homogenized samples were carefully portioned into individual 1.5 ml Eppendorf microcentrifuge tubes. Lysis buffer (1 M Tris-HCl, 0.5 M EDTA, SDS 10%) was preheated in the microwave oven for 2 minutes before adding 1 ml each into the individual tubes containing the sample material. The mixture was vortexed gently for 15 minutes to obtain a suspension and then incubated at 70 °C for 5 minutes.

After incubation the samples were then mixed gently and irradiated in full power for 30 to 40 seconds in a domestic microwave oven (Buffler, 1993). The tubes were left open to prevent any pressure build-up, which would cause the tubes to explode and to contaminate the samples. The samples were also placed in microwave oven-proof tube racks and distributed evenly, but far apart for equal optimal heating. Immediately after irradiation, the tubes were centrifuged at full speed for 5 minutes using an Eppendorf MiniSpin centrifuge and the supernatant transferred to new clean tubes before centrifuging for 3 minutes. The centrifugation step would be carried until a non-turbid solution is obtained which would usually be after a third centrifugation step. The cleared supernatant from each preparation was transferred into fresh 1.5 ml Eppendorf microcentrifuge tubes and stored at 4 °C until PCR was to be conducted.

Previously published PCR primers of the lectin gene to amplify a 118bp fragment was used due to its high specificity and its success in previous work, where lectin's PCR products were detected in food samples using a commercial kit for DNA extraction.

The forward and reverse primer sequences for soya-lectin detection were as follows:

5'-GCCCTCTACTCCACCCCATCC-3' and 5'-GCCCATCTGCAAGCCTTTTTGTG-3' respectively. For the detection of GM content in food samples, the primers for the detection of CaMV 35S promoter were used. In general, all the approved genetically engineered agricultural crops have been transformed with constructs containing this element which is from the cauliflower mosaic virus. The forward and reverse primer sequences for 35S promoter detection used were as the following: 5'-GCTCCTACAAATGCCATCA-3' (sense) and 5'-GATAGTGGGATTGTGCGTCA-3' (antisense) yielding a product of 195 bp (Thion et al., 2002). The primers were purchased from MWG Biotech GmbH, (Ebersberg, Germany). PCR amplifications were carried out using *Taq* DNA polymerase with Thermopol buffer (New England Biolabs, U.S.A) from a final reaction volume of 25  $\mu$ L Each reaction contained by Termonol buffer (10 mM KCL 10 mM (NH) SO -20 mM Tris HCL 2 mM

DNA extract. PCR reactions were performed with a Mastercycler Gradient Thermocycler (Eppendorf, Germany). After an initial denaturation step at 95 °C for 2 min, the cycling parameters were as the following: 34 cycles at 95 °C for 1 minute, 55 °C for 1 minute and 72 °C for 1 minute with a final extension at 72 °C for 10 minutes.

PCR products were analyzed on a 1.8 % high grade agarose gel electrophoresis containing ethidium bromide and visualized and printed using an Alpha imager gel analysis system (Alpha Innotech Corporation, USA). A molecular weight marker (100 bp DNA Ladder; New England Biolabs, USA) was used to identify the PCR products' sizes.

## Results

Amplification of lectin material was accomplished with primers that targeted the highly species-specific lectin gene fragment of 118 bp (Fig. 1-4). As expected, the absence of amplified product from non-soya containing samples gives evidence of the specificity of the lectin PCR test and that no cross and/or carryover contamination occurred during the procedure.

The success of the amplification of the lectin gene fragment shows that GM content in food can be detected as the lectin gene is the marker for the genome of soya (refer to Fig. 1-4). This is proven by the fact that two samples were tested positive for GMO from the amplification of the 195 bp CaMV 35S promoter as indicated in figures 5 and 6. The DNA samples were stored at -20 °C for future use. The amplification of the selected lectin gene fragment from a variety of food samples which contain PCR inhibitors shows the sensitivity and reliability of the method. The suitability of this approach for analysis of plant DNA (soya lectin) specific detection by PCR proves possible. For future considerations this method of DNA extraction would be further optimized by using 96-well microtitre plates from the initial stage of DNA extraction by microwave irradiation to the final stage of PCR to enable a large number of different samples for the detection of different genes and markers to be analyzed at the same time.

**Figure 1:** PCR products amplified from soya-containing processed foods endogene regions with primers Lec F/R. Most of the food samples above, which are highly processed foods, are detected positive for lectin gene at the fragment size of 118bp, dominant gene in soya. Lane 1: smooth tofu; Lane 2: rice cracker B; Lane 3: powdered dairy milk; Lane 4: tempura flour (band not visible, signifies the non-presence of soya in food sample); Lane 5: anchovies cooking base; Lane 6: coffee cream; Lane 7: orange cream sandwich.



**Figure 2:** PCR products amplified from soya-containing processed foods endogene regions with primers Lec F/R. Most of the food samples above, which are highly processed foods, are detected positive for lectin gene at the fragment size of 118 bp, dominant gene in soya. Lane 1: traditional tofu; Lane 2: cereal; Lane 3: noodle seasoning; Lane 4: soya beverage; Lane 5: noodle soup seasoning; Lane 6: deep fry flour; Lane 7: grated wasabi.



**Figure 3:** PCR products amplified from soya-containing processed foods endogene regions with primers Lec F/R. Most of the food samples above, which are highly processed foods, are detected positive for lectin gene at the fragment size of 118 bp, dominant gene in soya. Lane 1: butter biscuit; Lane 2: vegetable salted fish (light band); Lane 3: dried soyabean (light band); Lane 4: gushu tofu; Lane 5: Australian raw soya bean; Lane 6: tofu puff; Lane 7: chicken stock cube.



**Figure 4:** PCR products amplified from soya-containing processed foods endogene regions with primers Lec F/R. Most of the food samples above, which are highly processed foods, are detected positive for lectin gene at the fragment size of 118 bp, dominant gene in soya. Lane 1: Canadian raw soyabean; Lane 2: wheat biscuit; Lane 3: vegetable cracker; Lane 4: tofu soyabean curd; Lane 5: chocolate (no or undetected soya or lectin gene); Lane 6: rice cracker C; Lane 7: noodle soup base.



**Figure 5:** PCR products of 35S sequence from a butter biscuit containing soya. Lane 1: 118 bp of lectin amplification; Lane 2: 195 bp amplification of CaMV 35S promoter (1:10 dilution of DNA); Lane 3: 195 bp amplification of CaMV 35S promoter (1:5 dilution of DNA); Lane 4: 195 bp amplification of CaMV 35S promoter (no dilution of DNA), Lane 6: 100 bp DNA ladder.



**Figure 6:** PCR products of 35S sequences from a baby food containing soya. Lane 1: 118 bp of Lectin amplification, Lane 2: 195 bp amplification of CaMV 35S promoter (1:10 dilution of DNA); Lane 3: 195bp amplification of CaMV 35S promoter (1:5 dilution of DNA); Lane 4: 195 bp amplification of CaMV 35S promoter (no dilution of DNA); Lane 6: 100bp DNA ladder.



### Discussion

Processed food products contain high levels of proteins and fats which can act as PCR inhibitors; therefore different commercial protocols (Qiagen, Biokits and Promega) have been tested to prepare good DNA extracts with minimal inhibitors for efficient PCR. From previous work, we found that the use of the commercial Qiagen Tissue Kit (Germany) was an easy, rapid and a reliable method to obtain sample extracts suitable to be used for the study of DNA by PCR assays (Greiner, 2004; Greiner and Konietzny, 1997; Miraglia, 2004). The microwave irradiation procedure used for sample production, did not compromise the quality of the DNA recovered by this method, as shown by the result from PCR analysis.

In general, the microwave-based DNA extraction method is simple, has a lower contamination risk, and results in a yield of DNA comparable with other commercial methods of DNA extraction for plant material. The basis of microwave dielectric heating uses the ability of some liquids and solids to transform electromagnetic radiation into heat to drive chemical reactions (Anklam et al., 2002). In the electromagnetic spectrum, the microwave radiation region is located between and infrared radiation and radiowave. Mostly all domestic microwaves use a frequency of 2.45 GHz for operation (Bernard et al., 2002). In one study, bacteria obtained from growth culture was treated with microwave irradiation and then directly subjected to a multiplex PCR technique to accurately and rapidly identify the presence of *mecA* and *femB* genes which characterize MRSA (Menon and Nagendra, 2001). Together with rapid microwave-stimulated processing, better DNA recovery in higher amount is achieved if compared to DNA extracted from formalin-fixed tissue, particularly

protocols. This method provided template DNA suitable for PCR assay, without any purification steps. The costs were much lower and time consumed was lower compared to conventional methods or using commercial DNA extraction kits. The alternative procedures were much faster, but were equally sensitive for this particular screening. Moreover, in this particular application the procedures are easily adaptable to the routine processing of high number of clinical samples.

As microwave irradiation of DNA extraction is temperature based, heating tissues at a higher temperature and at pH 6-9 gave higher yields of DNA (Angelini et al., 2003). However compared to normal boiling methodologies for DNA extraction, the boiling points of solvents can be raised up to 26 °C above their conventional values (Shi. et al., 2002). In this case, the lysed sample emulsion can be raised above its boiling point for faster DNA extraction. Another observation was that the actual geometry as well the volume of the sample are important for the production of uniform heat, thus, the need for placing the tubes equally apart and of equal volume (Gabriel, 1998; Galema, 1997).

Combining the specificity and sensitivity of the PCR together with the DNA extraction by microwave irradiation, allows rapid detection of lectin gene fragment from processed food samples as a molecular detection (Wang and Fang, 2005) method for lectin contents in food products from which the detection of genetically modified material was done.

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#### A HISTORY OF NEGATIVE NUMBERS

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#### Introduction

It may be surprising to learn that negative numbers troubled mathematicians till 1600 AD, with few exceptions. The reason is that mathematicians totally ignored negative numbers, as they did not think they would have any utility in the future. This paper gives a survey of the "struggle" of the negative numbers for their existence and universal acceptance.

#### **Ancient Period**

In India, negative numbers were introduced in practice only to represent "debts". The 6<sup>th</sup> century mathematician Brahmagupta stated the rules of the four fundamental operations in practice only. He used abbreviations for each of the several unknowns, squares, square roots, but used a dot to represent a negative number as .3 for -3.

Bhaskaracharya (1114-1185), obtained the solutions of linear equations in terms of negative numbers, but said that such a solution was "inadequate". He said that negative roots exist, but without giving any evidence, and hence people did not accept negative numbers until much later.

In the 12th century, the Islamic mathematician 'Ibna Alsama used negative numbers in mathematical operations in his text *Al Sabir Baisaba*. Neither al-Khwarizmi (9th century) nor other Arabic scholars countenanced negative numbers. al-Khwarizmi solved the equation  $x^2 - 10x + 21 = 0$ , but could not solve  $x^2 - 4x - 21 = 0$ , because one root is -3.

Omar Khayyam (1045-1123) tried to solve cubic equations by using conics. He could not find all positive roots, and rejected all negative roots because they were "unwanted".

Although the Arabs rejected negative numbers, and absolute negative magnitudes, they were familiar with the rules governing signed numbers. There seems to be a remote possibility that negative numbers were represented in texts by the Babylonians, although they had little conception of negative numbers.

Earlier, the Chinese used red rods for positive numbers and black rods for negative numbers, for computation. The 3<sup>rd</sup> century mathematician Lui Hui used red dots for negative numbers in his manuscript *Jiuzbang Suanshu* ("Nine Chapters on Mathematical Art"), but the Chinese did not accept the notion of negative numbers for the solutions of an equation.

The Greek mathematician Diophantus declared the equation x + 7 = 0 as unsolvable. He had no notion of negative numbers. All he knew was that "2x - 8 stands for 2x > 8".

The Europeans eventually acknowledged negative numbers, which appeared in Islamic texts, but they continued for centuries to think that negative numbers had no use or no Leonardo of Pisa (1180-1250), also known as Fibonacci, wrote *Liber Abaci* ("The Book of the Abacus"). There, he solved quadratic and cubic equations. But he did not take any cognizance of negatives and imaginary roots because they were unintelligible.

The 15-16<sup>th</sup> century French mathematicians Nicholas Chuquet (c. 1500) and Michael Stifel (1487-1567) regarded the negative numbers as "absurd". In his book *Triparty en la Science des Nombres*, Stifel used x + 5 = 0 to represent -5 !

The Germans used symbols for the numbers; the Italians used p for positive numbers and m for the negatives. Stifel was familiar with negative coefficients, but called negative numbers as "*numeri absurdi*", and irrationals as "hidden under infinitude".

An Englishman, G. Peacock (1791-1858), represented the negative numbers by the number-couple as: [m, m] for zero, [m, m + n] for -n, [m + n, m] for n, etc. Augustus Mobius (1790-1868) used AB for the positive sign and BA for the negative sign to show directions.

In the 16<sup>th</sup> century, G. Cardano (1501-1576) was willing to accept the negative numbers as roots of equations, but could not persuade the academicians to accept them because of mounting pressure from religious leaders who claimed that the concept of negativeness was "anti religion". In his work *Ars Magna* he stated that "minus times minus is plus" as an independent preposition. He recognized the negative numbers as existing but, on the evidence, thought they were "doubtful and fictitious".

Francois Viete (1540-1603) discarded the negative numbers, saying that such an idea was purely imaginary. Viete was considered to be an important mathematician in his time. His significant contributions were in algebra. In fact, he introduced and popularized some of the useful notations we use today such as + for positive, – for negative, a dot (.) for the decimal point,  $A_n$  as the nth element in a sequence, and a / b for a fraction.

It is ironic that Viete would not let literal coefficients, such as  $A_n$ , represent negative numbers. The rules for operating with negative numbers had been in existence for a hundred years, but Viete rejected these numbers because he thought that negative numbers did not possess the intuitive and physical meaning which the positive numbers had.

In 1657, the Dutch mathematician Johann Hudde (1629-1704) was the first to start using literal coefficients in an equation to represent negative numbers. The final step in the process of generalizing the notation of Viete in the theory of equations was published in Descartes` *Geometry*.

#### Development.

Rene Descartes (1596-1650) accepted the concept of negative numbers, but he called them "false" because negative numbers represent numbers "less than nothing". Whien stating the rule of signs for the zeroes of a polynomial, he used the word "true" for the positive roots and "false" for the negative roots. He said that "negative numbers offer more difficulties because they are not readily approximated, as positive numbers".

In the 17<sup>th</sup> century, negative numbers started to come into use more and more, but it took some time for them to establish themselves. A large section of mathematicians continued to protest against negative numbers for being "unfit in the row of gentlemen", that is, positive numbers. They continued to be wary of negative numbers.

The theologian and mathematician Antoine Arnuld (17<sup>th</sup> century), an associate of Pascal (1623-1662), presented an intriguing and surprising argument against negative numbers. He did not accept the proportion -1:1 = 1: -1. He asked, "How can 'smaller to a greater' be equal to 'greater to a smaller'?"

mathematical texts of the  $18^{th}$  century were confused about using the negative numbers, rejecting the multiplication of negative numbers, and the sign – for minus and subtraction. Their argument was: "how is adding –6 the same as subtracting the number 6?" They expected to use a different sign for the subtraction.

Finally, Euler (1707-1783) successfully established the process of subtraction by -x and addition by +x. He was the first to use the - sign for negative numbers, which later on was universally accepted. He said "-x is debt, and x is recovery" so that the treasury of numbers remains unchanged.

Today, the minus or - symbol plays three roles: as subtraction, the opposite, and the additive inverse. Many other notations are used for negative numbers, such as -a or a <sup>-1</sup> or a < 0, all parallel to the concept of -a. The additive inverse of a is -a. It is commonly used in algebra or arithmetic. It should be noted that -a has no meaning without mentioning zero.

Also interesting was the major opposition to the statement:

 $(-1) \cdot (-1) = 1.$ Euler proved it as follows:  $(-1) \cdot (-1) = (-1) \cdot (-1) + 0.1$  $= (-1) \cdot (-1) + [(-1) + 1].1$  $= (-1) \cdot (-1) + (-1) \cdot 1 + 1.1$  $= (-1) \cdot [(-1) + 1] + 1$  $= (-1) \cdot 0 + 1$ = 0 + 1= 1

but this proof was ridiculed by the academicians with the comments that it was Euler's "jugglery". After more protests from the critics, Euler explained the above proof by saying that "the enemy's enemy is our friend"; and the proof was accepted !

From this time on, zero and negative numbers have joined the family of positive numbers so as to make our number system consistent and logical. This happened long after the early Mayans and Hindu mathematicians began working with the concept of zero.

Euclid (300 BC) gave an elegant proof showing that the number of primes is infinite, but did not mention negative primes. It was Eduard Lucas in 1876 who made a significant contribution to the theory of primes, and defined the primes explicitly to be positive and rejected negative primes because they were "rough" and might "spoil" the number family.

An Englishman, George Boole (1815-1864), concentrated on mathematical logic in the construction of algorithmic systems. In his work *Investigation of the laws of thought* (1854), Boole established the foundations of symbolic logic, which gave birth to "Boolean Algebra". In this algebra, the negation of a statement p was denoted by -p, which was later changed to  $\sim p$ , because -p represents the opposite of p. He used -x to generate a new concept "the law of the excluded middle", which was expressed by x + (1 - x) = 1. This law was later on stated as x + x' = U, where U is the universal set.

#### **Imaginary Numbers**

The real test for mathematicians was in solving the equation  $x^2 + 1 = 0$ , or  $x^2 = -1$ . Now -1 was defined as opposite to 1 and also as the additive inverse of 1. Where -1 was difficult to digest, it was even harder to accept  $\sqrt{-1}$ . Euler introduced the symbol "i" for  $\sqrt{-1}$ . But he could not find a place for  $\sqrt{-1}$  on the real number line. So he termed  $\sqrt{-1}$  "imaginary", and

easily found. Then mathematicians were forced to include  $\sqrt{-1}$  in the family of numbers in order to complement the obvious real solutions of a quadratic equation.

Cauchy (1789-1857) rejected "i" for  $\sqrt{-1}$ , saying that "we refuse to acknowledge this symbol completely and we abandon it without any regret because one does not know what this alleged sign signifies, nor what meaning should we attribute to it". Later he said, "such number is purely imaginary", which was literally to come true in the future.

Bhaskaracharya wrote: "There is no square root of a negative number because a negative square does not exist." The Italian mathematician Cardano described the square root of a negative number as "beyond imagination". He could not find the solutions to the equations x + y = 10,  $x \cdot y = 40$  because  $x = 5 + \sqrt{-15}$ ,  $y = 5 - \sqrt{-15}$  were "fictitious".

Interestingly,  $\sqrt{a}$ .  $\sqrt{b} = \sqrt{(ab)}$  is true in the real number system, but not in complex numbers, as  $\sqrt{-4}$ .  $\sqrt{-9}$  is not 6 but it is equal to 2i.3i = -6.

Jean Argand (1768-1822) gave a geometrical interpretation to a + ib, and this planar representation became the "Argand Plane"; now the mathematicians were motivated to find the nth roots of  $\sqrt{-1}$  and could plot them on the Argand plane.

It is undoubtedly true that number theory was the inspiration for the two important algebraic concepts: Rings and Fields. The first difficult step towards abstract algebra was the introduction of negative numbers.

Mathematicians, for many centuries from Diophantus to Descartes, lived halfway. Negative numbers were partially accepted, or sometimes admitted with hesitation, in intermediate calculations. But negative numbers were not allowed in answers or solutions.

The first level of abstraction was the creation of an inverse for addition, which appeared unconsciously and was generally accepted in the form –a for the additive inverse of a. At this point, zero was accepted as the additive identity. Thus Z, the ring of integers, was created. Similarly, Q, the field of rational numbers, came into existence. Then were constructed, in the 19<sup>th</sup> century, the axioms for Z, Q, and other structures.

We write numbers with base 10. It is thanks to earlier mathematicians that we can express any number in the base of any negative number as for example:

$$(11110)_{-2} = 1(-2)^{-4} + 1(-2)^{-3} + 1(-2)^{-2} + 1(-2)^{-1} + 0(-2)$$
  
= 16 - 8 + 4 - 2 + 0  
= 10

This is how the negative numbers gave rise to a new branch of mathematics, known as Algebraic Number Theory. The negative numbers appeared in their own right on a parity with positive numbers. Thus negative numbers proved their *existence* and importance beyond the earlier concept of mere "numbers to the left of the origin" on the number line.

#### **Conclusion**:

The history of negative numbers, described above, shows that mathematical concepts are man-made abstractions and were introduced out of necessity. These concepts were later developed depending on their usefulness.

Today, despite all historical, social and religious resistance, the negative numbers play such a major role that we cannot afford to lose them. They are used in every walk of life, even in temperature charts or in accountancy.

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#### MATERIALS FOR DISCHARGE LIGHT SOURCES

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**Abstract:** This paper reviews the materials used for low and high pressure discharge light sources. In particular, the development that led to the introduction of energy saving high pressure sodium, ceramic metal halide lamps and compact fluorescent (CFLs) lamps are discussed. A wide range of materials are needed in the lighting industries, including glasses and glass ceramics, ceramics, metallic materials, polymers, composites as well as various types of gases. The paper focuses on the development of ceramic envelope, ceramic-metal composites and sealing materials for high intensity discharge light sources; and on special phosphor materials for the conversion of the ultraviolet to the visible for compact fluorescent lamps.

#### 1. Introduction

In this paper, the materials development for various types of discharge light sources will be discussed. The high intensity discharge light sources such as in high pressure sodium and metal halide lamps operate at relatively high temperatures and pressures compared with fluorescent lamps. The materials requirements for high intensity light sources are more demanding and stringent.

The first discharge lamp was shown at the Royal Society of London way back in 1860. The lamp induced a brilliant white light by the discharge of high voltage through carbon dioxide at low pressure. Around the 1870s, Europe was well ahead in electrical arc light. Thomas Edison in the States went on to demonstrate his electric incandescent lamp made with carbon filament in 1879.

#### 2. Generation of Radiation

It is well known that an air gap kept at ambient between two spherical conductors gives a spark if the voltage between the two conductors is sufficiently high to induce the breakdown of the air. It is also known that when some UV light is in the vicinity of the two conductors, the voltages needed to generate the sparks are considerably reduces. Also well established is the lower voltage required to cause the gaseous discharge when the air pressure inside a glass tube is reduced. If there is some residual air, the gaseous discharge is pinkish. For different gases, the colour of the discharge will be different. Figure 1 summarises the phenomena of gas discharge in air at various pressures. Note that 1 bar is equivalent to 760 mm Hg or 10<sup>5</sup> Pa (Pascal); thus at 0.01 mm Hg the pressure in Pascal is 1.316 Pa approximately. In the lighting industry, the gas pressure inside the lamp envelope is often quoted in Pascal. Low pressure discharge light source, as in the case of low pressure sodium lamp, is of the order of 400 Pa to 2000 Pa

halide lamps, the pressure of the species is between 2500 Pa to 9000 Pa. However, during the operation of lamps, the pressure can attain several atmospheres in HPS lamps and up to tens of atmospheres in the case of metal halide (MH) lamps. The marked increase in pressure is due to the high vapour pressure generated by the very volatile halide species at elevated operating lamp temperatures. In these high intensity discharge light sources, the wall temperatures of the ceramic lamp envelope can reach 1300K and the centre of arc can attain 6000K. Thus the lamp materials have to withstand extremely hostile environments. The materials requirements become very stringent.

**Figure 1:** Gaseous discharge as the pressure is reduced. FDS – Faraday Dark Space; CDS – Crookes Dark Space. (Stirling, Textbook on Electicity & Magnetism).



**Figure 2:** Relationship between the current and the applied voltage. (Sears, Electricity and Magnetism, MIT Press).



#### 3. Fluorescent lamps

Although discharge light sources were known as early as 1860, practical fluorescent lamps were introduced commercially in the 1940's. Fluorescent lamp fill consists of small amount of mercury and argon. The colour of the light sources is characteristic of the gas in the tube. In the case of a fluorescent lamp containing mercury and argon, the generation of the UV radiation of 253.7 nm comes from the de-excitation of the mercury atoms as shown in Figure 3. Over 50% of the electrical power is converted into UV radiation. The luminous efficacy of the lamp without any phosphor is only about 6 lumens per watt. Obviously, this is very low compared with the 683 lumens per watt for monochromatic yellow-green light of 555 nm that can be generated theoretically. The corresponding efficiency for a white light with a wide distribution is around 250 lumens per watt.

**Figure 3:** (a) Simplified energy level showing the excitation and de-excitation of mercury atoms giving ultraviolet resonance radiation of 253.7nm. (Cayless and Marsden, 1983). (b) A more detailed energy level diagram for mercury showing the origin of the 253nm and 197 nm radiations. (American Institute of Physics Handbook, Third Edition, 1972).



Using special narrow band phosphor materials in the form of coatings inside the glass envelope, efficacy of the order of 90 lumens per watt and colour rendering index (CRI) over 80 have been achieved (Ranby, 1983). The recently developed compact fluorescent lamps (CFLs) can replace directly the incandescent lamps invented by Edison and Swan. These CFLs have become very popular due to its lower power consumption leading to considerable saving in energy and also reducing the cost of lightly. These energy saving lamps are therefore making important contributions in reduction of carbon dioxide to the environment.

The phoshors used for conversion of UV radiation to the visible is known as the Stoke phosphors as these obey Stoke's law. The tasks of the lamps scientists and engineers are to devise means of converting the UV into the visible efficiently, develop low cost and reliable electronic starter, eliminate flicker, and improve the lamp efficacy and lamp life.

#### 4. Phosphors

A phosphor is basically a material that exhibits the phenomenon of phosphorescence. This means that the material sustains continued glowing in the dark after exposure to light or to energized particles such as the electrons. The term phosphors originated from the glow of the element phosphorus in the dark. The glow observed in phosphorus is now known to be due to the oxidation of phosphorus.

Both inorganic and organic materials are known to exhibit some glow or emission of light. So the term luminescence is now more commonly used. Now a prefix is used, for instance to distinguish the origin of the luminescence. However, the term phosphors has been kept for historical reasons, and has been applied to substances that exhibits light emission stimulated by various means.

For example, glow arising from chemical reaction is termed chemi-luminescence, whilst that from heating a material is known as thermo-luminescence. Luminescence arising from electric field is known as electro-luminescence. The luminescence arising from interaction with light is known as photoluminescence.

In the lighting industries, the term phosphors are used for materials that exhibit luminescence when exposed to UV radiation. The term phosphors are kept for all materials that exhibit luminescence when subjected to not only uv radiation, but also radiation of shorter wavelength like X-rays, gamma rays, or to a stream of electrons.

#### 4.1 Phosphors for Cathode Ray Tubes

To date, a wide range of materials have been used as phosphors in the Cathode Ray Tubes. These include the zinc sulphide doped with various activators particularly from the rare earth elements. Some examples are ZnS doped with Ag, Cu, Al;  $Zn_2SiO_4$  containing Mn and As activators;  $Y_2 SiO_5$  with Ce, Tb; ZnO with Ag;  $Gd_2O_2$  with S and Tb; YAG with Ce, Tb; InBo with Th, Eu. These phosphors, with some modifications, have also been used for X-Ray screens, neutron detectors, alpha particle scintillators, and photomultipliers for electrons.

**Figure 4:** Various mechanisms of luminescence: (a) de-excitation to the ground state; (b) de - excitation with relaxation in a metastable state; (c) de –excitation with trapping. (Cayless and Marsden. 1983).



#### 4.2 Phosphors for Lighting Applications

In the lighting industries, it is well known that the fluorescent lamps require special materials known as phosphors to convert the uv radiation produced by the gaseous discharge into visible light sources. Phosphors that convert the uv into the visible are known as Stoke

conversion of higher wavelength of the infra-red radiation into the visible (lower wavelength as shown in Figure 5(b).

Figure 5: (a) Stoke and (b) Anti-Stoke Phosphors. (Cayless and Marsden, 1983).



The conversion of infrared into the visible is a more difficult process, requiring very special materials. Currently the efficiency of anti-Stoke phosphors is very poor, typically well below 1%. Research in this area is useful for improving the efficiency of the conversion from infra-red to the visible.

The following phosphors have been used in trichromatic fluorescent lamps: Barium and Europium doped magnesium aluminate (Ba, Eu),  $MgAl_{16}O_{27}$  as blue phosphors; (Ce, Tb) Mg  $Al_{11}O_{19}$  as green;  $Y_2O_3$ .Eu as red phosphors. These rare earth based phosphor materials are generally more expensive than phosphors based on the silicates, phosphates, tungstate, arsenates, vanadates etc.

#### 5. Mechanisms of Conversion UV to Visible using Phosphors

Basically uv radiation has short wavelengths, of the order of about 100 nm to 350 nm, and thus are more energetic than visible light with wavelength of the order of 450 to 600 nm. This is because the energy associated with a photon of light is in the form of quanta of energy,  $E = hv \text{ or } hc/\lambda$ , where h is the Planck's constant, v the frequency, c the velocity of light, and  $\lambda$ , the wavelength of the radiation. Thus the uv radiation is able to excite the activators (dopants) in the phosphor matrix to higher energy states. The total efficiency  $\eta$  of the phosphor is given by [1]

$$\eta = \eta_{qe} \frac{\lambda_{ex}}{\lambda_{em}}$$

where  $\eta_{qe}$  is the quantum efficiency,  $\lambda_{ex}$  is the wavelength of the radiation used to excite the phosphors, and  $\lambda_{em}$  the wavelength of the emitted radiation. Even if  $\eta_{qe}$  attains 100 % (every quantum of radiation absorbed by the phosphors gives a quantum of visible radiation), the overall efficiency would be less than 100% as the ratio of  $\lambda_{ex}$  and  $\lambda_{em}$  is less than one. material. Thus the environment where the activators are located and its oxidation states play an important role in the efficiency of the energy conversion. Phosphors are basically characterized by their emission colour and the length of time the emission lasts.

The combination of red, blue and green emission from the phosphor or combination of phosphors produces white light. The so called tri-phosphors is exploited in fluorescent lamps. Often other halo-phosphate phosphors are added as the first layer coating to improve the colour of the white light generated by the triphosphors.

Linear and circular type of fluorescent lamps for indoor applications are widely used in offices, condominiums, public and commercial buildings. These fluorescent lamps are also popular for indoor lighting of kitchen areas. A typical tubular fluorescent lamp is shown on the top part of Figure 8 reproduced from the March 2008 issue of Scientific American (Fischetti, 2008).

An early design of energy saving compact fluorescent lamp developed at Thorn Lighting as early as 1980's is shown in Figure 6.

**Figure 6:** Early designs of energy saving compact fluorescent lamps. These lamps are for direct replacement of the incandescent lamps. (Cayless and Marsden, 1983).



The white light source is used extensively for general lighting applications. There are various grades of white light ranging from warm to very cool white, the difference in the white colour depends on the amount of red component of light generated from the conversion, and hence from the type and amount of phosphors used.

#### 5. Energy Saving Compact Fluorescent Lamps (CFLs)

For over half a century now, scientists have been developing all types of phosphors for lighting applications. Today, with the latest development of tri-phosphors, it now possible to achieve routinely over 80 lumens per watt of white light in compact fluorescent and energy saving lamps. The efficiency is still low compared with the 250 lumens per watt of white light that can be generated theoretically, or 683 lumens per watt of monochromatic radiation at 555 nm.

Nonetheless, the current efficiency of fluorescent lamps represents a marked improvement of some 5 to 6 times that of the general incandescent lamps, and this is translated into considerable energy saving. The incandescent lamps, shown in Figures 7 & 9

**Figure 7:** Various types of lamps reported in an article in March 2008 Issue of the Scientific American (Fischetti, 2008).



Energy conservation today is therefore a very important strategy to minimize the use of

replacing these lamps and contribute to marked energy saving and cost. Today there are commercially numerous designs of these energy saving fluorescent lamps ranging from a few watt to 60 watt; some typical shapes are shown in Figures 7 and 8. Use of these compact energy saving lamps not only save energy but in the long run also reduce the cost of lighting drastically. A 26 Watt Compact Fluorescent Lamps CFLs is equivalent to a 100 watt incandescent lamp.

The introduction of narrow band phosphors has increased not only the efficiency but also the colour rendering index of the light source. In view of the contributions of these special phosphor materials, it is useful to study the behaviour and characteristics of these materials, and hopefully understand the mechanisms involved in the energy conversion.

Although the subject of luminescence is an active area of research covering bioluminescence, chemo-luminescence, thermal luminescence, electro -luminescence, photo luminescence, it is therefore pertinent to devote some discussion on photoluminescence using phophors that lead to the development of efficient light sources. It may be of interest to note that in the case of fluorescent discharge light sources, both electroluminescence and photoluminescence are involved. Electroluminescence in the sense that electrical voltage is used to generate and maintain the discharge. Photo luminescence is closely involved as a suitable phosphor material or several layers of different phosphors are used to convert uv (shorter wavelengths) radiation into the visible (longer wavelengths).

**Figure 8:** Some designs of energy saving Compact Fluorescent Lamps (CFLs) available on the market. (Fischetti, 2008).



#### 6. Energy Conservation and Sustainable Development in Lighting

The age of cheap oil is most likely over for good. Oil has been hovering around 100 US dollar a barrel for some time now. Some traders are even speculating that price of oil could well double in the not too distant future. Most of the easily recoverable oil has been recovered and used up at an alarming rate of over 80 million barrels a day. Oil must now be pumped from greater depth and also in hostile offshore environment. Such scale of

tons of oil can be saved annually. This will have an impact on the reduction of the carbon dioxide emitted to the atmosphere. Moreover, the saving in electricity bill is substantial as the 100 watt incandescent lamp can be replaced by a 25 watt compact fluorescent lamp and gives the same lumen output.

There is also now a greater awareness of climate change and global warming from increasing emission of green house gases, in particular, increasing amount of carbon dioxide from human activities. Efforts world wide are being implemented to reduce the green house gases, and minimise mankind carbon foot print. Thus, there is a very strong emphasis to use more efficient form of lamps, such as the direct replacement compact energy saving fluorescent lamps in the home and offices. The manufacturing of incandescent lamps is being phased out in Europe.

Figure 9: The incandescent lamps invented by Edison and Swan.



The energy saving fluorescent lamps, however, contain small amount of mercury. This requires careful disposal of the used lamps and greater awareness and publicity needs to be given to this issue. Mercury is a toxic chemical. It is also being debated whether its continued use in the future is sustainable. There is also intense research to develop low cost reliable white light solid state lamps from semiconductors such as gallium nitride and from organic polymers light emitting diodes (OLD). Novel materials are being investigated to produce white light source without the use of the expensive phosphors.

# 7. Transparent Sintered Ceramics for High Pressure Sodium and Ceramic Metal Metal Halide Lamps

Considerable research and development were conducted to improve the sinterability, the inline transmission, chemical resistance, thermal shock resistance, thermo-mechanical properties, and the relationship between processing, properties (Hing, 2006; Hing, 1990; Hing et al., 2007; Hing et al. 1979; Evans and Day, 1983; Chiang et al., 1997) and performance in actual high intensity discharge lamps, namely High pressure Sodium (HPS) and in the experimental Ceramic Metal Halide (CMH) lamps (Brown et al., 1982; Chalmers and Brown, 1982; Hing, 2003).

A range of ceramic materials investigated for the ceramic discharge lamps include single crystal sapphire, polycrystalline alumina, magnesium aluminate spinel, MgAl<sub>2</sub>O<sub>4</sub> yttrium

It is found that the most suitable arc tube materials in terms of chemical resistance, refractoriness, thermal shock resistance, dimensional reproducibility, cost effectiveness, manufacturability, availability of starting materials, and reliability is sintered translucent polycrystalline alumina.

**Figure 10:** Hexagonal crystal structure of  $\alpha$ -alumina. Unfilled circle – O<sup>2-</sup> ions Filled circles – Al<sup>3+</sup> ions occupying 2/3 of the octahedral sites. Broken unfilled circle is vacsnt octahedral site. (Anderson et al., 1991).



Note that the crystal structure of alpha alumina is shown in Figure 10. Alpha alumina is also known as corundum and exists in various crystalline form like kappa and gamma alumina. The latter has a cubic structure. The most stable form of alumina is the alpha alumina with a hexagonal crystal structure. The packing factor is about 0.84.

Figure 11: Sintered translucent alumina arc tube for high intensity discharge lamps.



#### 8. Processing of Alumina to Translucency

From a materials' processing point, high purity alumina powders starting from alum (aluminium sulphate dodecahydrate) precursors is preferred as ultra high purity and reactive alpha alumina powders can be obtained. The starting materials should have surface areas of the order of preferably about 30 m<sup>2</sup>/gram, and submicron size powders. In particular, the purity should be at least 99.98% or higher.

For instance, it was found that alumina slurry containing soluble precursors of magnesia and yttria, as dopants have good spray drying characteristics due to the high solid content, typically above about 65% by weight of alumina in the alumina slurry. Moreover, low form of soluble precursors had enabled both a small concentration of dopants, such as magnesia and yttria, typically in the region of 500 ppm to be uniformly dispersed in the alumina slurry.

These dopants, moreover, had enabled the sintering of the polycrystalline alumina to high density, minimal residual porosity and high translucency at temperatures as low as 1700C°C. The best in-line transmission was achieved when sintering was conducted at about 1900°C. This was achieved at the expense of reducing the grain boundary areas and appreciable grain growth and abnormal grain growth (Figure 12). There is evidence of segregation of chemical species, and formation of second phases at the grain boundaries such as yttrium aluminate on the internal and external surfaces of the sintered polycrystalline alumina arc tubes. At very high temperatures, these second phases on the surface of the samples tend to disappear presumably due to volatilization.

**Figure 12:** Grain structure of sintered translucent alumina used as envelope for High Pressure Sodium (HPS) and recently introduced Ceramic Metal Halide (CMH) lamps.



Very high temperatures operating temperatures around 1900C in a hydrogen atmosphere usually reduces the life of the molybdenum furnace, and increased the embrittlement of not only the refractory metal shields, but also the tubular molybdenum shields used as sample holder for supporting the alumina tube during the sintering process. Trials conducted using experimental refractory cermets (metal-alumina composites) as shields for the tubular components were also successfully conducted. These cermet tubes, moreover, proved to be lasting and did not suffer embrittlement compared with 100% molydenum metal shields.

Wet and Dry bag compaction were used for the fabrication of the arc tubes for ceramic lamp applications. In the wet bag processes, the polymer moulds or polymer colostomy were in contact with the pressure transmitting fluids during compaction. In the dry bag compaction, the polymer moulds did not make contact with the pressure transmitting fluids. This development, coupled with the use of the free flowing spray dried ceramic granules, has enabled the large volume manufacturing of the ceramic arc tubes to be carried out. Up to now, the most widely used material for the high intensity ceramic discharge light source is still alumina. The polycrystalline alumina has been sintered to very high translucency using pressureless sintering in a reducing atmosphere such as hydrogen.

Studies also shown that cheaper gamma alumina powders of similar purity could also be used as starting materials for the fabrication of the sintered translucent alumina. However, the lower cost of starting materials and lower sintering temperature did not justify changes in the manufacturing process.

Experimental studies were also conducted on rapid sintering. Primary aims are to reduce the sintering time and increases the volume throughput. Although it was shown possible to sinter the green body to fully dense and translucent envelope within several minutes close to 1950C and above, the distortion in the sintered components was dramatic.

However, the distortion was removed with longer sintering and holding time. The capital cost of changing the continuous high temperature hydrogen furnaces to a very low thermal mass furnace with considerable saving on hydrogen was not studied further.

Similarly vacuum sintering of the polycrystalline alumina to translucency was conducted successfully. It was shown that the pay back would be about 3 years; this was considered too long in the industry, and thus combined with the high capital cost to replace existing continuous hydrogen furnaces was not pursued further. A large vacuum furnace using refractory tungsten mesh elements was constructed, and experiments were conducted successfully. The vacuum sintered components were also used in high pressure sodium lamps successfully.

We also tried sintering alumina in vacuum using carbon susceptor in an RF Furnace. We were able to solve the blackening problems in both the refractory W mesh furnace and also in Carbon tube used as susceptor and sample holder. The experimental studies carried out at Balzers, Westlar, Germany, using large diameter vacuum furnaces showed that volume vacuum production of sintered translucent polycrystalline alumina was successful.

Cost analysis showed that the return on investment (ROI) was achievable within 2 years. Vacuum sintering was not pursued further due to huge capital investment in furnaces and the need to maintain and replace expensive refractory metal parts. The problems associated with darkening of the sintered translucent ceramics using carbon susceptors and samples holders were considered too risky as the thermodynamics of the processes need to be very carefully controlled. In particular the need to control or eliminate traces of moisture trapped inside the furnace parts such as carbon felt for insulation was vital. Similarly, the need to avoid reactions between the water vapour and the refractory metal such as molybdenum and tungsten in vacuum furnaces was necessary. This causes contamination in the sintered translucent alumina tubes. We have been able to control and fine tune the processing.

The development and continued refining of the ceramic processing and implementation of strict quality control in the manufacturing operation enabled elimination of various factors contributing to the residual porosity, cylindrical porosity, white specks, black specks, surface roughness and so on. This enabled volume production of these highly refractory, chemically resistant, with high in-line transmission, and a satisfactory high surface finish.

It was necessary to develop very high surface finish in the interior and external surfaces of the tubular polycrystalline alumina arc tube. The development of effective materials processes contributed tremendously to the widespread use of the alumina ceramic envelopes, not only for the normal high pressure sodium lamps but also in the improved Colour Rending Index (CRI) HPS lamps which requires higher wall loading.

The use of the polycrystalline alumina arc tube was extended to the development of compact high pressure, high intensity Ceramic Metal Halide (CMH) lamps containing highly chemically reactive and volatile halide species.

The internal pressure of the lamp fill volatile species in the compact Ceramic Metal Halide lamps can reach tens of atmospheres as the thin walled alumina tube was observed to bulge out over prolonged operation (private communication AG Chalmers and K Brown)

Besides the development of the straight highly translucent polycrystalline alumina arc tubes, research were conducted on the development of highly translucent complex shape hollow ceramic tubular components with tapered ends using dry compaction techniques as well as wet forming units such as traditional slip casting and the more demanding blow moulding technique adopted from the glass and plastic industries.

**Figure 13:** Fracture surface of sintered translucent alumina sintered at 1850C (LHS) and 1900C (RHS). [ \_\_\_\_\_\_ 20 µm ]



**Figure 14:** The phase diagram of  $Al_2O_3 - Y_2O_3$  showing eutectic at 1860C >



Examples highlighting the important processes developed, and some critical problems solved during the development of compact high pressure sodium (HPS) and ceramic metal halide (CMH) lamps are outline below:

- 3. Development of compact bulbous shaped translucent arc tubes by blow moulding and the slip casting by the author at Thorn Lighting Ltd.
- 4. Development of dense sintered translucent polycrystalline alumina by rapid vacuum and reducing atmosphere sintering.
- 5. Development of alumina arc tubes with external grooves for effecting quick start of the HPS and CMH lamps.
- 6. Development of leak tight, strong, tough, electrically conducting alumina-metal (cermet) composites with matching thermal expansion with alumina.

#### 9. Ceramic Metal (Cermets) Composites for High Intensity Discharge Lamps.

The high pressure sodium lamps using sintered translucent sintered alumina as the envelope requires a niobium insert. The latter carries a tungsten electrode. Each ends of the alumina tube is closed with the niobium insert. The reason niobium is used is because of the close thermal expansion between niobium and alumina. To obtain a hermetic seal, a silica free glass ceramic is used as the intermediate sealing materials. This type of sealing material is covered in patents by lighting companies such as General Electric and GTE Sylvania in the United States, Philips in the Netherlands, Osram in Germany, and Mashushita, Kyocera, Toshiba, Panasonic in Japan.

The use of the niobium metal insert with the intermediate glass ceramic seals provides a close match in thermal coefficient of expansion with the dense translucent polycrystalline alumina arc tube. The end closure can thus be thermally cycled over the life of the lamps without losing its hermeticity. The latter is vital for the containment of the gaseous vapour species inside the arc tubes. These could be alkali and mercury vapours together with some inert gases such as argon, krypton, xenon. The seals for the HPS lamps are generally highly reliable. Failure of the HPS lamps usually occur by loss of emitter materials on the tungsten electrodes and due to the blackening inside the surfaces of the alumina arc tubes, particularly near the electrode regions. This leads to drastic reduction in lamp efficacy and lamp life.

It is of import to note that the use of niobium in contact with the highly reactive metal halide vapours such as sodium and tin iodide, bromide, chloride and fluoride leads to severe chemical attack of the electrodes and niobium. The silica envelope was also found to be attacked by the metal halide vapours. To overcome these materials's problems, it was first reported by Philips in Eindhoven, that a new design of end closure material such as alumina-molydenum cermet in place of the niobium-metal insert for the ceramic metal halide lamps could enable the experimental ceramic metal halide lamps to be investigated.

Following this announcement by Philips in the 80's, Thorn Lighting embarked on the development of ceramic-metal composites with different arrangement of the dispersed phases. This development was led by the author, and resulted in several patents. The key to the development of the novel alumina – refractory metal cermets was the development of processes that enabled the refractory metallic particles in the micron range to coat the alumina granules and other types of ceramic granules formed either by pan granulation and other spherodisation processes, including spray drying. Coatings can be effected by mixing the ceramic and metallic phases in a suitable manner that minimizes the aggregation of the micron size metallic phases. The economical processes developed could be adopted for large scale processing of ceramic-metal composites. The development of the ceramic-metal composites enabled Thorn Lighting to have a substantial lead in the early development of very compact high intensity and afficiency.

Whilst cermets containing dispersed match phases and well known in the patent literature and journal publications, the development of electrically conducting cermets with low volume fraction of metallic phases and with closely matching thermal expansion with the polycrystalline alumina arc tubes were novel. The cost effective technique of fabricating dense strong and tough composites with dispersed phases and a distribution of free flowing spray dried ceramic granules were pursued aggressively.

The author led the development of the ceramic-metal composite based on coating the ceramic granules with a layer of metallic phase. Two types of granules were investigated; those fabricated by spray drying of the high purity alumina slurry; and alumina granules prepared by mechanically rolling the mixture of the metal and ceramic phases. In this way, ceramic granules ranging from less than ten micron to several hundreds of microns can be produced. The processes were optimized and led to the development of very strong and tough cermet materials capable of carrying sintered—in tungsten electrodes. Such designs of enclosure with the cermet materials led to granting of a US Patent (Hing et al., 1979).

It was also found that the spray dried alumina granules developed relatively smooth surface finish and also had marked "dimpled" appearance like doughnut. The spray dried granules were more difficult to coat with refractory metallic particles. Moreover, the metallic particles were found to lodge in the dimples in the spray dried alumina. This led to higher metal loading for electrical conduction. Some roughening of the surfaces were , however, found to be effective to fill the dimples with alumina debris first and this is found to assist the metallization processes.

**Figure 15:** Microstructure of sintered alumina – tungsten cermets. Note the metallic tungsten surrounding the alumina granules. The ceramic -metal composites (known as cermet) was developed for the experimental ceramic metal halide lamps. (Hing et al., 1979).

## MICROSTRUCTURE OF ELECTRICALLY CONDUCTING AL<sub>2</sub>O<sub>3</sub> CERMETS



However, when the granules prepared by the simple granulation process we used at the

of the metallization process was attributed partly to the mechanical entrapment of the micron size metallic particles on the rough surfaces. However, the physics of the adhesion processes were not investigated. This would be worth studying in more detail. In particular the nature of the charge and charge distribution on the various size of the granules, and the effect of residual moisture, humidity effect, additives etc. would be worth investigating in more detail to elucidate the mechanisms of sticktion of the metallic particles on the various types of ceramic granules.

Moreover, further enhancement in the thermo mechanical properties of the ceramicmetal composites were obtained by dispersing discrete metallic phases in the alumina granules. This resulted in stronger and tougher dense sintered cermets. The improvement in the mechanical strengths was attributed to the inhibition of grain growth due to the dispersed metallic particles. The spatial distribution of the metallic phases on the sinterability and thermo mechanical properties of the ceramic metal composites were reported in the several Proceedings of The Science of Ceramics (Hing, 1980; Hing, 1984).

#### 10. Development of Glass Ceramic Sealing Materials for the Ceramic Halide lamps

The construction of the seals for the HPS lamps consists of a niobium tube as an insert into the alumina arc tube. The niobium carries a coiled tungsten electrode coated with emitter materials to reduce the work function. Niobium is chosen as an insert because of the fairly close match in thermal expansion with the alumina. Hermetic seal is obtained using a silica free glass ceramics. The composition is proprietary as each lamp company has their own compositions. Basically it contains magnesia, alumina, barium oxide and boric oxide with some other alkaline earth like strontium oxide. The sealing glasses for the HPS lamps were found to be inadequate for the sealing the ceramic metal halide lamps as the metal halides attack these glasses during the lamp operation.

Thus there was a need to develop sealing materials capable of withstanding the highly reactive metal halide species inside the alumina arc tubes. There is a growing trend to use ceramic arc tube instead of the fused silica as envelope as the ceramic are more refractory, and also capable of withstanding higher internal pressures of the gases during the lamp operation.

Conventional ceramic powder processing route, sol gel processing and melt formed routes have been used to prepare experimental materials for evaluation as potential sealing materials. One simple test was to cold press first the chemically prepared glass compositions and premelt on the sintered translucent alumina discs in air to study the melting characteristics and its wettability on the alumina substrate. The glass compositions should preferably melt below 1500°C and exhibit good wettability. The molten glass should spread easily with low contact with the substrate. Nucleation and crystallization heat treatments of the premelted glasses were also carried in a muffle furnace in air. Sealing compositions resulting in crack free substrates and glass ceramics after nucleation and crystallization heat treatments are selected for further studies.

#### 11. Development of Compact White Light High Pressure Sodium Lamps

Figure 16 depicts typical energy level of sodium atom. As the pressure of the lamp fill in the high pressure sodium lamp is increased, the spectrum is broadened as shown in Figure 17.





**Figure 17:** Spectral distribution in HPS lamps at different pressures: (a) 250watt at 104 Pa; (b) 250watt at 4x104 Pa. Note improved colour rendition at higher pressure – Ra about 20. Golden yellow white light – motorways, buildings, airports etc. Note the predominant feature of HPS lamp is self reversal of Na D lines (bottom spectrum).



**Figure 18:** High Pressure Sodium (HPS) lamps (400 watt) using inner alumina arc tube. Note different types of outer glass envelope. (a) fused quartz linear outer envelope; (b) larger linear borosilicate glass outer envelope; (c) bulbous outer glass envelope with inner surface coated with phosphor.



#### 12. Development of Seals for the Ceramic Metal Halide (CMH) lamps.

Figures 19, 20, and 21 show experimental Metal Halide lamps constructed with alumina ceramic envelope. The seal consists of a cermet end plug joined to an alumina ceramic arc tube. A glass ceramic sealing compound is used to join the cermet end to the translucent alumina tube. The cermet carries an electrode that is sintered inside the cermet enclosure at both ends.

The CMH lamp shown in Figure 19 is a double ended lamp meaning that there is an electrode at each end of the tube. The seals must be fully hermetic, implying that gases inside the sealed arc tube necessary for the operation of the lamp must not leak out, nor should any gas in the outer envelope of the lamp leak in externally through the interfaces of the glass ceramic seals.

Hermeticity tests were usually conducted on sealed arc tube containing argon and metal halides pellets. The seals were thermally cycled at various temperatures. One technique of assessing quickly whether the seals were leaky was to observe the colour of the cold discharge produced using a Tesla coil applied at one end of the sealed ceramic envelope.

Figure 19: Experimental Ceramic Metal Halide (CMH) lamp. (Chalmers et al. 1989).



A leaky seal produced a distinct violet/purple discharge; a hermetic seal gives a distinct bluish discharge. This bluish discharge must be maintained for months under various cycling conditions. A bluish discharge indicates a leak tight seal. The sealed arc tube can be thermally cycled to simulate service performance of the lamp. Maintenance of the bluish discharge over prolonged thermal cycling indicates that the seals are fully hermetic.

The chemical resistance was also tested in experimental metal halide lamps under actual service conditions required. The techniques of containing the metal halides at relatively high sealing temperatures without loss of metal halides required special sealing techniques. The technology will be discussed separately.

**Figure 20:** Ceramic Metal Halide Lamps developed at Thorn Lighting Ltd, UK using proprietary ceramic–metal (cermet) composite end closure and glass sealing compositions.



Figure 21: Spectrum of white light source in experiment CMH lamp.



13. Materials systems investigated to produce refractory and metal halide resistant glass seals

MgO.Al<sub>2</sub>O<sub>3</sub>. SiO<sub>2</sub> SrO-ZrO<sub>2</sub>.SiO<sub>2</sub> Y<sub>2</sub>O<sub>3</sub>.Al<sub>2</sub>O3.SiO<sub>2</sub> SrO.MgO.SiO<sub>2</sub>

To make the seals, frit rings were fabricated by uniaxial compaction using specially designed stainless steel dies. In all the four systems investigated we have identified various compositions that have potential for the construction of experimental ceramic halide lamps. These will be discussed. The development of the glass ceramic sealing had involved extensive research. It is more appropriate to discuss this aspect of the materials development in more detail in a separate article.

#### 14. Conclusions

In this review it is demonstrated that the development and use of functional ceramics and ceramic composites as well as glass ceramic seals were necessary in the construction of high intensity discharge light sources such as compact high pressure sodium and ceramic metal halide lamps. Whilst these high intensity ceramic discharge lamps are now available commercially, the early development required extensive materials development and design.

This material development has enabled the commercialisation of these compact high efficacy and high colour rendering high intensity discharge light sources. These lamps can be operated cost effectively, safely and reliably. Even today, there is still a lot of R&D needed to effect continuous improvement in the evolution of these light sources. This is because most of these light sources are far from achieving their theoretical 683 lumens per watt for monochromatic radiation, and about 250 lumens per watt for the white light distribution. Even the most efficient high pressure sodium lamp is of the order of 140 lumens per watt, and the ceramic metal halide white light source of the order of 100 to 120 lumens per watt.

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