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ON THE GEOMETRY OF ALHAMBRAN TILINGS

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1. Introduction

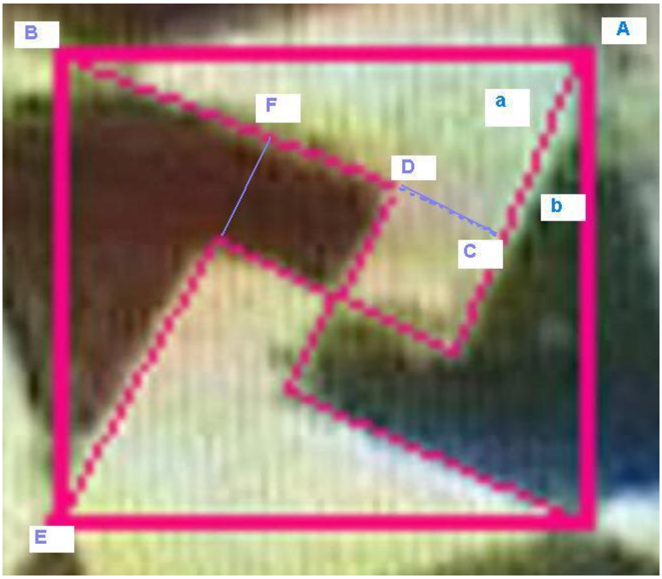
The walls of the harem of the Alhambra Palace in Spain are decorated with rotationally symmetric tilings (Gallian, 1998). In Figure 1, the upper tiling is a 6-fold symmetry tiling of sinuous triangles and the lower tiling is a 4-fold symmetry tiling of arrow shapes (Bronowski, 1976). The Alhambra was built in the 9th Century, but most of its decorations date from the 13th Century (Calvert, 1907).

Figure 1 Rotationally symmetric tilings in the Alhambra harem

How did the artist-geometers of the day discover these symmetries? Tesselating a plane by triangles is straightforward, but where did the idea of tesselating with arrow shapes come from? Lu and Steinhardt (2007) state: “The conventional view holds that girih (geometric star-and-polygon, or strapwork) patterns in medieval Islamic architecture were conceived by their designers as a network of zigzagging lines, where the lines were drafted directly with a straightedge and a compass. We show that by 1200 C.E. a conceptual breakthrough occurred in which girih patterns were reconceived as tessellations of a special set of equilateral polygons (“girih tiles”) decorated with lines.”

As shown in Figure 2, the arrow tiling of the lower part of the harem wall derives from a tesselation of the plane by swastikas. The artists that designed the Alhambra harem tiling must have known its underlying geometry. The pattern could have been etched on square girih tiles, but the cementwork of the Alhambra harem wall decoration indicates clearly that it is a tesselation by arrow-shaped tiles, which are more complex shapes than equilateral polygons, thus extending the conceptual breakthough observed by Lu and Steinhardt (2007).

Figure 2 The underlying symmetry



2. Geometry

The pattern of Figure 2 is one example of a range of figures that can be constructed from any value of the angle *a* between 0 and 90, as:

$$b = 90 - a$$
$$BC = AB \sin(a)$$
$$AC = AB \sin(b)$$

The figures are rotationally symmetric about the point D at the midpoint of CF, because

$$\Delta\, ABC \equiv \Delta\, BEF$$
$$\text{so } AC = BF$$
$$\text{so } CF = BC - AC$$
$$\text{and } CD = FD$$

The LOGO (Papert, 1993) program in Figure 3 produces a semi-tesselation of the plane of *r* rows and *c* columns of arrows of size *s* whose shape is determined by angle *a*. The program draws rows of pairs of arrows filled with blue flood-colour, the second an inverted form of the first, placed underneath it. Successive rows of arrow pairs are displaced by one arrow-width so that the patterns fit together. Because of the swastika-symmetry upon which the tesselation is based, the spaces between the blue arrows have an identical arrow-shape, as can be seen in Figure 4, produced from $a = 20°$.

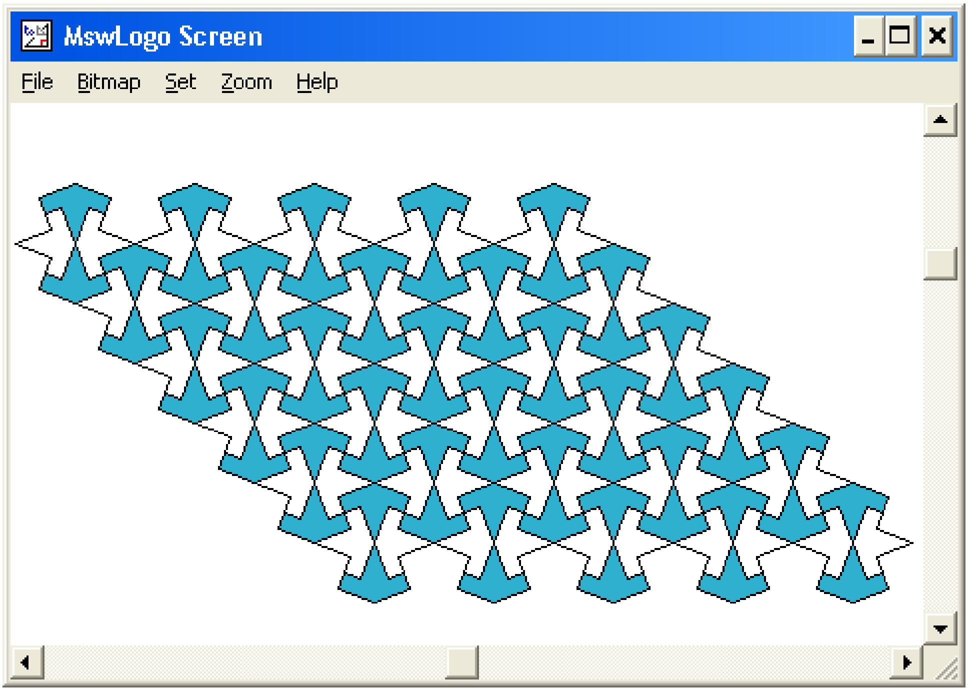
Figure 3 A program to tile the plane with arrows

```
to arrows :s :a :r :c
  setfloodcolor [45 177 206]
  cs pu setxy -200 250 pd
  ; work out the lengths
  make "ac :s * sin :a
  make "b 90 - :a
  make "bc :s * sin :b
  make "cd 0.5 * (:bc - :ac)
  make "bd :ac + :cd

  repeat :r ~
       [repeat :c ~
          [repeat 2 ~
             [arrow :a pu lt 90 bk :s pd
             arrow :a pu fd :s lt 90 pd]
           pu fd 3 fill bk 6 fill fd 3 pd
           pu rt 90 fd 2*:s lt 90 pd]
           pu bk :s lt 90 fd (2*:c-1)*:s rt 90]
end

to arrow :a
  lt :a fd :bd
  lt 90 fd :cd
  rt 90 fd :cd
  rt 90 fd :bd
  rt 2*:a fd :bd
  rt 90 fd :cd
  rt 90 fd :cd
  lt 90 fd :bd
  lt :a+180 pu fd 3 bk 3 pd
end arrow
```

Figure 4 The image produced by `arrows 30 20 6 5`

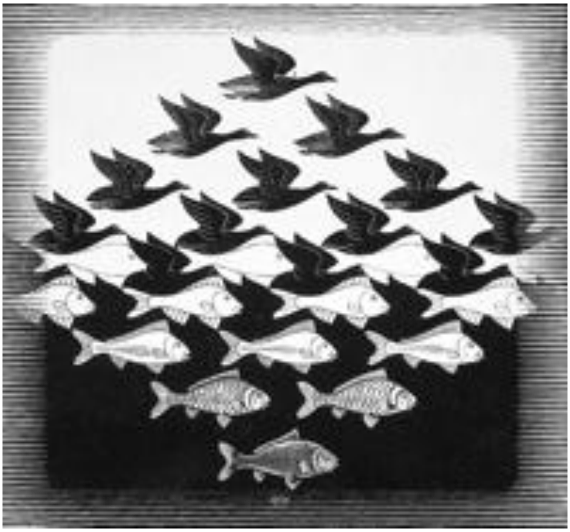


3. Semiotics

It is usually remarked that mediaeval Islamic art was purely abstract and aniconist (Burckhardt, 1976; Dodds, 1992; Ettinghausen and Grabar, 1994).

The noted painter M C Escher was inspired by the Alhambra's designs' interplay of similar light and shadow. His woodcut, shown in Figure 5, apparently depicts real things (fish and birds) but actually alludes to the duality of light and shadow (Koptsik, 2003); in exact contrast, the Alhambra tilings are apparently abstract geometric shapes. The intellects that created Escher's and the Alhambran artworks are equally worthy of admiration – both are masters of geometry and allusion.

Figure 5 *Sky and Water I* by M C Escher, 1938 (woodcut)



4. Acknowledgements

M.R. Anderson recognised the underlying geometry of the swastika tesselation.

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GENERALIZATIONS AND VARIATIONS OF THE MONTY HALL PROBLEM

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Abstract: The Monty Hall problem is a probability puzzle based on the American television game show *Let's Make a Deal* that was made famous by its appearance in Marilyn vos Savant's *Ask Marilyn* column in *Parade* magazine. Some generalizations of the original problem are considered here. The probability distribution is changed from the equal likelihood case to a given prior distribution, and the number of doors is increased to an arbitrary *n*. Variations of the original problem are also considered.

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1. The Monty Hall Problem

During the period from 1963 to 1976, US television networks NBC and ABC aired a game show called *Let's Make a Deal*, hosted by Monty Hall. In the Eighties, the show received a facelift, and further modified versions of the show appeared on the networks during the 1990's and 2000's. The latest version debuted on CBS in October 2009 under the original name, hosted by Wayne Brady, with Monty Hall as a creative consultant.

The core idea of the show is to give the contestants a chance to trade one prize of medium value for another of considerably higher value at the risk of getting a junk prize, otherwise known as a zonk. The contestants try to make their choices in order to minimize their chances of being zonked. Though the problem was originally posed by Steve Selvin in 1975 in a letter to the *American Statistician*, the probabilistic aspects of the matter, and the optimization strategies, became an issue of debate after it was discussed in a column called *Ask Marilyn*, a Sunday column in *Parade* magazine.

Marilyn vos Savant is an American magazine columnist who became famous through her listing in the *Guinness Book of World Records* under the "Highest IQ" category. Her fame spread further as the author of *Ask Marilyn*, in which she solves puzzles and answers questions from readers on a variety of subjects. After the vos Savant article was published and the raging debate that followed, the puzzle became well-known under the name *the Monty Hall Problem*. The scenario of this probabilistic puzzle is described below:

You are given the choice of three doors. Behind one door is a car; behind the others, goats (zonks). You pick a door, say No. 1, and the host, who knows what is behind the doors, opens another door, say No. 3, which has a goat. He then says to you, "Do you want to pick door No. 2?" The question is whether it is to your advantage to switch your choice. The host will never open the door containing the prize, and he will never open the door you chose originally.

A high percent of respondents answered that it makes no difference whether one switches or not – either way one has 50% chance of winning. But Vos Savant wrote in her column that it matters – that switching considerably improves your chances of winning the car. According to vos Savant, if the contestant does not switch doors, her probability of winning the car is $\frac{1}{3}$, whereas if she switches, the probability increases to $\frac{2}{3}$. When the solution of the problem appeared in *Parade*, approximately 10,000 readers, including nearly

1,000 with Ph.D.s, wrote to the magazine claiming the published solution was wrong. The debate raged on for quite some time.

According to Wikipedia, some of the controversy was because the *Parade* version of the problem is technically ambiguous, since it leaves certain aspects of the host's behavior unstated. But much of the response can be attributed to poor understanding of probability principles.

2. The Generalization

In this article, boxes are used instead of doors, and the number of boxes is changed to a general n. The probability of a box containing the prize is changed from equal likelihood to a general probability distribution, and randomization of the behaviour of the host is incorporated. Also given is allowance for randomization of initial choice and switching for the contestant. Precise assumptions are listed below.

- There are n boxes where $n \geq 3$ and exactly one of these boxes contains a prize. These boxes are numbered 1 to n.

- The probability that Box i contains the prize is p_i. Let $\mathbf{p} = (p_1, p_2, \ldots, p_n)$. The value of $\mathbf{p}$ is known to all.

- The host enters the value of $\mathbf{p}$ into a computer. The computer then assigns the prize into one of the boxes at random according to this probability law, and informs the host secretly.

- The contestant selects a box, either deterministically, or randomly. She can enter her own choice probability vector $\mathbf{q} = (q_1, q_2, \ldots, q_n)$ into the computer and it will do the choosing for her.

- The host opens every box except two. One of the two that remain closed is the one that the contestant has selected.

- The host will never open the box that contains the prize, and he will never open the box chosen by the contestant. If the contestant selected an incorrect box, the host leaves that box and the correct box unopened.

- If the contestant selected the correct box, the host uses a probability distribution to choose at random the other box that will be left closed. This is given by an $n \times n$ stochastic matrix
$$R = ((r_{ij}))$$
where r_{ij} represents the probability that the host will leave Box j closed if Box i contains the prize and the contestant has selected that box. For each i, $r_{ii} = 0$ and $\sum_{j=1}^{n} r_{ij} = 1$. The value of R is unknown to the contestant.

- The contestant now has an option – to stay with the original choice, or to switch to the other unopened box.

For $n \in \mathbb{N}$, let $N_n = \{i : 1 \leq i \leq n\}$. The contestant can also randomize her decision on whether or not to switch. If the initial choice is Box i and the alternative is Box j, let p_{ij}

denote the probability that the contestant switches from *i* to *j*, where $i, j \in N_n$, $i \neq j$. We define $p_{ii} = 0$, $1 \le i \le n$ for completeness, though the value of p_{ii} is irrelevant for all the derivations and arguments that follow. This gives us the $n \times n$ matrix

$$P = ((p_{ij}))$$

Note that *P* is *not* a stochastic matrix. If the contestant has a never-switch policy, *P* will be the zero matrix, and if she has an always-switch policy, all off-diagonal elements of *P* will be 1.

If Box *i* contains the prize, the probability that the contestant wins it is a function of the choice probability vector **q** and the switching probability matrix *P*. Here we treat **p** as a fixed constant. We denote this function by $f(P, \mathbf{q})$. We now compute the form of *f* explicitly.

Proposition 2.1

$$f(P,\mathbf{q}) = \sum_{i=1}^{n} q_i \left[p_i + \sum_{j \neq i} (p_j - p_i r_{ij}) p_{ij} \right]$$

Proof:

Let *W* denote the event that the contestant wins the prize and B_i denote the event that Box *i* has the prize. Given that Box *i* has the prize, there are two ways she can win it. Either choose Box *i* initially and do not switch, or choose another box and switch to Box *i*. If Box *j*, $j \neq i$, is initially chosen, then the probability of winning is equal to p_{ji}. On the other hand, if Box *i* is initially chosen, then the probability of winning is given by

$$\sum_{j \neq i} r_{ij} \bar{p}_{ij} \ ,$$

where for any probability *p*, $\bar{p} = 1 - p$, the probability of the complement. So the conditional probability of winning the prize given Box *i* has the prize is given by

$$P(W \mid B_i) = q_i \sum_{j \neq i} r_{ij} \bar{p}_{ij} + \sum_{j \neq i} q_j p_{ji}$$

Thus by Bayes' theorem, the winning probability is given by

$$\begin{aligned} f(P,\mathbf{q}) = P(W) &= \sum_{i=1}^{n} P(B_i) P(W \mid B_i) \\ &= \sum_{i=1}^{n} p_i \left[q_i \sum_{j \neq i} r_{ij} \bar{p}_{ij} + \sum_{j \neq i} q_j p_{ji} \right] \\ &= \sum_{i=1}^{n} p_i \sum_{j \neq i} (q_i r_{ij} \bar{p}_{ij} + q_j p_{ji}) \end{aligned}$$

For $i \neq j$, the sum of the $(i, j)^{\text{th}}$ and the $(j, i)^{\text{th}}$ terms in the double sum above is given by

$$\begin{aligned} & p_i q_i r_{ij} \bar{p}_{ij} + p_i q_j p_{ji} + p_j q_j r_{ji} \bar{p}_{ji} + p_j q_i p_{ij} \\ &= p_j q_i p_{ij} + p_i q_i r_{ij} (1 - p_{ij}) + p_i q_j p_{ji} + p_j q_j r_{ji} (1 - p_{ji}) \\ &= (p_j - p_i r_{ij}) q_i p_{ij} + p_i q_i r_{ij} + (p_i - p_j r_{ji}) q_j p_{ji} + p_j q_j r_{ji} \end{aligned}$$

Note that, in the above expression, the sum of the last two terms is obtained by interchanging the roles of *i* and j in the sum of the first two terms. Hence

$$\begin{aligned} f(P,\mathbf{q}) &= \sum_{i=1}^{n} \sum_{j \neq i} [(p_j - p_i r_{ij}) q_i p_{ij} + p_i q_i r_{ij}] \\ &= \sum_{i=1}^{n} q_i \left[p_i + \sum_{j \neq i} (p_j - p_i r_{ij}) p_{ij} \right] \end{aligned}$$

because $\sum_{j \neq i} r_{ij} = 1$. □

In order to determine the contestant's best strategy, we need to find **q** and P that maximize $f(P,\mathbf{q})$. The following lemma provides the optimal values.

Lemma 2.1

For $i \in N_n$, let $C_i = \{j : 1 \le j \le n, j \ne i, p_j \ge p_i r_{ij}\}$ and let $\beta_i = p_i + \sum_{j \in C_i}(p_j - p_i r_{ij})$. If i_0 is such that $p_{i_0} = \min_{1 \le i \le n} p_i$, then $\beta_{i_0} = \max_{1 \le i \le n} \beta_i = 1 - p_{i_0}$.

Proof:

For any $i \in N_n$,

$$\begin{aligned}\beta_i &= p_i + \sum_{j \in C_i}(p_j - p_i r_{ij}) \\ &\ge p_i + \sum_{j \ne i}(p_j - p_i r_{ij}) \\ &= \sum_{j=1}^{n} p_j - p_i \sum_{j \ne i} r_{ij} \\ &= 1 - p_i\end{aligned}$$

Also, for any i such that $C_i = N_n \setminus \{i\}$,

$$\begin{aligned}\beta_i &= p_i + \sum_{j \ne i}(p_j - p_i r_{ij}) \\ &= \sum_{j=1}^{n} p_j - p_i \sum_{j \ne i} r_{ij} \\ &= 1 - p_i\end{aligned}$$

In addition, $C_{i_0} = N_n \setminus \{i_0\}$ because if $\exists j$ such that $p_j < p_{i_0} r_{i_0 j} \le p_{i_0}$, it contradicts the fact that $p_{i_0} = \min_{1 \le i \le n} p_i$. Consequently, $\beta_{i_0} = 1 - p_{i_0}$.

Now it remains to show that for all $i \in N_n$, $\beta_i \le 1 - p_{i_0}$. Suppose $\exists i$ such $\beta_i > 1 - p_{i_0}$ and hence $\beta_i > 1 - p_i$. Now C_i Ø $N_n \setminus \{i\}$, so there exists $k \ne i$ such that $k \notin C_i$. Then $\beta_i \le p_i + \sum_{j \in C_i} p_j \le 1 - p_k \le 1 - p_{i_0}$ and we arrive at a contradiction. □

Thus we have the following theorem:

Theorem 2.1

1. *For any value of R, the optimal strategy is to choose the box with the least probability and then switch to the other closed box after the host has opened all other boxes.*
2. *The winning probability is given by* $1 - \min_{1 \le i \le n} p_i$.
3. *The winning probability is always at least* $1 - \frac{1}{n}$. *The minimum is attained when and only when* $\mathbf{p} = (\frac{1}{n}, \frac{1}{n}, \ldots, \frac{1}{n})$.

Proof: Since the contestant can choose her switching probabilities without any constraints, for any **q**, $f(P, \mathbf{q})$ is maximized when she sets $p_{ij} = 1$ for all i and j such that $p_j \ge p_i r_{ij}$ and $p_{ij} = 0$ for all others. Note that this is independent of the choice probability vector **q**. Denote this optimal switching probability matrix by $\hat{P}$. Then for any **q**, the maximized value of $f(P, \mathbf{q})$ is given by

$$f(\hat{P},\mathbf{q}) = \sum_{i=1}^{n} q_i \left[p_i + \sum_{j\in C_i} (p_j - p_i r_{ij}) \right]$$
$$= \sum_{i=1}^{n} q_i \beta_i$$

Clearly the above expression is maximized by finding i that maximizes β_i and choosing the corresponding $q_{i_0} = 1$ with all the other components zero.

Thus $f(\hat{P},\hat{\mathbf{q}}) = \max_{1\le i\le n} \beta_i$. Now the proofs of Part 1 and Part 2 follow from Lemma 2.1. Part 3 follows from the fact that $\min_{1\le i\le n} p_i \le \frac{1}{n}$ and that $\min_{1\le i\le n} p_i = \frac{1}{n}$ if and only if $\mathbf{p} = (\frac{1}{n}, \frac{1}{n}, \ldots, \frac{1}{n})$. ☐

3. A Variation

The following is a well-known variation of the original version. Two contestants are selected instead of one. Each of them picks a box, and they cannot choose the same box. One of the selected boxes is opened, revealing an empty space. The host dismisses the contestant who selected that box, turns to the other one, and gives him or her the option of switching.

The existence of the second contestant changes the problem dramatically, as explained below. Assume Contestant 1 selects Box *A* and Contestant 2 selects Box *B*.

Case 1: The prize is hidden behind one of the two selected boxes, say, *A*. The host must open Box *B* to dismiss Contestant 2 and presents Contestant 1 with the choice. Since he has selected the box with the prize, he should not switch. This case has probability $\frac{2}{3}$.

Case 2: Neither contestant has selected the prize. No matter which of the two contestants is presented with the choice, he or she should switch because they will be faced with a choice between their original selection and Box *C*, which contains the prize. Case 2 has probability $\frac{1}{3}$.

We find that in this setting the contestant should not switch. The probabilities are the reverse of those in the classical problem – switching leads to probability $\frac{1}{3}$ of winning and not switching to $\frac{2}{3}$.

4. Unification and Generalization

First, note that the variation given above is equivalent to the following. There is only one contestant, but she gets to select two boxes. The host opens one of those two and shows it is empty, then gives the contestant a chance to switch. The optimal strategy is never to switch.

Once the problem is thus transformed, we can generalize it as follows. There are n boxes, of which exactly one has the prize behind it. The contestant is asked to select n_1 of these, where $1 \le n_1 \le n - 1$. After the contestant has selected the boxes, the host opens k_1 of the n_1 that were selected and k_2 of the $n_2 = n - n_1$ that were not selected, showing that all of them are empty, where $0 \le k_1 \le n_1 - 1$ and $0 \le k_2 \le n_2 - 1$. (Again, the host knows where the prize is and will not open that box.) The contestant is now given a chance to select one of the unopened boxes. We assume that n_1, n_2, k_1, k_2 are all fixed beforehand.

All that matters is whether the final selection is within or outside the first set of boxes selected. The final choice of the box is done completely at random, as no further information is available. The only question is whether the contestant should stay with the original set or switch to the other group.

To understand the situation at hand, look at it from the point before the boxes are opened. If you stayed within, you are in a group whose probability (of containing the prize) is $\frac{n_1}{n}$ and the switch will take you to a group whose probability is $\frac{n_2}{n}$. Now we incorporate the additional information provided by the open boxes and see that, given that we are in the correct group, the probability of correctly identifying the box is $\frac{1}{n_1-k_1}$ or $\frac{1}{n_2-k_2}$ depending on whether it is in the first or second group.

Now, assume that you use a random mechanism to decide to switch or not with *p* being the probability of switching. With this strategy, the probability of winning the prize is given by

$$\begin{aligned} P(W) &= \left(\frac{n_1}{n}\right)(1-p)\left(\frac{1}{n_1-k_1}\right)+\left(\frac{n_2}{n}\right)p\left(\frac{1}{n_2-k_2}\right) \\ &= \frac{n_1}{n(n_1-k_1)}+\frac{1}{n}\left(\frac{n_2}{n_2-k_2}-\frac{n_1}{n_1-k_1}\right)p \end{aligned}$$

Clearly *p* should be taken as 1 if $\frac{n_2}{n_2-k_2} > \frac{n_1}{n_1-k_1}$, or equivalently decide to switch if $\frac{k_1}{n_1} < \frac{k_2}{n_2}$. The winning probability then becomes

$$\frac{1}{n}\max\left(\frac{n_1}{n_1-k_1}, \frac{n_2}{n_2-k_2}\right)$$

The classical problem is the special case with $n_1 = 1$, $n_2 = 2$, $k_1 = 0$ and $k_2 = 1$. Thus $p = 1$ and $P(W) = \max(\frac{1}{3}, \frac{2}{3}) = \frac{2}{3}$.

The variation is the case with $n_1 = 2$, $n_2 = 1$, $k_1 = 1$ and $k_2 = 0$. Thus $p = 0$ and $P(W) = \max(\frac{2}{3}, \frac{1}{3}) = \frac{2}{3}$.

As an example, suppose there are 10 boxes and the contestant is asked to select 3 boxes. The host then opens one of the selected boxes and 4 of the unselected boxes. Here $n = 10$, $n_1 = 3$, $n_2 = 7$, $k_1 = 1$ and $k_2 = 4$. $\frac{k_1}{n_1} = \frac{1}{3} < \frac{4}{7} = \frac{k_2}{n_2}$ so one should switch. The winning probability is $\frac{1}{n}(\frac{n_2}{n_2-k_2}) = \frac{1}{10}(\frac{7}{3}) = \frac{7}{30}$. If the host opens two out of the three selected boxes, with everything else unchanged, then $\frac{k_1}{n_1} = \frac{2}{3} > \frac{4}{7} = \frac{k_2}{n_2}$, so one should not switch. The probability of winning will then be $\frac{1}{10}(\frac{3}{1}) = \frac{3}{10}$.

5. Extension to Unequal Likelihood

Now consider the situation where the assumption of equal likelihood is removed and is replaced by an arbitrary probability vector **p**. As earlier, you select n_1 boxes, and after you have selected the boxes, the host opens k_1 of the n_1 you selected and k_2 of the $n_2 = n - n_1$ you did not select, all of which are empty. For the sake of simplicity, we shall assume that for the host, all the choices of opening boxes would be equally likely. Otherwise the problem will change into one of a game-theoretic nature, and we have to decide whether or not the host is to be considered as a malevolent opponent.

From the discussions and the conclusions of Section 2, it is clear that the contestant's best strategy is one of the two given below:

1. Select the n_1 boxes with the highest probabilities, choose not to switch to the other group, and after the host is done with opening boxes, pick the box within the closed ones of the originally selected group with the highest probability.

2. Select the n_1 boxes with the lowest probabilities, then switch to the other group, and pick the box within the closed ones of the new group with the highest probability.

For the sake of convenience, assume that the probabilities are in ascending order, that is $p_1 \le p_2 \le \cdots \le p_n$.

Suppose the contestant uses Strategy 1. If the prize is in the unselected group, the winning probability is zero. If the prize is in the selected group, assuming it is in Box $n_2 + i$, $1 \le i \le n_1$, the contestant wins if and only if the numbers of the boxes that the host leaves closed are all less than $n_2 + i$. As the choices of open boxes in the unselected group are immaterial, the probability of this happening, given the prize is in Box $n_2 + i$, is $\binom{i-1}{n_1-k_1-1}/\binom{n_1-1}{k_1}$, where $\binom{m}{r}$ is taken to be zero whenever $m < r$. Thus, if the contestant uses Strategy 1, the probability of winning is given by

$$P_1 = \frac{1}{\binom{n_1-1}{k_1}} \sum_{i=1}^{n_1} \binom{i-1}{n_1-k_1-1} p_{n_2+1}.$$

Similarly, it is easy to show that if the contestant uses Strategy 2 the probability of winning is given by

$$P_2 = \frac{1}{\binom{n_2-1}{k_2}} \sum_{i=1}^{n_2} \binom{i-1}{n_2-k_2-1} p_{n_1+1}$$

If $P_1 > P_2$ use Strategy 1; otherwise use Strategy 2. The probability of winning the prize is given by $\max(P_1, P_2)$.

When all the p_i's are $\frac{1}{n}$,

$$P_1 = \frac{1}{n\binom{n_1-1}{k_1}} \sum_{i=1}^{n_1} \binom{i-1}{n_1-k_1-1}$$

The sum, by a simple application of the binomial theorem, reduces to $\binom{n_1}{n_1-k_1}$, and hence

$$P_1 = \frac{\binom{n_1}{n_1-k_1}}{n\binom{n_1-1}{k_1}} = \frac{n_1}{n(n_1-k_1)}$$

as seen in Section 4. Similarly, P_2 reduces to $\frac{n_2}{n(n_2-k_2)}$ when all the p_i's are $\frac{1}{n}$.

Note that if the host is given free choice of doors to be opened, he can deliberately thwart the contestant's strategy by always leaving the highest probability box closed. If this happens, the contestant will invariably fail to win the prize except when it is in the highest probability box. If the host is trying to lower the probability that the prize is won, the contestant must try to out-guess him. This gives the problem a game-theoretic flavour and complicates the issue, which is why it was decided that the choice of doors to be opened is made completely at random.

5.1. An example

Let $n = 13$, $n_1 = 5$, $n_2 = 8$, $k_1 = 3$ and $k_2 = 5$. Then

$$P_1 = \tfrac{1}{4}(p_{10} + 2p_{11} + 3p_{12} + 4p_{13})$$

and

$$P_2 = \tfrac{1}{21}(p_8 + 3p_9 + 6p_{10} + 10p_{11} + 15p_{12} + 21p_{13})$$

so that

$$P_2 - P_1 = \tfrac{1}{84}(4p_8 + 12p_9 + 3p_{10} - 2p_{11} - 3p_{12})$$

Let

$$\mathbf{p_1} = (.021,.022,.023,.024,.025,.026,.029,.03,.04,.05,.2,.25,.26)$$

and

$$\mathbf{p}_2 = (.021,.022,.023,.024,.025,.026,.029,.135,.136,.137,.138,.139,.145)$$

Then for $\mathbf{p} = \mathbf{p_1}$, $P_1 = \frac{14}{25} > P_2 = \frac{583}{1050}$ and for $\mathbf{p} = \mathbf{p}_2$, $P_1 = \frac{141}{400} < P_2 = \frac{3}{8}$. So in the first case the contestant should select Boxes 11, 12 and 13 (the three boxes with the highest probabilities) and not switch when the opportunity is offered later; the winning probability is $\frac{14}{25}$. In the second case she should select Boxes 1, 2 and 3 (the three boxes with the lowest probabilities) and switch later; the winning probability in this case is $\frac{3}{8}$.

6. Concluding Remarks

A natural extension that springs to mind is to allow more than one box to contain a prize. But such a case can be reduced to an already covered case by combining theses boxes into a single box and assigning to it the sum of the probabilities of the original distinct boxes that are combined.

Here is another possible variation to the case where one box is chosen and all but two boxes are opened by the host. Suppose the contestant is given the choice probability set $Q = \{q_1, q_2, \ldots, q_n\}$ and is required to use it. It is up to her to assign these probabilities to the boxes as she sees fit. This time it is difficult to avoid the computation of the β_i's. $\sum_{i=1}^{n} q_i \beta_i$ is maximized by associating the largest q_i with the largest β_i, the second largest q_i with the second largest β_i, etc. and the smallest q_i with the smallest β_i.

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EFFECTS OF SAMPLE CONDITIONS ON MULTI-PARTICLE SIZE ANALYSIS USING LASER DIFFRACTION TECHNIQUE

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Abstract: The basis of laser diffraction for particle size analysis is that the samples are subjected to an intense, monochromatic, coherent light source, which they diffract, where the resulting scattering data reflects their sizes. This paper looks at the experimental conditions when conducting analysis of samples to attain accurate and reproducible results. As trivial as they may seem, these little details are vital to attaining consistency and reproducible data. Examples of these conditions are: circulation on/off, circulation speed, turbulent or laminar flow, ultrasonic on/off, ultrasonic strength, sampling and sample preparation prior to analysis.

Keywords: Particle size analysis, Light diffraction, Sample preparation and analysis.

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1. Introduction

The basis of laser diffraction-based particle size analysis is the scattering of incident light upon the particles. The light scattered off the particles will be at various angles, which are detected at these angles. The intensity of the respective scatterings indicates the particle size. The larger the particles, the narrower the scattering angles and more intensely the light scatters, as opposed to smaller particles that scatter light at wider angles producing less intense illumination as shown in Figure 1. It is with these very well known trends that instruments based on laser diffraction are used to determine particle sizes and their distribution.

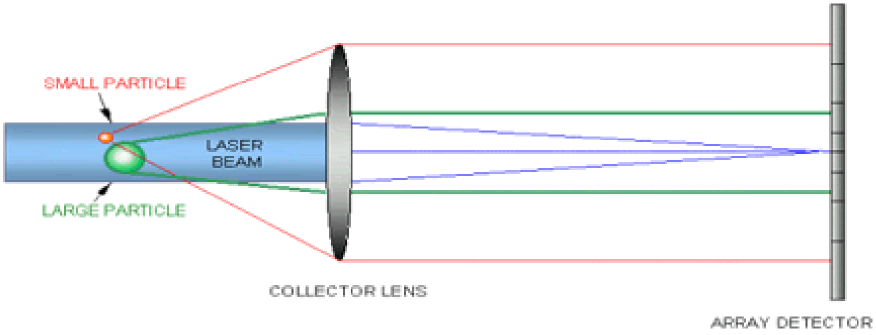
A typical system consists of a laser and/or light source to provide the source of monochromatic, coherent intense light; a series of detectors to detect the scattered light (noting the angle at which they scatter and the intensity) and some sort of analysis and sample presentation system to ensure that the material under test passes through the laser beam as a homogeneous stream of particles with a reproducible state of dispersion.

In laser diffraction, particle size distributions are calculated by comparing a sample's scattering pattern with an appropriate optical model. The two most recognized models are based on the Fraunhofer theory and the MIE theory. Today, the latter model is preferred as IT technology has improved tremendously (Horiba, Instruction Manual).

The type of Low Angle Laser Light Scattering (LALLS) implemented for the HORIBA LA-920, the particle size analyzer used for this paper, is one of static diffraction.

The intensity of the scattered light indicates the relative amount of particles present. As the particle size gets closer to or less than the wavelength of light incident upon it, more of the light is scattered to higher angles, which causes backscatter. The MIE theory takes into consideration this factor and requires the use of information about the particles' optical properties (to be more specific, its refractive index). In order to make particle size measurements, the light intensity pattern over the full angular range is required. When the particle size is larger than the wavelength of the light incident upon the particle, the MIE theory converges with the Fraunhofer approximation (Kelly and Etzler).

Figure 1 Static light scattering (Horiba, 2009).



2. Sampling and Decision

Ensuring that an adequate representative sample is obtained from a batch is extremely important. To obtain a good representation of the available sample, the batch must be well or evenly mixed or dispersed. The particle size distribution in the small sample (usually in mg) should be similar to the particle distribution held in the larger/original batch. It is a well-known fact that the particle size distribution of the particles on the surface of a batch might well be different from the particle sizes within or at the bottom of the batch. Particles of smaller sizes and heavier weights are expected to settle at the bottom of the batch, an occurrence termed ‘segregation’. There are a number of dispersive methods: sieving (for solid particles); using better dispersants (for suspensions); ultrasonification (for suspensions) and using circulation (for suspensions). Two examples of dispersants used for this paper are distilled/de-ionized water or NaHMPo (Sodium Hexa-Meta-Phosphate).

The function of a dispersant is to thoroughly disperse the powder sample. It should also act to prevent any form of coagulation and clumping of the powder samples. It should act to increase interaction between the powder and itself to fully disperse the individual particles. One of the available dispersion methods provided by LA-920 is the ultrasonic energy from the internal ultrasonic unit under the dispersion bath. This acts to disperse the particles. How long the ultrasonic unit is switched on must be controlled to make sure that it doesn’t cause damage to the particles. To determine the suitable time required calls for a trial and error run varying the time at which the ultrasonic probe is left on.

One of the more basic ‘glitches’ that is more likely to occur is the measurement of agglomerates instead of the individual particles. In order to achieve a more accurate result, optimum dispersion must be attained, and to do so factors such as the dispersant used and the dispersion method must be chosen carefully.

In practice, for consistent and reliable data measurement, there must be a development and hence universal application of Standard Operatiing Procedures (SOP). The importance of such procedures is evident in industries such as the pharmaceutical manufacturing areas. Laser diffraction, as has been mentioned numerous times, operates as a sizing technique by predicting the light scattering behavior of particles; the quality of this prediction determines the accuracy of the size determination (Jones, 2003).

3. Statistical Information

The mean is an average of some data. There are a number of ways a 'mean' can be calculated. The method used for the purpose of this paper is the D[4,3] method. D[4,3] is the De Brouckere Mean Diameter, which represents the Volume or Mass Moment Mean. Equation (1) below can be used to compute the mean. This formula indicates around which central point of the frequency the volume or mass will rotate. This represents the center of gravity of the distribution concerned. The main advantage of the formula is that it doesn't require the actual number of particles present in the sample. The LA-920 particle size analyzer calculates the distribution based around volume terms and the D[4,3] is reported in a distinct and prominent form.

$$D[4,3]=\frac{\sum d^4}{\sum d^3}=\frac{1^4+2^4+3^4}{1^3+2^3+3^3}=2.72 \qquad (1)$$

The median is the value of the particle size that splits the whole population into two even or equal groups, such that there will be 50% of the population on the left and right of the dividing line. The mode is the most common value of the frequency distribution, on the graph it will correspond to the peak. For a normal (Gaussian) distribution the mode will coincide with the median. Results however can be bi-modal, where the median does not coincide with the mode at all. Standard Deviation (S.D.) from the mean is used widely in statistics to indicate the degree of dispersion (how far the value strays from the mean or central value). The smaller the value of the S.D. the better, as this shows how precise the results are. A deviation is calculated by taking the difference between each deviated point with the mean or central value. This difference is then squared. The average of the squared values taken for the whole measurement is termed the variance.

4. Equipment

The equipment used is the Horiba LA-920 (Laser Scattering) with the accompanying software. The principle of measurement is based on MIE theory, and the range of particles that can be measured is from 0.02μm to 2000μm. The optical system consists of two light sources and photocell detectors. The detectors are placed in a range from in front of the light sources (0°) rotating through an angle of approximately 140° towards the light sources. The light source is a 632.8nm Helium-Neon laser and a 1mW Tungsten lamp. The photocell is made up of a 75-division, ring shaped silicon photo-detector array and 12 Silicon photo-detectors. The sample ultrasonic system consists of an Ultrasonic probe of 30W and frequency 22.5kHz. It also features a centrifugal pump that is capable of discharging 8L/min (for mediums which are distilled water). The circulation strength of the centrifugal pump can be varied from 1-15.

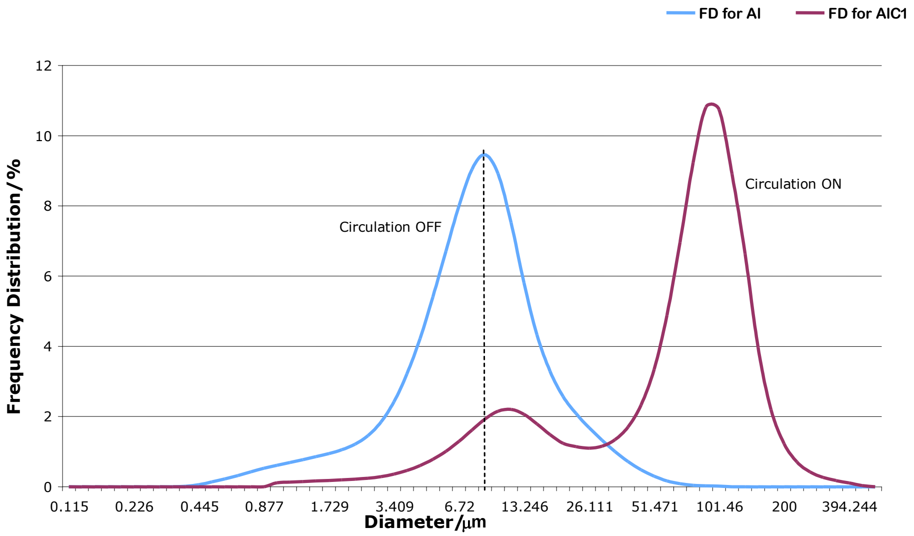
5. Results and Discussion

5.1 Alumina powder

In this paper, the powder used for analysis is alumina (Riedel Deltoen 11028, Lot. 22250). The sample was subjected to different experimental conditions to see which conditions yielded the best result or gave the truest value of the particle sizes being measured. The different conditions are: different ultrasonification time (external ultrasonification), different circulation speed (internal circulation), stirring before particle size analysis, different dispersants, and whether or not settling time is allowed after ultrasonification prior to particle size analysis.

Figure 2 shows the first result of analyzing alumina taken straight from its container. It shows the frequency distribution for the samples that have undergone internal circulation and no internal circulation during measurement. Both samples are not subjected to any sample preparation (ultrasonification or magnetic stirring) beforehand. After circulation, it appears that the particles sizes get bigger. One can understand that after circulation one can get a bimodal or even trimodal distribution, as it could be argued that the motion breaks the agglomerates into smaller size range. It seems that in the example presented it is doing both - circulation seems to be breaking the agglomerates into two distinct sizes and then agglomerating again. This observation needs to be further investigated.

Figure 2 Frequency distribution for alumina without sample preparation with internal circulation on and off during measurement.



5.2 Sample preparation through ultrasonification

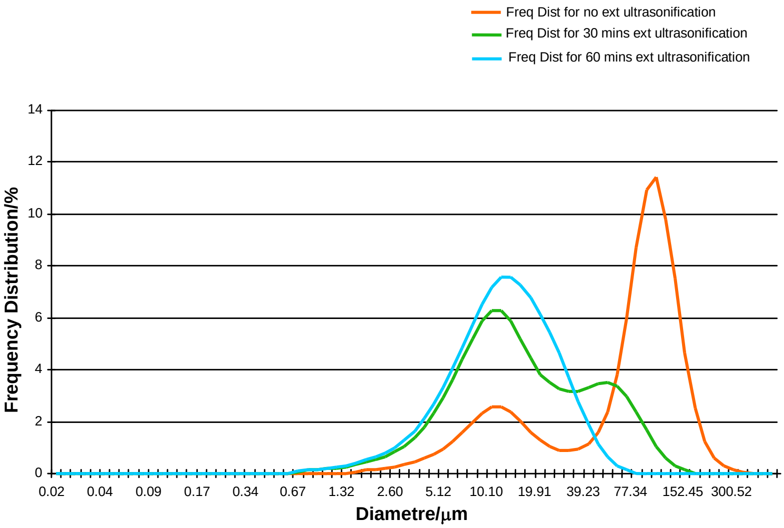
Figure 3 shows the sample subjected to different external ultrasonification durations. The different durations were: no external ultrasonification, 30 minutes and 60 minutes external ultrasonification. The distributions obtained were compared to see which ultrasonification duration gave the best result/representation of the sample size distribution. For alumina, with increasing ultrasonification duration an improvement is shown in the distribution. Subjecting the sample to no external ultrasonification and 30 minutes external ultrasonification didn't yield a true enough representation of the sample particle sizes.

It can be seen that increasing the external ultrasonification time from 0 to 60 minutes gave a significant difference in particle dispersion. It was found that increasing the ultrasonification time from 60 minutes to 90 minutes did yield a slightly wider distribution (indication of better dispersion). It should be noted however from the graph that 60 minutes is a sufficient enough duration to obtain optimum dispersion using ultrasonification.

5.3 Internal circulation speed applied to sample

Figure 4 shows the results obtained from analyzing a sample of alumina dispersed in distilled water subjected to external ultrasonification for 60 minutes but with 3 different internal circulation speeds (5, 7 and 9) and without circulation. It can be seen that with increasing circulation speed the distribution becomes narrower (higher peak), the standard deviation is less and a more accurate mean of the true representation of the mode of the particle size is obtained. The narrowing effect of the curve is due to the centrifugal effect provided by the centrifugal pump. With higher circulation speeds, the particles are better circulated around the system and hence the detection of the particles is more thorough.

Figure 3 Frequency distribution of particles size distribution of alumina with different ultrasonification times of no external ultrasonification (bottom curve on left), and 30 and 60 minutes (top curve on left) external ultrasonification. Circulation speed was 5.



5.4 Standing after external ultrasonification

A bi-modal distribution is obtained when the sample is allowed to stand after external ultrasonification before stirring and feeding to the particle size analyzer. What is basically done during this part of the analysis is that first the sample is put in an ultrasonic bath for 30 minutes, and then is allowed to stand for 30 minutes before it is magnetically stirred, after which it is fed into the particle size analyzer. The distribution indicates the presence of two extreme sizes in the sample. If the sample is stirred immediately and fed into the particle size analyzer after ultrasonification, the distribution obtained consists of a single mode. This result indicates the presence of a wider range of particles as oppose to just two extreme sizes as shown in Figure 5.

Figure 4 Frequency distribution for alumina with internal circulation speeds 5, 7 and 9 (top curve).

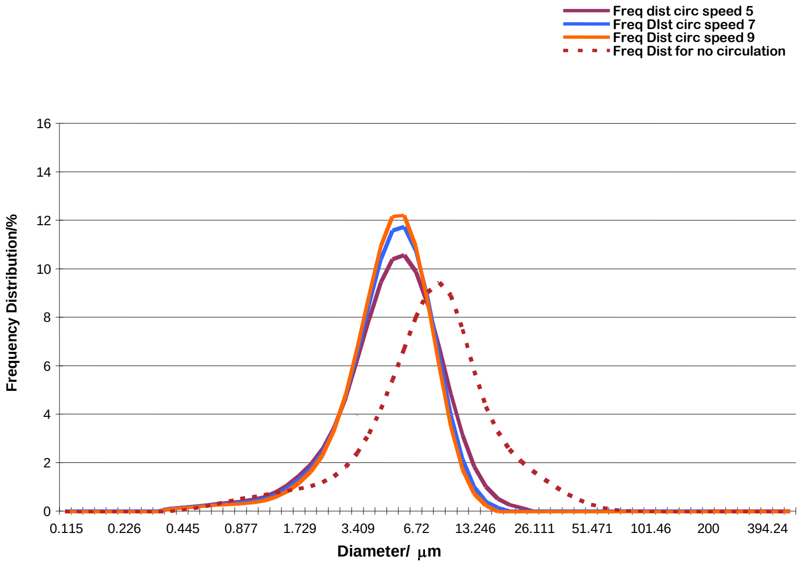
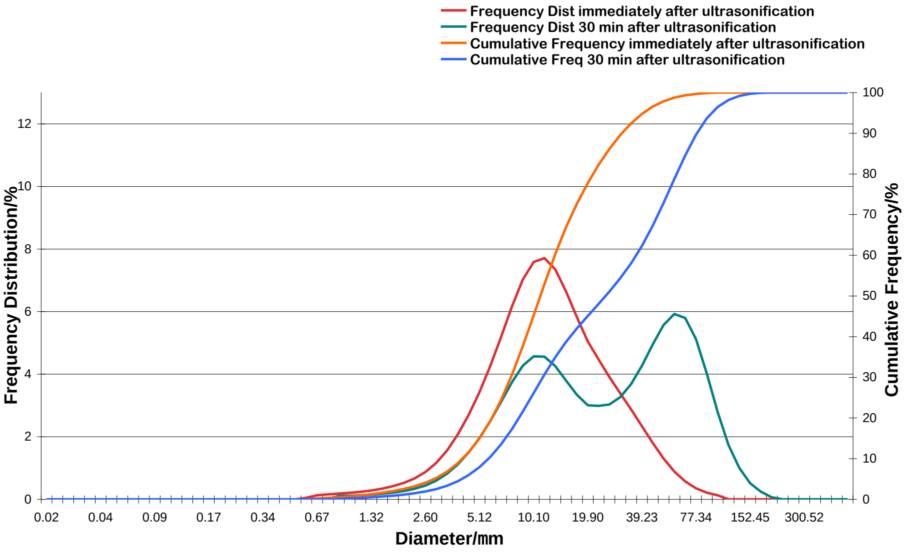


Figure 5 Frequency distribution and cumulative frequency of alumina for samples (subjected to 30 minutes of external ultrasonification) that were allowed to stand (lower curves) and instantly fed to the particle size analyzer (upper curves)) with internal circulation speed 5.



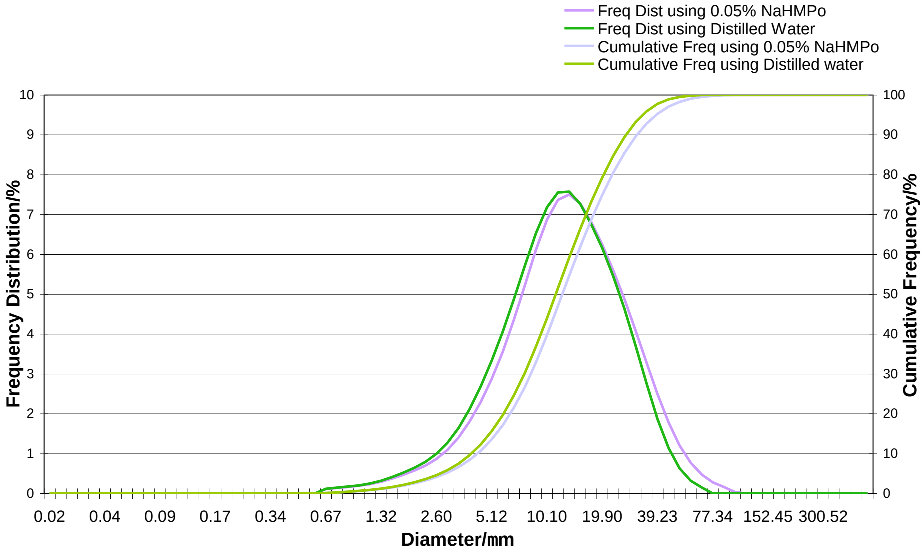
5.5 Different concentrations of NaHMPo

Different concentrations by weight of NaHMPo were used to disperse the alumina. The concentrations were: 0.01%, 0.05%, 0.1%, 0.15% and 0.20%. The concentration that yielded the smallest particle size distribution was compared against the result obtained using distilled water. The best concentrations when using NaHMPo as a dispersant is 0.05% wt NaHMPo. Figure 6 shows that distilled water has a distribution that is similar to the distribution that 0.05% wt NaHMPo yields with the exception that it is shifted slightly to the left. This indicates that it is slightly more efficient in separating the alumina particles.

6. Conclusion

One of the objectives of carrying out the analysis on alumina was to form or develop the most systematic and effective way to investigate a distribution of particle sizes. The most effective series of steps is: to use appropriate dispersant (distilled water in this case), to subject the sample to an ultrasonic bath for at least one session (60 minutes in this case), to thoroughly stir the sample, and to have internal circulation speed on during analysis. It is also important to note that, prior to stirring the sample in suspension, it should not be allowed to stand.

Figure 6 Frequency distribution and cumulative frequency of particles size distribution of alumina with 0.05% NaHMPo (lower curves) and distilled water (upper curves). Internal circulation speed is 5.



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ELECTROCHEMICAL SENSORS: SOME RECENT DEVELOPMENTS

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Abstract: Development of reliable detection methods for various substances in our environment, such as food contaminants, water pollutants and toxic gases has taken a significant place in science and technology. Among various sensors available for such detections, electrochemical sensors, which measure concentration-dependent electrical properties, preferably potential or current, have become very attractive. The unique advantages of these sensors are selectivity, sensitivity, simplicity, ability of automation, cost-effectiveness, etc. Therefore, electrochemical sensors are fast gaining popularity in many fields, including medicine, environmental monitoring and corrosion. Recently designed electrochemical sensors include implantable glucose sensors for continuous monitoring of glucose levels in humans, probing cancer cells, accurate measurement of dissolved gases in water and toxic gases in the environment, and wireless corrosion sensors to monitor corrosion in concrete structures, airplanes and heavy-duty automobiles. The physical size, shape and components used in their construction depend on the intended use. These sensors are marketed in many attractive designs, yet their functioning and performance are based on the fundamentals of electrochemistry.

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1. Introduction

An electrochemical sensor is a device which quantitatively determines an analyte by measuring the electrical signal produced by the sensor. The electrochemical measurement should therefore be concentration-dependent, such as current or potential. Amperometric sensors and potentiometric sensors are two common types of electrochemical sensors. The former measures current at a constant potential, while potential is measured as the electrical property in the latter type. It is not necessary that the magnitude of the electrical property is linearly related to concentration. However, a linearized relationship between a derivative of the concentration of the analyte and a derivative of the electric property is often used in calibrating a sensor. Calibration of the sensor is an essential step in quantitative analysis, which is performed to check whether the device properly functions according to the theoretical or known relationship.

Electrochemical techniques offer many advantages such as selectivity, sensitivity, simplicity, ability of automation, ability of hyphenation and cost-effectiveness (Kissinger and Heinemann, 1996; Xiao et al., 2007). Electrochemical sensors have electrode arrays with two, three or more electrodes, which are called the auxiliary electrode, reference electrode, and working electrodes. These sensors are used to determine the concentrations of various analytes in testing samples, mainly fluids and dissolved solid materials. Further, electrochemical gas sensors are well known for detecting and quantifying concentrations of toxic gases such as carbon monoxide, hydrogen sulphide, nitrogen oxides, chlorine and sulphur dioxide (Jasinski, 2006; Miura et al., 2003; Wama, 2007). Recent applications of electrochemical sensors are found in many sectors: Occupational safety, medical technology, process measuring engineering, environmental analysis, agriculture, etc.

The electrodes of an electrochemical sensor provide a surface at which an oxidation or a reduction reaction occurs to provide a mechanism whereby the ionic conduction of an electrolyte solution in contact with the electrodes is coupled with the electron conduction of each electrode to provide a complete circuit. In an electrochemical gas sensor, the gas to be quantitatively analyzed typically passes from the atmosphere into the sensor through a gas-porous or gas-permeable membrane to the working electrode where a chemical reaction occurs. Electrochemical sensors are equipped with electrical conductors to allow electrical signals to be transmitted to and from electrodes contained within the sensor. An electrochemical sensor is typically comprised of three parts: a specimen-sensing ion electrode (referred to as the working electrode in certain applications), a reference electrode, and an amplifier that translates the signal into useable information that can be read.

This paper mainly focuses on some recent advances in electrochemical sensors with emphasis on the use of basic chemistry for such developments together with a summary of common electrochemical sensors that have been in use for many decades. In addition to amperometric and potentiometric sensors, the discussion of this paper also includes resistance/impedance sensors, which are becoming increasingly popular.

2. Potentiometric Sensors

2.1 The pH electrode (sensor)

The degree of acidity (or alkalinity) of aqueous solutions may be expressed as pH, which is defined by the equation:

$$pH = -\log a_{H^+} \qquad (1)$$

where a_{H^+} is the hydrogen ion (H^+) activity, which is approximately equal to the concentration of H^+ for dilute solutions. The pH of a given solution is obtained by measuring the potential (voltage) E of the electrode, and converting it to pH using equation (2).

$$E = E^o + 0.059 \log [H^+] = E^o - 0.059\, pH \qquad (2)$$

The simplest device for measuring pH is a strip of paper impregnated with a dye whose color changes with pH. For more precise measurements of pH (to 0.01 of a unit), the reversible electromotive force (emf) cell is used. It consists of two electrodes, the pH-sensing electrode and a reference electrode. The glass membrane at the tip of the electrode assembly allows the transfer of H^+ ions between the measuring solution and a standard solution of H^+ placed inside the electrode.

The pH electrode is one of the most commonly used electrochemical sensors. It finds applications in medicine (biological fluids, pharmaceutical preparations), agriculture (soil pH), industry (quality control), environmental science (quality of water), etc. (Spitzer and Meinrath, 2002).

Traditional pH electrodes have a glass bulb at the tip where the glass membrane is sensitive to pH changes. Flat surface pH electrodes are now available for measurements in viscous solutions.

2.2 Ion-selective electrodes (ISE)

Ion-selective electrodes have a special membrane to improve selectivity toward a particular analyte in the presence of others. These membranes allow a particular ion to go through, generating a potential difference, which is quantitatively related to the activity of the selective ion according the Nernst relationship as shown in equation (3),

$$E = k + (0.059/Z_i) \log C_i \qquad (3)$$

where E is the potential measured, Z_i is the charge of the ion, k is a constant and C_i is the concentration of the selective ion. Ion-selective electrodes are available for many anions such as F^-, Br^-, Cl^-, NO_3^-, CO_3^{2-} and S^{2-}, and many cations such as Na^+, Pb^{2+}, Cd^{2+} and NH_3 (Buck and Lindner, 2001; Rundle, 2006). Their applications are thus unlimited.

Ion-selective electrodes offer many advantages as stated below. It should be noted that the *p*H electrode is also an ion-selective electrode although it is usually treated separately.

1. They can function in turbid solutions.
2. They are sensitive to only free ions.
3. They offer broad linear dynamic ranges, as a logarithmic scale [equations (2) and (3)] is used in the construction of calibration curves.

Nevertheless, disadvantages of these electrodes include not providing specific, temperature-independent measurements, and frequent calibrations.

3. Amperometric Sensors

3.1 Introduction

Selectivity of amperometric measurements can be achieved by the selection of a suitable operational potential. Chemical modification of the surface of the sensor electrode also improves the selectivity of the sensor. Further, ion-selective electrode technology has been combined with amperometric detection to further improve the performance of electrochemical sensors (Senda et al., 1999).

The O_2 sensor, a common amperometric device, is presented in this section in order to clarify basic practical aspects of amperometric sensing. Different types of amperomertic sensors are presented in latter chapters under specific themes.

3.2 Determination of oxygen

Measurements of oxygen are important in many diverse fields as shown below:

1. Assessment and monitoring of patient health (by monitoring partial pressure of O_2)
2. Monitoring of activity of sewage treatment plants
3. Spoilage determination and quality control of food
4. Biosynthetic pathways of some pharmaceuticals
5. Environmental control

Before the adventure of polarography, only classical wet-chemistry techniques were available for the determination of O_2. The first standard test for dissolved O_2 was reported in 1888 by L.W. Winkler (Winkler, 1888). It is a titrimetric method based on three redox reactions: Reduction of oxygen with concomitant oxidation of Mn^{2+} to Mn^{4+}; re-reduction of Mn^{4+} to Mn^{2+} with oxidation of I^- to I_2; and the $I_2/S_2O_3^{2-}$ standard reaction. The volume of a standard thiosulfate solution required to complete the reaction is then quantitatively related to the concentration of dissolved O_2. This method was susceptible to many errors because ions such as Fe^{2+} and SO_3^{2-}, suspended solids, and organics would interfere. Consequently, there have been many modifications over the years to improve the accuracy of detection (American Public Health Association, 1971).

Heyrovsky's work with the dropping mercury electrode (in polarography) in the early 1920's was conducive to the acceptance of electrochemistry as a routine analytical tool. He was able to identify the direct relationship between dissolved O_2 concentration and limiting current (Kolthoff and Lingane, 1952).

3.3 Clark electrode

This is one of the first membrane-covered amperometric oxygen sensors, invented by Clark in 1954, and patented in 1956 (Clark, 1993). It consists of a permeable membrane selective to O_2. The mechanism of detection is amperometry, where the current associated with the electrochemical reduction of O_2 (*i.e.*, the extent of the reaction)

$$O_2 + 2H^+ + 2e \rightarrow H_2O_2$$

at a constant potential is used for the determination of the concentration of O_2 in water, while the extent of the chemical reduction of O_2 is used in the classical method.

There are many advantages of using the O_2 electrode as compared to the chemical method. It can be used as a sensor, recommended for field measurements, is fast and simple and can be interfaced to a computer. However, the chemical method is more reliable, and still in use.

The major components of the oxygen electrode are the O_2 permeable membrane (*e.g.*, Teflon, polyethylene, silicone rubber, cellophane or silica gel), a filter paper soaked with the electrolyte, a platinum working electrode and a silver reference electrode (Sawyer et al., 1959; Falck, 1997). Two-point calibration is usually performed for accurate measurements of dissolved O_2 using an air-saturated solution (maximum response) and a deoxygenated solution (minimum response). In certain instances, oxygen meters are calibrated against air.

Many O_2 sensors have been developed using a variety of materials and configurations, yet using the same chemical principle used by Clark. Modern commercial models have digital display of the measurement in ppm O_2 or % O_2 saturation with a high resolution of 0.01 mg dm^{-3} together with automatic temperature compensation. Water-resistant electrodes are also available with 4 to 10 m long cables for direct measurements of O_2 in water at different depths.

4. Electrochemical Sensors in Medicine

Many potentiometric and amperometric sensors are used in medicine for monitoring the health of humans/patients. This section mainly focuses on some recent advances in electrochemical sensors in the field of medicine.

4.1 Three-function blood gas analyzer

This is a single device to measure *p*H, O_2 and CO_2 in biological fluids. CO_2 and *p*H are measured with the *p*H electrode while O_2 is measured with the Clark electrode.

Initial discovery of CO_2 measurements was made in the 1950's by observing the change in *p*H when the glass bulb of the *p*H electrode was wrapped with a rubber glove and CO_2 was allowed to diffuse through it (Stow and Randall, 1954; Severinghaus and Bradley, 1958). Optimization of CO_2 sensing for levels similar to that of blood and spinal fluid (10-25 mM) was performed by the addition of HCO_3, which improves stability and increases sensitivity. The basic theory used in the 1950's is still used in modern designs of blood gas analyzers (Severinghaus, 2004).

4.2 Glucose sensor

Electrochemical detection of glucose at platinum was a unique finding, which used the catalytic activity of Pt electrodes. Pt shows complicated electrochemistry such as formation of platinum oxide and subsequent reduction, hydrogen adsorption followed by its desorption.

There is only a very thin potential window where Pt does not show its surface chemistry. Oxidation of glucose under certain experimental conditions within this potential region made it possible for its electrochemical detection (Castro Luna, 1991).

G. S. Wilson is a pioneer in the development of a glucose sensor based on the above reaction. For sensitive detection, oxidation of glucose to gluconolactone is mediated by the flavine adininie dinucleotide active site of the glucose oxidase enzyme. Many advances have been developed over the past years by different research groups (Bott, 1998; Zhu et al., 2002), such as a glucose microsensor designed for *in vitro* and short-term *in vivo* evaluation (Ward et al., 2002). This design has an inner membrane and an outer membrane (polyurethane with hydrophilic segments and polyethersulfone membrane stabilized with a trimethoxysilane or polyurethane without hydrophilic segments with cellulose acetate and nafion). The function of the inner membrane is selectivity, while that of the outer membrane is specificity. Glucose meters based on this chemistry are now commercially available.

A recent advancement is the design of an implantable glucose sensor that is able to continuously monitor the glucose level with a reasonable accuracy. This sensor provides an ideal size and geometry for optional long term implantation and linear responses over the concentration ranges of interest. The sensor includes an elongated body to support the working electrode within which the enzyme is immobilized to form an enzymatic indicating surface. A permeable synthetic polymer membrane is applied over the sensor body to protect the enzyme and regulate diffusion of the analyte through it, to ensure linearity of sensor response. These sensors are of flexible design and can be implanted using a catheter (Wilson et al., 1991).

A novel, attractive design is the development of an implantable glucose sensor with an insulin pump. The sensor monitors the blood glucose level, and sends data continuously to a device attached to the sensor. It then controls the insulin pump wirelessly, which delivers insulin as needed through a soft tube attached to the pump, which can be inserted under the skin. With these developments, new-generation, fully-integrated glucose monitoring systems for the management of diabetes will soon be commercially available.

4.3 Sensors for cancer cell detection

Electrochemical technology has advanced to a level that amperometric detection of cancer cells has been possible. In one application, the redox reactions occurring in individual cancer cells have been probed using scanning electrochemical microscopy (SECM) with an ultramicroelectrode (UME) (Liu et al., 2002; Feng et al., 2003). For this purpose, rate constants of the redox reactions in cancer cells which are in contact with the chemical mediator, menadione, where the dione and the diol form are at equilibrium at a constant potential under controlled *p*H, are measured. The magnitudes of the rate constants depend on the activity of protein kinase Cα, an enzyme involved in cancer cell formation, and hence they are different for cancerous and non-transformed cells. SECM allows the tip of the UME to be scanned over the entire area at the potential required for the redox reaction to occur to obtain the rate constants over a selected region. These are then transformed to obtain a 2-D image, called a redox map. The variation in intensity of the redox map can be used to identify cancer cells (Rotenberg and Mirkin, 2004).

Future applications of this type of research would be to search for better mediators to maximize the differences between the rate constants of cancerous and non-transformed cells for more sensitive detection.

5. Sensors for Pesticide Monitoring

Most pesticides are organic in nature, causing some limitations in electrochemcal detection. They show sluggish electrochemical kinetics and solubility problems in aqueous media. These limitations can be addressed by using suitable catalytic materials to modify the electrode surface and using mixed aqueous/nonaqueous solvent systems, respectively. Chemical modification of electrodes would also overcome undesirable surface reactivities (*e.g.*, oxide formation on metallic electrodes such as Pt), high background noise, electrode fouling and lack of selectivity in certain instances. Thus, modification of bare electrode surfaces has become crucial for pesticide sensing.

Some amperometric devices developed based on modified electrodes for detection of pesticides commonly used in agriculture are shown in Table 1 (Priyantha and Weerabahu, 1996; Priyantha and Weliwegamage, 2002; Priyantha et al., 2004; Priyantha and Weliwegamage, 2005; Priyantha et al., 2007; Priyantha and Weliwegamage, 2008). Biosensors have also been successfully used for amperometric detection of pesticides recently (Andreescu et al., 2002; Mazzeia et al., 2004; Hleli et al., 2006). As detection methodologies for many pesticides have already been developed and successfully applied, electrochemical sensors for pesticides will be available in the future.

Table 1 Analytical characteristics of electrochemical detection of some pesticides.

Pesticide	Electrode	Sensitivity (μA/ppm)	LDR (ppm)	MDL (PPM)	Lifetime
Gramoxone	Bare GCE	0.089	32.0 - 65.0	26.8	-
Gramoxone	Stearic acid-coated GCE	0.042	20.0 - 150	6.0	5 weeks
Propanil	CuO-modified CPE	0.003	65.0 - 80.0	45.5	3 weeks
Thriam	Bare GCE	0.004	4.0 - 20.0	26.8	-
Thiram	Stearic acid-coated GCE	0.002	5.0 - 45.0	4.5	5 weeks
3,4-DCA	Bare GCE	1.80	2.0 - 41.5	1.8	NA
3,4-DCA	Stearic acid-coated GCE	1.23	0.08 - 0.80	0.03	5 weeks

6. Corrosion Sensors

Corrosion, *i.e.* degradation or deterioration of metallic surfaces by an electrochemical process, is a natural process which occurs under certain environmental conditions. The rate of corrosion is usually enhanced by the following conditions: humidity, saltiness, dissolved gasses, dissolved ions and temperature. When corrosion proceeds upon exposure of an object to the above conditions, a current (called the corrosion current) is generated and the conductivity of the surface of the object increases. The quantitative relationship between the corrosion current or conductivity/resistance of the surface and the extent of corrosion, measured usually in millinches of the object lost per year (mpy), is used in designing nondestructive corrosion sensors. Additionally, indirect measurements such as the concentration of substances that promote corrosion (*e.g.*, chloride) or that are produced during corrosion can be used to design corrosion sensors.

6.1 Determination of the health of concrete structures

The *p*H of concrete is about 12.5, at which a passivating layer forms on the surface of steel. The passivating film is broken down by two processes: development of an acidic environment when CO_2 mixes with water in the concrete pores, and penetration of chloride ions through the passivating layer which initiates pitting corrosion. This is a worldwide problem because steel reinforcements cannot be inspected visually. Therefore, corrosion often remains undetected. Detection of corrosion in concrete, before structural damage occurs, would thus be important.

An early method of monitoring corrosion of reinforced concrete structures was to use a hand-held meter to measure the electrical potential between a reference electrode placed on the external surface of the concrete structure and the reinforcing steel (as the sensor electrode) by connecting them with a conducting wire. Potentials measured by moving the reference electrode over the surface of the concrete lead to a potential map, which can be used to probe the extent of corrosion (Moller, 1992). As the concrete layer between the reference electrode and steel shows variations in resistance and thickness, the results obtained with this device are not accurate.

This simple design was later improved by having a reference electrode embedded in concrete close to the reinforcing steel. Many designs of embeddable reference electrode assemblies, consisting of metal oxide electrodes in steel casings with alkaline, chloride-free gels are now commercially available (CESCOR srl). The PVC covered sensor used in this design is wrapped around the steel to be monitored. The potential between the steel and the electrode is then measured to determine the level of corrosion. This sensor offers great sensitivity, which makes the sensor suitable for measurements of pitting corrosion of large concrete structures (Ingenieurbüro Wietek).

Another improvement over this sensor is to measure the current due to corrosion (*i.e.* amperometric mode) or to measure fluctuations of potential or current generated spontaneously by the corrosion process (electrochemical noise measurements) (Bjegovic et al., 1999). Polarization measurements can also be employed for the amperometric mode to determine the rate of corrosion with the aid of Tafel slopes (Schiessl and Raupach, 1992).

6.2 Wireless corrosion sensors

Interior corrosion of heavy-duty vehicles, airplanes and concrete structures is usually difficult to detect because the actual damage is not visible. Embeddable wireless corrosion sensors have recently been developed for this purpose. One successful design is to use Zn plated rectangular-shaped metallic surfaces to monitor the extent of corrosion (Allahar et al., 2005). These sensors can be placed under the top paint coating, and then operated through remote-sensing devices. For accurate corrosion rate determination, Tafel slope measurements are made, and converted to the loss of material and decrease in material thickness using software. However, this technology is yet to be tested on a flight.

It has recently been reported that chloride levels in concrete can be determined using a wireless solid state potentiometric sensor. This sensor monitors the level of chloride ingress into concrete bridge decks. The sensor assembly contains a chloride indicator electrode and a radio-frequency identification (RFID) chip that can be made active remotely both to identify it and to indicate chloride concentration levels. This sensor is designed to be inserted in the bridge deck either during the initial construction (or during refurbishment) or in a back-filled core hole (Watters et al., 2003).

7. Solid-State Electrochemical Sensors

Many electrochemical sensors discussed in the earlier sections do have a liquid/solution component, which creates some drawbacks in real applications. Solid-state electrochemical sensors offer many advantages, because they are compact, robust, cost-effective in large-scale production, durable (their life-times can be about 10 years in clean environments) and versatile. On the other hand, they are susceptible to interferents (especially gases), requiring a filter medium to minimize this effect.

Many solid-state electrochemical sensors have been designed using semiconductors as sensing devices. Metal oxides having semiconductor properties can cause ionization of gases at high temperatures changing the conductance/resistance of the oxide. The magnitude of the change is directly proportional to the concentration. This basic theory, *i.e.* the sensitivity of *p-n* junctions of semiconductors to environmental components, has been utilized in designing sensors for gases.

The history of semiconductor-based electrochemical sensors dates back to the late sixties, and many novel designs with improved sensitivity and selectivity are still in development. Gases such as acetone, acetylene, ammonia, carbon monoxide, freons, hydrogen cyanide, etc. have been detected using conductance/resistance sensors made up of specific semiconductor metal oxides. Potentoimetric and amperometric solid-state sensors, consisting of various metal oxides were also later developed (Jasinski, 2006; Miura, 2008). The lambda sensor used to control the air-fuel ratio to maximize the efficiency of combustion in automobiles is a good example of a solid-state potentiometric sensor

8. Conclusion

Electrochemical sensors have been devised to detect toxic gases generated by industrial and anthropogenic activities and vapors from explosives, various biological molecules in the human body, pesticides in water reservoirs, interior corrosion of bridges and concrete structures, etc. The beauty of electrochemical sensors is that they are nondestructive in nature. Many diverse easy-to-use wireless electrochemical sensors are currently being developed. These are designed for the continuous monitoring of the health of humans, of the structural health of concrete constructions, automobiles and airplanes, and of minute levels of toxic gases in the environment. Consequently, electrochemical sensors no doubt have a promising future

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QUALITY OF ORGANIC FERTILIZERS AND RESPONSE STUDIES IN VEGETABLES

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Abstract: A survey study conducted to assess the quality of the different organic fertilizers commonly being used by farmers and others in Brunei Darussalam indicates that the nutrient composition varies among the fertilizers depending on the source. Among the surveyed fertilizers, 8 were found to be purely organic whereas the remaining 11 were not pure but blended with chemical fertilizers in order to increase the content of nitrogen, phosphorus and potassium. Also, the blended organic fertilizers did not meet the specifications with respect to nutrient and organic matter content declared by the manufacturer. Pure organic fertilizers were found to contain high organic matter compared with blended fertilizers. Among pure organic fertilizers, indusol – a bi-product from soybean, cotton seed and sugar beet waste – contains the highest content of nitrogen, followed by quail manure, whereas the lowest was in vermi-compost produced by using sawdust.

Regarding crop response studies conducted in pot, it was found that the growth and nutrient content of tomatoes was higher after the use of organic fertilizers like cattle manure, compost and indusol over control. Similarly, field studies conducted at Kilanas and Lumapus research stations showed an increase in the yield of sweet corn and cucumber after the application of different types of organic fertilizers and a residual effect on subsequent crops tested, namely okra and long bean. It was observed that the organic matter status in soil was improved by organic fertilizer application and the improvement was more marked with the use of pure organic fertilizers – namely poultry manure, indusol and sheep manure – than with blended organic fertilizers.

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1. Introduction

Just like chemical fertilizers, organic fertilizers are used to supply the amount of plant nutrients needed to maximize crop production and net return in agriculture. The major factors that affect the performance of organic fertilizers are composition, source, soil characteristics and management, fertilizer placement and carryover effects. It has been found that microbial activity and biomass are higher in fields with organic amendments than fields with conventional fertilizers (Drinkwater et al., 1995). Incorporating moderate amounts of animal and other organic materials into the field is an established agricultural practice generally recognized to have beneficial effects on physical, chemical and microbiological properties of the soil, as has been documented by research findings (Lin et al., 1973; Chao et al., 1966). Organic matter increases the available phosphorus in the soil through organic anions, preventing P fixation and replacing the P bound to the soil (Nagaraja et al., 1970; Kafkafi et al., 1998).

Manure application in excess of crop needs can cause significant build up of N, P and other ions and salts in the soil. Dormar and Chang (1995) showed that cattle feedlot manure application for 20 years resulted in a significant increase in soil P levels from 9ppm to 1200ppm.

Agriculture and agro-industries produce high quantities of organic wastes that are typically rich in nutrients, which can be well used in agriculture to conserve nutrients as well as to reduce waste discharge and the use of chemical fertilizers. However, use of industrial and municipal wastes as organic amendments may result in undesirable levels of heavy metals. Conacher and Conacher (1998) reported that more than 10% of fruit and vegetable samples such as carrots, potatoes and safflower contained cadmium residues in excess of maximum permitted concentrations in Victoria, Australia. Increasing recognition of the impact of continuous use of chemical fertilizers on sustainability in crop production and on soil properties, has led tp more thrust being given to the use of organic fertilizers under the concept of integrated nutrient management in many developing and developed countries.

In the context of Brunei, poultry manure is the common organic fertilizer used by farmers because of its cheap price and availability. But the use of organic fertilizers from other sources like cattle, sheep, etc. is insignificant due to the non-availability and higher price of imported manures. Besides, the country also imports a variety of pure and blended organic fertilizers, but their quality and safety for use in crop production are unknown due to the non-existence of a Fertilizer Act. In the past, no crop response studies on the use of organic fertilizers have been documented. With this background in mind, a study on evaluating the quality of different organic fertilizers and their crop response was conducted.

2. Materials and Methods

Around 19 types of different organic fertilizers (including both locally produced and imported) were selected to evaluate the quality of nutrients and related parameters through laboratory analysis by following standard methods. Organic matter content was estimated by ashing using a furnace as outlined by Schulte and Hopkins (1996). Total nitrogen was analysed by the kjeldhal digestion and distillation method. For rest of the nutrients, samples were digested using di-acid mixture (nitric acid and perchloric acid) followed by analysis using a UV-spectrophotometer and an Atomic Absorption Spectrometer.

Pot and field trials were conducted on different vegetable crops to study the effect of organic fertilizers on yield. The crops tested include tomatoes under pot study. Two field trials were conducted using sweet corn (*Zea mays*) and cucumber (*Cucumis sativus*) at Kilanas and Lumapus. In this study, 8 different fertilizers including pure and blended organic fertilizers were tested besides chemical fertilizers with a total of 10 treatments and replicated 3 times in RCBD. Subsequently, after the harvest, okra (*Hibiscus esculentus*) and long bean (*Vigna sinensis var. sesquipedalis*) were planted to study the residual effect of previously applied fertilizers.

Yields were recorded and a statistical analysis was made to measure the effects of the treatments. Furthermo

re, an analysis was made of the physico-chemical properties of the soil before planting and after harvest on a treatment-wise basis using standard analytical methods.

3. Results and Discussion

3.1 Evaluation of Organic Fertilizers

The fertilizer analysis in Table 1 indicates that among natural organic fertilizers like compost, vermin-compost, cattle, poultry, sheep and quail manure, the organic matter content was a maximum (83.9%) in cattle manure, followed by sheep manure (81.8%), whereas the minimum of 43.1% was found in compost. Nutrients such as nitrogen, phosphorus and potassium varied from 0.7-8.1%, 0.5-3.8% and 1.3-6.9% respectively. Similarly, the calcium status was in the range 0.7-10.1% while magnesium was 0.5-1.3%. The high content of calcium present in poultry manure was due to the use of lime in the bed material during the rearing process. The lowest calcium content was found in vermin-compost produced using sawdust. With respect to the micronutrients content, the iron content was at a relatively higher level (0.1-0.7%) compared with other micronutrients, except in poultry manure which contained the lowest content of 0.03%. The content of zinc, manganese and copper varied from 41-1635ppm, 45-972ppm and 16-125ppm respectively among the different manures. It can be observed from the analysis results that poultry manure and quail manure contained the maximum levels of zinc and copper. This might be due to the use of trace minerals in feed supplements for the poultry, which was thereby transferred into the faeces being used as fertilizer. The nutrient content of these pure organic fertilizers varies depending upon the source of feeds supplied to the birds or animals and the source of plant materials used in the case of compost and vermin-compost. The study also shows that the compost produced by using vegetable waste contained relatively higher level of nutrients compared with the vermi-compost produced by using sawdust. These results are in conformity with the findings of Titiloye et al. (1985).

Regarding the quality of imported organic fertilizers –those claimed by the producers to be organic with a declared nutrients content – the analysis results indicate that most of them did not meet the standards, except the sheep manure imported from New Zealand. The criteria on which fertilizers are declared to be organic are the content of organic matter and the NPK levels, which are generally never more than 4-5% except in the case of soybean-based fertilizers. The imported fertilizers evaluated in this study were found to contain high levels of N, P, and K whereas some of them had lower than the declared values. This shows that these fertilizers are blended with chemical fertilizers to increase the nutrient status as part of their marketing strategy. The source of organic materials used in production is unknown in 6 fertilizers, whereas in 3 fertilizers the sources of organic material used were poultry droppings, soybean waste and forest/coffee plantation waste. Although some of the imported organic fertilizers mentioned the micronutrients content in traces, the analysis results indicate that their content could not be detected. The copper content in Humicgro, Deliver and Growel was nil. Similarly, other nutrients like zinc and manganese in Growel and Complehumus were also nil. This indicates that the type of raw organic material and its nutrient content used for manufacturing determine the quality of the organic fertilizer with respect to its composition. Around half of the imported organic fertilizers surveyed did not mention the source of material used in production.

Table 1 Composition of different organic fertilizers.

Sl No	Name of the fertilizer	Physical status	Organic matter	Nitrogen	Phosphorus (P_2O_5)	Potassium (K_2O)	Calcium (CaO)	Magnesium (MgO)	Iron	Zinc	Manganese	Copper	Remarks
			%							ppm			
I. LOCAL													
1	Poultry	Powder	44.6	2.4	2.6	3.9	10.1	0.6	0.03	1635	972	125	Pure
2	Quail	Powder	48.0	7.17	1.73	2.46	9.64	0.62	0.11	1365	567	162	Pure
3	Cattle	Powder	83.9	2.1	1.0	1.3	4.2	0.5	0.13	215	941	37	Pure
4	Horse	Powder	74.5	2.0	0.90	2.3	6.0	0.6	0.15	241	517	59	Pure
5	Compost	Powder	43.1	2.3	2.9	6.9	3.7	2.6	0.48	148	805	37	Pure from vegetable waste and green weeds
6	Vermicompost	Powder	48.8	0.7	0.5	2.2	0.7	0.9	0.70	41	193	16	Pure (sawdust)
II. IMPORTED													
7	Sheep	Pellets	81.8	3.3 (4.0)	1.8 (1.4)	3.6 (3.0)	1.4	1.3	0.45 (T)	90 (T)	280 (T)	30 (T)	Pure (sheep)
8	INDUSOL	Pellets	80.9 (64.0)	8.1 (9.0)	3.8 (3.0)	1.9 (3.0)	1.1 (3.0)	0.3 (2.0)	0.10 (0.25)	51 (60)	0.0 (137)	49 (17)	Pure(soybean, cotton seed & sugar beet cake)
9	OMIX Bio-organic	Powder	73.7 (92.1)	4.7 (4.2)	7.9 (5-6)	5.2 (7-9)	8.4 (6.1)	0.1 (0.1)	0.12	67 (50)	nd	40 (13)	Forest/coffee plantation waste material blended with fertilizer
10	AMINA	Pellets	68.6	5.0 (5.5)	5.8 (5.5)	4.7 (5.5)	3.92	4.61 (1.5)	0.11 (T)	132 (T)	45 (T)	19 (T)	Tobacco residue /poultry manure blended with fertilizer

Note: Values in parenthesis indicates the content mentioned by the producer/manufacturer;
(T): Traces; nd: not detected

3.2 Crop Response Studies

3.2.1 Pot studies

A pot study conducted on cherry tomatoes using sandy soil with the application of vermi-compost, cattle manure and indusol was compared with chemical fertilizer and control. It was found from Table 2 that the plant height was a maximum in treatment with cattle manure followed by indusol and vermi-compost, while the minimum occurred in control. Increase in fruit yield was significant with organic fertilizer treatment over control but among the different organic fertilizers, including chemical fertilizer, there was no significant difference. The nutrient content in tomato leaves (Table 3) indicates that nitrogen content was highest in treatments with indusol (5.27%), followed by vermin-compost (4.18%), while the lowest among them was 2.53% in cattle manure. Similar trends were also observed with respect to potassium and phosphorus content, which were significantly improved over control. This is due to the presence of nutrients in the soil because of the application of organic manures and their availability and uptake by the plants.

3.2.2. Field Studies

Initial soil analysis results of the field trial sites are presented in Table 4, which shows that the soil pH was optimumally suitable for the plants, as evidenced by the low level of exchangeable aluminium. The phosphorus content was relatively higher (227ppm) in Lumapus, which was due to the regular use of NPK fertilizers in the past as common practice. The iron content in both locations was higher, which is an inherent property of Brunei soils which were formed from the weathering of iron-rich rocks and minerals. The other micronutrients such as manganese, zinc and copper were also at optimum levels.

Table 2 Effect of different organic manures on growth parameters and yield of tomato in pot studies.

Treatments	**Plant height (cm)**	**No. of branches/ plant**	**Fruit yield/ plant (g)**
Control	106.6	6	90.6
Chemical fertilizer	129.0	18	357.3
Vermicompost @10ton/ha	155.0	19	372.3
Cattle manure @10ton/ha	171.0	29	335.3
Indusol @3ton/ha	155.0	33	349.3
F-Test	*	*	*
LSD 5%	18.1	8.0	33.2
CV %	10.1	9.4	12.1
SE (+)	9.254	0.523	13.120

LSD: Least significance Difference; CV: Coefficient of variation
SE (+): Standard Error

Table 3 Effect of different organic manures on nutrients content in leaves of tomato.

Treatments	**N**	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**Fe**	**Cu**
	%					**ppm**			
Control	1.99	0.17	0.97	0.29	0.41	89	39	279	nd
Chemical fertilizer	5.62	0.57	3.11	2.12	0.71	60	117	537	3
Vermicompost @10ton/ha	4.18	0.43	5.32	1.22	0.70	125	63	399	14
Cattle manure @10ton/ha	2.53	0.46	1.64	1.22	0.79	34	35	351	21
Indusol @3ton/ha	5.27	0.55	2.48	1.25	0.47	43	77	398	11
F-Test	*	*	*	*	*	NS	*	*	*
LSD 5%	4.0	0.2	2.2	0.4	0.3	-	4.3	35.2	1.8
CV %	18.5	17.1	15.4	18.1	15.8	14.9	19.2	19.4	13.2
SE (+)	0.214	0.002	0.192	0.001	0.005	4.123	3.254	12.12	0.420

LSD: Least significance Difference; CV: Coefficient of variation
SE (+): Standard Error

Table 4 Soil physico-chemical properties at the experimental field sites.

Parameters	**Kilanas**	**Lumapus**
pH	6.3	6.2
EC (dS/m^2)	0.2	0.2
Organic matter (%)	2.6	1.59
Total Nitrogen (%)	0.07	0.07
Ex. Aluminium (me/100g)	3.3	3.0
Ex. Calcium (me/100g)	4.0	1.0
Ex. Magnesium (me/100g)	0.8	0.8
Ex. Potasssium(me/100g)	0.19	0.18
Available Phosphorus(ppm)	90	227
Available Manganese(ppm)	`10.0	5.5
Available Zinc(ppm)	7.0	8.9
Available Iron (ppm)	409	303
Available Copper (ppm)	5.8	8.1`

The results of the field studies conducted at Lumapus and Kilanas show a significant increase in the yields of sweet corn and cucumber over control and chemical fertilizer (Table 5). The increase in yield was due to the increased availability of nutrients in the soil and their uptake by the plants at optimum level. In cucumber, the application of indusol at 3 ton/ha recorded the highest yield of 35.1 ton/ha, followed by poultry manure (33.5 ton/ha) and sheep manure (28.7 ton/ha), whereas the lowest yield of 19.5 ton/ha was recorded with the control treatment. In the case of sweet corn at Kilanas, application of Humicgro at 2 ton/ha gave the recorded highest yield of 9.5 ton/ha. However, the use of sheep manure, poultry manure and indusol also resulted in relatively higher yields of 7.0, 7.7 and 7.9 ton/ha, which represent statistically significant increases over control and chemical fertilizer. Among the organic fertilizers treatment with Organic Top recorded the lowest yield of 3.8 tons/ha, which reflects the quality of the organic matter. These findings are in conformity with findings of Rosenani et al. (2006), who observed an increase in yields of the leafy vegetable *Ipomea reptans* after application of organic fertilizer produced from oil palm waste. Vimla et al. (2001) reported from their field trials that poultry manure was the most suitable organic fertilizer for organic vegetable cultivation. The poor performance of organic fertilizers blended with chemical fertilizers, such as Organic Top and Omex-Bioorganic, over pure organic fertilizers was due to the low nutrient content coupled with the leaching of nutrients from the fertilizer sources which were commonly blended.

Table 5 Effect of application of different types of organic fertilizers on the yield of cucumber and sweet corn and the residual effect on long bean and okra.

Sl No	**Treatments**	**Direct application**		**Residual Effect**	
		Cucumber	**Sweet corn**	**Long bean**	**Okra**
		Yield (ton/hectare)			
1	Control	18.4	1.7	4.8	3.6
2	Chemical Fertilizer	19.5	3.0	9.1	5.4
3	Sheep manure@6 ton/ha	28.7	7.0	10.8	5.9
4	Poultry manure@ 6ton/ha	33.5	7.7	11.5	5.1
5	Indusol@3 ton/ha	35.1	7.9	11.3	6.4
6	Omex-Bioorganic @3ton/ha	32.0	7.0	8.8	5.1
7	Comple-humus @2ton/ha	32.5	8.9	8.2	5.0
8	Humic-gro@2ton/ha	29.5	9.5	9.2	4.8
9	Green life @2ton/ha	34.4	5.3	11.3	5.6
10	Organic Top @2ton/ha	27.9	3.8	10.3	5.4
F-Test		*	*	*	*
LSD 5%		7.5	1.4	1.9	0.4
CV %		18.2	17.2	19.4	10.1
SE (±)		0.9852	0.3225	0.2965	0.1432

Note: Trials for cucumber and long bean were at Agricultural Station, Lumapus whereas sweet corn and okra trials were conducted at BARC, Kilanas
LSD: Least significance Difference; CV: Coefficient of variation
SE (±): Standard Error

Subsequent residual studies in the same experimental plots using long beans (Lumapus) and okra (Kilanas) showed an increase in yield with residual organic fertilizers over control (Table 5). The highest yield of 11.5 ton/ha was recorded with poultry manure treatment in the case of long beans while indusol treatment recorded the maximum yield of 6.4 ton/ha for okra. This may be due to the availability of nutrients in the soil as a residual from the fertilizer which was applied for the previous crop, and which is released slowly because of its organic nature and smaller leaching losses. The long-term effects of the use of organic fertilizers in improving soil fertility and contributing towards yield have been reported in Lin et al., 1973 and Chao et al., 1966.

Soil analysis for organic matter content after the harvest of cucumber and sweet corn found a higher but statistically insignificant increase with the application of organic fertilizers (Table 6). Treatment with indusol recorded the highest organic matter content of 1.54% and 1.69% in the soils at Kilanas and Lumapus trial respectively. This trend indicates that application of organic fertilizers increases the content of organic matter as well as supplying nutrients for the plant in a sustainable way. Similar findings were also reported by Rosenani et al. (2006).

Table 6 Organic matter content in soil due to the application of different organic fertilizers.

Sl No	**Treatments**	**Kilanas**	**Lumapus**
		%	
1	Control	1.26	1.54
2	Chemical Fertilizer	1.28	1.63
3	Sheep manure@6 ton/ha	1.46	1.64
4	Poultry manure@ 6ton/ha	1.52	1.67
5	Indusol@3 ton/ha	1.54	1.69
6	Omex-Bioorganic @3ton/ha	1.51	1.54
7	Comple-humus @2ton/ha	1.49	1.53
8	Humic-gro@2ton/ha	1.47	1.46
9	Green life @2ton/ha	1.52	1.65
10	Organic Top @2ton/ha	1.45	1.68
	F-Test	NS	NS
	LSD 5%	-	-
	CV %	16.4	15.7
	SE (±)	0.1225	0.1025

LSD: Least significance Difference; CV: Coefficient of variation
SE (±): Standard Error

4. Conclusion

The studies described in this paper show that the quality of organic fertilizers with respect to nutrients and organic matter content varies widely depending on the source from which it is derived and is not consistent. The majority of the organic fertilizers imported, with a few exceptions, did not meet the specifications declared by the manufacturer and most of them were blended with chemical fertilizers in order to increase their nutrient content and to facilitate an immediate response for the users as part of their marketing strategy. Pot and field trials showed a good response to the use of organic fertilizers in vegetables and also a residual effect for the succeeding crop. However, further investigation is needed, in the form of response studies for other crops and the screening of imported organic fertilizers for heavy metals status, which is becoming more controversial within the scientific community because of its effects on human health and soil contamination.

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COOLING TOWERS OF MARINE SNAILS: IS HIGHER BETTER?

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Abstract: Under extremely hot conditions, air-exposed eulittoral fringe *Echinolittorina* snails lift their shells off the rocky surface and sometimes climb onto their neighbor's 'backs' to form a stack. In the general context of predicting how ectothermic animals might alleviate the effects of climate warming through behavioural and physiological adaptations, we assessed the effectiveness of stacking behaviour in lowering the maximum body temperatures of these snails. Using a biophysical model, we found that the uppermost snail in a three-tier stack had the lowest operative body temperature at the highest temperatures experienced. However, this was only slightly lower than that of the middle snail, whereas both the upper and middle snails had much lower body temperatures than the bottom snail. This suggests that the uppermost position presents a compromise between improved convective cooling at the higher elevation and increased heating due to greater exposure to direct solar irradiance (the lower snails are partially shaded by the upper one). Although shell stacking clearly lowers body temperature, the discussion concerns whether all aspects of this behaviour are driven by evolutionary selection – the data suggest rather that a three-tier stack might be incidental.

1. Introduction

The body temperatures of ectothermic animals generally relate to ambient environmental temperature, which is usually the air temperature. A current concern of ecologists is to understand how ectothermic animals are likely to respond to the future predicted global elevation of air temperature associated with the greenhouse effect (climate warming). It has been shown that through their behaviour, many ectothermic animals can reduce the effects of climate warming, especially terrestrial animals that can move to cooler, shady places (Kearney et al., 2009). Another recent study suggests that some ectotherms may not be immediately affected by climate warming because they have adapted to heat sources that are unrelated to climate (Marshall et al., 2010a). This study shows that the maximum body temperature of marine rocky eulittoral fringe snails (*Echinolittorina* – which spend most of their lifetime in air) is determined by solar and re-radiative heating from the rock surface. Intertidal rocks heat up considerably, and these snails can survive exposure to body temperatures of 56.5°C, which well exceeds the maximum climatically-relevant air temperature for the region (around 34.5 °C; Marshall et al. 2010a).

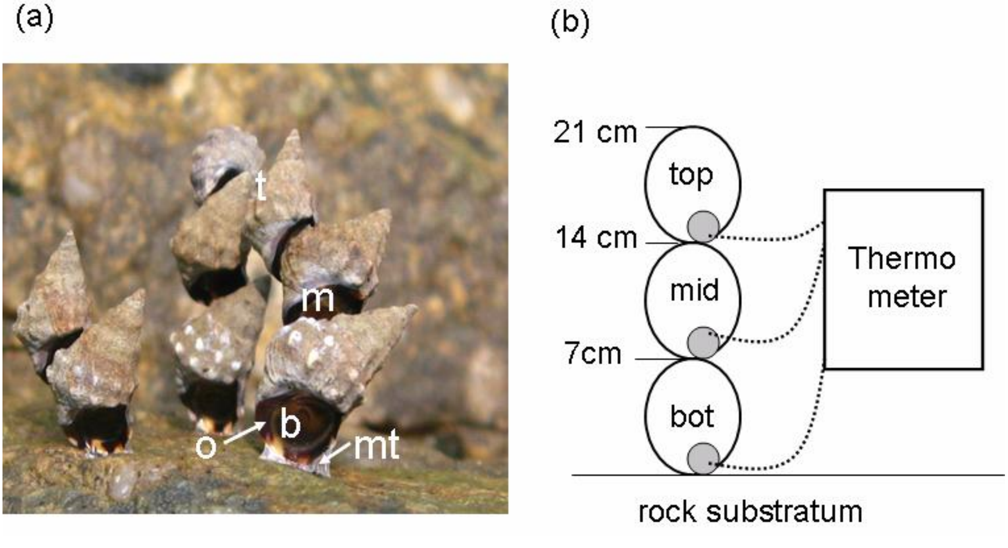
Echinolittorina snails typically seek shade in crevices or under rocks, where this is available. However, where shady refuges are unavailable, they are known to exhibit a behavioural response to extreme heating in the form of raising their shells off the hot rocky surface and cementing them in an upright position with mucus, or they climb onto their neighbour's 'backs' (referred to hereafter as stacking behaviour; see Figure 1a). Although these behaviour patterns are well known, there are no empirical data to assess their effectiveness in terms of reducing body temperature.

Because the uppermost snail in a stack is furthest from the surface of re-radiation, it might be expected to have lowest body temperature. However, this snail also experiences greater exposure to direct solar irradiance compared to the snails below it, which it partially shades. Using a simple biophysical model (Figure 1b), we aimed to determine in particular the effectiveness of stacking behaviour in lowering the body temperature in *Echinolittorina* snails. It was hoped that this study would also provide insights into the evolutionary selective advantage of stacking behaviour.

2. Materials and Methods

Echinolittorina malacanna is a tropical eulittoral fringe snail occurring throughout South East Asia and parts of Australia (Reid, 2007). We studied a population found between 2 to 5 m above Chart Datum (mean high water level ~ 2.5 m CD) on artificial seawalls at Jerudong in Brunei Darussalam (4°32’N, 114°43’E). During aerial emersion this snail typically withdraws its foot into the shell, cements its shell to the rock surface with mucus and seals the aperture with its operculum. Such behaviour prevents heat conduction (by excluding tissue contact with the rock substratum) and limits evaporative cooling. Thus, all heat gain is by means of convection and solar radiation, and heat loss is by means of convection.

Figure 1 (a) Shell lifting and stacking behaviour of *Echinolittorina malacanna. t, m, b* indicate the top, middle and bottom snails in a three-tiered stack. *mt* indicates the mucus thread used for attachment of the lifted shell, and *o* the operculum which closes the aperture in air-exposed snails. (b) The design of the biophysical model used in experiments representing a three-tiered stack.



Biophysical models have been used to assess the operative body temperatures (T_bs) of several other intertidal organisms (Helmuth et al., 2005; Helmuth et al., 2006; Denny and Harley, 2006). In the present study, a biophysical model was constructed comprising three shells of *Echinolittorina* (shell length ~ 7 mm) glued on top of one another and to a rock surface (Figure 1b). Prior to gluing, the shells were plugged with silicone and a thermocouple was inserted into each shell. The T_b within each shell was recorded simultaneously in the field (FOS rooftop) over 6 days. $T_{b(\mathrm{top})}$, $T_{b(\mathrm{mid})}$ and $T_{b(\mathrm{bot})}$ indicate temperatures inside the uppermost, middle and bottom shells, respectively (Figure 1b). Because there was a close correlation between the T_bs of model snails glued directly to rocks and live snails (determined in their natural habitat at Jerudong), we regarded the model to reflect the real situation ($r = 0.9086$, $p < 0.001$, $n = 60$).

Temperatures were recorded every 1 min between 12.00 and 14.00 (the hottest period of the day) using fine K-type thermocouples and two dual channel Fluke 54 Series II recording thermometers. The data were downloaded to Fluke Viewforms software and transferred to Excel files. The maximum and mean temperatures for the period 12.00 to 14.00 were determined for each treatment (top, middle, bottom snails) for each day.

3. Results

Maximum daily T_bs (both absolute maximum and mean) were found to differ consistently among the snails in relation to their position in the three-tiered stack (see Table 1 and Figure 2 – for clarity of representation only the data for the last 3 days are given). The highest T_bs were recorded in the bottom snail and the lowest T_bs in the uppermost snail ($T_{b(\mathrm{bot})} > T_{b(\mathrm{mid})} > T_{b(\mathrm{top})}$; see Table 1 and Figure 2). There was however a marked difference in the differential of $T_{b(\mathrm{bot})}$ and $T_{b(\mathrm{mid})}$, and of $T_{b(\mathrm{mid})}$ and $T_{b(\mathrm{top})}$, with that for the latter becoming increasingly smaller as temperatures rose (Figure 2 and Figure 3 – the plot of $T_{b(\mathrm{mid})}$ and $T_{b(\mathrm{top})}$ against $T_{b(\mathrm{bot})}$ for a single day). A plot of the differential for $T_{b(\mathrm{top})}$ and $T_{b(\mathrm{bot})}$ against $T_{b(\mathrm{bot})}$ for the last three days further shows that the difference between $T_{b(\mathrm{top})}$ and $T_{b(\mathrm{bot})}$ increases during heating (Figure 4).

Table 1 Mean, maximum and minimum T_bs for days 4, 5 and 6 for between 12.00 and 14.00.

	Day 4			**Day 5**			**Day 6**		
	$T_{b(\mathrm{bot})}$	$T_{b(\mathrm{mid})}$	$T_{b(\mathrm{top})}$	$T_{b(\mathrm{bot})}$	$T_{b(\mathrm{mid})}$	$T_{b(\mathrm{top})}$	$T_{b(\mathrm{bot})}$	$T_{b(\mathrm{mid})}$	$T_{b(\mathrm{top})}$
Mean	41.02	36.34	35.49	46.26	40.15	39.11	35.43	32.58	31.39
Max	44	40.4	39.1	48.8	42.7	41.5	39.4	35.9	34.5
Min	38.2	33.4	32.8	41.8	41.8	37.3	31.8	30.2	29.2

4. Discussion

Tropical *Echinolittorina* snails experience some of the highest body temperatures of metazoan animals aside from those associated with hydrothermal and hot springs. Although their general behaviour is extremely complex (they form dense aggregations and follow trials), much of their behaviour seemingly relates to reducing the effects of solar heating of the rock surfaces on which they live (Garrity, 1984; McMahon, 1990). This study shows that stacking effectively reduces body temperatures. However, as temperatures rose there was a reduced difference between the maximum body temperatures of the middle and upper snail, suggesting a tradeoff by the upper snail between improved convective cooling at a higher elevation and enhanced heating through greater exposure to direct solar irradiance. The observation that elevation becomes increasingly more effective in reducing body temperature at the highest temperatures (a steep positive relationship between the differential between the temperatures of the bottom and top snails as temperatures rise), suggests that re-radiative heating could be more important to body temperature than direct solar radiation at these temperatures. However, this interpretation is made with reservation, given that the model design caused shading of the bottom snail.

Figure 2 Temperature recordings made between 12.00 and 14.00 on days 4, 5, 6. The thin line represents $T_{b(\mathrm{bot})}$, the thick line, $T_{b(\mathrm{mid})}$ and the line with open circles, $T_{b(\mathrm{top})}$. Note that on hotter days (4 and 5) $T_{b(\mathrm{top})}$ and $T_{b(\mathrm{mid})}$ become similar.

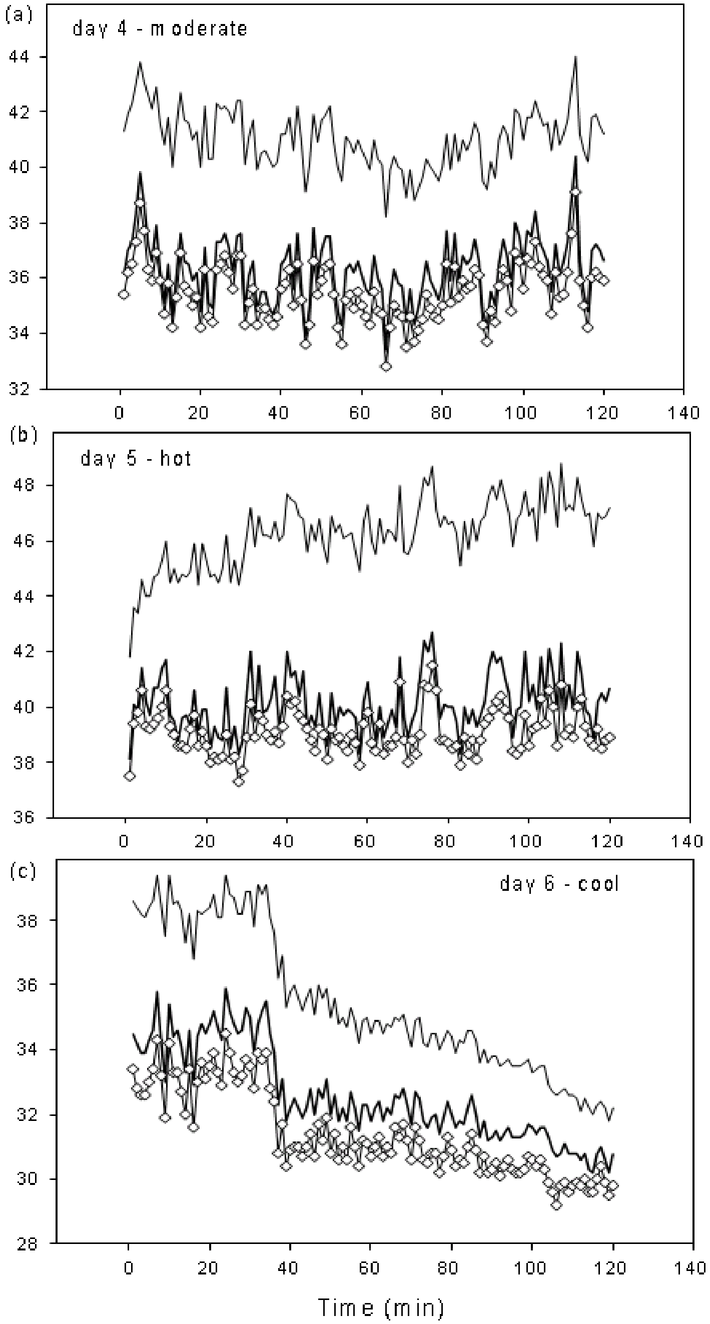


Figure 3 Linear regressions for $T_{b(\text{top})}$ (closed circles) and $T_{b(\text{mid})}$ (open circles) against $T_{b(\text{bot})}$ for day 4. There was a significant difference between $T_{b(\text{top})}$ and $T_{b(\text{mid})}$ ($T_{b(\text{top})}$: $r^2 = 0.9032$; $p = 0.001$; $y = -3.22 + 0.94x$; $T_{b(\text{mid})}$: $r^2 = 0.8491$; $p < 0.001$; $y = -5.86 + 1.02x$; ANCOVA, $F = 936$, $p < 0.001$.) Reference data for $T_{b(\text{bot})}$ are indicated by triangles.

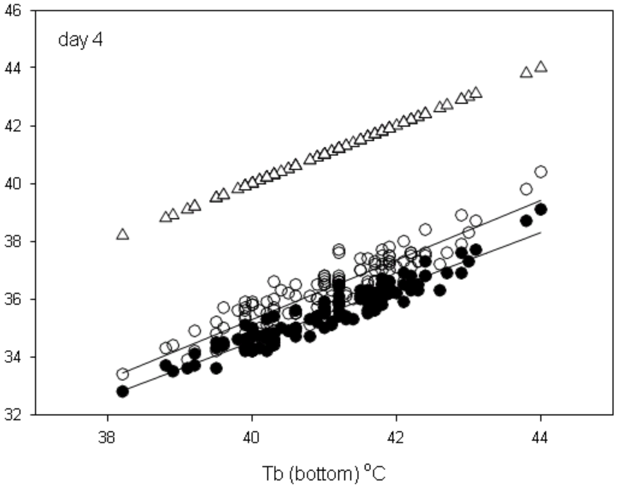
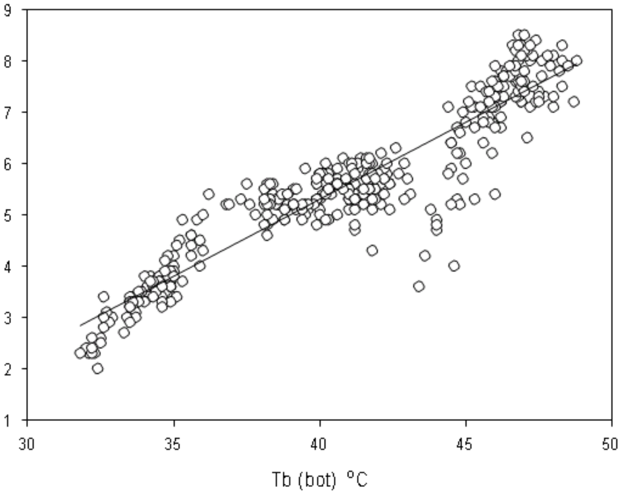


Figure 4 Linear regression for the difference between $T_{b(\text{bot})}$ and $T_{b(\text{top})}$ against $T_{b(\text{bot})}$ for days 4, 5, 6. ($y = -6.7 + 0.3x$, $r^2 = 0.865$, $n = 360$). This shows that the difference between $T_{b(\text{top})}$ and $T_{b(\text{bot})}$ increases with increasing temperature.



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UPDATED CHECKLIST OF THE AMPHIBIANS OF THE ULU TEMBURONG NATIONAL PARK AND BRUNEI DARUSSALAM

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Abstract: Seven new records of amphibians are reported for Brunei Darussalam bringing the total number of species to 80. Two of the new records are Bornean endemic frogs with restricted ranges. Within the Ulu Temburong National Park, two new records are reported with the total species number now at 68. *Philautus ingeri* was found in the lower montane forest on Bukit Belalong, an area not well sampled and likely to reveal additional diversity. Furthermore, *Hylarana glandulosa*, a common peat swamp denizen, was found to have recently invaded the park, suggesting turnover of species assemblages within rainforest environments.

1. Introduction

The description and functional analysis of biological diversity lies at the core of ecological and evolutionary investigations. Biodiversity is fundamental to human well-being in agriculture, livestock, forestry, economic output and for its ecological services and functions (Wilson, 1988). A better understanding of biodiversity helps to focus conservation efforts and political policies, as well as enabling us to address existing environmental problems such as the impacts of foreign species introduced to native ecosystems and the effects of climate change. Updating and expanding the level of knowledge about species diversity and species richness is central to these efforts.

Rain forests of northwestern Borneo have been identified as one of the world’s biodiversity hotspots that are exceptional in high species richness and levels of endemism (Meyers, 1988). Borneo is home to over 160 amphibians (Das. 1995; Manthey and Grossmann, 1997; Malkmus et al., 2002; Veith et al., 2004; Inger and Stuebing, 2005; Das, 2007; Dehling, 2008; Grafe and Keller, 2009).

In 2007, Das reported 62 species for Brunei Darussalam. However, comprehensive surveys carried out in the Ulu Temburong National Park (UTNP, Temburong District; Cranbrook and Edwards, 1994) revealed that the amphibian inventory of Bruneian forests was far from complete (Grafe and Keller, 2009). Grafe and Keller (2009) reported 66 species within the park, 15 species were new records for the UTNP and 12 species were new records for Brunei Darussalam.

In this study, we provide an updated list of the amphibian fauna of Brunei Darussalam based on recent extensive surveys in two key areas of the country: the UTNP and the Bukit Teraja Protection Forest (BTPF, Belait District; Ashton, 2010). In addition, this study provides longitudinal data on the presence or absence of amphibian species that will aid in evaluating long-term trends in population dynamics and regional extinctions. Furthermore, since amphibians are good indicators of forest condition as they are sensitive to forest degradation and climate change, this inventory provides insights into larger ecological processes.

2. Materials and Methods

Standardized visual and acoustic transect sampling and encounter surveys were conducted at least once a month in 2009 and between January and April 2010 within the UTNP and in April 2010 within the BTPF in both riparian and forest leaf litter habitats. In addition, a one-night survey was conducted in the lower montane forest of Bukit Belalong in March 2009 (UTNP). Chance observations were also made in urban and disturbed habitats in other areas of Brunei. Further details of survey methods are provided by Grafe and Keller (2009).

3. Results and Discussion

We found seven new records of amphibians for Brunei Darussalam during our surveys (Tables 1 and 2). Two species, *Duttaphrynus melanostictus* and *Kaloula pulchra* had not been previously reported although they are widespread in urban and disturbed habitats. Their numbers appear to be on the increase in parallel to urban development. *Kaloula baleata* is also a commensal species. We found males of this species calling from stagnant pools near the Teraja longhouse. *Limnonectes malesianus* was found twice along the forest trails in the BTPF. Two additional species were encountered in the BTPF in 2010: *Microhyla perparva* (an adult gravid female) and *Hylarana nicobariensis* (adult male). *Philautus ingeri* was found about 2 m above ground in the lower montane forest of Bukit Belalong (UTNP) at approximately 900 m above sea level in March 2009. These records bring the total number of amphibian species in Brunei Darussalam to 80 (Tables 1 and 2).

Two new records are reported for the UTNP (Table 1): *Philautus ingeri* from Bukit Belalong and *Hylarana glandulosa* that has moved into the park from the buffer zone. *H. glandulosa* breeds in ponds and ditches and can be regarded as a pioneer species that invades disturbed areas. We first encountered this species in 2009 within the buffer zone of the UTNP. Since then it has become quite common in the buffer zone and has invaded adjacent areas of the park. It remains to be seen whether it can establish itself within the core community of rainforest frogs. These new records bring the total number of amphibians within the park to 68 species. Thus, the UTNP harbours a remarkable 85% of the amphibian species richness of Brunei.

Our most recent surveys within the UTNP also confirm the presence of 52 out of the 68 species reported (Table 1), suggesting that the vast majority of species still have large enough populations even within the limited area of the park surveyed. Those species not encountered recently were never previously seen in large numbers and appear to have low population sizes.

Over half of Brunei's amphibian fauna (55%) are Bornean endemics, suggesting a high degree of vulnerability and a need for conservation. Of the seven species newly recorded, two are Bornean endemics, *Microhyla perparva* and *Philautus ingeri*. The latter species is vulnerable, whereas the other six species are either classified as least concern or threatened (Tables 1 and 2).

Table 1 Amphibian species recorded from the Ulu Temburong National Park (UTNP). Species names follow Frost et al. (2006). Data source (D = Das, 2007, GK = Grafe and Keller, 2009, N = new record). Country codes of known species distribution: BD = Bangladesh, BN = Brunei Darussalam, CN = China, ID = Indonesia, IN = India, KH = Cambodia, LA = Lao People's Democratic Republic, MM = Myanmar, MY = Malaysia, NP = Nepal, PH = Philippines, SG = Singapore, TH = Thailand and VN = Vietnam. Conservation status follows the Global Amphibian Assessment (2007) listings of IUCN red list categories (LC = Least Concern, NT = Near Threatened, VU = Vulnerable, EN = Endangered). New records are shown in bold.

Species	Data source	Presence confirmed 2009-2010	Country code	Conservation Status
Ichthyophiidae				
Caudacaecilia nigroflava	D, GK	+	BN, MY	DD
Bufonidae				
Ansonia sp.	GK	-	BN	DD
Ansonia albomaculata	D, GK	+	BN, ID, MY	NT
Ansonia leptopus	D, GK	+	BN, ID, MY	NT
Ansonia longidigita	D, GK	+	BN, ID, MY	NT
Ansonia platysoma	GK	-	BN, MY	EN
Ingerophrynus divergens	D, GK	+	BN, ID, MY	LC
Pedostibes hosii	D, GK	+	BN, ID, MY, TH	LC
Pedostibes rugosus	D, GK	+	BN, ID, MY	NT
Pelophryne signata	D, GK	+	BN, MY	NT
Phrynoidis aspera	D, GK	+	BN, ID, MM, MY, TH	LC
Phrynoidis juxtaspera	D, GK	+	BN, ID, MY	LC
Ceratobatrachidae				
Ingerana baluensis	D, GK	+	BN, ID, MY	LC
Ingerana sp	GK	-		-
Dicroglossidae				
Limnonectes ibanorum	D	+	BN, ID, MY	NT
Limnonectes ingeri	D, GK	+	BN, ID, MY	NT
Limnonectes kuhlii	D, GK	+	BN, CN, ID, IN, LA, MM, MY, TH, VN	LC
Limnonectes laticeps	D	-	BN, ID, IN, MM, MY, TH	LC
Limnonectes leporinus	D, GK	+	BN, ID, MY	LC
Limnonectes palavanensis	D, GK	+	BN, ID, MY, PH	LC

Limnonectes paramacrodon	GK	-	BN, ID, MY, SG, TH	NT
Occidozyga baluensis	D, GK	+	BN, ID, MY	NT
Occidozyga laevis	D, GK	+	BN, ID, MY, PH, SG, TH	LC
Megophryidae				
Leptobrachella mjobergi	D, GK	+	BN, ID, MY	LC
Leptobrachella parva	GK	+	BN, ID, MY	NT
Leptobrachium abbotti	D, GK	+	BN, ID, MY	LC
Leptobrachium montanum	D, GK	+	BN, ID, MY	LC
Leptolalax dringi	GK	+	BN, ID, MY	NT
Leptolalax gracilis	D, GK	+	BN, ID, MY	NT
Leptolalax pictus	GK	-	BN, ID, MY	VU
Megophrys edwardinae	D	+	BN, MY	VU
Megophrys nasuta	D, GK	+	BN, ID, MY, SG, TH	LC
Microhylidae				
Chaperina fusca	D, GK	+	ID, MY, PH, TH	LC
Kalophrynus intermedius	D, GK	-	BN, ID, MY	VU
Kalophrynus pleurostigma	D, GK	+	BN, ID, MY, PH, SG, TH	LC
Kalophrynus subterrestris	GK	-	BN, ID, MY	NT
Metaphrynella sundana	D, GK	+	BN, ID, MY	LC
Microhyla borneensis	D, GK	-	BN, ID, MY, SG, TH	LC
Microhyla petrigena	D, GK	-	BN, ID, MY, PH	NT
Ranidae				
Hylarana erythraea	D	-	BN, ID, KH, LA, MM, MY, SG, TH, VN	LC
Hylarana glandulosa	N	+	BN, ID, MM, MY, TH	LC
Hylarana megalonesa[1]	D, GK	+	BN, ID, MY	LC
Hylaran picturata	D, GK	+	BN, ID, MY	LC
Hylarana signata	D, GK	+	BN, ID, MY, TH	LC
Meristogenys jerboa	GK	+	BN, MY	VU
Meristogenys orphnocnemis	D	-	BN, ID, MY	LC
Meristogenys poecilus	D, GK	+	BN, ID, MY	NT
Odorrana hosii	D,GK	+	BN, ID, MY, TH	LC
Staurois latopalmatus	D,GK	+	BN, ID, MY	LC
Staurois guttatus	D,GK	+	BN, ID, MY, PH	LC

Staurois tuberilinguis	D,GK	+	BN, ID, MY	NT
Rhacophoridae				
Nyctixalus pictus	D	+	BN, ID, MY, PH, SG, TH	NT
Philautus hosii	GK	+	BN, ID, MY	NT
Philautus ingeri	N	+	BN, ID, MY	VU
Philautus tectus	D, GK	+	BN, MY	VU
Polypedates leucomystax	D, GK	+	BD, BN, CN, ID, IN, KH, LA, MM, MY, NP, PH, SG, TH, VN	LC
Polypedates macrotis	D, GK	+	BN, ID, MY, PH, TH	LC
Polypedates otilophus	D, GK	+	BN, ID, MY	LC
Rhacophorus belalongensis	GK	+	BN, MY[2]	-
Rhacophorus cyanopunctatus	GK	-	BN, ID, MY, SG, TH, VN	LC
Rhacophorus dulitensis	D	-	BN, ID, MY	NT
Rhacophorus fasciatus	D	-	BN, ID, MY	VU
Rhacophorus harrissoni	D	+	BN, ID, MY	NT
Rhacophorus kajau	D	+	BN, ID, MY	NT
Rhacophorus nigropalmatus	D	+	BN, ID, MY, TH	LC
Rhacophorus pardalis	D, GK	+	BN, ID, MY, PH	LC
Rhacophorus rufipes	GK	+	BN, ID, MY	NT
Theloderma sp.	D	-	-	-

[1] Replaces *Hylarana raniceps* in Brunei, Inger et al (2009). [2] Also found in Gunung Mulu (Sarawak; TU Grafe unpublished).

Table 2 Additional species found in Brunei Darussalam not found in the Ulu Temburong National Park (UTNP). See Table 1 for meaning of abbreviations. New records are shown in bold.

Species	Data source	Presence confirmed 2009-2010	Country code	Conservation Status
Bufonidae				
Ingerophrynus quadriporcatus	D	+	BN, ID, MY, SG	LC
Duttaphrynus melanostictus	N	+	Widespread throughout East, South and SE Asia	LC
Dicroglossidae				
Fejervaya cancrivora	D	+	Widespread throughout SE Asia	LC
Fejervaya limnocharis	D	+	Widespread	LC

			throughout SE Asia	
Limnonectes malesianus	N	+	BN, ID, MY, SG, TH	NT
Microhylidae				
Kaloula baleata	N	+	Widespread throughout East, South and SE Asia	LC
Kaloula pulchra	N	+	Widespread throughout East, South and SE Asia	LC
Microhyla perparva	N	+	BN, ID, MY	NT
Ranidae				
Hylarana baramica	D	+	BN, ID, MY, SG	LC
Hylarrana nicobariensis	N	+	BN, ID, IN, MY, PH, TH	LC
Rhacophoridae				
Polypedates colletti	D	+	BN, ID, MY, TH	LC
Rhacophorus appendiculatus	D	+	BN, ID, IN, MY, PH	LC

In conclusion, this study confirms the high species richness of amphibians found in Brunei and especially in the UTNP. The UTNP remains one of Borneo’s amphibian hotspots (Grafe and Keller, 2009). However, other areas within Brunei have not received the degree of survey intensity and may well have similar species richness. Our amphibian data clearly supports the assessment by Ashton (2010) that the Bukit Teraja area merits the highest priority for exploration and biodiversity assessment. Recent surveys in the BTPF suggest that substantial amphibian diversity remains undetected there and, from what is known, exhibits significant low complementarity to the UTNP. Other conservation areas within Brunei that have not been sampled extensively may hold similar biodiversity value, especially heath forests, peatswamp forests and montane forests. It is hoped that the BTPF and other areas within the Heart of Borneo Project, such as the Sg. Ingei Conservation Area, will come under the same degree of protection as the UTNP.

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